

Rediscovery of four narrow endemic *Didymocarpus* species (Gesneriaceae) from Mizoram, India, with revised species descriptions and lectotypifications

Naibi Shrungeshwara Prasanna¹, Vinita Gowda¹

¹ *Tropical Ecology and Evolution (TrEE) Lab, Department of Biological Sciences, Indian Institute of Science Education and Research, Bhopal, 462066, India*

Corresponding author: Vinita Gowda (gowdav@iiserb.ac.in)

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Abstract

Here we report the rediscovery of four endemic gesneriads from the state of Mizoram, India, after a span of 86 to 90 years since their last collection. The four species belong to the genus *Didymocarpus* Wall. and they are: *D. adenocarpus*, *D. lineicapsa*, *D. parryorum*, and *D. wengeri*. We present revised morphological descriptions, photographs, and designate lectotypes for *D. parryorum* and *D. wengeri*. During our study we came across several discrepancies between morphological characters assigned to these four species in the protologue and morphological characters present (or absent) in the type specimens and in plants recollected by us. We list these discrepancies in a section titled ‘amendments to protologue’. Based on the high endemism and critical conservation status of all the four rediscovered species, we suggest that floristic studies along with large-scale biogeographic studies should be prioritized in the Indo-Burmese region.

Keywords

C.E.C. Fischer, gesneriads, Lushai hills, nomenclature, Northeast India, pair-flowered cyme, taxonomy

Introduction

The genus *Didymocarpus* Wall. was recently redefined by Weber and Burtt (1998) and it now consists of approximately 100 species that are distributed in India, Nepal, Bhutan, southern China, Myanmar, Thailand, Vietnam, Laos, Cambodia, Peninsular Malaysia,

and Sumatra (Möller 2019). Phylogenetic studies by Palee et al. (2006) suggested the geographic origin of the genus to be Malay Peninsula, although the northeast region of India along with southern China accounts for more than half of known *Didymocarpus* species (Möller et al. 2017; Möller 2019). India is known to have about 25 species of *Didymocarpus* most of which are narrow endemics, restricted to relatively unexplored areas of Northeast India (Prasanna et al. 2020). Within India, they are mainly distributed in the Indo-Burmese and Eastern Himalayan region with one species extending into the Western Himalayas (Möller et al. 2017; Roy 2017).

Mizoram, formerly known as Lushai hills, is a small state in the northeast region of India and was part of the state of Assam until 1972. It is bordered to the west by Bangladesh and to its east and south by Myanmar, and it is part of the Indo-Burmese biogeographic region. Two of the earliest colonial-era plant collectors who can be credited with collecting gesneriads in this region are Mrs Anne Parry (commonly cited as Mrs N.E. Parry, *pers. comm.* H. Noltie) and Rev. W.J.L. Wenger. Parry is known to have accompanied her husband, a British officer, who was appointed as the Superintendent of Lushai hills, Mizoram (then Assam) from 1924–1928 (Parry 1932). Wenger was a Baptist missionary who was sent to work at the Baptist church of Lunglei, Mizoram from 1922 to 1933 (Lalzama 1990). During their stay in Mizoram, both Parry and Wenger independently made extensive plant collections within the northeast region of India and they regularly sent their collections to Kew, where C. E. C. Fischer identified and described these species and published floristic studies (Fischer 1928a–c, 1938). Here we report rediscoveries of four of these species which were first collected by either Parry or Wenger and later described by Fischer. All four gesneriads reported here have not been collected since either Parry or Wenger collected them in the early 1900s, despite recent revisionary, taxonomic, or floristic studies carried out in the Northeast of India (Sinha 2012; Sinha and Datta 2016; Roy 2017). Thus, our rediscovery of the four species is clocked at approximately 86 to 90 years since their last collection. Our observations suggest that all four species are narrow endemics within the state of Mizoram, India, which was also noted by earlier botanists like Wenger (Fischer 1928a) in the type description (Fig. 1).

The rediscovery of the four *Didymocarpus* species is an outcome of our concerted effort to collect gesneriads from the Northeast of India for our ongoing revision of the genus using a molecular phylogenetic approach. Although there have been several revisionary studies of *Didymocarpus* from India (Sinha 2012; Sinha and Datta 2016; Roy 2017), these studies either omitted to list a species (e.g., *D. lineicapsa*) rediscovered by us or we found errors in their taxonomic descriptions. We therefore utilized freshly collected material which allowed us to make an in-depth exploration of morphological characters that are taxonomically important but may be difficult to study in herbarium specimens. We have made several amendments to the current taxonomic descriptions of the four rediscovered species and these are given in a separate section titled ‘amendments to protologue’ (see materials and methods below). Finally, our description and taxonomic study of *D. parryorum* C.E.C.Fisch. and *D. wengeri* C.E.C.Fisch. also resulted in our discovery that Fischer did not assign unambiguous types to both these taxa. We therefore also assign lectotypes for both these species here.

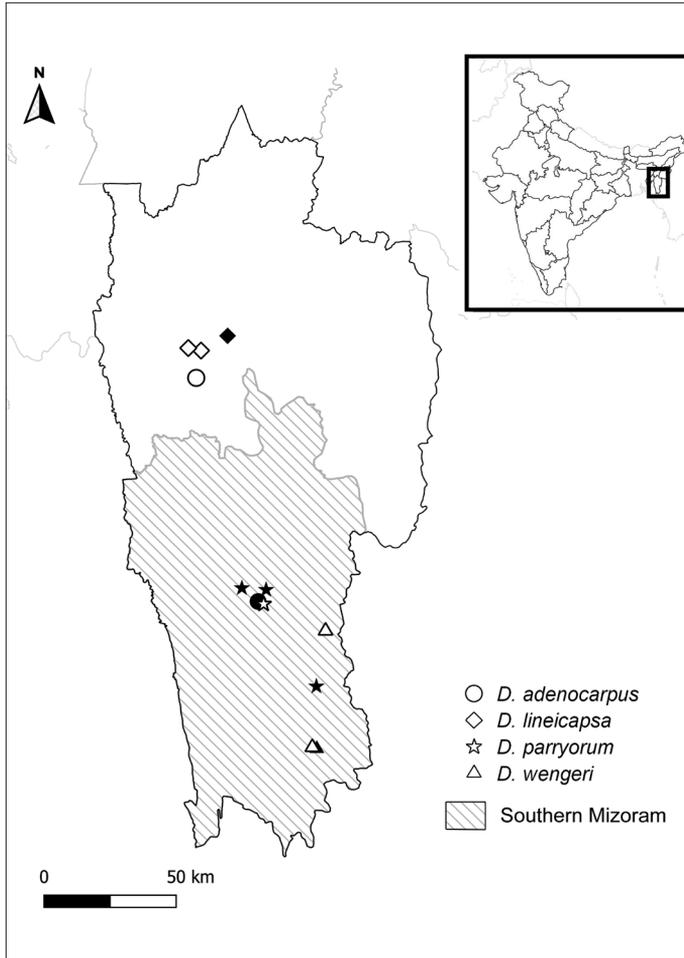


Figure 1. Map showing distribution records of the four rediscovered species of *Didymocarpus* Wall. (Gesneriaceae) in Mizoram, India. Solid symbols indicate historical collection sites and open symbols indicate extant populations. Hatched area represents southern Mizoram which is referred to as ‘South Lushai hills’ in the protologues and historical collections.

Materials and methods

Field expeditions were carried out throughout the monsoon season (July to September), when the plants are known to flower, in the years 2017 and 2018. Type localities of all *Didymocarpus* species in Mizoram and surrounding areas, including neighboring states, were visited. Due to logistic and financial constraints, we were unable to visit the field sites in the dry season and therefore the taxonomic descriptions listed here are based on rainy season forms only, although some species of *Didymocarpus* have been known to have morphologically distinct seasonal forms (Weber and Burt 1998; Nangngam and Maxwell 2013). We carried out extensive metadata collection

for each species which included scoring of morphological, phenological, reproductive and ecological characters such as flower opening time, pollinator visitation, and fruit set. Morphological measurements of five to six freshly dissected samples of each species were taken using both a ruler and a digital calliper. Plant materials used in this study include herbarium vouchers, spirit samples, and leaf tissues in silica for ongoing molecular phylogenetic studies. All herbarium vouchers collected by us are deposited at BHPL and duplicates will be deposited at ASSAM.

For taxonomic and nomenclatural work, we studied relevant *Didymocarpus* protologues along with following research materials: Kanjilal et al. 1939; Sinha 2012; Sinha and Datta 2016; Roy 2017. Herbarium collections including type specimens were consulted at ARUN, ASSAM, BHPL, CAL, E, K and BM and in online databases (Chinese Virtual Herbarium: <http://www.cvh.ac.cn/en>; Global Plants: <https://plants.jstor.org/>; Kew Herbarium Catalogue: <http://apps.kew.org/herbcat/>; Muséum National d'Histoire Naturelle: <https://science.mnhn.fr/>; Smithsonian Institution: <https://www.si.edu/>; The Linnaean Collections: <http://linnaean-online.org/>; see Appendix 1). We evaluated the conservation status for the four rediscovered species according to the latest International Union for Conservation of Nature guidelines (IUCN 2019) using species distribution ranges and size of the populations we encountered during fieldwork.

In this study, we have modified the terms that describe bracteoles and glands in all the four species and we provide rationale wherever a different term from the protologue has been used. Within Gesneriaceae, many authors including Fischer (1928a–c, Spare and Fischer 1929) have used the term ‘bracts’ to indicate modified leaves present in the inflorescence. However, in pair-flowered cymes of *Didymocarpus*, the morphology of the bracts present at the base of the primary fork may vary from those subtending the subsequent forks. To bring taxonomic clarity, we find it necessary to differentiate between these two structures and here, we use the term ‘primary bracteoles’ to indicate the modified leaves subtending the primary forks and ‘secondary bracteoles’ to indicate all the bract-like structures subtending the subsequent forks within the inflorescence.

We have added a new section ‘amendments to protologue’ which is an important part of the updated taxonomic description for all the four rediscovered species. This section has been added because we found that the morphological description of all four rediscovered species of *Didymocarpus* did not match those given in their respective protologues. Listing these discrepancies in a separate section allows us to avoid ambiguities in the description and therefore avoid future taxonomic confusions. In this section, we list all morphological differences we have noted between the protologue, type specimen and fresh specimens of the same species. Descriptive discrepancies may have resulted because Fischer wrote his taxonomic descriptions only from a limited number of herbarium material shipped to him by Parry and/or Wenger from Northeast India. Thus, the quality of the specimens may have resulted in ambiguous or erroneous descriptions (e.g., refer to discrepancy in stem color of *D. adenocarpus* and indumentum on corolla in *D. lineicapsa*).

Results

Didymocarpus adenocarpus C.E.C.Fisch., *Bull. Misc. Inform. Kew* 1929: 253. 1929. Fig. 2A–G, Suppl. material 1: Fig. S1A, B

Holotype. INDIA. Assam (= Mizoram): Southern Lushai Hills, 4500 ft., Sept. 1928, Rev. W.J.L. Wenger 239, K (K000820546!).

Revised description. Terrestrial or epilithic herbs, up to 35 cm tall. Stem 16 × 6 mm, terete, light green, sparsely pubescent with 4–10 celled eglandular hairs. Leaves 2–6 pairs, opposite and anisophyllous, decussate, terminal pair smaller in size, membranous, exstipulate; petioles 1–8 cm long, pubescent with multicellular eglandular hairs as on stem; lamina 9–15 × 5–8 cm, oblong to orbicular, lamina separated unequally by midrib, base cordate to obliquely cordate, apex acute to acuminate, margin coarsely crenate-dentate; dorsal surface green, sparsely pubescent with multicellular eglandular hairs; ventral surface pale green, densely pubescent along veins but sparsely pubescent otherwise; densely dotted with minute globose, pale-brown glistening pigment glands (in dried specimen); midrib with 6–10 lateral veins on either side, sunken above, raised below, secondary veins more prominent. Inflorescence 1 to 4, pedunculate, axillary, pair-flowered cymes (many-flowered), usually arising only from the axils of the 1–2 uppermost pairs of leaves, cyme with up to 20 flowers; primary bracteoles present, 4 × 7 mm, opposite, suborbicular, apex mucronate, glabrous, translucent white, veins visible when dried; secondary bracteoles (within the cyme) present at each dichotomous fork, 4 × 6 mm, suborbicular, apex mucronate glabrous, whitish, veins visible when dried. Inflorescence usually hidden below the leaves, pendent; peduncle 3–4 cm long, light green, lower part sparsely pubescent with multicellular eglandular hairs, upper part glabrous; pedicel ca. 5 mm long, slender, glabrous; Calyx 0.8–1 mm long, fused, narrowly funnel shaped, with 5–9 short, broadly triangular teeth with a visible vein running into each, glabrous, whitish, translucent; Corolla 2.8–3.5 cm × 0.5–0.8 cm, tubular with a slight bend, infundibuliform towards mouth; tube whitish at base, purple towards lobes; corolla bi-lipped, total 5 lobes, 0.5 × 0.5 cm, suborbicular, glabrous, purplish with whitish outer edge, the 3 lower lobes larger than the 2 upper lobes, ventral part of the corolla tube and lobes striated. Stamens 2, inserted at 1/3rd of the length of the tube from the mouth of the corolla, anthers dorsifixed, coherent by adaxial surfaces; filaments 1–1.2 cm, glabrous, whitish; staminodes 2 or 3, inserted lower than the stamens, tip bifurcated, the third when present below the others and much shorter. Disc up to 2 mm long, tubular with undulating upper margin, glabrous, persistent. Gynoecium 2.1–2.2 cm, ovary linear, slightly widened upwards, glabrous, covered with globose yellow glands; stigma peltate, glabrous. Capsule linear, brown, 3–3.5 cm long, dotted with glistening yellowish glands. Seeds very minute, pale-reddish-brown, fusiform, acute at both ends.

Amendments to protologue. Upon examining fresh specimens (Fig. 2 and Suppl. material 1: Fig. S1A, B), we noted that the stem color is light green (brown in proto-

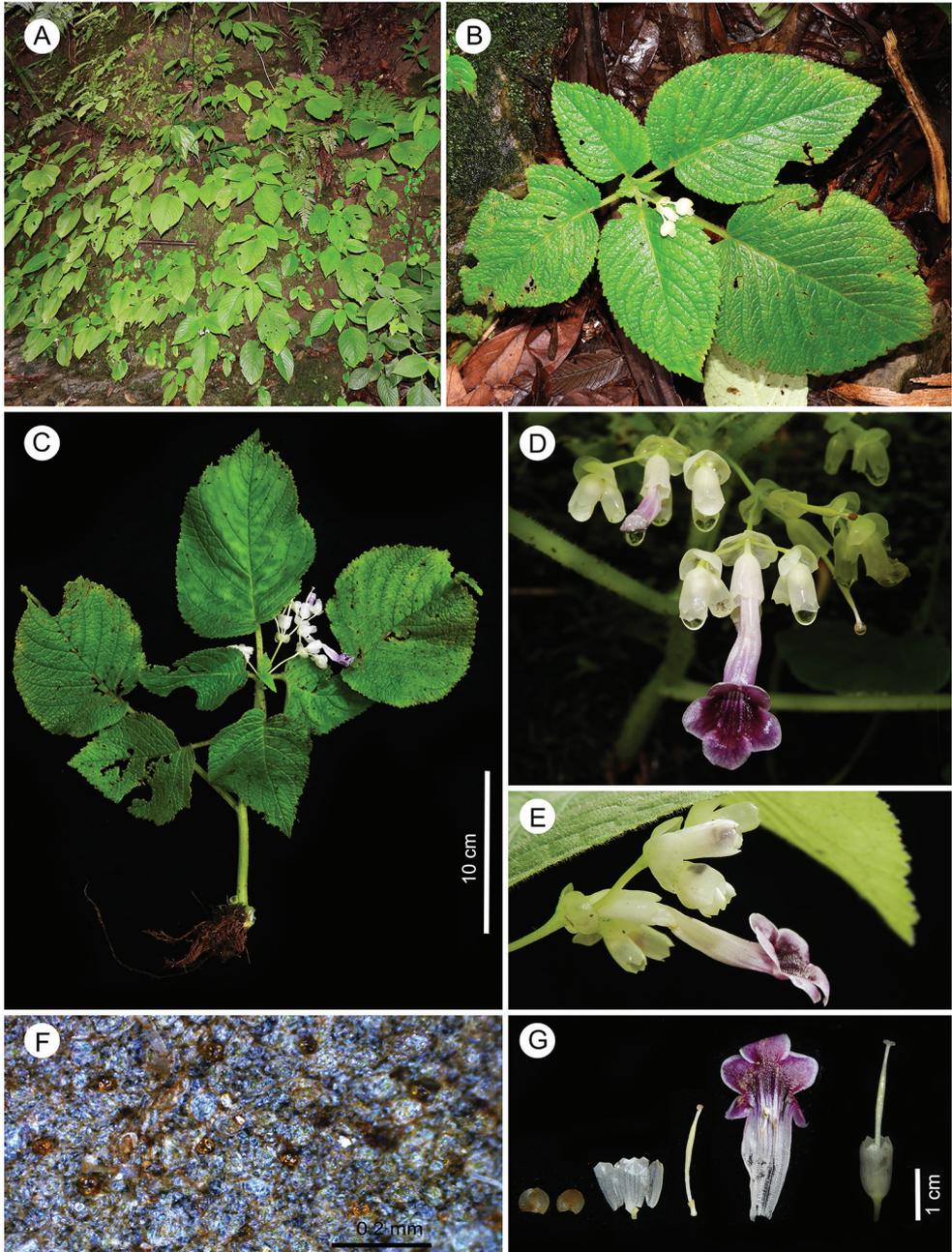


Figure 2. *Didymocarpus adenocarpus* C.E.C.Fisch. **A** habitat **B** habit **C** complete plant with inflorescence **D** pair-flowered cyme **E** flower, side view **F** glands on abaxial surface of leaves **G** floral dissection (from left to right): primary bracteole, secondary bracteole, sepal, gynoecium, open floral tube showing fused anthers, gynoecium surrounded by persistent calyx. Photographs by NSP.

logue), corolla tube is whitish at base, purple towards lobes (white tinged with pink in protologue), and bracteoles are translucent white (reddish brown in protologue). Leaf apex is acute to acuminate (acute or abruptly acutely cuspidate in protologue) and inflorescence is typical pair-flowered cyme (central 1-flower and trichotomous branching in protologue). We found stigma to be glabrous (pubescent in protologue).

Note. *D. adenocarpus* is similar to *D. purpureobracteatus* W.W.Sm. but differs from it in having slightly cordate leaves (rounded or oblique in *D. purpureobracteatus*), sparsely pubescent peduncle (glabrous in *D. purpureobracteatus*), and glabrous pistil (sparsely puberulent in *D. purpureobracteatus*).

Distribution. Historically, *D. adenocarpus* is known from southern Mizoram. However, in this study we located one extant population at Reiek Tlang in Mamit district of northern Mizoram (specimen numbers: VG2018MZ2589, VG2018MZ2590, VG2018MZ2592).

Habitat. Grows on moist loamy banks in partially shaded areas of tropical wet evergreen forests.

Phenology. Flowering in August to September, fruiting in September to December.

Ecology. We observed that *D. adenocarpus* has a tubular calyx which can retain and immerse the buds in water (see Suppl. material 1: Fig. S1B). In other gesneriads such as *Aeschynanthus* and *Chrysothemis*, a similar character was referred to as watery calyces, and was suggested as a mechanism to reduce florivory by insects (Carlson and Harms 2007).

Conservation status and preliminary IUCN assessment. *D. adenocarpus* is known from only four specimens collected from southern Mizoram, India. To the best of our knowledge there have been no further collections of *D. adenocarpus* until this study, which brings the time until its current rediscovery up to 87 years. We surveyed multiple potential locations in Mizoram and we could not locate any population in southern Mizoram. The extant population is limited to an area of about 15 km² in Reiek Tlang hills, Mamit district, which is in northern Mizoram. Although it is a community protected forest, with limited anthropogenic disturbance, the population has only 300 mature individuals. Therefore, based on the criterion C2a(i) of IUCN guidelines (IUCN 2019), we propose that the species should be considered as endangered (EN).

***Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 21(4): 187. 1954.**

Fig. 3, Suppl. material 1: Fig. S1D

Basionym. *Trisepalum lineicapsa* C.E.C.Fisch., Bull. Misc. Inform. Kew 1928 (7): 276 (1928).

Holotype. INDIA. Assam (= Mizoram): Lushai Hills, Aijal (= Aizawl), 1225 m, September 1927, Mrs N.E. Parry No.79, K (K000820539!).

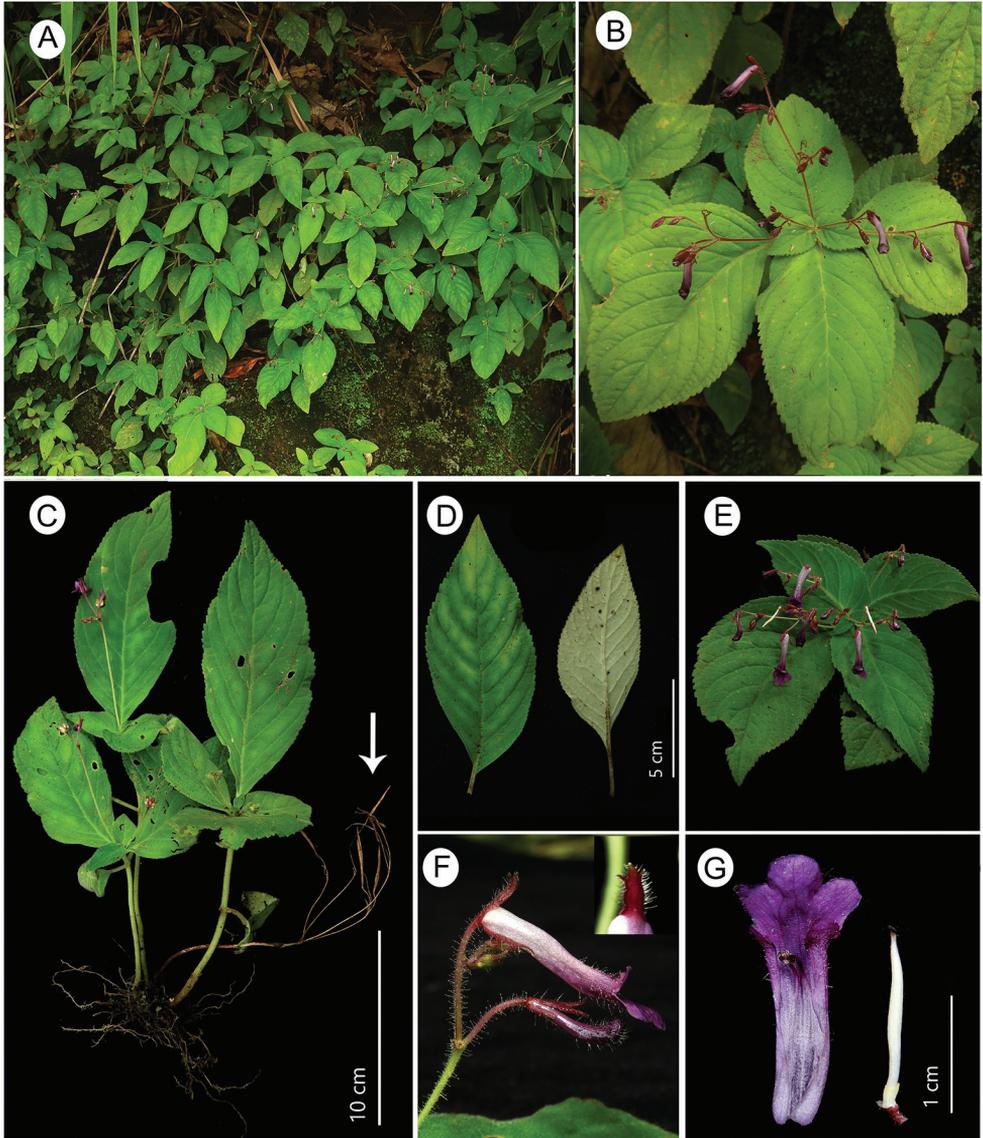


Figure 3. *Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt **A** habitat **B** habit **C** complete plant with emerging inflorescence. Old stem with dehisced capsules from previous season indicated by arrow **D** leaf adaxial and abaxial surface **E** inflorescence **F** flower (inset - tridentate calyx lobe) **G** floral dissection from left to right: open floral tube showing fused anthers, gynoeceium with disc and ovary. Photographs by NSP.

Revised description. Terrestrial or epilithic herbs, to 15 cm tall, 1 to 4 stems arising from the same rhizome. Stems 3 to 15 cm long, 2–4 mm wide at base, erect, dark green, terete, densely tomentose with 3 to 4 celled glandular hairs and sparsely interspersed globular, yellow pigment glands. Leaves 4–6 pairs, opposite and anisophyllous, decussate, often whorled at the top; petioles up to 2.7 cm long, terete, densely

tomentose as on stem, sparsely covered with globular, yellow pigment glands; lamina 3–10 cm × 1.5–3.5 cm, lanceolate to narrowly elliptic, lamina separated unequally by midrib, base oblique, apex acute; margin dentate, often entire towards the base, dorsal surface dark green, densely strigose with short eglandular hairs, ventral surface light green, strigose with yellow-glandular (colour as observed in dried specimen) and eglandular hairs, hairs more dense along the veins; midrib with 8–10 secondary veins on each side, sunken above, raised below. Inflorescence 1 to 4, axillary, spreading from upper leaves forming the whorl, erect, pair-flowered cymes (many-flowered), usually arising only from the axils of the 1–2 uppermost pairs of leaves; peduncle 1.5–6 cm long, up to 5 mm thickness (slender), sparsely covered with multicellular glandular and eglandular hairs; pedicel up to 2 cm long, pale pink, covered with multicellular glandular and eglandular hairs; bracteoles absent. Calyx 5–6.5 mm long, maroon coloured, tripartite; two segments up to 0.5 mm wide, linear-lanceolate, tip acute, free to base, held ventrally along the lower side of the corolla tube; third segment tridentate, up to 1.2 mm wide, held dorsal to the corolla tube, central tooth wider than the two lateral teeth; dorsal surface glandular-pubescent; ventral surface glabrous. Calyx not persistent. Corolla 1.5–1.8 cm long, ca. 2.2 mm wide, tubular, light purple at base but dark purple towards throat and lobes. Corolla tube usually held perpendicular to the pedicel; corolla tube glabrous at base but with multicellular glandular hairs below the lobes, hairs sometimes present also on lower part of the lobes, corolla tube glabrous on the inside; corolla bi-lipped, total 5 lobes; upper lobes 2, 1.6 × 3.1 mm, apices rounded; lower lobes 3, 6.5–7.5 × 3.5–4.5 mm, spreading at right angles to the upper lobes, middle lobe apex rounded, lateral lobes apices obtuse. Stamens 2, filament inserted at about 1/3rd of the length of the corolla tube; filaments 5–6 mm, glabrous, filament dark purple near the anthers, anthers dorsifixed, coherent by adaxial surfaces, glabrous; staminodes absent. Disc up to 2 mm, tubular, yellowish, glabrous, upper margin undulate, persistent. Gynoecium 10–11 mm, ovary white, linear, indistinct from stipe, glabrous; style ca. 2 mm glabrous; stigma dark purple, capitate. Capsule 1.5–2.5 cm long, linear/straight, glabrous, longitudinal dehiscence. Seeds data not available.

Amendments to protologue. The protologue by Fischer indicates that *D. lineicapsa* has bracts at each inflorescence fork (“*bractea ad furcas*”). However, we observed that the holotype and other subsequent collections by Parry, Wenger as well as our own collections (Fig. 3), do not have any bracts or bracteoles within the inflorescence. The protologue also mentions that *D. lineicapsa* has a glabrous corolla tube, however all specimens including the type specimen have been found to be sparsely covered with multicellular, glandular hairs towards the lobes.

Note. *D. lineicapsa* is similar to *D. graciliflorus* R.W.MacGregor & W.W.Sm. in its vegetative morphology but differs due to the absence of bracteoles (ovate bracteoles present in *D. graciliflorus*) and linear-lanceolate, tripartite calyx lobes (oblong 5-partite lobes in *D. graciliflorus*).

Distribution. The type locality of *D. lineicapsa* is near Aizawl in northern Mizoram and subsequent collections are known from throughout the state. In our expeditions, we could not locate any populations in its type locality or historical collection

sites. However, we found three scattered populations in Mamit district of northern Mizoram which is at least 40 km away from its type locality (specimen numbers: VG-2018MZ2581, VG2018MZ2584, VG2018MZ2585, VG2018MZ2596).

Habitat. These plants grow on steep clayey banks along the roads in partially shaded, tropical wet evergreen forests.

Phenology. Flowering in August to September, fruiting in October to January.

Conservation status and preliminary IUCN assessment. *D. lineicapsa* is known from only seven specimens collected from Mizoram, India, and it has not been recollected for the past 89 years. We carried out collection expeditions in the years 2017 and 2018 to the type location (Aizawl, Mizoram) as well as other historical collection sites (Fig. 1). All of the historical locations have undergone dramatic urbanization in the last eight decades and we could not find any population of *D. lineicapsa* in any of these sites. Instead, we found only three disjunct populations of *D. lineicapsa* with a total of less than 1000 individuals, in Mamit district, Mizoram. All the extant populations are located in rapidly degrading, fragmented forests that do not fall under federally protected areas, and therefore we propose the conservation status of this species as vulnerable (VU) following the criteria D2 of IUCN guidelines (IUCN 2019).

***Didymocarpus parryorum* C.E.C.Fisch., Bull. Misc. Inform. Kew. 1928(4): 142. 1928.**

Fig. 4, Suppl. material 1: Fig. S1C, E

Lectotype (designated here). INDIA. Assam (= Mizoram), Lunglei district, Lushai hills at Sairep, 5000 ft., July 1926, Mrs N.E. Parry 7, K (K000820535!).

Lectotypification. The protologue by Fischer indicates the specimen that was studied for the description of the species as “INDIA. Assam, South Lushai Hills at Sairep, 1700 m. July, *Mrs N E. Parry 7*”. During our study we located five different sheets at Kew herbarium having the same collection number and locality as quoted above. Hilliard and Burtt (1995) noted that Parry’s numbers do not refer to individual collections, but instead they refer to unique species that she had recognized in the field. Weber et al. (2000) recognized three of these specimens as type material, but failed to designate a lectotype. One of these specimens, with a barcode number K000820535, has the collector’s original label which mentions ‘July 1926’ as the collection date. The author’s note on the label matches the note that Fischer has quoted in the protologue: “grows on rocky cliffs, leaves pale-green, silvered when dry, calyx light yellow, corolla orange red”. Since this is the only specimen where the collection number, month, and the author’s note matches the protologue, we designate K000820535 as the lectotype here.

Revised description. Terrestrial or epilithic herbs, up to 20 cm tall, total height including inflorescence ca. 25 cm. Rhizome 1–2 × 0.5–1.0 cm. Stem 3–10 × 3.5–8 mm, erect, dark brown to light green, terete, pubescent with eglandular hairs, interspersed

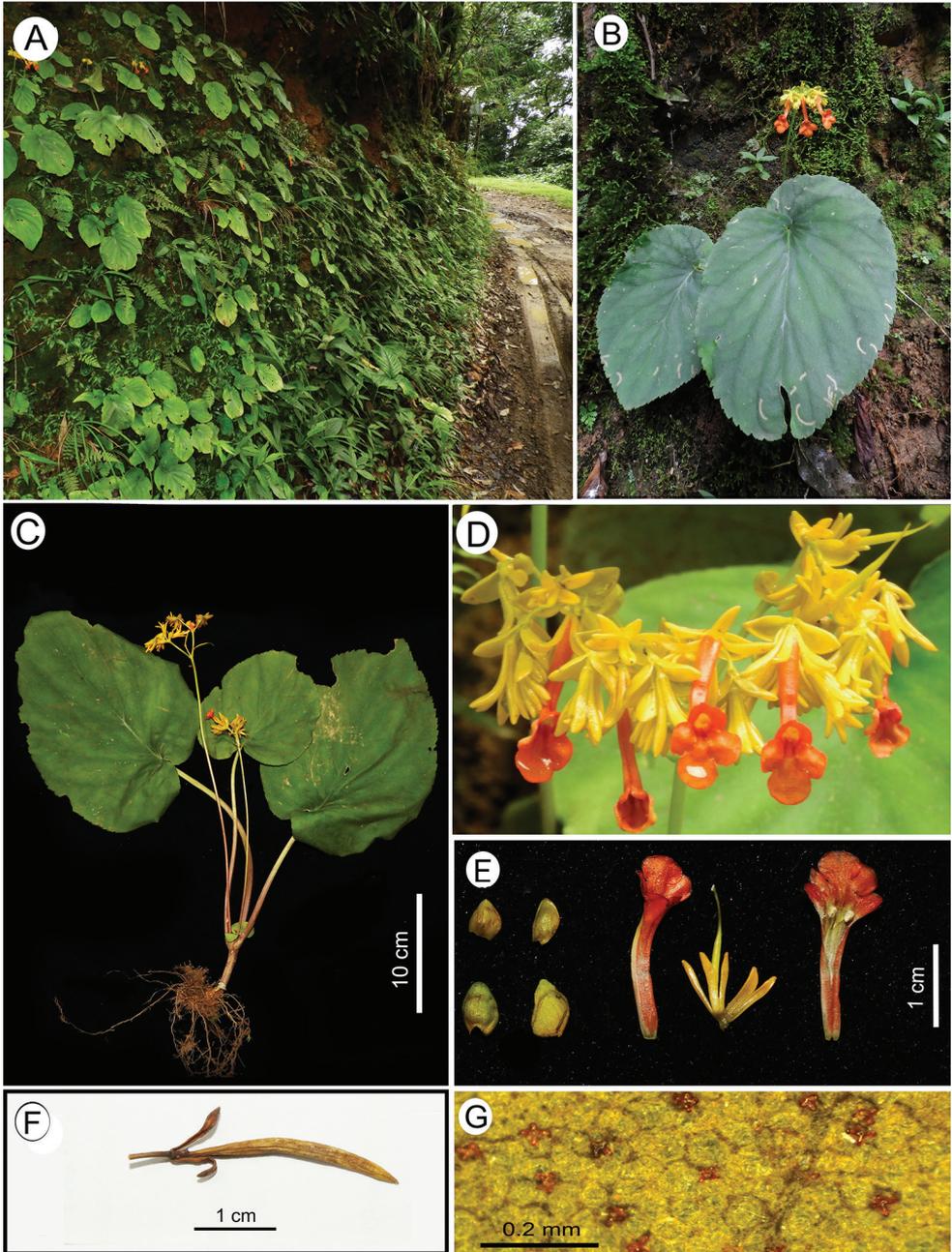


Figure 4. *Didymocarpus parryorum* C.E.C. Fisch. **A** habitat **B** habit **C** complete plant with inflorescence **D** inflorescence **E** floral dissection from left to right: primary bracteoles (above), secondary bracteoles (below), corolla tube, calyx with gynoecium, open floral tube with anther (fusion of anther lost in dissection of flower) **F** mature fruit **G** glands on the lower surface of leaves. Photographs by NSP.

with yellowish cruciform pigment glands (in dried specimen). Leaves 1 – 4 pairs, opposite, anisophyllous, decussate, arrangement tufted in close pairs, terminal pair is reduced, exstipulate; petioles 4–9 cm long, light brown to light green, pubescent as on stem, interspersed with yellowish cruciform pigment glands (in dried specimen); lamina 6–12 × 5–10 cm, orbicular to ovate, base cordate to obliquely cordate, apex acute to subobtuse, margins crenate to serrate, dorsal surface dark green, pubescent with eglandular hairs, ventral surface light green, veins pubescent and intervals sparsely pilose, hairs eglandular, abaxial surface is covered with yellow to brownish cruciform pigment glands (in dry specimen); midrib with 6–8 secondary veins in either side, basal 3–4 pairs palmate, sunken above, raised below. Inflorescence 1–4, pedunculate, erect, axillary, pair-flowered dichasial cymes, arising from the axils of the 1–2 uppermost pairs of leaves, cyme with 12–16 flowers; primary bracteoles present, 8 × 4 mm, greenish-yellow, opposite, ovate, apex subacute, glabrous, abaxial surface covered with small cruciform pigment glands, secondary bracteoles yellow, present at the base of each cyme unit, 6 × 3 mm, thick, veins visible in dried specimens; orange flowers contrast against yellow calyces; peduncle 10–25 cm long, up to 2 mm wide, brownish at the base, light green towards apex, pubescent with eglandular hairs, sparsely covered with minute cruciform pigment glands as on bracteoles; pedicel 4–12 mm long, greenish-yellow, glabrous, pigment glands absent. Calyx up to 1 cm long, bright yellow, linear lanceolate, apex acute, lobes 5, free to base, conspicuously veined, glabrous, thick, persistent. Corolla 1.3–2.2 cm long, ca. 1.5 mm wide, tubular, tip infundibuliform, glabrous, corolla tube dorsally orange while ventral section of the corolla tube with a light yellow stripe running along the length of the tube, corolla lobes bilabiate, orange, glabrous, orbicular, upper lobes 2, 1.5 × 2 mm; lower lobes 3, 4 × 3 mm, spreading at right angles to the upper lobes, central lobe wider than the 2 lateral lobes. Stamens 2, oblong, glabrous, inserted near the throat of the corolla tube, anthers dorsifixed, coherent by adaxial surfaces; filaments 4–5 mm long, yellowish orange, glabrous; staminodes 2, 2 mm long, linear. Disc up to 1.2 mm, tubular with undulating upper margin, yellowish, glabrous, persistent. Gynoecium ca. 2 cm, ovary greenish yellow, linear, glabrous; style ca. 0.5 cm, glabrous; stigma capitate, green. Capsules 17–24 mm, linear, glabrous, orthocarpic. Seeds minute, ellipsoid, glabrous, muricate.

Amendments to protologue. Upon examining the historical specimens and our fresh collections (Fig. 4) we believe that the indumentum on the stem is better described as pubescent rather than as strigose. Similarly, we describe corolla lobe shape as orbicular and not suborbicular (as mentioned in the protologue).

Note. Differs from *D. tristis* Craib in having larger, bright yellow colored bracteoles and sepals respectively (maroon bracteoles and sepals in *D. tristis*). In addition, *D. parryorum* has orbicular to ovate leaves (oblong to lanceolate in *D. tristis*) and smaller corolla (1.3–2.2 cm in *D. parryorum*, 2–2.4 cm in *D. tristis*).

Distribution and vernacular name. Historically, *D. parryorum* is known from only two districts (Lunglei and Lawngtlai) of southern Mizoram. We could locate the species only from the type locality (Sairep village in Lunglei district, specimen numbers: VG2018MZ2522, VG2018MZ2528, VG2018MZ2529, VG2018MZ2546). In Sairep, the plant is known as ‘*Chhakzhau*’ in local Mizo language.

Habitat. This plant is generally found growing on moist loamy banks in partially shaded tropical wet evergreen forests.

Phenology. Flowering in July to September, fruiting in August to December.

Conservation status and preliminary IUCN assessment. *Didymocarpus parryorum* is historically known from only two localities in southern Mizoram, India: Lunglei and Lawngtlai districts. It has not been collected for the past 90 years, until this study in 2018, when we found it growing in its type locality. The extant population is restricted to a small patch of less than 10 km² in a rapidly degrading forest and it has about 500 mature individuals. A village road passes through the plant's habitat, further threatening its population. Therefore we propose that the species should be considered as critically endangered (CR) as per the B2ab criteria of IUCN guidelines (IUCN 2019).

Didymocarpus wengeri C.E.C.Fisch., *Bull. Misc. Inform. Kew.* 1928(2): 74. 1928. Fig. 5, Suppl. material 1: Fig. S1F

Lectotype (designated here). INDIA, Assam (= Mizoram): South Lushai Hills, 2500 ft, comm. September 1927, Rev. W. J. L. Wenger 1, K (K000820530!).

Lectotypification. There are only four historical collections of *D. wengeri*, two of which are specimens collected by Wenger, and the third by Parry. There is a fourth specimen at CAL, collected from southern Mizoram, which does not have the collectors' details, but there is a possibility that it may be from Wenger or Parry's collection, as the specimen was received from Kew herbaria and the script matches Fischer's writing. In the protologue, Fischer indicated the specimen he studied for the description of the species as "Assam, South Lushai Hills, 2400 ft., Rev. W.J.L. Wenger", but he did not mention any specific collection date or number. We could not locate any specimen collected by Wenger at South Lushai hills at 2400 feet elevation in any herbaria (ARUN, ASSAM, CAL, E, K and BM), where Wenger's specimens are known to exist. There is a specimen collected by Wenger (Wenger 1) at Kew (K000820530), without a collection date but with a note "comm. Sept 1927", presumably written by Fischer. The label clearly mentions that the specimen was collected at '2500 ft.' In their study, Weber et al. (2000) considered K000820530 as a type but they did not designate the status of the type: "Type: INDIA, Mizoram (previously Assam), South Lushai Hills, 2500 ft., IX. 1927. Wenger (K)". We suggest that the '2400 ft.' in Fischer's protologue possibly is a typographical error, which has also been suggested by Weber et al. (2000), wherein the elevation has been cited as '2500 ft.' by them and not as '2400 ft.', as featured in the protologue. Given these observations, we designate K000820530 as the lectotype here.

Revised description. Terrestrial or epilithic herbs, 7 cm tall, total height including inflorescence ca. 10 cm. Stem 5–60 × 3 mm, subacaulascent to 6 cm, terete, light green to dark maroon, villose with 4–10 celled glandular hairs (rarely eglandular), densely covered with cruciform pigment glands. Leaves 1–4 pairs, opposite and anisophyllous, decussate, terminal pair smaller in size, arrangement tufted in close pairs, exstipulate; petioles 2–5 cm long, villose with 4–10 celled eglandular hairs, glands cruciform, den-

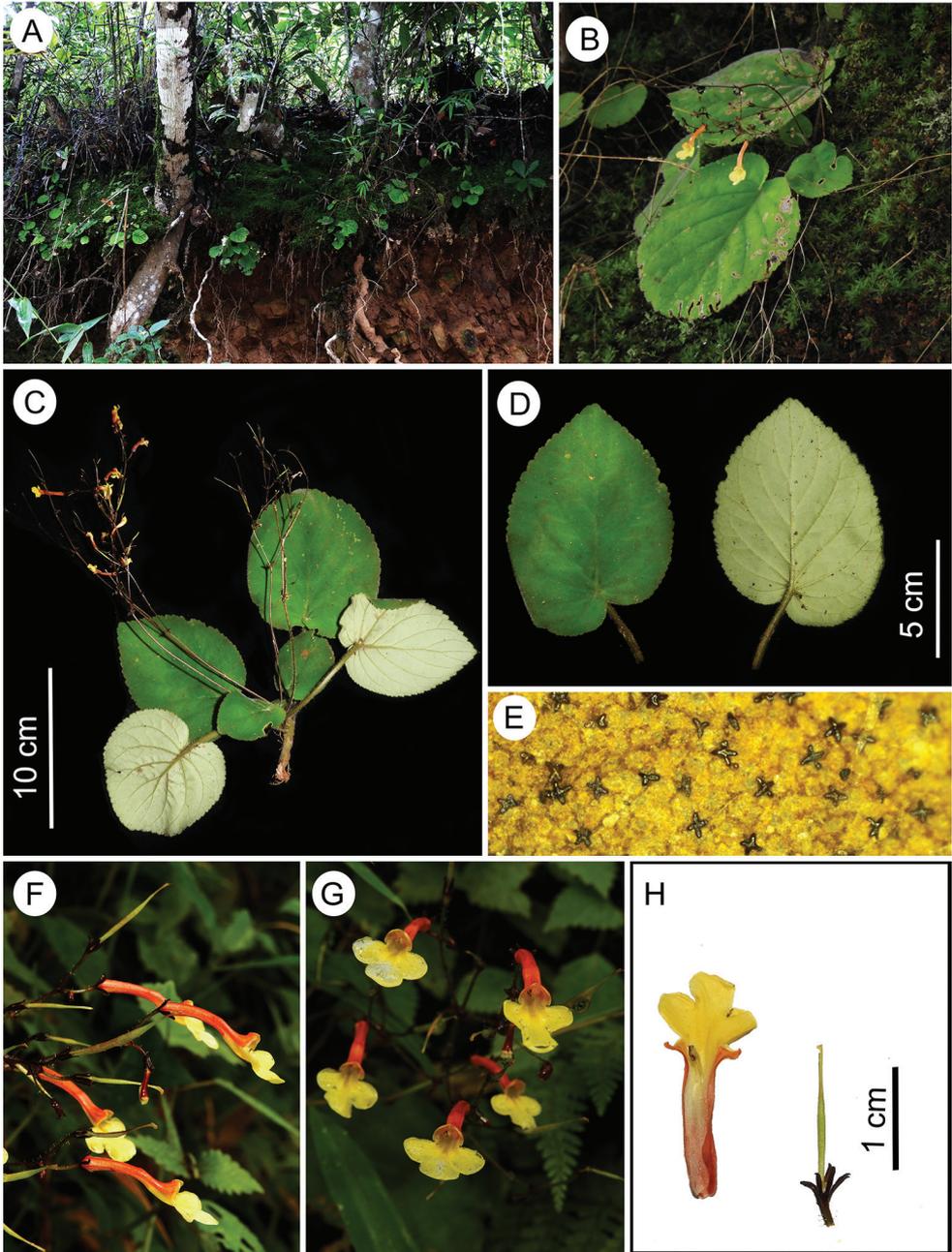


Figure 5. *Didymocarpus wengeri* C.E.C. Fisch. **A** habitat **B** habit **C** plant with inflorescence **D** leaf adaxial and abaxial surface **E** glands on the lower surface of leaves **F** flowers (lateral view) **G** flowers (frontal view) **H** floral dissection from left to right: opened floral tube, calyx with gynoecium. Photographs by NSP.

sity and structure similar to the ones on stem; lamina 1.8–6 × 1.5–6 cm, orbicular, base cordate and often unequal, apex sub-obtuse, margin crenate to serrate with multicellular hairs; dorsal surface dark green, villous with eglandular hairs, ventral surface,

densely villose along veins but sparsely villose otherwise; lower lamina covered with cruciform, dark brown pigment glands (in dried specimen), dense along the (midrib) and veins; midrib with 5–8 lateral veins on either side, basal 3–5 veins palmate, sunken above, raised below. Inflorescence 1 to 5, pedunculate, axillary, pair-flowered cymes usually arising only from the axils of the 1–2 uppermost pairs of leaves, cyme with 6–16 flowers; primary bracteoles present, 2 × 1 mm, opposite, lanceolate, reddish brown, sometimes with eglandular hairs, below densely covered with cruciform glands; secondary bracteoles (within the cyme) present at each dichotomous fork, 1–2 mm in diam., orbicular, reddish brown, sparsely covered with eglandular hairs, hirsute on upper surface, glandular hairs on lower surface; peduncle 5–15 cm long, dark maroon, primary axis is sparsely covered with both glandular and eglandular hairs, secondary axis with glandular hairs, cruciform pigment glands present near the base of the primary axis and at the fork; pedicel 2–10 mm long, glandular hairs present. Calyx 5 lobes, 1.5–3.5 mm long, free to base, linear lanceolate, glabrous, dark brown to maroon, persistent. Corolla 0.8–2.2 cm long, ca. 2–3 mm wide, tubular, tip infundibuliform, orange-red at the base and yellow ventrally from throat to mouth, yellow extending into the lower lobes, corolla bi-lipped, total 5 lobes, yellow, upper 2 lobes fused with 2 × 2 mm, orbicular; lower lobes 3, 7 × 4 mm, orbicular, spreading at right angles to the upper lobes, middle/central lobe wider than the 2 lateral lobes, glabrous. Stamens 2, inserted near the throat of the corolla tube; filaments 3–4 mm long, glabrous, filament yellowish, anthers oblong, dorsifixed, coherent by adaxial surfaces, pubescent; staminodes absent. Disc up to 1.5 mm, tubular with undulating upper margin, greenish yellow, glabrous, persistent. Gynoecium 10–12 mm, ovary greenish yellow, linear, indistinct from stipe, glabrous; style ca. 4 mm glabrous; stigma yellow, capitate. Capsule 1.5–2.5 cm long, linear, glabrous, dehiscence longitudinal. Seeds data deficient.

Amendments to protologue. The protologue mentions that disc is absent at the base of the ovary. Fresh specimens show the presence of small tubular disc at the base of the ovary (Fig. 5 H).

Note. This species is similar to *D. margaritae* W.W.Sm., but differs from it in having cruciform brownish glands on abaxial surface of the leaves (glands absent in *D. margaritae*) and has yellow colored corolla lobes (orange corolla lobes in *D. margaritae*). Peduncle with glandular hairs in *D. wengeri*, whereas peduncle of *D. margaritae* is glabrous.

Distribution. All collections of *D. wengeri*, including the type specimen, are from southern Mizoram. In our study, we located a population from Tuipang in the Saiha district of southern Mizoram (specimen numbers: VG2018MZ2556, VG2018MZ2557, VG2018MZ2558). This locality corresponds to where Parry had collected specimens in 1928. In addition, we found a second population in the Lawngtlai district of southern Mizoram (specimen numbers: VG2018MZ2568, VG2018MZ2569, VG2018MZ2570).

Habitat. Growing on steep clay banks along the roads in partially shaded, tropical wet evergreen forests.

Phenology. Flowering in August to September, fruiting in October to January.

Conservation status and preliminary IUCN assessment. *D. wengeri* is currently known from only two locations in southern Mizoram, India: Saiha district and Lawngtlai district. Only one population each has been located in these two districts and they

are separated by a distance of 135 km. This rediscovery is after a span of 87 years and a total of 52 individuals were found during the flowering season of 2018. In the prologue, Fischer has quoted Wenger's (collector) note as "apparently scarce, at least in these hills, for I have only found one small patch on a steep clayey bank", indicating that these plants were very rare even when they were first collected. Considering the small, fragmented population and rapidly degrading habitat, the species should be considered as critically endangered (CR) as per C2a(i) of the IUCN guidelines (IUCN 2019).

Discussion

Northeast India, and the state of Mizoram in particular, has been floristically underexplored, and our study highlights the need for more careful and extensive biodiversity studies in this region. The rediscovery of three species close to their type localities and the collection of one away from its type locality (*D. adenocarpus*) suggests that conservation of the narrow endemics is critical and understanding their biology should be prioritized. The geographic placement of Mizoram (between Bangladesh and Myanmar) also indicates that cross-border biogeographic studies should be carried out to understand the dispersal and evolution of plants in this region.

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Appendix I

List of *Didymocarpus* specimens examined

***Didymocarpus adenocarpus* C.E.C.Fisch.:** INDIA, Mizoram. S. Lushai hills, September 1928, Rev. W.J.L. Wenger 239, K (K000820546); S. Lushai hills, September 1931, Rev. W.J.L. Wenger 347, K (K000820547); S. Lushai hills, September 1931, Rev. W.J.L. Wenger 347, E (E00627876); Sairep, September 1927, Mrs N.E. Parry, No.251, K; Reiek Tlang, 25 August 2018, Prasanna N.S., VG2018MZ2589, BHPL; Reiek Tlang, 25 August 2018, Prasanna N.S., VG2018MZ2590, BHPL; Reiek Tlang, 25 August 2018, Prasanna N.S., VG2018MZ2592, BHPL.

***Didymocarpus purpureobracteatus* W.W.Sm.:** CHINA, Yunnan. S. of Red River, without date, Henry 9746A, US (US00056528); San Teo Chen, without date, A. Henry 9189, NYBG (NY00063234).

***Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burt:** INDIA. Mizoram: S. Lushai, August 1928, Rev. W.J.L. Wenger, No.238, E (E00627917); Aizawl, September 1927, Mrs N.E. Parry, No.79, K (K000820539); Aizawl, March 1928, Mrs N.E. Parry, No.79, K (K000820540); Aizawl, 1928, Mrs N.E. Parry, No.79, K (K000820541); S. Lushai, August 1928, Rev. W.J.L. Wenger, No.238 K; Hmuifang, August 1927, Mrs N.E. Parry, No.262, K; Hmuifang, August 1927, Mrs N.E. Parry, No.259, K; Mamit, Reiek Village, August 2018, Prasanna N.S., VG2018MZ2581, BHPL; Mamit, Reiek Tlang, August 2018, Prasanna N.S., VG2018MZ2584, BHPL; Mamit, Reiek Tlang, August 2018, Prasanna N.S., VG2018MZ2585, BHPL; Mamit, Luanpawl to Dawpui road, August 2018, Prasanna N.S., VG2018MZ2496, BHPL.

***Didymocarpus graciliflorus* R.W.MacGregor & W.W.Sm.:** MYANMAR. S. Shan States: Keng Tung, August 1909, MacGregor, R.W., No.714, K, (K000820542).

***Didymocarpus parryorum* C.E.C.Fisch.:** INDIA. Mizoram. Lunglei, Sairep, July 1926, Mrs N.E. Parry No.7, K (K000820535); Sairep, 1928, Mrs N.E. Parry No.7, K, (K000820536); Sairep, December 1927, Mrs N.E. Parry No.7, K, (K000820537); Phongpui, March 1927, Mrs N.E. Parry No.NA (2 sheets) K; N.Vanlaphai, 'comm' January 1928, Mrs N.E. Parry CAL (CAL0000019176); Sairep, August 2018, Prasanna N.S., VG2018MZ2522, BHPL; Sairep, August 2018, Prasanna N.S., VG2018MZ2528, BHPL; Sairep, August 2018, Prasanna N.S., VG2018MZ2529, BHPL; Sairep, August 2018, Prasanna N.S., VG2018MZ2546, BHPL.

***Didymocarpus tristis* Craib:** THAILAND, Chanthaburi, Khao Khitchakut, 27 Aug 2012, Middleton et al, 5673, E.

***Didymocarpus wengeri* C.E.C.Fisch.:** INDIA. Mizoram. S. Lushai, August 1931, Rev. W.J.L. Wenger (collectors number not mentioned), E, (E00627988); South Lushai hills, (without date, comm. September 1927), Rev. W.J.L. Wenger, 1, K, (K000820530); Lushai hills, Tuipang, January 1928, Mrs N.E. Parry, 452, K, (K000820531); S. Lushai, without collectors name, and date, CAL (CAL0000019172); Saiha, August 2018, Prasanna N.S., VG2018MZ2556, BHPL; Saiha, August 2018, Prasanna N.S., VG2018MZ2557, BHPL; Saiha, August 2018, Prasanna N.S., VG-

2018MZ2558, BHPL; Lawngtlai, August 2018, Prasanna N.S., VG2018MZ2568, BHPL; Lawngtlai, August 2018, Prasanna N.S., VG2018MZ2569, BHPL; Lawngtlai, August 2018, Prasanna N.S., VG2018MZ2570, BHPL.

Didymocarpus margaritae W.W.Sm.: CHINA, Yunnan. Szemoa. Henry, A., #12380b, US, (US00056517).

Supplementary material I

Figure S1

Authors: Naibi Shrungeshwara Prasanna, Vinita Gowda

Data type: Multimedia

Explanation note: **A** Underside of *D. adenocarpus* C.E.C.Fisch. plant showing inflorescence hidden under leaves **B** Watery calyx of *D. adenocarpus* C.E.C.Fisch. **C** Lateral view of *D. parryorum* C.E.C.Fisch. flower showing color stripe along the floral tube **D** Tridentate calyx lobe of *D. lineicapsa* (C.E.C.Fisch.) B.L.Burt **E** Inflorescence of *D. parryorum* C.E.C.Fisch. **F** Inflorescence of *D. wengeri* C.E.C.Fisch. Photographs by NSP.

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Link: <https://doi.org/10.3897/phytokeys.148.49772.suppl1>

New combinations and updated descriptions in *Podagrostis* (Agrostidinae, Poaceae) from the Neotropics and Mexico

Steven P. Sylvester^{1,2}, Paul M. Peterson³, Konstantin Romaschenko³,
William J. Bravo-Pedraza⁴, Lia E. Cuta-Alarcon⁴, Robert J. Soreng³

1 College of Biology and the Environment, Nanjing Forestry University, Long Pan Road No. 159, Nanjing, 210037, China **2** Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK **3** Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington DC 20560, USA **4** Grupo Sistemática Biológica, Herbario UPTC, Escuela de Biología, Facultad de Ciencias, Universidad Pedagógica y Tecnológica de Colombia, Avenida Central del Norte 39-115, Tunja-Boyacá, Colombia

Corresponding author: Steven P. Sylvester (steven_sylvester@hotmail.com)

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Abstract

Based on morphological study and corroborated by unpublished molecular phylogenetic analyses, five grass species of high-mountain grasslands in Mexico, Central and South America, *Agrostis bacillata*, *A. exserta*, *A. liebmanni*, *A. rosei*, and *A. trichodes*, are transferred to *Podagrostis* and bring the number of species of this genus recognized in the New World to ten. The name *Apera liebmanni* is lectotypified and epitypified. We provide an updated genus description for *Podagrostis*, and updated species descriptions, images, and notes on the new combinations. The diagnostic characteristics differentiating *Podagrostis* from *Agrostis* are: a) palea that reaches from $(2/3) \frac{3}{4}$ to almost the apex of the lemma; b) florets that usually almost equal the length of the glumes or are at least $\frac{3}{4}$ the length of the glumes; c) rachilla extension present and emerging from under the base of the palea as a slender short stub (rudimentary or up to 1.4 mm long, sometimes obscure in most florets in *P. rosei*), smooth or scaberulous, glabrous or distally pilulose (hairs < 0.3 mm long); d) lemmas usually awnless, sometimes with a short straight awn 0.2–0.6 mm long, inserted medially or in the upper 1/3 of the lemma, not surpassing the glumes (awn well-developed, straight or geniculate and inserted in lower 1/3 of lemma, not or briefly surpassing glumes in *P. rosei*). We include a generic key to distinguish the species of *Podagrostis* from other similar genera in Latin America and a key to distinguish the species of *Podagrostis* now accepted as occurring in these areas.

Resumen

Basado en estudios morfológicos y corroborado por datos no publicados de análisis filogenéticos, las cinco gramíneas de alta montaña de los pastizales en México, Centroamérica y Sur América *Agrostis bacillata*, *A. exserta*, *A. liebmannii*, *A. rosei*, y *A. trichodes*, son transferidos a *Podagrostis* incrementando a diez el número de especies en el nuevo mundo de este reconocido género. El nombre de *Apera liebmannii* también es lectotipificado y epitipificado. Proporcionamos una descripción actualizada del género *Podagrostis*, y descripciones actualizadas de las especies, imágenes y comentarios sobre las nuevas combinaciones. Las características diagnósticas que diferencian a *Podagrostis* de *Agrostis* son: a) pálea de $2/3$ – $3/4$ hasta casi el ápice de la lemma; b) espiguillas, generalmente casi iguales en longitud a las glumas o de al menos $3/4$ la longitud de las glumas; c) extensión de la raquilla presente y emergiendo desde debajo de la base de la pálea como un trozo corto y delgado (desde rudimentario hasta de 1.4 mm de largo, a veces oscuro en la mayoría de los flósculos en *P. rosei*), liso o escabérulo, glabro o piloso distalmente (pelos < 0.3 mm de largo); d) lemmas generalmente sin arista, o a veces con arista corta y recta de 0.2–0.6 mm de largo, insertada medialmente o en el tercio superior de la lemma, sin sobrepasar las glumas (arista bien desarrollada, recta o geniculada e insertada en la parte inferior $1/3$ de lemma, no o superando brevemente las glumas en *P. rosei*). Incluimos una clave para distinguir *Podagrostis* de otros géneros similares presentes en América Latina y una clave para distinguir las especies de *Podagrostis* ahora aceptadas por estar presentes en estas áreas.

Keywords

Agrostis, Andes, Central America, Colombia, Ecuador, páramo, Peru, Venezuela

Introduction

Agrostis L. and *Podagrostis* (Griseb.) Scribn. & Merr. both belong to the tribe Poeae R.Br., subtribe Agrostidinae Fr. s.s. (Soreng et al. 2017), and share numerous characteristics, including paniculate, single-flowered laterally-compressed spikelets that disarticulate above the glumes. Delimitation of these two genera has been complicated by a lack of surety of the morphological discontinuities, with *Podagrostis* originally described as a section of *Agrostis*, and some accounts treating the former as a taxonomic synonym of the latter (e.g. Clayton and Renvoize 1986; Pohl and Davidse 1994). The distinction of *Podagrostis* was corroborated by molecular phylogenetic research, with plastid analyses finding *Podagrostis aequivalvis* (Trin.) Scribn. & Merr. to be placed with *Calamagrostis bolanderi* Thurb. in a maximally supported basal lineage sister to a clade comprising *Agrostis*, *Polypogon* Desf., and four Chinese species of “*Deyeuxia*” Clarion ex P.Beauv. plus *Agrostis rosei* Scribn. & Merr. from Mexico that did not resolve with *Calamagrostis* Adans. sensu Soreng et al. (2017) (Saarela et al. 2017). Current treatments recognize five species of *Podagrostis*, three from North America (Roché et al. 2007) and two from South America (Rúgolo de Agrasar 2012; Sylvester et al. 2019a).

Morphologically, these taxa of *Podagrostis* differ from currently circumscribed *Agrostis* taxa in the combination of a well-developed, relatively long palea that reaches from $(2/3)$ $3/4$ to almost the apex of the lemma, florets that usually almost equal the length of the glumes or which at least reach past $3/4$ the length of the glumes, the presence of a slender rachilla prolongation in all or most spikelets that is smooth or scaberulous, with hairs lacking or distally pilulose (hairs < 0.3 mm long), and lemmas

usually awnless or with a short straight awn 0.2–0.6 mm long, inserted medially or in the upper 1/3 of the lemma, not surpassing the glumes. These characters are also found in the species *Agrostis liebmannii* (E. Fourn.) Hitchc. and *A. rosei* (awn well-developed, straight or geniculate and inserted in lower 1/3 of lemma, usually not surpassing glumes in *A. rosei*) from mountains of central and southern Mexico, *A. exserta* Swallen from high-elevation grasslands of Guatemala, and *A. bacillata* Hack. and *A. trichodes* (Kunth) Roem. & Schult. from high-elevation páramo grasslands of Costa Rica and Panama, or northwest South America, respectively. The possible affinity of some of these species to *Podagrostis*, based on the presence of a rachilla prolongation, has been suggested (Pohl and Davidse 1994; Briceno 2010), and the placement of *A. rosei* in the plastid tree in Saarela et al. (2017) provided molecular support for this idea. All other *Agrostis* taxa with well-developed paleas in Mexico, Central America and northwest South America, with which these species could possibly be confused (because their paleas sometimes exceed 2/3 the length of their lemmas), are exotics introduced from the Old World (i.e. *A. alba* L.; *A. capillaris* L.; *A. castellana* Boiss. & Reut.; *A. gigantea* Roth; *A. stolonifera* L.) that lack a rachilla prolongation and have florets that are 1/3–3/4 the length of the glumes, and paleas ranging from 2/5 to 2/3 (rarely reaching to 3/4) the length of the lemmas. The other native *Agrostis* species from the region we are discussing have shorter, often rudimentary or absent paleas.

In a large unpublished molecular DNA sequence study using three plastid gene regions (*rpl32-trnL* spacer, *rps16-trnK* spacer, and *rps16* intron), Romaschenko et al. (pers. comm.) found that *A. bacillata* and *A. rosei* were closely related to other taxa of North American *Podagrostis* [*P. aequivalvis*; *P. humilis* (Vasey) Björkman; *P. thurberiana* (Hitchc.) Hultén]. These form a clade sister to *Agrostis* and *Polypogon*. Saarela et al. (2017) also reported a similar result for *A. rosei* in their plastid tree, which found the taxon to be part of a well-supported clade with four Chinese species of *Deyeuxia*, and *Calamagrostis bolanderi* + *Podagrostis aequivalvis*. Some of the aforementioned Chinese *Deyeuxia*'s and *C. bolanderi* from California remain to be sampled using the same markers used in this study to clarify their position. Although *C. bolanderi*'s placement in a strongly supported lineage with *Podagrostis aequivalvis* (Saarela et al. 2017) provides support for its transferal to *Podagrostis*, it may represent a separate hybrid between *Podagrostis* and *Calamagrostis*.

Nuclear ribosomal internal transcribed spacer DNA sequences of all these species resolved within *Calamagrostis* sensu Peterson et al. (2019), which is also congruent with placement of *P. aequivalvis* in the ITS and ITS+ETS tree and of *A. rosei* in the ITS tree in Saarela et al. (2017). Saarela et al. (2017) also mentioned *A. rosei* to be placed outside of two strongly supported clades that include all sampled species of *Agrostis*, *Polypogon* and one species of *Lachnagrostis* in their more well-resolved, albeit more poorly sampled, ITS-ETS tree (Saarela et al. 2017: 65, not shown in Saarela et al. 2017: fig. 7), further confirming the distinctiveness of this taxon. The contrasting nrDNA and plastid placements indicate *Podagrostis* is reticulate in origin between *Calamagrostis* and a sister lineage to *Agrostis* plus *Polypogon*, and may yet extend to Asian elements.

The molecular results support our transfer of these and other morphologically similar *Agrostis* species, *A. exserta*, *A. liebmannii*, and *A. trichodes*, to *Podagrostis*. We pro-

pose five new combinations, *Podagrostis bacillata*, *P. exserta*, *P. liebmannii*, *P. rosei*, and *P. trichodes*, and provide images, up-to-date descriptions, and notes for each species. We also present a key to distinguish *Podagrostis* from similar genera in the Neotropics and Mexico, and a key to distinguish *Podagrostis* species from these areas.

Materials and methods

Accepted species follow Soreng et al. (2003 and onwards). Herbarium acronyms follow Index Herbariorum (Thiers, continuously updated). In this treatment, unless otherwise stated, glabrous means without pubescence (in the sense of slender, relatively soft hairs). Smooth indicates no prickle-hairs with broad bases and/or hooked or pointed apices (i.e., pubescence can occur on a smooth surface, and a rough or scabrous surface can be glabrous). Specimens in the herbaria COL, FMB, K, UPTC, and US were reviewed for this study. Beyond types (some only seen in images), only material from herbaria where specimens have been checked and verified by the authors are cited. Parts of the generic key related to taxa of previously circumscribed *Calamagrostis* s.l. were adapted from Peterson et al. (2019).

Taxonomic treatment

Key to distinguish *Podagrostis* from other Latin American, one-flowered genera of tribe Poeae with both glumes as long or longer than the floret

- 1 Spikelets disarticulating below the glumes, the glumes and floret, and often part of the pedicel, falling together as a unit; glumes awned or muticous **2**
- Spikelets disarticulating above the glumes, the glumes remaining on the inflorescence after the florets have fallen; glumes acute to acuminate, not awned..... **5**
- 2 Palea < ½ the length of the lemma; glume apices lanceolate or lanceolate-subulate, muticous, mucronulate or awned ***Chaetotropis* Kunth**
- Palea equaling or subequaling the lemma; glume apices obtuse or bilobed (*Polygona*) or muticous (*Cinna*), awned or unawned..... **3**
- 3 Panicles condensed, generally spikelike; glumes much longer than the lemma; glume apices obtuse or bilobed, awned or unawned; rachilla extension absent ***Polygona* Desf.**
- Panicles open to loosely contracted, somewhat lax; glumes as long as the lemma; glume apices muticous, unawned; rachilla extension usually present as a short glabrous stub..... **4**
- 4 Lemma with a subapical awn 5–14.5 mm long; glumes coriaceous, rigid, hispid or scabrous throughout; anthers 3 ***Limnodea* L.H. Dewey**
- Lemma unawned or with a subapical awn < 1 mm long; glumes membranous, flexible, glabrous, scabrous only on the keel and sometimes lateral veins; anthers 1 or 2 ***Cinna* L.**

- 5 Rachilla extension absent; palea of varying length; callus glabrous or shortly pubescent with hairs to 0.5 mm long..... **6**
- Rachilla extension present (cases where it is sometimes rudimentary key both ways), of varying lengths (sometimes very short, and requiring the base of the palea be checked closely to distinguish the structure from hairs) smooth or scaberulous, glabrous or pilulose to pilose, NB. (absent in *Calamagrostis llanganatensis* Laegaard but then callus hairs reaching from $\frac{1}{2}$ to $\frac{3}{4}$ the length of the lemma); palea well-developed, generally $> \frac{2}{3}$ the length of the lemma; callus glabrous, shortly pubescent, or notably pubescent with long hairs..... **9**
- 6 Floret stipitate (lowermost rachilla internode distinctly elongated between the glumes and the floret), stipe 0.2–4 mm long, cylindrical, dilated towards its apex (can be seen at the base of the glumes after the floret has fallen); spikelets > 4 mm long; palea well-developed, subequalling the lemma
..... ***Deschampsia* P. Beauv.** (in part)
- Floret sessile (lowermost rachilla internode not, or not noticeably, prolonged between the glumes and the floret), stipe less than 0.2 mm long; spikelets commonly < 4 mm long; palea of varying length, well-developed and sub-equalling or equaling the lemma to minute/absent..... **7**
- 7 Lemma apex terminating in 4 scabrous setae; lemma surface often pilose; lemma with a well-developed geniculate awn inserted in the lower $\frac{1}{3}$ and surpassing the glumes; calluses pilulose; caryopsis thin, with liquid endosperm..... ***Bromidium* Nees & Meyen** (likely a synonym of *Agrostis*; Nogueira da Silva et al. 2020)
- Lemma apex entire or finely dentate with short teeth at the end of each lateral vein; lemma surface glabrous (rarely with a few hairs in *Agrostis castellana* L.); lemmas mucous, with a short straight awn 0.2–1 mm long, or with a long geniculate and twisted awn to 6+ mm long, inserted basally, medially or in the upper half of the lemma, not surpassing to greatly surpassing the glumes; calluses usually glabrous or with hairs restricted to lateral lines continuous with the basal lemma margins; caryopsis usually rounded, with hardened endosperm..... **8**
- 8 Floret equaling or subequalling the glumes, sometimes slightly shorter but reaching past $\frac{3}{4}$ the length of the glumes, usually with a short rachilla prolongation emerging behind the palea (sometimes absent in many florets of *P. rosei* so check many spikelets); paleas well-developed, usually reaching from ($\frac{2}{3}$) $\frac{3}{4}$ to almost the apex of the lemma; panicles 1–6.5(–11) cm long (up to 18 cm in the Mexican species *P. liebmannii* and *P. rosei*); lemmas mucous or with a short straight awn 0.2–0.6 mm long, inserted medially or in the upper half of the lemma, not surpassing the glumes (awn well-developed, 1.6–2 mm long, inserted in lower $\frac{1}{3}$ of lemma, straight or geniculate and usually not surpassing glumes in *P. rosei*) ***Podagrostis* (Griseb.) Scribn. & Merr.** (in part)
- Floret notably shorter than the glumes, usually $\frac{1}{3}$ – $\frac{3}{4}$ the length of the glumes, rarely longer, without a trace of a rachilla prolongation; paleas well-developed, poorly-developed, or absent, when well-developed reaching from

- $\frac{1}{2}$ – $\frac{3}{4}$ the length of the lemma; panicles often > 5 cm long; lemmas muticous, with a short straight awn 0.2–1 mm long, or with a long geniculate and twisted awn to 6+ mm long, inserted basally, medially or in the upper half of the lemma, not surpassing to greatly surpassing the glumes..... ***Agrostis* L.**
- 9 Lemmas densely pubescent, with rigid and abundant hairs that cover the lemma veins..... **10**
- Lemmas glabrous or pilose only at the basal margins, with veins evident at least in the upper part **11**
- 10 Culms fragile at maturity, inflorescence often disarticulating with age; panicles generally open, with divaricate branching..... ***Lachnagrostis* Trin.**
- Culms slender or stout, not disarticulating with age; panicles contracted or open, without divaricate branching (in species with hairy lemmas) ***Peyritschia* E. Fourn.** (in part)
- 11 Floret stipitate (lowermost rachilla internode distinctly elongated between the glumes and the floret), stipe 0.2–4 mm long, cylindrical, dilated towards its apex (can be seen at the base of the glumes after the floret has fallen); callus and rachilla glabrous (rarely with a few short callus hairs); ligules slightly to strongly decurrent, usually elongated, 4–20 mm long, acuminate, smooth or nearly so, glabrous, entire or laterally cleft ***Deschampsia* P. Beauv.** (in part)
- Floret sessile or subsessile (lowermost rachilla internode not, or not noticeably, prolonged between the glumes and the floret), stipe < 0.2 mm long; callus and rachilla glabrous or pubescent; ligules decurrent or not, 0.2–10 (–15) mm long, commonly less than 4 mm long (lateral lobes often exceeding the central part), often scabrous or pubescent, commonly truncate to obtuse..... **12**
- 12 Lemmas unawned or with a short straight awn, usually < 0.5 mm long, inserted in the upper half of the lemma, not or barely exceeding the glumes (awn well-developed, 1.6–2 mm long, inserted in lower 1/3 of lemma, straight or geniculate and usually not surpassing glumes in *Podagrostis rosei*, but then callus glabrous, rachilla very short, < 0.3 mm long, glabrous, plants from Mexico)..... **13**
- Lemmas with a well-developed usually geniculate and twisted awn (sometimes flexuose in e.g. *Apera*), > 1 mm long, inserted in the lower or upper half of the lemma, clearly exceeding the glumes..... **17**
- 13 Callus and rachilla glabrous [*Podagrostis sesquiflora* (E. Desv.) Parodi ex Nicora often with short hairs emerging from only the rachilla apex and the basal side-ridges of the callus] **14**
- Callus and rachilla hairy (NB. Rachilla absent in *Calamagrostis llanganatensis*) **15**
- 14 Anthers 3 in number, 0.4–1 mm long (to 2.2 mm long in *P. colombiana* Sylvester & Sorong); spikelets < 4 mm long (sometimes to 4.2 mm long in *P. colombiana*); panicles 1–6.5(–11) cm long in taxa from Central and South

- America, 7–17 cm long in *P. liebmannii* and *P. rosei* from Mexico; palea keels smooth ***Podagrostis* (Griseb.) Scribn. & Merr.** (in part)
- Anthers 3 or 1 in number, 1.2–3 mm long; spikelets 3.5–6+ mm long; panicles usually >11 cm long; palea keels often scabrous, at least in part, sometimes smooth throughout (e.g. *C. carchiensis* Lægaard).....
- ***Calamagrostis* Adans.** (in part)
- 15 Rachilla absent..... ***Calamagrostis llanganatensis* Laegaard**
- Rachilla present **16**
- 16 Florets with a short stipe (± 0.15 mm long) between the upper glume and the callus of the floret; both glumes 3-veined; caryopsis hard, hilum linear $\pm 1/3$ the grain in length; plants from the páramo in Ecuador.....
- ***Laegaardia* P.M. Peterson, Soreng, Romasch. & Barberá**
- Florets sessile; lower glumes 1-veined, upper glumes 1- or 3-veined; caryopses hard to pasty, hilum oval to punctiform $< 1/4$ the grain in length; plants from various locations, including Ecuadorian páramos.....
- ***Cinnagrostis* Griseb.** (in part)
- 17 Anthers 2, or 3 in plants from Mexico and Guatemala; lemma body strongly 5-veined, often puberulent in part ***Peyritschia* E. Fourn.** (in part)
- Anthers 1 or 3, if 3 then plants not from Mexico (*Calamagrostis guatemalensis* Hitchc. from Guatemala); lemma body variously veined, glabrous, sometimes scabrous..... **18**
- 18 Panicles open, diffuse; rachilla extension nearly as long as lemma, densely and evenly hairy with hairs 0.5–1.2 mm long; lemmas awned from middle, the awns 2–7.2 mm long, basally twisted, geniculate; callus hairs 0.2–0.8 mm long; leaf blades readily to tardily disarticulating from collars in age (tending to form a J at base after falling), involute, sometimes sinuous; caryopsis hard, hilum linear $\pm 1/3$ the grain in length; lower glumes 1-veined, upper glumes 3-veined and sometimes with 1 or 2 cross-veins between them; plants from páramos of Colombia, Ecuador, and Venezuela.....
- ***Paramochloa* P.M. Peterson, Soreng, Romasch. & Barberá**
- Panicles open (infrequently diffuse) or contracted; rachilla extension mostly less than $3/4$ the lemma in length, variously hairy, the hairs 0.5–4 mm long, sometimes reduced or absent proximally; lemmas awned from near base to upper $1/3$, the awns 1–10 mm or more long, straight, sinuous or geniculate; callus hairs absent to 4 mm long; leaf blades not disarticulating from collars in age, flat, folded or involute, not sinuous; caryopsis soft or hard; hilum elliptical, oval, round to punctiform $1/5$ – $1/3$ the grain in length; lower glumes 1–3-veined without cross-veins between them; plants of various habitats, from Mexico to Tierra del Fuego, Argentina **19**
- 19 Caryopses hard, distinctly sulcate, hilum $1/6$ – $1/3$ the grain in length; lemmatal awns straight or slightly bent, readily distinguished from callus hairs, in-

- serted from near base to middle, not or slightly exceeding the lemma apex; callus hairs 0.1–3 mm long 1/10–3/4 as long as the lemma in length; rachilla glabrous, or sparsely to densely hairy, hairs not reaching lemma apex; panicles contracted; anthers 1 or 3; lodicules entire and lanceolate, sometimes with an isolated lateral lobe, glabrous ***Calamagrostis* Adans.** (in part)
- Caryopses soft (liquid or semi-soft) or hard, sulcate (often shallowly) or not, hilum 1/6–1/4 the grain in length (often obscure in species with lipid); lemmatal awns sometimes indistinguishable from callus hairs, straight, sinuous or geniculate, capillary or stout, inserted from base to upper 1/3, usually exceeding the lemma and often exerted from the glumes; callus hairs shorter to exceeding the lemma in length; rachilla hairy, hairs often reaching 3/4 to exceeding the lemma length; panicles contracted or open; anthers 3; lodicules apically bilobate or bidentate, infrequently entire and lanceolate, apical margin sometimes ciliate or ciliate ***Cinnagrostis* Griseb.** (in part)

***Podagrostis* (Griseb.) Scribn. & Merr. Contr. U.S. Natl. Herb. 13(3): 58. 1910.**

Agrostis sect. *Podagrostis* Griseb. Fl. Ross. 4(13): 436. 1852.

Type. *Agrostis canina* var. *aequalivalvis* Trin. (lectotype designated by: Hitchcock 1920: 127).

Description. *Perennials*, loosely to densely tufted, sometimes forming small tussocks, sometimes subrhizomatous (North American, including Mexico, and Austral South American taxa). *Culms* 5–90 cm tall, slender. *Tillers* either extra- or intravaginal. *Leaves; ligules* 0.2–5.5 mm long, hyaline, glabrous, smooth or lightly scabrous, apices truncate, obtuse, acute or acuminate, entire to lacerate; *blades* involute, folded, or flat. *Inflorescence* 1–12 cm long, a panicle, lax and open to loosely to moderately densely contracted; *panicle branches and pedicels* glabrous, often smooth or infrequently scaberulous. *Spikelets* 1–4.2 mm long, 1-flowered, disarticulating above the glumes, weakly laterally compressed; *glumes* equal or subequal, the lower often longer than the upper, equaling or subequaling the length of the floret or slightly longer, persisting on the plant after the florets have fallen or sometimes readily caducous, glabrous, keel smooth or usually scabrous at least distally, lateral veins smooth or slightly scabrous distally, surfaces usually smooth, less often scabrous; lower glume 1- or 3-veined; upper glume 1- (2-) or 3-veined; *floret* 1 in number, sessile, subequaling to equaling the apex of the glumes; *lemmas* membranaceous, often slightly thicker than the glumes, dorsally rounded, 3- or 5-veined, lateral veins not evident to distinct, glabrous, smooth or scabrous, apex mucicous or with a short straight awn 0.2–0.6 mm long, inserted medially or in the upper 1/3 of the lemma, not surpassing the glumes, (awn well-developed, 1.6–2 mm long, inserted in lower 1/3 of lemma, straight or geniculate and usually not surpassing glumes in *P. rosei*); *paleas* well-developed, reaching from (2/3) 3/4 to subequaling the lemma, keels obscure to distinct, glabrous, smooth; *calluses*

rounded, blunt, usually glabrous, or with two short lateral tufts of hairs to 0.5 mm long in some species, abaxially smooth; **rachilla** prolongation present, slender, varying from rudimentary to $2/3$ the floret in length (obscure or absent in many *P. rosei* spikelets), glabrous or sometimes with short strict hairs to 0.3 mm long emerging only distally, smooth or scaberulous. **Flowers** perfect; **lodicules** 2 in number; **anthers** 3 in number, 0.3–1.6 mm long (–2.2 mm long in *P. colombiana*); **ovaries** glabrous. **Caryopses** slightly shorter to equaling the lemmas, concealed at maturity, subterete to fusiform, hardened, sulcus distinct; hilum punctiform to narrowly ovoid; embryo c. $1/4$ – $1/3$ length of the caryopsis; endosperm solid (information on caryopses taken from Harvey 2007, Rùgolo de Agrasar 2012 and *P. trichodes* specimens). $2n = 14$ (in *P. aequivalvis*, *P. humilis*, *P. thurberiana*, *P. rosei*) or 28 (*P. bacillata*).

Distribution and ecology. New World i.e. North, Central, and South America. Found in cold and wet, often high-elevation environments.

Notes. The taxonomic disposition of *Podagrostis* as part of *Agrostis* or a separate genus has long been an obstacle to transfer of the species to the genus. Now that molecular evidence has confirmed the independence of these two genera (Saarela et al. 2017; Konstantin Romaschenko unpublished data) for the type species as well as other species from North America, we feel confident that the genus can be expanded based on shared morphological characteristics. The genus is here considered to contain at least ten distinct species (*Podagrostis aequivalvis*, *P. bacillata*, *P. colombiana*, *P. exserta*, *P. humilis*, *P. liebmannii*, *P. rosei*, *P. sesquiflora*, *P. thurberiana*, *P. trichodes*). Aside from the characters mentioned in the key above, species of *Podagrostis* from Guatemala to NW South America can be easily distinguished from *Agrostis* species with well-developed paleas by the leaf blades being involute or convolute, while being generally flat in the *Agrostis* species with well-developed paleas (*A. capillaris* generally has basal blades involute and culm blades flat). Further distinction of *P. exserta* and *P. trichodes* from other species of *Agrostis* with well-developed paleas in Guatemala and NW South America can be made by the very short panicles, usually < 5 cm long, versus panicles > 5 cm long in the latter.

In high-elevation Guatemala and páramos of Central and NW South America, species are known to only have a densely tufted or tussock-forming habit with intravaginal innovations. A loosely tufted habit and extravaginal innovations, that often leads to a rhizomatous or subrhizomatous habit, is only found in species from Mexico (*P. liebmannii*, *P. rosei*), the USA and Canada (*P. aequivalvis*, *P. thurberiana*) and from Chile and Argentina (*P. sesquiflora*).

Calamagrostis meridensis (Luces) Briceño, a species from Venezuelan páramos that is stated to have a rhizomatous habit (Briceño 2010) and which Sylvester et al. (2019b) mentioned may possibly bear affinity to *Podagrostis*, does share certain characteristics with *P. sesquiflora*, such as the flat leaf blades and callus and rachilla apex with short hairs. However, stipitate florets and certain spikelets with a second, albeit reduced, floret at the end of the rachilla prolongation (Briceño 2010; Sylvester pers. observation) suggests this species more likely belongs in *Deschampsia* P. Beauv. The generic placement of *C. meridensis* needs to be confirmed with molecular data.

Key to the species of *Podagrostis* that are accepted in Latin America

- 1 Leaf blades flat; tillers extravaginal, with cataphyllous shoots present; spikelets (1.8–)1.9–2.5 mm long; from Argentina and Chile (*P. sesquiflora*) or Mexico (*P. liebmannii*, *P. rosei*)..... **2**
- Leaf blades involute or convolute, filiform or acicular; tillers intravaginal, cataphyllous shoots absent; spikelets 1–2 mm long, or 2.8–4.2 mm long in *P. colombiana*; from NW South America (*P. colombiana*, *P. trichodes*), Guatemala (*P. exserta*), or Costa Rica and Panama (*P. bacillata*) **4**
- 2 Panicle contracted and slender, 2–6.5 × 0.25–1 cm, spikelets present from near the base; callus with short hairs 0.1–0.2 mm long; rachilla extension distinct, 0.2–0.4 mm long, often with short hairs distally; from Argentina and Chile..... ***P. sesquiflora* (E. Desv.) Parodi ex Nicora**
- Panicle usually lax and open, less often contracted, 7–18 × (1–)2.5–7 cm, spikelets in the distal 1/3, the lower 2/3 naked; callus glabrous; rachilla extension distinct, 0.3–0.5 mm long, or rudimentary and sometimes absent, glabrous; from Mexico **3**
- 3 Lemmas with a well-developed awn inserted in the lower 1/3, straight or geniculate, reaching the apex of the lemma or glumes or briefly surpassing them; leaf blades usually dimorphic, those of the tillers filiform and narrow, 0.2–0.6(–1) mm wide when opened out, flat or folded, those of the culm 1–2.6 mm wide, flat or slightly convolute towards their apices.....
..... ***P. rosei* (Scribn. & Merr.) Sylvester & Soreng**
- Lemmas awnless; leaf blades not notably different between tillers and culm, filiform or flat, (0.5–)1–3.5 mm wide
..... ***P. liebmannii* (E. Fourn.) Sylvester & Soreng**
- 4 Panicle contracted and slender, < 1.2 cm wide, spikelets present from near the base; spikelets 2.8–4.2 mm long; upper glume 3-veined; plants forming small tussocks with a basal mat of leaves 5–26 cm tall.....
..... ***P. colombiana* Sylvester & Soreng**
- Panicle lax and open, 1–8 cm wide, spikelets in the distal 1/3, the lower 2/3 naked; spikelets 1–2 mm long; upper glume usually 1-veined, sometimes 3-veined in *P. trichodes*; plants forming short tufts with a basal mat of leaves often < 10 cm tall (to c. 17 cm tall in *P. bacillata*) **5**
- 5 Leaf blade abaxial surface finely to densely scabrous; culms usually with at least one elongated internode visible (not including the terminal culm segment below the panicle), with at least 1 node usually visible at flowering; basal mat of leaves usually 6–13 cm tall, to 17 cm tall; leaf blades 2–15 cm long; ligules 1.7–4.3 mm long; panicles 4–11 cm long, panicle branches and pedicels smooth; spikelets 1.7–2 mm long; glume keels scaberulous just in the distal 1/3, surfaces smooth; lemmas smooth; rachilla 0.3–1.4 mm long;

- from páramos of Costa Rica and Panama.....
***P. bacillata* (Hack.) Sylvester & Soreng**
- Leaf blade abaxial surface finely to densely scabrous (*P. trichodes*) or smooth (*P. exserta*); culms usually without visible elongated internodes (NB. this does not refer to the terminal culm segment below the panicle), with 0(–1) nodes visible at flowering; basal mat of leaves usually < 5 cm tall, to 10 cm tall; leaf blades 1–4 cm long, rarely longer, lightly to densely scabrous or smooth; ligules 1–2(–2.5) mm long; panicles 2–5(–6) cm long, panicle branches and pedicels smooth (*P. exserta*) or usually lightly to densely scabrous (*P. trichodes*); spikelets 1–2 mm long; glume keels scabrous just in the distal 1/3 to throughout their length, surfaces smooth or scabrous distally; lemmas smooth (*P. exserta*) or lightly to densely scabrous (*P. trichodes*); rachilla 0.2–0.5 mm long; from various localities, not found in Costa Rica or Panama **6**
- 6 Leaf blade abaxial surface moderately to usually densely scabrous; spikelets 1–1.5 mm long; glume keels scabrous just in the distal 1/3 to throughout their length, surfaces smooth or scabrous distally; lemmas moderately to densely scabrous; panicle branches usually lightly scabrous, infrequently smooth; pedicels usually lightly scabrous, rarely smooth; from Colombia and Venezuela to Peru.....***P. trichodes* (Kunth) Sylvester & Soreng**
- Leaf blade abaxial surface smooth; spikelets (1.2–)1.5–2 mm long; glume keels scaberulous just in the distal 1/3, surfaces smooth; lemmas smooth; panicle branches smooth; pedicels smooth; from Guatemala.....
 ***P. exserta* (Swallen) Sylvester & Soreng**

***Podagrostis bacillata* (Hack.) Sylvester & Soreng, comb. nov.**

urn:lsid:ipni.org:names:77209699-1

Fig. 1

Agrostis bacillata Hack., Oesterr. Bot. Z. 52(2): 59. 1902.

Type. COSTA RICA. Cerro de la Muerte, Jan. 1897, H. Pittier 10477 (**holotype**: W (W19160027240 [image!]); **isotypes**: BAB (BAB00000206 [image!]), BR (BR0000006595845 [image!]), G (G00192030 [image!]), US (US00131726 [not seen], US00131725 [not seen], US00131136 fragm. [not seen]).

Description. *Tufted perennial* forming dense tufts, with the basal mats reaching c. 6–17 cm tall and inflorescences usually well-exserted from the basal foliage. *Tillers* intravaginal. *Culms* 10–30(–50) cm tall, erect, simple, delicate; *nodes and internodes* terete, smooth, usually with at least 1 or 2 elongated internodes visible, with (0–)1–3 nodes exposed at flowering, uppermost internode c. 4–13 cm long, longer than the sheath. *Leaves* generally basal; *sheaths* terete, glabrous, smooth to lightly scabrous

with very short scabers; flag sheath 4–13 cm long; basal sheaths 1–4 cm long, striate, becoming fibrous; **ligules** 1.7–4.3 mm long, membranous, slightly to usually strongly decurrent with the sheath; upper culm ligules acute to acuminate with a obtuse to acuminate apex, sometimes slightly erose towards the apex; ligules of tillers similar to those of the culm, sometimes slightly shorter; **blades** 2–15 cm long, 0.2–0.3(–0.4) mm wide in diameter, involute or convolute, acicular to capillaceous and filiform, straight to slightly curved, abaxial surfaces glabrous, lightly to usually densely scabrous, adaxial surfaces glabrous, lightly to usually densely scabrous on the veins with scabers varying in size from short to long and robust. **Panicles** (3.5–)4–11.5 × (1–)3–5 cm, open to slightly congested when young, usually ovoid; **panicle branches** ascendant to patent, branched above the middle, filiform, with spikelets not present near the base, glabrous, smooth, longest branches 1–4.5 cm long; **pedicels** (1–)2–6 mm long, usually longer than the length of the spikelets, divaricate, glabrous, smooth. **Spikelets** 1.7–2 mm long; **glumes** remaining on the inflorescence at maturity, equal or subequal, the lower usually slightly longer than the upper, almost equaling the length of the floret to 0.5 mm longer, oblong–lanceolate, slightly keeled, apex broadly acute, glabrous, keels lightly scaberulous just in the distal 1/3, surfaces smooth; lower glume 1- (or 3-) veined, lateral veins usually vestigial; upper glume 1- (or 2- or 3-)veined, lateral veins usually vestigial; **lemmas** 1.4–1.6 mm long, glabrous, smooth, faintly 5-veined, apex obtuse, awn lacking or to 0.6 mm long, straight, inserted medially or in the upper 1/3; **paleas** well-developed, 0.8–1.5 mm long, usually reaching from $\frac{3}{4}$ to subequaling the lemma, keels slightly obscure, smooth, apex bifid and sometimes erose; **rachilla** 0.3–1.4 mm long, prolonged from the base of the floret, glabrous, rarely smooth, usually scabrous, sometimes with scabers extending into very short hairs < 0.2 mm long. **Calluses** 0.1–0.2 mm long, slightly elongated, glabrous, smooth. **Flowers; lodicules** c. 0.3–0.5 mm long, lanceolate with acute apices, not lobed; **anthers** 3 in number, 0.8–1 mm long. **Caryopses** not seen. $2n = 28$.

Distribution and ecology. Costa Rica and Panamá, páramo grasslands, 2200–3900 m alt. Pohl (1980) mentions that this species apparently flowers throughout the year.

Additional specimens examined. COSTA RICA. **Cartago:** Canyon of Rio Tiribi, 3 km NW of Llano Grande, 2200 m, 7 Feb. 1969, R.W. Pohl 11705A (US3096988). **Limón:** Cordillera de Talamanca, Atlantic slope, Kamuk Massif, páramo north-east of the main Kamuk peak, 9°1'–9°17'N, 83°00'–83°0'W, 3000–3300 m alt., 17–18 Sep. 1984, G. Davidse 29277 (US3041608). **San José:** Cerro Chirripo, treeless páramo SW of and around summit, 11000–12000 ft [3353–3658 m alt.], 24–26 Aug. 1967, A.M. Evans 145 (US2589766A); Cerro de la Vueltas, páramo, 2700–3000 m alt., 29 Dec. 1925–1 Jan. 1926, P.C. Standley 43625 (US1307153); P.C. Standley 43678 (US1307155); Cordillera de Talamanca, Cerro de la Muerte, Asuncion, 3250 m alt., 13 July 1968, R.W. Pohl 10693 (US3096655).

Notes. The possible affinity of *Agrostis bacillata* to *Podagrostis* was mentioned previously (Pohl and Davidse 1994; Briceño 2010), with Pohl and Davidse (1994) recommending transfer of *A. bacillata* to *Podagrostis*. The species is similar to *P. trichodes* and *P. exserta* (see notes under these taxa on how to differentiate them). The character

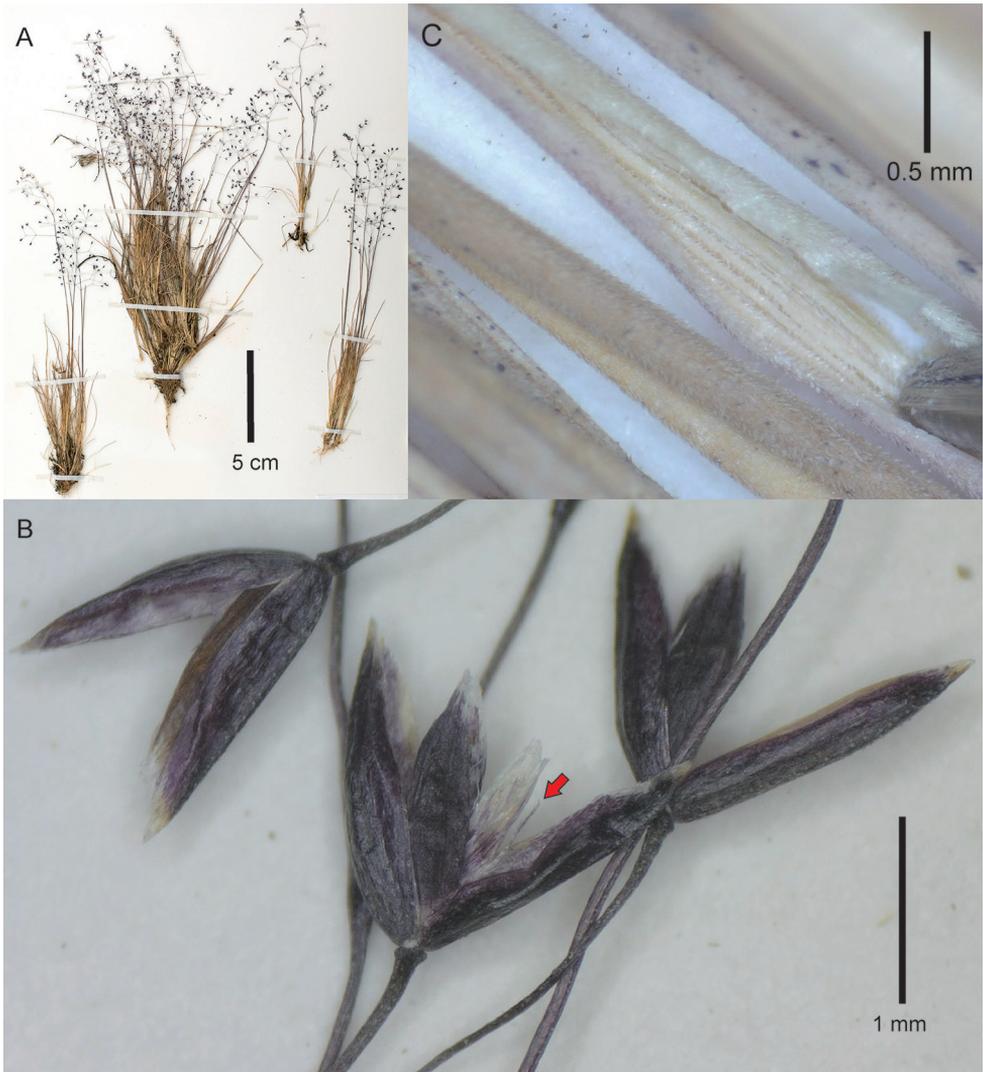


Figure 1. *Podagrostis bacillata*. **A** Whole plant **B** spikelets, the center spikelet at anthesis with the palea opened out and the rachilla prolongation visible (indicated with a red arrow) **C** leaf blades showing the densely scabrous abaxial surface and the central blade opened out to reveal the scabrous adaxial surface. **A, B** Images of specimen A.M. Evans 145 (US2589766A) courtesy of United States National Herbarium (US) **C** image of specimen R.W. Pohl 11705A (US3096988).

of adaxial leaf blade surface with conical trichomes intermixed with scabers across a scaberulous surface, mentioned by Pohl and Davidse (1994) to be a key character for differentiating *P. bacillata* from *P. exserta*, was not found in any of the Costa Rican specimens studied at US, including specimen Pohl 10693 that was cited by Pohl and Davidse (1994). All specimens studied of this species were generally densely scabrous on the veins of the adaxial blade surface, with short to long and robust scabers of simi-

lar consistency to the abaxial surface. This could be attributed to a difference in interpretation by the authors, although similar long scabers, in a similar density, were found on the adaxial leaf blade surface of specimens of *P. exserta*, meaning this character is not considered useful for species delimitation of these taxa. Instead, we found that a key character for differentiating the two species was the presence or absence of scabrocities on the abaxial leaf blade surface, with *P. bacillata* usually being densely scabrous with short hooks while *P. exserta* is smooth.

Although we have not studied characters of foliar anatomy, Pohl and Davidse (1994) mention these to be important in separation of *P. exserta* and *P. bacillata*. Pohl and Davidse (1994) also mention that spikelets are amply open at maturity in *P. exserta*, while those of *P. bacillata* are more closed, although this distinction is not always clear. Caryopsis shape is also mentioned to be distinct (Pohl and Davidse 1994), although no fruiting specimens were found among specimens at US.

Pohl (1980) mentions the glumes of *P. bacillata* to be 3-veined, although this is not mentioned by Pohl and Davidse (1994) or Morales-Quirós (2003). Most of the spikelets that we examined had 1-veined glumes, with vestigial lateral veins sometimes present.

***Podagrostis exserta* (Swallen) Sylvester & Soreng, comb. nov.**

urn:lsid:ipni.org:names:77209700-1

Fig. 2

Agrostis exserta Swallen, Contr. U.S. Natl. Herb. 29(9): 404. 1950.

Type. GUATEMALA. Dept. Huehuetenango: collected in alpine area, vicinity of Tojquia, Sierra de los Cuchumatanes, 3700 m alt., 5 Aug. 1942, J.A. Steyermark 50119 (**holotype**: US (US00131747 [not seen]); **isotype**: US (US00131748 [not seen])).

Description. *Tufted perennial* forming short dense tufts, with the basal mats reaching c. 3–6 cm tall and inflorescences usually well-exserted from the basal foliage. *Tillers* intravaginal. *Culms* 5–15(–30) cm tall, erect, simple, delicate; *nodes and internodes* terete, smooth, nodes usually hidden in the sheaths with 0(–1) nodes exposed at flowering, uppermost internode usually < 1 cm long (to 4 cm long in Steyermark 50216), usually not longer than the sheath. *Leaves* generally basal; *sheaths* terete, glabrous, smooth; flag sheath 2–5.6 cm long; basal sheaths 0.5–1.5 cm long, striate, becoming fibrous; *ligules* 0.8–2(–2.5) mm long, membranaceous, slightly to usually strongly decurrent with the sheath; upper culm ligules acute with a obtuse to truncate apex, usually slightly erose towards the apex; ligules of tillers similar to those of the culm; *blades* 1–4(–5.5) cm long, 0.3–0.4(–0.6) mm wide in diameter, involute or convolute, acicular to capillaceous and filiform, usually curved, abaxial surfaces glabrous, smooth, adaxial surfaces glabrous, lightly to usually densely scabrous with scabers usually short, less often long and robust, edges smooth to lightly scabrous with very short hooks. *Panicles* (1.2–)2–5 × (0.5–)1–2.5 cm, open to slightly congested

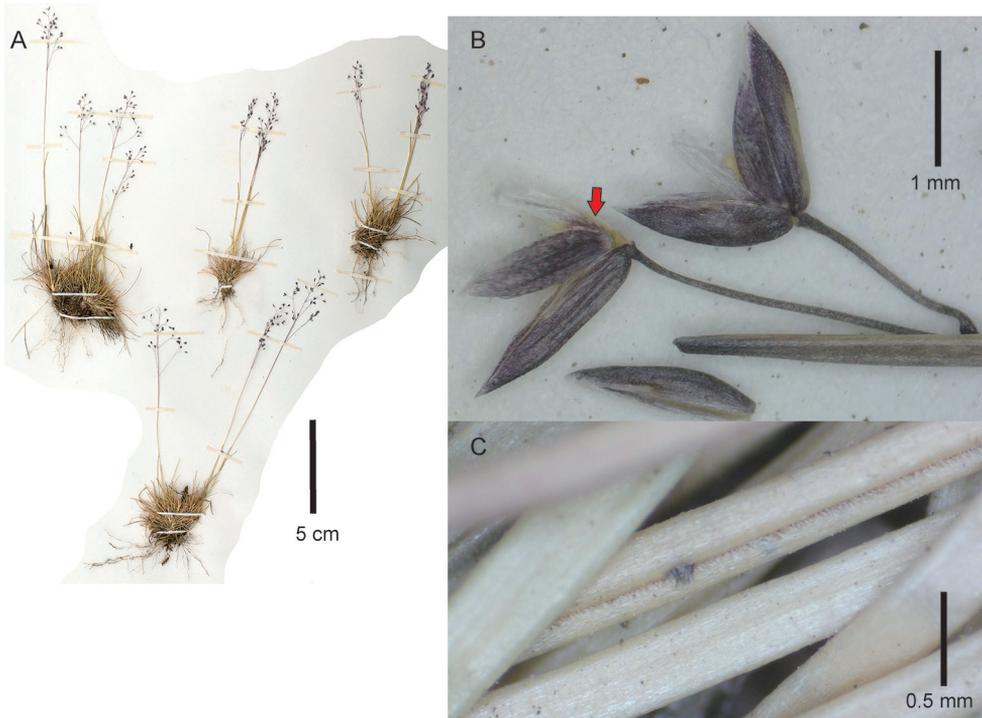


Figure 2. *Podagrostis exserta*. **A** Whole plant **B** Spikelets, the upper glume of the left-hand spikelet has been removed to reveal the rachilla prolongation (indicated with a red arrow) **C** leaf blade adaxial view, showing scabers on the adaxial surface and smooth abaxial surface of the leaf blade. **A** Digitized image of specimen J.H. Beaman 3873 (US01247246) courtesy of United States National Herbarium (US) **B, C** images of specimen G.L. Webster 11826 (US3336153).

when young, usually ovoid; **panicle branches** ascendant to patent, branched above the middle, filiform, with spikelets not present near the base, glabrous, smooth, longest branches 0.3–2 cm long; **pedicels** (1–)1.5–5 mm long, usually longer than the length of the spikelets, divaricate, glabrous, smooth. **Spikelets** (1.2–)1.5–2 mm long; **glumes** remaining on the inflorescence at maturity, equal or subequal, the lower usually slightly longer than the upper, subequaling the length of the floret or slightly longer, oblong–lanceolate, slightly keeled, apex broadly acute, glabrous, keels lightly scabrous just in the distal 1/3, surfaces smooth; lower glume 1- or 3-veined; upper glume 1- or 3-veined; **lemmas** (1.1–)1.4–1.6 mm long, glabrous, smooth, faintly to strongly 5-veined, apex obtuse, awn lacking or to 0.5 mm long, straight, inserted medially or in the upper 1/3; **paleas** 0.9–1.3 mm long, notable, usually reaching from $\frac{3}{4}$ to subequaling the lemma, infrequently reaching $\frac{2}{3}$ the length of the lemma, keels usually obscure, smooth, apex bifid and crose; **rachilla** 0.3–0.5 mm long, prolonged from the base of the floret (sometimes lacking in a small number of spikelets within the inflores-

cence), glabrous, smooth or scabrous. **Calluses** 0.1–0.2 mm long, slightly elongated, glabrous, smooth. **Flowers; lodicules** c. 0.5 mm long, lanceolate with acute apices, not lobed; **anthers** 3 in number, 0.7–1.1 mm long. **Caryopses** not seen. $2n$ = unknown.

Distribution and ecology. Guatemala, endemic. Grows in alpine grasslands on volcanic soils, 2900–3700 m.

Additional specimens examined. GUATEMALA. **Huehuetenango:** Sierra de los Cuchumatanes, alpine areas in vicinity of Tunima, 3400–3500 m alt., 7 July 1942, J.A. Steyermark 48321 (US1914763; US2208640); Sierra de los Cuchumatanes, between Tojiah and Chemal at km 319.5 on Ruta Nacional 9N, 3380 m alt., 31 July 1960, J.H. Beaman 3873 (US01247246); Sierra de los Cuchumatanes, between Tojquia and Caxin bluff, summit of Sierra de los Cuchumatanes, 3700 m alt., 6 Aug. 1942, J.A. Steyermark 50216 (US2181354). **Solola:** Woods 11 miles SE of Tonicapán, 3200 m alt., 27 June 1962, G.L. Webster 11826 (US3336153). **Tonicapán:** Desconsuelo, potrero natural, 3100 m alt., Aug. 1954, M. de Koninck 201 (US2153266); On the Tecum Uman Ridge at km 154 on Ruta Nacional No. 1, ca. 20 km east of Tonicapán, 3340 m alt., 13 Aug. 1960, J.H. Beaman 4154 (US2381726).

Notes. Similar in general habit to *P. trichodes* (see notes under this species for how to distinguish them). *Podagrostis bacillata* also has smooth panicle branches and pedicels and bears very similar spikelet characteristics to *P. exserta*, with both having spikelets usually measuring > 1.5 mm long, with smooth glumes apart from the keel being lightly scaberulous, and smooth lemmas. *Podagrostis bacillata* can be distinguished from *P. exserta* principally by the culms having at least one visible elongated internode, 4–13 cm long, with usually at least one node exerted from the sheaths (vs. usually no visible elongated internodes or nodes, with the distalmost internode usually < 1 cm long in *P. exserta*), leaf blade abaxial surface lightly to usually densely scabrous (vs. smooth in *P. exserta*), leaf blades usually longer, 2–15 cm long, forming a basal mat reaching c. 6–17 cm tall (vs. usually < 4 cm long, forming a basal mat reaching c. 3–6 cm tall in *P. exserta*), ligules 1.7–4.3 mm long (vs. 0.8–2.5 mm long in *P. exserta*), and a rachilla prolongation 0.3–1.4 mm long (vs. 0.3–0.5 mm long in *P. exserta*), (also see notes under *P. bacillata*). However, one specimen (Steyermark 50216) did have a moderately long internode at c. 4 cm long, as well as slightly longer leaf blades to 5.5 cm long, but could be differentiated based on the short ligules, < 1.4 mm long, short panicles < 3 cm long, and, crucially, the leaf blade abaxial surface being smooth, with scabers only present on the adaxial surface.

The possible affinity of *A. exserta* to *Podagrostis* was mentioned previously (Pohl and Davidse 1994; Briceño 2010), with Pohl and Davidse (1994) recommending transfer of *A. exserta* to *Podagrostis*. Despite repeated efforts by different researchers (e.g. P. Barbera, Y. Herrera, P.M. Peterson, J. R. Reichman, K. Romaschenko, L.S. Watrud, pers. comm.) to sequence leaf samples from herbarium specimens of this species, none have been successful to date largely due to the available specimens having mostly aged, brownish leaves. This may be a characteristic of the species in general, with only new fresh shoots being found very early in the growing season and becoming brown at maturity. Successful molecular sampling of this species is necessary.

***Podagrostis liebmannii* (E. Fourn.) Sylvester & Soreng, comb. nov.**

urn:lsid:ipni.org:names:77209701-1

Fig. 3

Agrostis liebmannii (E. Fourn.) Hitchc., N. Amer. Fl. 17(7): 519. 1937. *Apera liebmannii* E. Fourn., Mexic. Pl. 2: 97. 1886.

Type. MEXICO. [Veracruz:] Orizaba, M. Botteri 93 in part (**lectotype**, designated here: P (P00740547 [image!]); isolotypes: MSC fragm. ex P; **epitype**, designated here: Mexico. [Puebla:] Chinantla, May 1841, F.M. Liebmann s.n. "Plantae mexicanae Liebmann #12591" {handwritten on label} (US (US00595641!)). **Syntype:** Mexico. [Puebla:] Museum Botanicum Hauniense | Plantae mexicanae Liebmann | 1841–43 {printed label}, Gramineae N. 710 | *Apera liebmannii* Fourn. | [determ.] Fournier | Chinantla. May 1841, {handwritten on label} (NY (NY00345814 [image!]), US [not seen]).

Description. *Tufted perennial* forming lax tufts, with the basal foliage reaching c. 11 cm tall and inflorescences well-exserted from the basal foliage. **Tillers** extravaginal. **Culms** 35–80 cm tall, erect, simple, delicate; **nodes and internodes** terete, nodes smooth, internodes and segment below the panicle smooth throughout (or smooth proximally and lightly scaberulous towards their apices in Moore 3339), usually with at least 1 or 2 elongated internodes visible, with 1–2 nodes exposed at flowering, uppermost internode c. 5.5–11.2 cm long, longer than the sheath. **Leaves** basal and cauline; **sheaths** terete, glabrous, lower sheaths tending to be smooth, upper sheaths lightly to densely scabrous with short hooks; flag sheath 8–11.5 cm long; basal sheaths 0.5–1 cm long, striate, becoming fibrous, smooth; **ligules** c. 0.5–4 mm long, membranaceous or scarious, usually strongly decurrent with the sheath, abaxially scabrous; upper culm ligules 1.5–4 mm long, obtuse to acute, sometimes slightly erose towards the apex; ligules of tillers shorter to those of the culm, c. 0.5–1 mm long, truncate to acute; **blades** 2–8.6 cm long, (0.5–)1–3.5 mm wide in diameter, flat, flaccid to firm, basal blades sometimes very narrow, abaxial surfaces glabrous, smooth to lightly scabrous, or usually more densely scabrous further up the culm, adaxial surfaces glabrous, smooth to lightly scabrous on the veins, scaberulous further up the culm, edges scaberulous. **Panicles** 7–18 × (1–)2.5–7 cm, open to slightly congested following anthesis, usually ovoid; **panicle branches** ascendant to patent, branched above the middle, filiform, with spikelets not present near the base, smooth, longest branches 2–4.7 cm long; **pedicels** 1.5–5 mm long, usually longer than the length of the spikelets, divaricate, smooth or lightly scaberulous. **Spikelets** (1.8–)2–2.1 mm long; **glumes** remaining on the inflorescence at maturity, equal or subequal, the lower usually slightly longer than the upper, subequaling the length of the floret to 0.4 mm longer, lanceolate, slightly keeled, apices acute, glabrous, keels lightly scaberulous just in the distal 1/3, surfaces smooth; lower glume 1- (or 3-)veined, lateral veins, if present, vestigial; upper glume 1- (or 3-)veined, lateral veins, if present, vestigial; **lemmas** 1.7–2 mm long, glabrous, smooth, strongly 5-veined with excurrent prominent veins, apex broadly acute, awn

absent; *paleas* well-developed, 1.4–1.9 mm long, usually reaching from $\frac{3}{4}$ to sub-equaling the lemma, keels obscure, smooth, apex bifid and sometimes erose; *rachilla* prolonged from the base of the floret, 0.3–0.5 mm long, glabrous, smooth. *Calluses* not or slightly elongated, 0.05–0.1 mm long, glabrous, smooth. *Flowers; lodicules* c. 0.3–0.4 mm long, lanceolate with acuminate apices, not lobed; *anthers* 3 in number, 0.6–1 mm long. *Caryopses* not seen. $2n =$ unknown.

Distribution and ecology. Mexico, endemic. The authors have only verified specimens from Hidalgo, Puebla and Veracruz states of central Mexico, with Beetle (1983), Dávila et al. (2018), and Sánchez-Ken (2019) mentioning the species to range from Durango state in the north to Oaxaca state in southern Mexico. Found in humid areas of pine and fir forests, *Sphagnum* bogs, and by streams, 2100–2300 m (Beetle 1983).

Other specimens examined. MEXICO. **Hidalgo:** Distrito Zacualtipan, pine woods and *Sphagnum* bogs about 3 miles from Zacualtipan on road to Tianguistengo, 2100 m alt., 4 July 1947, H.E. Moore, Jr. 3339 (US00486609).

Notes. Beetle (1983) appears to consider *Agrostis durangensis* Mez a synonym of *A. liebmannii*, and states *A. liebmannii* to be distributed as far north as Durango state based on the type locality of *A. durangensis*. However, we consider *A. durangensis* to be a synonym of *A. exarata* Trin. and have only verified specimens from as far north as Hidalgo state. Herrera-Arrieta (2001) cite *A. liebmannii* for Durango state based on specimen Palmer 190 (US00486604), which is treated here as *A. exarata*. Herrera-Arrieta (2014), Dávila et al. (2018) and Sánchez-Ken (2019) also mention *Agrostis liebmannii* to be found as far north as Durango state, but do not include voucher specimens and may have based this on Beetle's (1983) and Herrera-Arrieta's (2001) treatments. Certain characteristics in Beetle's (1983: 82) description of *A. liebmannii* also do not fit the specimens examined, which may be due to the author's inclusion of *A. durangensis* in the species circumscription. Spikelet size of 2.5 mm mentioned by Beetle (1983: 82) does not fit the specimens studied which had spikelets (1.8–)2–2.1 mm long, although Beetle's (1983: 73) key to species separates *A. liebmannii* from *A. hiemalis* var. *laxiflora* (Michx.) Beetle (= *Agrostis scabra* Willd.) based on spikelets being c. 2 mm long and shorter than 2.5 mm long. Beetle's (1983) mention that the tiller blades are more-or-less involute was also not seen, although specimen Moore 3339 (US00486609) was intermixed with another species with involute blades, which might explain this.

Podagrostis liebmannii bears close affinity to *P. thurberiana*, a North American species that is found as far south as California (Hitchcock et al. 1969; Harvey 2007). These similarities include a) the overall habit, with tall culms, loosely tufted and subrhizomatous habit with extravaginal shoots; b) spikelet morphology, with spikelets usually < 2.3 mm long, lemmas with excurrent prominent veins and paleas almost subequalling the lemma; c) panicles open and becoming slightly congested following anthesis; d) flat leaf blades. *Podagrostis liebmannii* can be distinguished from the aforementioned species by a) panicles generally much larger, 8–18 × (1–)2.5–7 cm, with patent panicle branches at anthesis (vs. 5–14 × 0.2–3 cm, panicle branches usually ascending at anthesis in *P. thurberiana*); b) callus glabrous (vs. with short hairs to 0.5 mm long emerging from the basal side-ridges of the callus in *P. thurberiana*); c) rachilla glabrous, smooth (vs. short hairs to 0.3 mm long emerging from the apex of the rachilla in *P. thurberiana*).



Figure 3. *Podagrostis liebmannii*. **A** Whole plant **B** ligular area of flag leaf **C** section of inflorescence showing spikelets **D** floret, ventral view, with the rachilla prolongation indicated with a red arrow. Images of epitype specimen Liebmann s.n. (US00595641) courtesy of the United States National Herbarium (US).

Fournier (1886: 97) cited two specimens, Liebmann 710 and Botteri 93, in the protologue but only indicated the P herbarium for the Botteri 93 specimen. As we are sure that Fournier saw the Botteri 93 specimen at P, we lectotypify on this collection.

Because both the Botteri 93 and Liebmann 710 material included just an inflorescence culm and part of the flag leaf and did not show the basal parts of the plant, we also epitypify the lectotype on Liebmann s.n. (US00595641) that was collected on the same date and at the same locality as the Liebmann 710 collection and includes the basal portion of the plant to help interpret the lectotype. The epitype Liebmann s.n. (US00595641) cited here may in fact be a duplicate of the Liebmann 710 syntype that Fournier (if he ever saw it) did not annotate at the Copenhagen herbarium. The US specimen identified as *Agrostis liebmannii* (“*Apera*” not mentioned) was catalogued as *Plantae mexicanae* Liebmann #12591; these catalogue numbers are often followed by Liebmann’s field numbers such as the handwritten “710”. The Copenhagen herbarium does not have a Liebmann duplicate bearing either of these numbers, or either of the synonyms.

***Podagrostis rosei* (Scribn. & Merr.) Sylvester & Soreng, comb. nov.**

urn:lsid:ipni.org:names:77209702-1

Fig. 4

Agrostis rosei Scribn. & Merr., Bull. Div. Agrostol., U.S.D.A. 24: 21, f. 5. 1901.

Type. MEXICO. **Zacatecas:** Sierra Madre mountains, [between Huasemote, Durango, and San Juan Capistrano], 18 Aug. 1897, J.N. Rose 2373 (**holotype:** US-301286 [not seen]; **isotype:** NY (NY00327649 [image!])).

Description. *Tufted perennial* forming lax tufts, with the basal foliage reaching c. 4–9 cm tall and inflorescences well-exserted from the basal foliage. *Tillers* extravaginal. *Culms* 34–65 cm tall, erect or geniculate at the base, simple, delicate; *nodes and internodes* terete, nodes smooth, internodes and segment below the panicle smooth throughout, usually with at least 2 elongated internodes visible, with 1–3 nodes exposed at flowering, uppermost internode c. 5.6–9 cm long, longer than the sheath. *Leaves* basal and cauline, somewhat dimorphic with basal leaf blades filiform, flat or folded, while culm blades are wider and flat; *sheaths* terete, glabrous, lower sheaths smooth, upper sheaths smooth to very lightly scaberulous with short hooks; flag sheath 9–11.5 cm long; basal sheaths c. 1–2 cm long, striate, becoming fibrous, smooth; *ligules* c. 1–3 mm long, membranaceous to scarious, strongly decurrent with the sheath, sometimes lacerated, abaxially smooth or very lightly scaberulous; upper culm ligules 1.5–3 mm long, obtuse, sometimes deeply lacerated or erose towards the apex; ligules of tillers shorter to those of the culm, c. 1–1.3 mm long, truncate to obtuse; *blades* 1.5–9 cm long, 0.2–2.6 mm wide when opened out, basal blades 1.5–7 cm long, 0.2–0.6(–1) mm wide when opened out, usually narrower than the culm blades, filiform, flat or folded, flaccid to slightly firm, culm blades (2.8–)3.5–9 cm long, 1–2.6 mm wide, flat or sometimes slightly convolute towards the apices, flaccid to moderately firm, abaxial and adaxial surfaces glabrous, smooth or usually lightly to moderately scabrous on the veins with short hooks, edges scaberulous to scabrous. *Panicles* 8–14 × (1–)2.5–9 cm, open to slightly congested when immature, usually ovoid; *panicle branches* ascendant

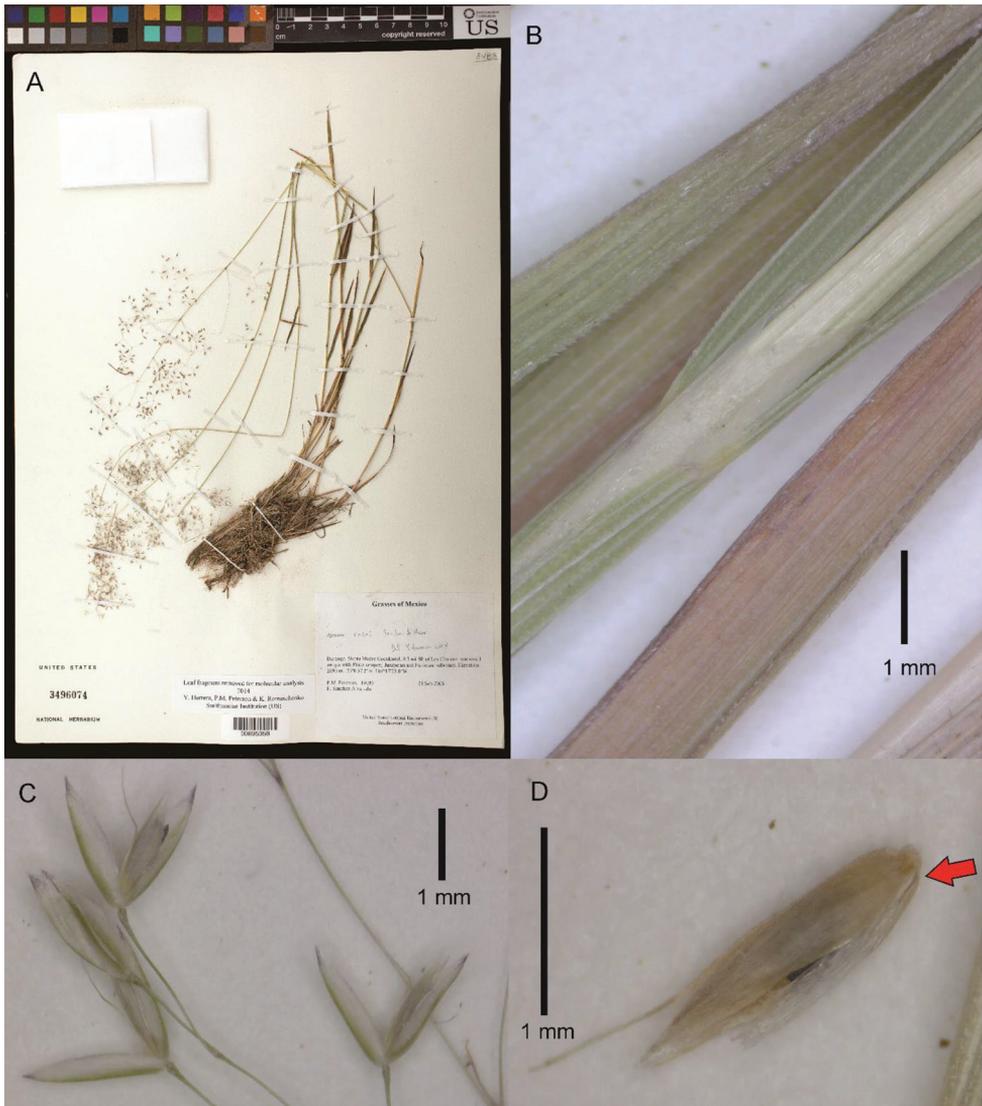


Figure 4. *Podagrostis rosei*. **A** Whole plant **B** ligular area of upper culm leaf **C** section of inflorescence showing spikelets. **D** Floret, lateral view, with the rachilla prolongation indicated with a red arrow. **A** Digitized image of specimen P.M. Peterson 19503 (US00895358) courtesy of United States National Herbarium (US) **B, C** image of specimen P.M. Peterson 19124 (US00900682) **D** image of specimen J.R. Reeder 4662 (US00486613).

to patent, branched above the middle, filiform, with spikelets not present near the base, smooth, longest branches 2.5–7 cm long; *pedicels* 1–2.5 mm long, shorter or longer than the length of the spikelets, divaricate, smooth or lightly scaberulous. *Spikelets* 1.9–2.3 mm long; *glumes* remaining on the inflorescence at maturity, equal or subequal, the lower usually slightly longer than the upper by up to 0.2 mm, subequaling the length of the floret or to 0.2 mm longer, lanceolate, slightly keeled, apices acute,

glabrous, keels completely smooth or scaberulous just in distal 1/3, surfaces smooth; lower glume 1- (or 3-)veined, lateral veins, if present, vestigial; upper glume 1- (or 3-)veined, lateral veins, if present, vestigial; **lemmas** 1.6–1.8 mm long, glabrous, smooth, strongly 5-veined with slightly excurrent prominent veins distally, apex broadly acute with 4 deltoid teeth, awn present, 1.6–2 mm long, straight, flexuous or geniculate, inserted in the lower 1/3 of the lemma, sometimes inserted basally c. 0.3 mm from the base, reaching the apex of the lemma, the glumes or sometimes briefly surpassing the glumes, glabrous, smooth proximally, scabrous distally; **paleas** well-developed, 1.3–1.7 mm long, usually reaching at least $\frac{3}{4}$ the length of the lemma to subequalling the lemma apex, keels obscure, smooth, apex bifid and sometimes erose; **rachilla** rudimentary or prolonged from the base of the floret, to 0.3 mm long, glabrous, smooth. **Calluses** not or slightly elongated, 0.05–0.1 mm long, glabrous, smooth. **Flowers; lodicules** c. 0.4–0.5 mm long, lanceolate with acute apices, not lobed; **anthers** 3 in number, 0.8–1 mm long. **Caryopses** not seen. $2n = 14$ (based on Reeder 4662).

Distribution and ecology. Mexico, endemic. Ranges from Durango state in the north to Zacatecas in the south. Found in open forests at high elevations, 2600–2750 m alt. The authors have only verified specimens from Durango and Zacatecas states, with Beetle (1983), Villaseñor Ríos (2016), Dávila et al. (2018) and Sánchez-Ken (2019) variously mentioning the species to also occur in the states of Distrito Federal, Hidalgo, Jalisco, Mexico, Querétaro, and San Luis Potosí.

Other specimens examined. MEXICO. **Durango:** Pine forest with scattered oaks and occasional junipers, about 2 miles E of Puerto Buenos Aires, 9000 ft [2743 m alt.], 11 Oct. 1966, J.R. Reeder 4662 (US00486613); Sierra Madre Occidental, 0.5 miles SE of Los Charcos near small arroyo with *Pinus cooperi*, *Juniperus* and *Panicum bulbosum*, 23°0'57.5"N, 104°17'23.0"W, 2690 m alt., 21 Sep. 2005, P.M. Peterson 19053 (US00895358); Sierra Madre Occidental, 2.3 miles E of Los Mimbres along ridgetop with *Pinus*, *Muhlenbergia* and *Quercus*, 23°28'31.4"N, 104°55'3.6"W, 2630 m alt., 25 Sep. 2005, P.M. Peterson 19124 (US00900682).

Notes. This species is distinct from all other species of *Podagrostis* currently circumscribed by the presence of a well-developed awn inserted in the lower dorsal surface of the lemma. Molecular data supports its inclusion in *Podagrostis* (Konstantin Romaschenko, pers. communication) with morphological attributes also corroborating this such as the florets subequalling the apices of the glumes, a well-developed palea > $\frac{3}{4}$ the length of the lemma, and completely glabrous spikelets. The lax and open, large panicles, and completely glabrous and mostly smooth spikelets, pedicels and panicle branches places it very close to *P. liebmannii*.

***Podagrostis trichodes* (Kunth) Sylvester & Soreng, comb. nov.**

urn:lsid:ipni.org:names:77209703-1

Fig. 5

Aira trichodes (Kunth) Spreng., Syst. Veg. [Sprengel] 1: 276. 1825[1824]. *Agrostis trichodes* (Kunth) Roem. & Schult., Systema Vegetabilium 2: 361. 1817.

Vilfa trichodes Kunth, Nova Genera et Species Plantarum (quarto ed.) 1: 139. 1815[1816].

= *Agrostis bogotensis* Hack., Repert. Nov. Sp. Fedde 8: 518. 1910. Type: COLOMBIA. S. Cristobal prope Bogota [près de Bogota], [2500–3000 m alt.], 13 July 1908, *F. Apollinaire s.n.* (holotype: W (W19160027256 [image!]); isotypes: BM (BM000938528 [image!]), MPU (MPU027104 [image!]), SI (SI000495 [image!] fragm. ex US), US (US75365 fragm. [not seen])).

Type. PERU. Crescit in crepidinibus Andium Peruvianum justa Montan, Santa Cruz et Guambos, alt. 1350 hexap. [2469 m alt.], floret Augusto, *F.W.H.A. Humboldt & A.J.A. Bonpland s.n.* (**holotype:** P [not seen]; **isotypes:** HAL (HAL0106929 [image!]), US (US75364! fragm. ex P)).

Description. *Tufted perennial* forming short dense tufts, with the basal mats reaching c. 4–11 cm tall and inflorescences well-exserted from the basal foliage. **Tillers** intravaginal. **Culms** 7–20(–30) cm tall, erect, simple, delicate; **nodes and internodes** terete, smooth, nodes usually hidden in the sheaths with 0(–1) nodes exposed at flowering, uppermost internode usually < 1 cm long, usually not longer than the sheath. **Leaves** generally basal; **sheaths** terete, glabrous, finely to densely scabrous; flag sheath 2–5.6 cm long; basal sheaths 0.7–1.5 cm long, striate, becoming fibrous; **ligules** 0.7–1.7(–2.5) mm long, membranaceous, slightly to usually strongly decurrent with the sheath; flag ligules acute with a obtuse to truncate apex, usually slightly erose towards the apex; ligules of tillers 0.7–1.2 mm long, truncate; **blades** 1–4 cm long, 0.3–0.4 mm wide in diameter, involute or convolute, acicular to capillaceous and filiform, usually curved, abaxial surface glabrous, finely to densely scabrous, adaxial surface glabrous, lightly to usually densely scabrous with prickle hairs usually short, less often long and robust. **Panicles** 2–5(–6) × 1–2(–3) cm, open, ovoid; **panicle branches** ascendant to patent, branched above the middle, filiform, with spikelets not present near the base, smooth to usually scaberulous, longest branches 0.8–3 cm long; **pedicels** 1–2 mm long, usually longer than the length of the spikelets, divaricate, smooth to usually lightly scabrous. **Spikelets** 1–1.5 mm long; **glumes** remaining on the inflorescence at maturity or one or both readily caducous at maturity and falling before the floret, equal or subequal, the lower often slightly longer than the upper or less often vice versa, almost equaling the length of the floret or slightly longer, oblong–lanceolate, slightly to distinctly keeled, apex obtuse to acute, glabrous, keels scabrous just in the distal 1/3 to throughout their length, surfaces smooth a scabrous distally; lower glume 1-veined; upper glume 1- or 3-veined; **lemmas** 1–1.5 mm long, glabrous, moderately to densely scabrous ('smooth' possibly mentioned by Tovar 1993!), sometimes granulose, faintly to strongly 5-veined, apex obtuse, awn lacking or to 0.5 mm long, straight, inserted medially or in the upper half of the lemma; **paleas** (0.7–)0.9–1.3 mm long, usually reaching from ¾ to subequaling the lemma, less often reaching 2/3 the length of the lemma, keels obscure to fairly prominent, smooth, apex bifid and erose; **rachilla** absent or prolonged from the base of the floret (sometimes lacking in a small number of spikelets within the inflorescence), 0.2–0.5 mm long, glabrous, smooth to scabrous. **Calluses** 0.05–0.1 mm long, slightly elongated or not, glabrous. **Flowers; lodicules**

c. 0.4 mm long, lanceolate with acute apices, not lobed; **anthers** 3 in number, 0.4–1 mm long. **Caryopses** c. 1 mm long, subterete, sulcus distinct, dark brown with apex dark; hilum 0.25 mm long, narrowly ovoid; endosperm solid. $2n =$ unknown.

Distribution and ecology. Bolivia?, Colombia, Ecuador?, Peru, Venezuela, 2800–4500 m alt. Relatively humid high-Andean puna grasslands of southern and central Peru and páramo grasslands of Ecuador, Colombia and Venezuela. Tovar (1993) mentions that the species may also occur in Bolivia, presumably in high-elevation cool and humid sites such as the Bolivian Yungas which have been referred to as páramo (García and Beck 2006), although no specimens have been verified by the authors. No specimens at the US herbarium were found from Ecuador after careful searching by the first author, although it is mentioned to occur there (Hitchcock 1927; Tovar 1993; Jørgensen and Ulloa-Ulloa 1994; Jørgensen and León-Yáñez 1999; Luteyn 1999). In Colombia, the taxon is known from multiple collections from páramos of the Cordillera Oriental of the Colombian Andes, belonging to Departamentos Cundinamarca, Boyacá, Santander, Santander Norte and Cesar. We present new regional records of the species for Departamentos Santander Norte and Cesar which are not mentioned in the recent checklist (Giraldo-Cañas et al. 2016). Giraldo-Cañas et al. (2016) also cite *Agrostis trichodes* for Departamento Meta, in the southernmost part of the Cordillera Oriental, and Departamento Magdalena, which contains páramos of the Sierra Nevada de Santa Marta, although no specimens have been verified. In Venezuela, the species is found in páramos of the Cordillera de Merida.

Usually found in frequently grazed areas where its short basal tufts of leaves are difficult for grazers to reach. Specimens from Peru appear to be found in humid habitats, with the specimens studied by Tovar (1993) collected from the Abra Malaga of the Cusco region which is relatively humid and receives updrafts of moisture-laden air from the Amazon (Sylvester et al. 2014, 2017). While *P. trichodes* is relatively common in páramos of Colombia and Venezuela, it may be that this species is much rarer further south and, in Peru, belongs to a thin band of humid páramo-like vegetation that extends from the Peruvian Jalca down through southern Peru and into the Bolivian Yungas (Antoine Cleef, pers. communication).

Other specimens examined. COLOMBIA. **Boyacá:** Munic. Chiscas, Vereda Rechiniga, Páramo de la Mesa, área húmeda semiperturbada de Páramo, con *Espeletia*, *Puya*, *Ageratina* e *Hypericum*, 6.59515N, 72.44359W, 3741 m alt., 3 Mar. 2018, S.P. Sylvester 3091 (K, US, FMB); Munic. Chiscas, Páramo de Chacaritas, límites entre páramo y superpáramo, 6.62865N, 72.39440W, 4064 m alt., 4 Mar. 2018, S.P. Sylvester 3103 (K., US); Munic. Chiscas, Páramo el Peñón, borde de bosque de *Polylepis*, 6.60119N, 72.43715W, 3917 m alt., 5 Mar. 2018, S.P. Sylvester 3157 (K, US, FMB, COL, UPTC, SI); Munic. Duitama, Páramo de la Rusia, en la carretera que conduce a la Peña Negra, páramo con rocas expuestas, 5,58389N, 73,053263W, 3970 m alt., 21 Nov. 2017, M. Vorontsova 2218 (K, US, FMB, SI). Munic. Duitama, Páramo de la Rusia, vía que conduce a la Vereda Avendaños, 5.93247N, 73.0798W, 3726 m alt., 4 Oct. 2017, S.P. Sylvester 3038 (K, US, FMB, UPTC); Munic. Duitama, Páramo de Agueros, en la vía que conduce a la vereda Avendaños, Se observa evidencia de fuego y

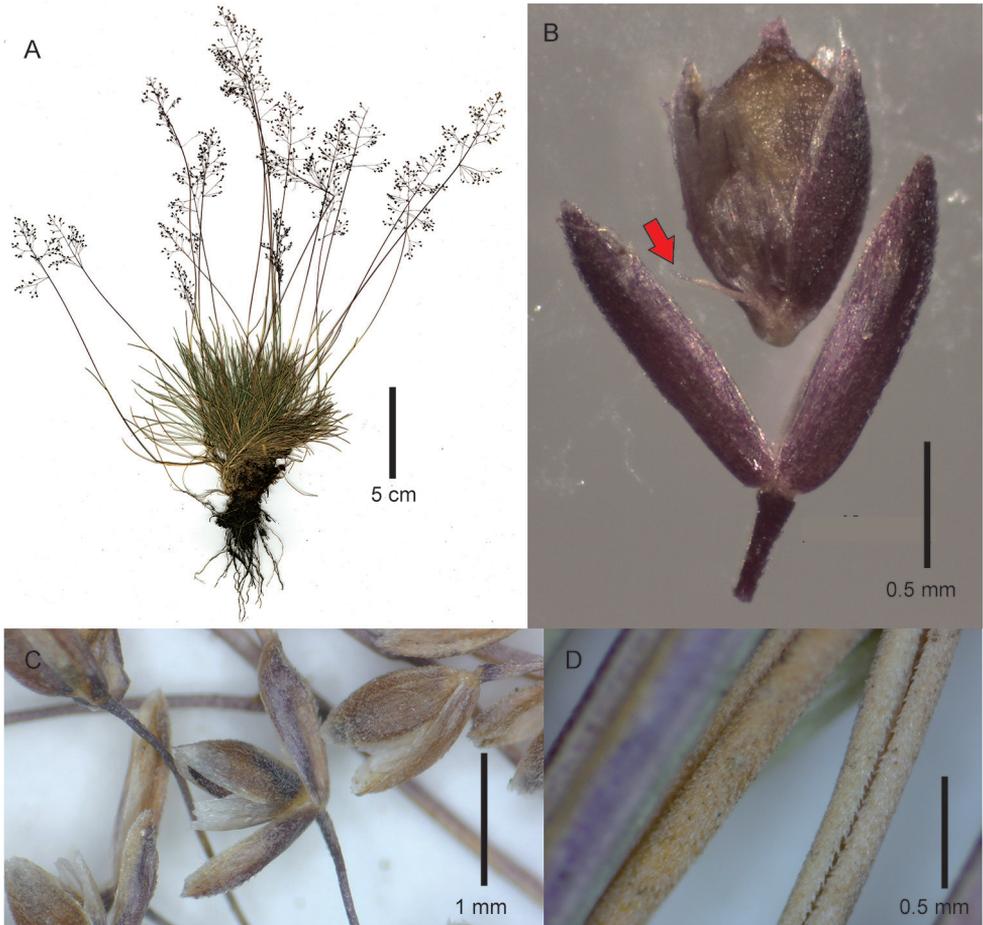


Figure 5. *Podagrostis trichodes*. **A** Whole plant **B** spikelets, with the floret detached and raised above the glumes so that the rachilla prolongation (indicated with a red arrow) can be seen **C** section of inflorescence **D** leaf blade, showing abaxial surface. **A, B** images of specimen L.E. Cuta-Alarcón 362 (FMB) **C, D** images of specimen M.C. Gomez 1 (US3534984).

pastoreo, 5.91464N, 73.07114W, 3445W m alt., 28 Oct. 2017, S.P. Sylvester 3067^a (K, US, FMB); Munic. Mongua, Páramo de Oceta, Valle de Laguna Negra, vegetación de pajonal frailejónal con presencia de pastoreo de vacunos, 5.69525N, 72.79133W, 3694 m alt., 29 Nov. 2017, L.E. Cuta-Alarcón 354 (K, US, FMB). **Santander:** Paramo de la Angostura, Vereda El Mortino, 06°57'30"N, 72°43'30"W, 3605 m alt., 17 Nov. 2007, M.C. Gomez 1 (US-3534984). **Santander Norte & Cesar:** Limites entre Santander Norte y Cesar jurisdicciones, Cerro de Oroque, 3700–3900 m alt., 22–27 July 1974, H. Garcia-Barriga 20588 (US2966591; US2966621).

VENEZUELA. **Mérida:** Sierra Nevada, 9000 ft, 1847, Funck & Schlim 1630 (US); Sierra Nevada de Santo Domingo, between partaderos and Timotes, Paramo

de Mucuchies, Pico Aguila, 4118 m alt., 21–26 Nov. 1959, H.G. Barclay 9685 (US3044346); Sierra Nevada de Santo Domingo, Paramo de Mucubaji, alrededores de la Laguna Grande, 3560–3600 m alt., 19 Nov. 1959, H.G. Barclay 9546 (US3096576); Sierra Nevada de Santo Domingo, Paramo Laguna de Mucubaji, carretera Barinas-Merida, 4200 m alt., 15 Nov. 1958, B. Trujillo 4072 (US3652663). **Trujillo:** Munic. Bocono, Laguna Eco to Pico Guarigay (summit), Monumento Natural Teta de Niquitao-Guirigay, summit of Pico Guarigay, 3600–3870 m alt., 16 Sep. 2003, B. Stergios 20450 (US00772686); Munic. Bocono, Laguna Larga, via Laguna Las Parias to Laguna Eco, Paramo de Motumbo, Monumento Natural Teta de Niquitao-Guirigay, 3400–3600 m alt., 15 Sep. 2003, B. Stergios 20420 (US00772685); Along the border with Merida state, 3400 m alt., 14 Sep. 2003, B. Stergios 20315 (US00772683); B. Stergios 20358 (US00772684); Monumento Natural Teta de Niquitao-Guirigay, sector Las Veguitas, 3060–3080 m alt., 20–21 Aug. 2002, L.J. Dorr 9157 (US00728039); Monumento Natural Teta de Niquitao-Guirigay, Paramo Guirigay, 3400–3600 m alt., 2–3 Aug. 2002, B. Stergios 19851 (US00728050).

Notes. Briceño (2010) noted the possible relationship of *Agrostis trichodes* to *Podagrostis* based on the rachilla prolongation. While studying specimens of *A. trichodes* from páramos of Colombia and Venezuela, SPS noted certain characteristics differed from the type collected in Peru, the protologue, and the description in the treatment of grasses of Peru (Tovar 1993). These characteristics, including presence of a rachilla prolongation emerging from the base of the florets, and lemmas sometimes with a short dorsally inserted awn, are also shared by *A. bacillata* and *A. exserta*, and highlight the connection of this species to *Podagrostis*.

The character of awn presence was not noted for this species by Hitchcock (1927) nor Tovar (1993), although Briceño (2010) mentions this for Venezuelan material. While Hitchcock (1927) highlights the rachilla prolongation as a crucial character for distinguishing this species from other *Agrostis*, Tovar (1993) did not mention it. This information is also lacking from the protologues of both *Vilfa trichodes* and *Agrostis bogotensis*. The *Vilfa trichodes* isotype at HAL bears spikelets which lack a rachilla extension, and lemmas that lack awns (Marcus Lehnert and Natalia Tkach, pers. communication). It appears that Oscar Tovar, when preparing his treatment of the grasses of Peru (Tovar 1993), had only seen the US isotype fragment, which lacks florets. His mention that the glumes are ‘glabrous’ (by which Tovar meant glabrous and smooth) raises ambiguity, although most other characters found in the description and illustration match. The flag leaf ligule of the US isotype fragment reached 1.5 mm long, while Tovar (1993) mentions the ligule to measure 2–2.5 mm long, with most material studied from Colombia and Venezuela having flag leaf ligules to 1.7 mm long, with those of the tillers c. 0.5 mm long. Tovar’s (1993) description seems to have been based largely on Tovar and Rivas-Martínez 8076, 8080 from Abra Malaga of the Cusco region of southern Peru, which were not seen by us. The first author visited the Abra Malaga site to conduct extensive field surveys and botanical collecting during different seasons from 2010–2013 (Sylvester et al. 2014, 2017) but no specimens were encoun-

tered. Aside from the type, no specimens from Peru have been located despite careful searching through the US herbarium.

Podagrostis trichodes closely resembles *P. exserta* and *P. bacillata*, considered endemic to alpine grasslands of Guatemala or páramos of Costa Rica and Panama, respectively (Pohl and Davidse 1994). Key similarities include: a) an overall similar habit (i.e. short tufted herbs with exserted open panicles); b) involute or convolute, acicular or filiform leaf blades; c) presence of a short glabrous rachilla extension emerging from the base of the floret; and d) a short awn often found inserted medially on the lemma dorsal surface. Both *P. bacillata* and *P. exserta* have smooth panicle branches, pedicels, glume surfaces (with only the keels being lightly scaberulous), and lemma surfaces while these are usually lightly to densely scabrous in *P. trichodes*, although specimens have been encountered with almost smooth panicle branches and pedicels [e.g., M.C. Gomez 1 (US3534984), H.G. Barclay 9685 (US3044346), 9546 (US3096576)]. The overall habit of *P. exserta* more closely resembles that of *P. trichodes* than *P. bacillata*, in lacking a visible elongated culm internode and having a shorter panicle (< 5 cm long vs. 4–11 cm long in *P. bacillata*). However, *P. exserta* can be differentiated from *P. trichodes* in having smooth leaf blade abaxial surfaces, lemma surfaces, panicle branches, and pedicels (vs. usually scaberulous to densely scabrous, panicle branches and pedicels infrequently smooth in *P. trichodes*), its glume keels and surfaces being mostly smooth with only few prickly hairs found on the keel distally (vs. glume keels often densely scabrous for most their length with surfaces often scabrous distally in *P. trichodes*), and larger spikelets (usually 1.5–2 mm long vs. 1–1.5 mm long in *P. trichodes*).

Podagrostis bacillata can be differentiated from *P. trichodes* in having culms with at least one visible elongated internode and an exserted node (vs. usually without a visible elongated internode and exserted node in *P. trichodes*), panicles usually larger, 4–11 cm long (vs. 2.5–6 cm long in *P. trichodes*), panicle branches and pedicels generally smooth (vs. usually lightly to densely scabrous, infrequently smooth in *P. trichodes*), longer spikelets, 1.7–2 mm long (vs. 1–1.5 mm long in *P. trichodes*), glumes smooth apart from the lightly scaberulous keel (vs. glume keels often densely scabrous for most their length, with surfaces often scabrous distally in *P. trichodes*), lemmas smooth (vs. lightly to densely scabrous in *P. trichodes*), and rachilla prolongation 3–1.4 mm long (vs. 0.2–0.5 mm long in *P. trichodes*).

Specimens from páramos of Departamento Boyacá, Colombia, were noted to have the unusual character of glumes being readily caducous at maturity and falling before the floret, with mature inflorescences lacking glumes and only the florets remaining on the pedicels. It is not clear whether this may be a reaction to a pathogen or whether it is taxonomically informative since other specimens sometimes lack this character.

Certain specimens of Freire Apollinaire are annotated as isotypes of *Agrostis bogotensis* at P (P00740431 [image!]) and NY (NY00327650 [image!], NY00688633 [image!]) that differ in collection dates, collection numbers, and/or localities from the holotype, with NY00688633 also obviously not the same species. These should be disregarded as type material and reexamined. Apollinaire s.n. K000308373 may be an isotype but the full collection date is missing to help clarify this.

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Sedum formosanum subsp. *miyakojimense* (Crassulaceae), a new subspecies from Miyako-jima Island of the Ryukyu Islands, Japan

Takuro Ito^{1,2}, Chih-Chieh Yu³, Masatsugu Yokota⁴, Goro Kokubugata²

1 Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan **2** Department of Botany, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan **3** CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China **4** Laboratory of Ecology and Systematics, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan

Corresponding author: Takuro Ito (896wfbt5@gmail.com)

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Abstract

We re-examined the taxonomic status of plants treated as *Sedum formosanum* (Crassulaceae) from Miyako-jima Island of the Ryukyu Islands, Japan, using morphological comparison and molecular phylogenetic analyses with related species. In morphology, plants from Miyako-jima Island bore a close resemblance to the other plants of *S. formosanum*, but differed in being perennial, polycarpic, and having lateral axillary branches. Molecular analyses based on ITS of nrDNA and six regions of cpDNA sequencing indicated that the Miyako-jima plants formed a distinct subclade. This subclade was part of a polytomy with three other subclades comprising nine taxa endemic to Taiwan and *S. formosanum* from other areas, including the type locality. Therefore, we propose and describe the Miyako-jima plants as a new subspecies, *Sedum formosanum* subsp. *miyakojimense*.

Keywords

Miyako Islands, phylogeny, stone crop, succulent plants, taxonomy

Introduction

The genus *Sedum* L. (Crassulaceae) comprises about 470 succulent herbaceous species (Thiede and Eggli 2007). Species within this genus are widely distributed in the Northern Hemisphere, and are most diverse in the Mediterranean Sea, Central America, the Himalayas, and East Asia (Stephenson 1994; Thiede and Eggli 2007). A previous phylogenetic study indicated that *Sedum* is a polyphyletic group within seven American genera (Carrillo-Reyes et al. 2009). However, in East Asia, *Sedum* has been shown to be monophyletic (Mayuzumi and Ohba 2004; Carrillo-Reyes et al. 2009). The Flora of China (Fu and Ohba 2001) divides East Asian *Sedum* species into three sections (sects.); *Sedum*, *Oreades* (Fröderström) K.T. Fu, and *Filipes* (Fröderström) S.H. Fu. Section *Sedum* is distinguished from sects. *Oreades* and *Filipes* by adaxially gibbous carpels and follicles, and sect. *Oreades* is differentiated from sect. *Filipes* by the absence of spurred leaves at the base. Additionally, species of sect. *Oreades* generally have yellow or purple-red (rarely red) petals, whereas members of sect. *Filipes* have white or reddish purple (rarely yellow) petals (Fu and Ohba 2001). Seventeen species of *Sedum* are reported from Japan, including four subspecies and four varieties within sect. *Sedum*, and one species within sect. *Filipes* (Ohba 2001).

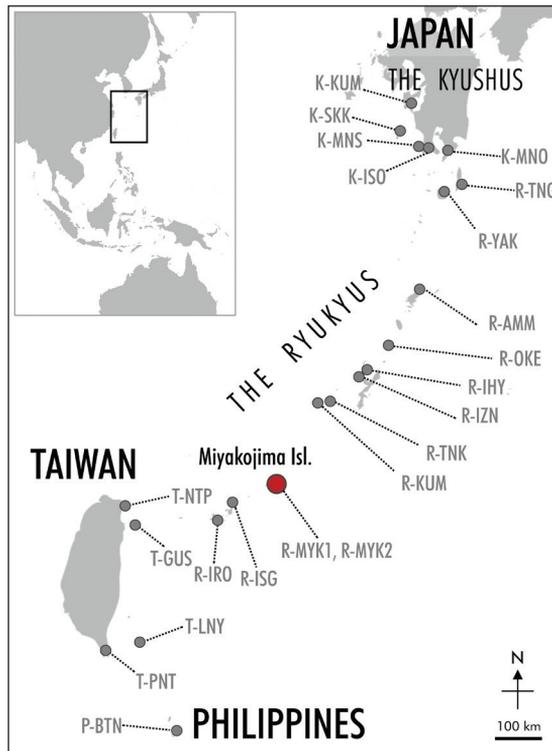


Figure 1. Map showing the location of Miyakojima Island and the adjacent area. The red circle indicates location of Miyakojima Island. The gray circle indicates the others sample localities of *S. formosanum* (see Table 2 for abbreviations for collection localities).

Sedum formosanum N. E. Brown, described based on a type specimen collected from Taiwan (Brown 1885), occurs on rocky seashore slopes in the southern part of Kyushu in the Ryukyu Islands of Japan, in Taiwan, and on Batan Island in the Philippines (Hatusima 1975; Lin 1999; Ohba 2001; Hotta 2013; Shiuchi and Hotta 2015; Ryukyu Plant Research Group 2018). *Sedum formosanum*, a monocarpic biennial herb, is one of the few species of East Asian *Sedum* characterized by a trichotomous branching form (Ohba 2001). In Japan, populations of *S. formosanum* are scattered on the Ryukyu Islands (the Ryukyus), which comprise approximately 140 islands in a 1,300-km-long stretch between Kyushu and Taiwan (Fig. 1). Owing to its scarcity, this species is classified as 'Near Threatened' (NT) on the Red List of Threatened Species of Japan (Japanese Ministry of the Environment 2019). However, accurate identification of *Sedum* species can be hindered by high morphological similarity and plasticity. Therefore, there is a lack of clarity in the taxonomic identity of *S. formosanum* (Ito et al. 2017a). In fact, *Sedum* plants distributed on the Danjo Islands, Japan, which had historically been treated as *S. formosanum*, were recently described as a distinct taxon, *S. danjoense* Takuro Ito, H. Nakanishi & G. Kokub. (Ito et al. 2017a).

Based on previous field surveys, we noted that plants treated as *S. formosanum* on Miyako-jima of the Ryukyus differed morphologically from other populations. In this study, we conducted morphological comparisons and molecular phylogenetic analyses to elucidate the taxonomic status of plants treated as *S. formosanum* on Miyako-jima Island.

Materials and methods

DNA Sample collection

The plants treated as *S. formosanum* are only known from one locality on Miyako-jima Island. We collected two individuals of the plants from the island for DNA samples. To clarify the phylogenetic position of *S. formosanum* growing on Miyako-jima Island, we utilized ITS (Internal Transcribed Spacer region of nuclear ribosomal DNA) sequences of 50 taxa (72 accessions) of *Sedum* in Asia including *S. formosanum* from 20 localities in Kyushu, the Ryukyus, Taiwan and the Philippines as ingroup reported by previous study (Mayuzumi and Ohba 2004; Ito et al. 2014, 2017a, 2017b) (Tables 1, 2). Additionally, we sequenced one species of the eastern Asian species, *S. emarginatum* (Table 1). Following previously reported phylogenetic study of Crassulaceae (Mayuzumi and Ohba 2004), *Aeonium castello-paivae* Bolle, *A. gomerense* Praeger, *A. lancerottense* Praeger, *A. viscatum* Bolle, and *Greenovia aizoon* Bolle, which were collected by Mort et al. (2002) and stored in GenBank were selected as outgroups (Table 1). In total, 80 operational taxonomic units (OTUs) were included in our molecular phylogenetic analysis based on ITS (Tables 1, 2). Subsequently, we conducted molecular phylogenetic analysis based on six cpDNA (Chloroplast DNA) regions with *S. formosanum* and its close relatives to clarify the detailed phylogenetic relationships. Following Ito et al. (2017b), nine Taiwanese taxa were selected as ingroup, *S. alfredii* Hance, and *S. sekiteiense* Yamam.

Table 1. Plant materials of eastern Asian *Sedum* taxa and five outgroup taxa with their collection locality, voucher information, and accession numbers of ITS sequences.

Taxon	Locality	Voucher (Herbarium)	Accession No.	Taxon	Locality	Voucher (Herbarium)	Accession No.
<i>S. actinocarpum</i>	Taiwan	TI 1749 (TNS)	¹ LC229265	<i>S. polytrichoides</i> ssp. <i>polytrichoides</i>	Japan	TI 2247 (TNS)	¹ LC229252
<i>S. alfredii</i>	China	GK 17190 (IBSC)	² AB930259	<i>S. polytrichoides</i> ssp. <i>yabeaenum</i> var. <i>yabeaenum</i>	Japan	TI 396 (TNS)	² AB906490
<i>S. arisanense</i>	Taiwan	TI 1836 (TNS)	¹ LC229272	<i>S. polytrichoides</i> ssp. <i>yabeaenum</i> var. <i>setouchiense</i>	Japan	TI 2298 (TNS)	¹ LC229253
<i>S. boninense</i>	Japan	TI 2371 (TNS)	¹ LC229242	<i>S. rupifragum</i>	Japan	TI 2070 (TNS)	¹ LC229254
<i>S. bulbiferum</i>	Japan	TI 416 (TNS)	¹ LC229234	<i>S. sarmentosum</i>	China	TI 978 (TNS)	¹ LC229255
<i>S. brachyrinchium</i>	Taiwan	TI 3118 (TNS)	¹ LC229277	<i>S. satsumense</i>	Japan	TI 2295 (TNS)	¹ LC229256
<i>S. danjinense</i>	Japan	TI 3658 (TNS)	³ LC260127	<i>S. setakiense</i>	Taiwan	TI 1456 (TNS)	¹ LC229295
<i>S. emarginatum</i>	China	TI 1062 (TNS)	LC530833	<i>S. subtile</i>	Japan	TI 2259 (TNS)	¹ LC229257
<i>S. erici-magnusii</i>	China	TI 2077 (TNS)	¹ LC229235	<i>S. taiwananpinum</i>	Taiwan	TI 1823 (TNS)	¹ LC229278
<i>S. hakonense</i>	China	TI 623 (TNS)	² AB930278	<i>S. taiwanianum</i>	Taiwan	TI 2523 (TNS)	¹ LC229296
<i>S. bangsboense</i>	China	TI 2604 (TNS)	³ LC260130	<i>S. tarokoense</i>	Taiwan	TI 2025 (TNS)	¹ LC229298
<i>S. japonicum</i> var. <i>japonicum</i>	Japan	TI 723 (TNS)	¹ LC229237	<i>S. tennactinum</i>	China	TI 3623 (TNS)	³ LC260135
<i>S. japonicum</i> ssp. <i>japonicum</i> var. <i>japonicum</i>	Japan	TI 2200 (TNS)	¹ LC229238	<i>S. tiammubhanense</i>	China	LP 67 (TNS)	¹ LC229261
<i>S. japonicum</i> ssp. <i>japonicum</i> var. <i>senanense</i>	Japan	TI 2285 (TNS)	¹ LC229239	<i>S. tosenense</i>	Japan	TI 655 (TNS)	¹ LC229258
<i>S. japonicum</i> ssp. <i>oryzifolium</i> var. <i>oryzifolium</i>	Japan	TI 2287 (TNS)	¹ LC229240	<i>S. triactina</i>	Nepal	TI 9596091 (TI)	⁴ AB088629
<i>S. japonicum</i> ssp. <i>oryzifolium</i> var. <i>pumilum</i>	China	GMQ 76 (TNS)	¹ LC229243	<i>S. triangulosepalum</i>	Taiwan	TI 2508 (TNS)	¹ LC229299
<i>S. jiujiangbanense</i>	China	TI 1030 (TNS)	¹ LC229244	<i>S. triararpum</i>	Taiwan	TI 2269 (TNS)	¹ LC229259
<i>S. kiangnanense</i>	China	TI 2440 (TNS)	¹ LC229293		China	TI 3597 (TNS)	³ LC260134
<i>S. kuanwanense</i>	Taiwan	TI 2440 (TNS)	¹ LC229293		China	TI 3597 (TNS)	³ LC260134
<i>S. lineare</i>	Japan	HU 667 (TNS)	¹ LC229245	<i>S. trullipetalum</i>	Nepal	TI 9420132 (TI)	⁴ AB088630
<i>S. lungsuwanense</i>	China	TI 3563 (TNS)	³ LC260131	<i>S. truncastignum</i>	Taiwan	TI 2766 (TNS)	¹ LC229305
<i>S. makinoi</i>	Japan	TI 2325 (TNS)	¹ LC229246	<i>S. uniflorum</i>	Taiwan	TI 447 (TNS)	¹ LC229241
<i>S. mexicanum</i>	Japan	TI 647 (TNS)	¹ LC229247	<i>S. zenataro-tashiroi</i>	Japan	TI 355 (TNS)	² AB906491
<i>S. microsepalum</i>	Taiwan	TI 2771 (TNS)	¹ LC229282	<i>Sedum</i> sp.	China	JP 404 (TNS)	¹ LC229262
<i>S. morrissonense</i>	Taiwan	TI 2348 (TNS)	¹ LC229289				
<i>S. multicaule</i>	China	TI 625 (TNS)	¹ LC229248	Outgroup			
<i>S. noboense</i>	Taiwan	TI 3196 (TNS)	¹ LC229294	<i>Aeonium castello-paiuae</i>	Canary	MEM 1519 (WS)	⁵ AY082236
<i>S. nagasakiarum</i>	Japan	TI 2064 (TNS)	¹ LC229249	<i>Aeonium gomerense</i>	Canary	MEM 1454 (WS)	⁵ AY082242
<i>S. oligospermum</i>	China	GMQ 74 (TNS)	¹ LC229250	<i>Aeonium lanceotense</i>	Canary	MEM 1518 (WS)	⁵ AY082143
<i>S. oreades</i>	Nepal	TI 9420140 (TI)	⁴ AB088632	<i>Aeonium viscatum</i>	Canary	MEM 1432 (WS)	⁵ AY082154
<i>S. polytrichoides</i> ssp. <i>polytrichoides</i>	China	TI 1057 (TNS)	¹ LC229251	<i>Greenovia aizoon</i>	Canary	MEM 1425 (WS)	⁵ AY082112

Reported by ¹Ito et al. (2017b), ²Ito et al. (2014), ³Ito et al. (2017a), ⁴Mayuzumi and Ohba (2004) and ⁵Morr et al. (2002).

and *S. tricarpum* Makino were selected as outgroups (Table 3). In total, 27 OTUs were included in our molecular phylogenetic analysis based on cpDNA (Tables 2, 3). Taxonomic treatments tentatively followed Ohba (2001) and Ito et al. (2018) for Japanese taxa, Lin (1999) and Lu et al. (2019) for Taiwanese taxa, and Fu and Ohba (2001) for Chinese taxa. Voucher specimens for our collections were primarily deposited in the herbarium of the National Museum of Nature and Science, Japan (TNS).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from dried leaves using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA), in accordance with the manufacturer's protocols. The ITS region containing the ITS1, 5.8S rDNA, and ITS2 and six regions of cpDNA (*matK-trnK*, *ndhA*, *psbM-ycf6*, *rpS16*, *trnD-psbM* and *trnL-F*) sequences were amplified by polymerase chain reaction (PCR) with an iCycler (Bio-Rad, Hercules, CA, USA). The ITS and six regions of cpDNA sequences were amplified using EmeraldAmp PCR Master Mix dye (Takara, Otsu, Japan) and the following forward and reverse primers, respectively: ITS, primers ITS1 and ITS4 (White et al. 1990); *matK-trnK* intron primers *matKAF* and *trnK2R*; *ndhA* intron, primers *ndh*×1 and *ndh*×2 (Shaw et al. 2007); *psbM-ycf6* intron, primers *psbMR* and *ycf6F*; *rpS16* intron, primers *rpS16F* and *rpS16R*; *trnD-psbM* intron, primers *psbMF* and *trnD* (Shaw et al., 2005); and *trnL-F*, primers *trnLc* and *trnLf* (Taberlet et al. 1991) by an iCycler (Bio-Rad, Hercules, CA). The PCR profile consisted of an initial 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C for the ITS sequence or 55°C for the cpDNA sequence, and 90 s at 72°C. The PCR product were purified by ExoStar clean-up kit (USB, Cleveland, OH). Cycle sequencing was performed using a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA) and the PCR primers mentioned above for the ITS and cpDNA sequences. The Sanger sequencing products were then purified by ethanol precipitation. Automated sequencing was carried out with an Applied Biosystems 3130xl Genetic Analyzer. The electropherograms were assembled using ATGC ver. 6 (GENETYX, Tokyo, Japan). The sequence data obtained in this study were deposited in the DDBJ/EMBL/GenBank database (<http://www.ncbi.nlm.nih.gov/gquery/>).

Phylogenetic analysis using ITS and cpDNA sequences

The ITS and cpDNA sequences were aligned using ClustalW 1.8 (Thompson et al. 1994) and then adjusted manually. Phylogenetic analyses were conducted with a Bayesian approach using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and maximum-likelihood (ML) phylogenetic analysis using RAxML (Stamatakis 2014). In the Bayesian phylogenetic analysis, we used Akaike's Information Criterion (AIC) implemented in MrModeltest 2.2 (Nylander 2004) to obtain an appropriate evo-

Table 2. Plant materials of *Sedum formosanum* with their collection locality, voucher information, and accession numbers of ITS and cpDNA sequences.

Abbreviation	Locality	Voucher (Herbarium)	nrDNA		cpDNA				
			ITS	matK-trnK	ndhA	psbM-ygf6	rpS16	trnD-psbM	tmL-F
P-BTN	Philippines: Batanes, Bataan Isl.	GK 15715 (TNS)	'AB930273	'LC258201	'LC229400	'LC258337	'LC229468	'LC258269	'LC229536
K-MNS	Japan: Kyushu, Minami-satsuma.	GK 16768 (TNS)	'AB930262	'LC258200	'LC229399	'LC258336	'LC229467	'LC258268	'LC229535
K-MNO	Japan: Kyushu, Kagoshima, Minami-Osumi.	TI 3238 (TNS)	'LC260123	-	-	-	-	-	-
K-ISO	Japan: Kyushu, Kagoshima, Mt. Isoma.	TI 2296 (TNS)	'LC260124	-	-	-	-	-	-
K-SKK	Japan: Kyushu, Kagoshima, Shimo-Koshiki Isl.	TI 3200 (TNS)	'LC260125	-	-	-	-	-	-
K-KUM	Japan: Kyushu, Kumamoto, Reihoku.	TI 637 (TNS)	'LC260126	-	-	-	-	-	-
R-IHY	Japan: Ryukyus, Iheya Isl.	GK 10726 (TNS)	'AB930267	'LC258193	'LC229392	'LC258329	'LC229460	'LC258261	'LC229528
R-ISO	Japan: Ryukyus, Ishigaki Isl.	GK 11775 (TNS)	'AB906474	'LC258194	'LC229393	'LC258330	'LC229461	'LC258262	'LC229529
R-IZN	Japan: Ryukyus, Izena Isl.	GK 12224 (TNS)	'AB930266	'LC258195	'LC229394	'LC258331	'LC229462	'LC258263	'LC229530
R-KUM	Japan: Ryukyus, Kume Isl.	GK 12755 (TNS)	'AB930269	'LC258196	'LC229395	'LC258332	'LC229463	'LC258264	'LC229531
R-TNK	Japan: Ryukyus, Tonaki Isl.	GK 13049 (TNS)	'AB930268	'LC258197	'LC229396	'LC258333	'LC229464	'LC258265	'LC229532
R-TNG	Japan: Ryukyus, Tanegashima Isl.	GK 15602 (TNS)	'AB930265	'LC258198	'LC229397	'LC258334	'LC229465	'LC258266	'LC229533
R-AMM	Japan: Ryukyus, Amami Isl.	GK 16712 (TNS)	'AB930264	'LC258199	'LC229398	'LC258335	'LC229466	'LC258267	'LC229534
R-IRO	Japan: Ryukyus, Iriomote Isl.	TI 598 (TNS)	'AB930270	-	-	-	-	-	-
R-MYK1	Japan: Ryukyus, Miyako-jima Isl.	TI 1115 (TNS)	LC530813	LC530834	LC530836	LC530838	LC530840	LC530842	LC530844
R-MYK2	Japan: Ryukyus, Miyako-jima Isl.	TI 1120 (TNS)	LC530814	LC530835	LC530837	LC530839	LC530841	LC530843	LC530845
R-OKE	Japan: Ryukyus, Kagoshima, Okinoerabu Isl.	TI 2671 (TNS)	'LC260128	-	-	-	-	-	-
R-YAK	Japan: Ryukyus, Kagoshima, Yakushima Isl.	TI 2648 (TNS)	'LC260129	-	-	-	-	-	-
T-LNY	Taiwan: Lanyu, Lanyu Isl.	GK 6132 (TNS)	'AB930271	'LC258202	'LC229401	'LC258338	'LC229469	'LC258270	'LC229537
T-NTP	Taiwan: New Taipei.	GK 16446 (TNS)	'AB930272	'LC258203	'LC229402	'LC258339	'LC229470	'LC258271	'LC229538
T-GUS	Taiwan: Yilan, Gueishan Isl.	TI 1260 (TNS)	'LC229279	'LC258204	'LC229403	'LC258340	'LC229471	'LC258272	'LC229539
T-PNT	Taiwan: Pingtung, Sheding.	TI 1921 (TNS)	'LC229280	'LC258205	'LC229404	'LC258341	'LC229472	'LC258273	'LC229540

Reported by ¹Ito et al. (2014), ²Ito et al. (2017a), ³Ito et al. (2017b).

Table 3. Plant materials of Nine Taiwanese *Sedum* species and three outgroups which are closely relatives of *S. formosanum* with their collection locality, voucher information, and accession numbers of cpDNA sequences reported by Ito et al. (2017b).

Taxon	Locality	Voucher (Herbarium)	cpDNA					
			<i>matK-trnK</i>	<i>ndhA</i>	<i>psbM-ycf6</i>	<i>rpS16</i>	<i>trnD-psbM</i>	<i>trnL-F</i>
<i>S. actinocarpum</i>	Taiwan	TI 1749 (TNS)	LC258179	LC229378	LC258315	LC229446	LC258247	LC229514
<i>S. arisanense</i>	Taiwan	TI 1836 (TNS)	LC258186	LC229385	LC258322	LC229453	LC258254	LC229521
<i>S. brachyrinchum</i>	Taiwan	TI 3118 (TNS)	LC258191	LC229390	LC258327	LC229458	LC258259	LC229526
<i>S. kuanwuense</i>	Taiwan	TI 2440 (TNS)	LC258218	LC229417	LC258354	LC229485	LC258286	LC229553
<i>S. microsepalum</i>	Taiwan	TI 2771 (TNS)	LC258207	LC229406	LC258343	LC229474	LC258275	LC229542
<i>S. nokoense</i>	Taiwan	TI 3196 (TNS)	LC258219	LC229418	LC258355	LC229486	LC258287	LC229554
<i>S. taiwanalpinum</i>	Taiwan	TI1823 (TNS)	LC258192	LC229391	LC258328	LC229459	LC258260	LC229527
<i>S. tarokoense</i>	Taiwan	TI2025 (TNS)	LC258223	LC229422	LC258359	LC229490	LC258291	LC229558
<i>S. triangulosepalum</i>	Taiwan	TI2508 (TNS)	LC258224	LC229423	LC258360	LC229491	LC258292	LC229559
Outgroup								
<i>S. alfredii</i>	China	GK 17190 (TNS)	LC258164	LC229363	LC258300	LC229431	LC258232	LC229499
<i>S. sekiteiense</i>	Taiwan	TI1456 (TNS)	LC258220	LC229419	LC258356	LC229487	LC258288	LC229555
<i>S. tricarpum</i>	Japan	TI2269 (TNS)	LC258175	LC229374	LC258311	LC229442	LC258243	LC229510

lutionary model of nucleotide substitutions. And then we performed two separate runs of Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis, each with a random starting tree and four chains (one cold and three hot) based on the selected model. The MCMCMC length was one million generations, and the chain was sampled every one hundredth generation from the cold chain. The first 2,500 sample trees (25% of the total 10,000 sample trees) were discarded as burn-in after checking that the average standard deviation of split frequencies (ASDSF) reached a stationary state at < 0.01 thereafter. A 50% majority consensus tree of the output tree file from MrBayes was generated using FigTree ver. 1.3.1 (Rambaut 2009). The ML phylogenetic analyses were implemented in RAxML 8 (Stamatakis 2014) with a GTRGAMMA substitution model. The ML bootstrap proportions (BPs) and trees were obtained by simultaneously running rapid bootstrapping with 1,000 iterations followed by a search for the most likely tree.

Intraspecific morphological comparison

The plants known as *S. formosanum* from Miyako-jima Island (*T. Ito* 1115, 1120, 2402 and 2408, TNS) were used for morphological comparisons. Herbarium specimens of *S. formosanum* deposited in the Kagoshima University Museum (KAG), the University of the Ryukyus (RYU), the National Museum of Nature and Science (TNS), the National Taiwan University (TAI) and the Taiwan Forestry Research Institute (TAIF) were examined. By field survey, the phenotypic plasticity of leaf shape in response to environmental changes was observed. Therefore, we also have cultivated the plants from Miyako-jima Island and from Taiwan, where the type locality of the species is, in Tsukuba Botanical Garden to compare their leaf shape and life cycle during 2015–2017.

Results and discussion

Phylogenetic analyses using ITS and cpDNA

We used 80 operational taxonomic units (OTUs), including 75 as ingroup accessions and 5 as outgroup accessions in the Bayesian and ML analyses based on ITS sequences (Tables 1, 2). Following alignment, we obtained a matrix of 629 base pairs (bp) and selected GTR+I+G for the Bayesian analysis. The 50% majority rule consensus tree of all post burn-in trees is shown with Bayesian posterior probabilities (PPs) in Fig. 2A. The topology of the ML tree was highly compatible with that of the Bayesian tree (Fig. 2A). In both the Bayesian and ML analyses based on ITS sequences, *S. formosanum* and nine taxa endemic to Taiwan formed a well-supported clade (PP/BS = 1.00/93). Within this clade, four subclades that formed a polytomy were recognized: nine taxa endemic to Taiwan (0.87/67, Clade A1), *S. formosanum* from Miyako-jima Island (1.00/100, Clade B1), *S. formosanum* from Izena Island and Iheya Island (1.00/100, Clade C1-I), and *S. formosanum* from 18 accessions from Japan (excluding Miyako-jima Island, Iheya Island, and Izena Island), Taiwan, and the Philippines (0.98/78, Clade C1-II).

We used 29 OTUs, including 26 accessions as ingroups and 3 as outgroups in the Bayesian and ML analyses based on combined six regions of cpDNA sequence (Tables 2, 3). Following alignment, we obtained a matrix of 5,115 bp. In the resulting Bayesian and ML phylogenetic trees, we observed a topology similar to the trees formed using ITS data. We again observed strong evidence that *S. formosanum* and nine taxa endemic to Taiwan formed a well-supported clade with four subclades (1.00/100; Fig. 2B). However, these four subclades formed a polytomy that differed from that suggested by the ITS tree. Although *S. formosanum* from Miyako-jima Island was again supported as forming a subclade (1.00/100, Clade B11), we found that the nine Taiwanese endemics were divided into two subclades (1.00/93, Clade A11-I; 0.95/61, Clade A11-II), and *S. formosanum* on Izena Island and Iheya Island formed a subclade with the 18 accessions from Japan (excluding Miyako-jima Island), Taiwan and the Philippines (1.00/99, Clade C11).

Morphological comparison

We observed a similar flower morphology among the herbarium specimens from Miyako-jima Island (TNS; *T. Ito* 1115, 1120, 2402, and 2408) and those from other regions in Japan, Taiwan, and the Philippines. Generally, *S. formosanum* displays trichotomous branching at the shoot tip and does not produce lateral branches. The Miyako-jima plants also displayed trichotomous branching at the shoot tips, but they often developed lateral branches in the leaf axils of long shoots. Additionally, we found similar plants of *S. formosanum* that also produce axillary lateral branches on Ishigaki Island, part of the Yaeyama Islands, on Gaja-jima Island and Akuseki-jima Island in the Tokara Islands, and on Yoron Island in the Amami Islands by specimen survey.

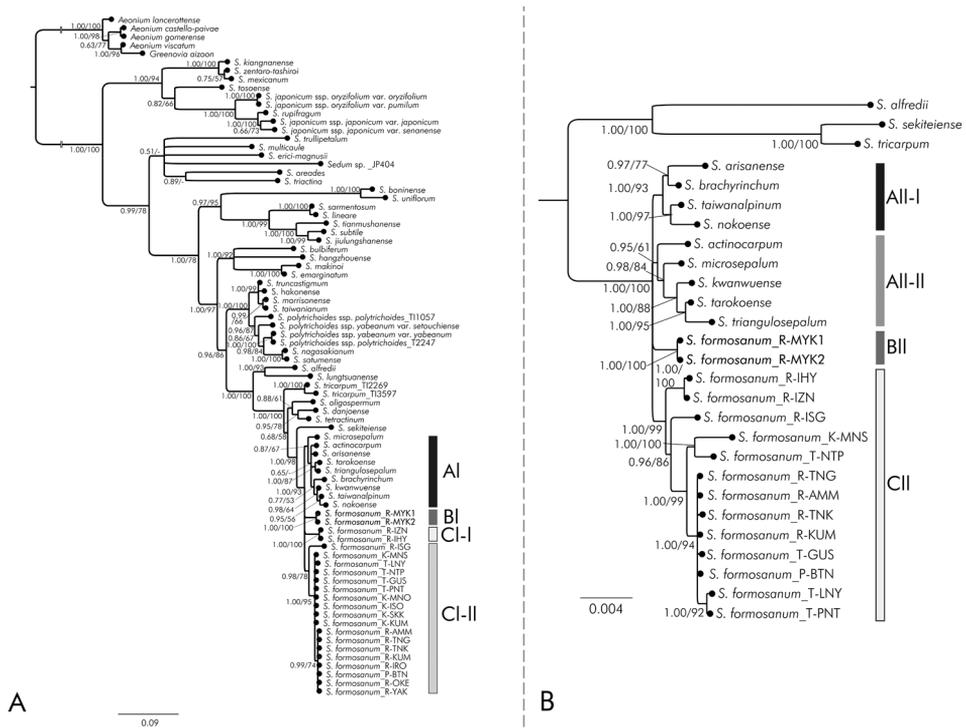


Figure 2. Bayesian phylogenetic tree based on ITS sequences for Eastern Asian *Sedum* (A), and Bayesian phylogenetic tree based on cpDNA for sequences *S. formosanum* and its closely relatives (B). The topology of the maximum likelihood (ML) tree was highly compatible with the Bayesian tree. Bayesian posterior probabilities (PPs: left) and bootstrap percentages from ML analysis (BP: right) are shown (See Tables 1, 2 and 3 for the abbreviations of localities).

In terms of leaf morphology, we observed high variation and no clear difference between the Miyako-jima plants and those from other locations. To remove the potentially confounding influence of environmental factors on leaf morphology, we cultivated plants from both Miyako-jima Island and Taiwan (obtained from the type locality) and compared them. Using this approach, we detected slight differences in leaf shape. Plants from Miyako-jima Island had spatulate to oblanceolate leaves, whereas plants from Taiwan had leaves that were spatulate to widely obovate. Most notably, plants from Miyako-jima Island were perennial and polycarpic, whereas plants from Taiwan were biennial and monocarpic.

Intraspecific taxonomy of *S. formosanum*

The molecular phylogenetic analyses based on both ITS and cpDNA indicated that the *Sedum* species from Miyako-jima Island, which are currently considered as *S. formosanum*,

formed a well-supported clade. This clade was distinct from that of *S. formosanum* collected from other regions of Japan, Taiwan (including the type locality), and the Philippines (Fig. 2). Morphologically, plants from Miyako-jima Island were distinguishable from plants from other areas due to the presence of axillary lateral branches and by life cycle, i.e., perennial and polycarpic versus biennial and monocarpic (Figs 3, 4). Leaf shape differed slightly between the Miyako-jima plants and those from other locations, i.e., spatulate to oblanceolate versus spatulate to widely obovate (Figs 3, 4). Therefore, we concluded that *S. formosanum* from Miyako-jima Island should be considered a distinct taxonomic entity and have thus described a new subspecies in this study.

Additionally, molecular phylogenetic trees based on both ITS and cpDNA suggested that *S. formosanum* on Iheya Island and Izena Island part of the Okinawa Islands formed a distinct clade (Fig. 2). Samples from Ishigaki Island in the Yaeyama Islands were also genetically distinct from the individuals from other islands (Fig. 2). However, no clear morphological differences could be observed between plants from Iheya Island and Izena Island and plants from Taiwan (including type locality). Plants from both Ishigaki Island and Miyako-jima Island had axillary lateral branches, however the life cycle and leaf morphology of the samples collected from Ishigaki Island were not in the focus of this study. Furthermore, plants from Akuseki Island, Gaja Island, and Yoron Island also have axillary lateral branches. However, we observed plants from all three islands developing flowering stems between August and October by specimen survey. Thus, they are likely autumn-flowering. Among Japanese *Sedum*, autumn-flowering is only reported in *S. danjoense*, which had been treated as *S. formosanum* and was described as an independent species recently (Ito et al. 2017a). Although the phylogenetic position of the populations on Akuseki Island, Gaja Island, and Yoron Island is uncertain, the plant may be closely related to *S. danjoense*. Therefore, further reconsideration of *S. formosanum* at the species and infraspecific level is needed to establish the circumscription of the species.

Taxonomic treatment

Sedum formosanum N.E. Brown., subsp. *formosanum*

Fig. 3E–G

≡ *Sedum mariae* Raym.-Hamet, Repert. Spec. Nov. Regni Veg. 8: 143. 1910. Type: Japan. Insula Oshima (Liukiu): Jul 1900, Faurie, U. J. 3923 (holotype: G [G00356298]).

Type. TAIWAN. Keelung City, date unknown, *C. Ford s.n.* (lectotype, designated by Byalt, V. V.: K [K000838648]; isotype, designated by N. E. Br. 1885, pg. 134: GH [GH00042587]).

Description. Usually biennial herb, fleshy, glabrous. First year stem stout, erect, partly woody, 1 or 2 trifurcate, 3–10 cm tall, with lax rosettes; rosettes 3–18 cm wide with 15–45 leaves. Flowering stems fleshy, 10–30 cm tall, base ca. 5 mm broad, usually

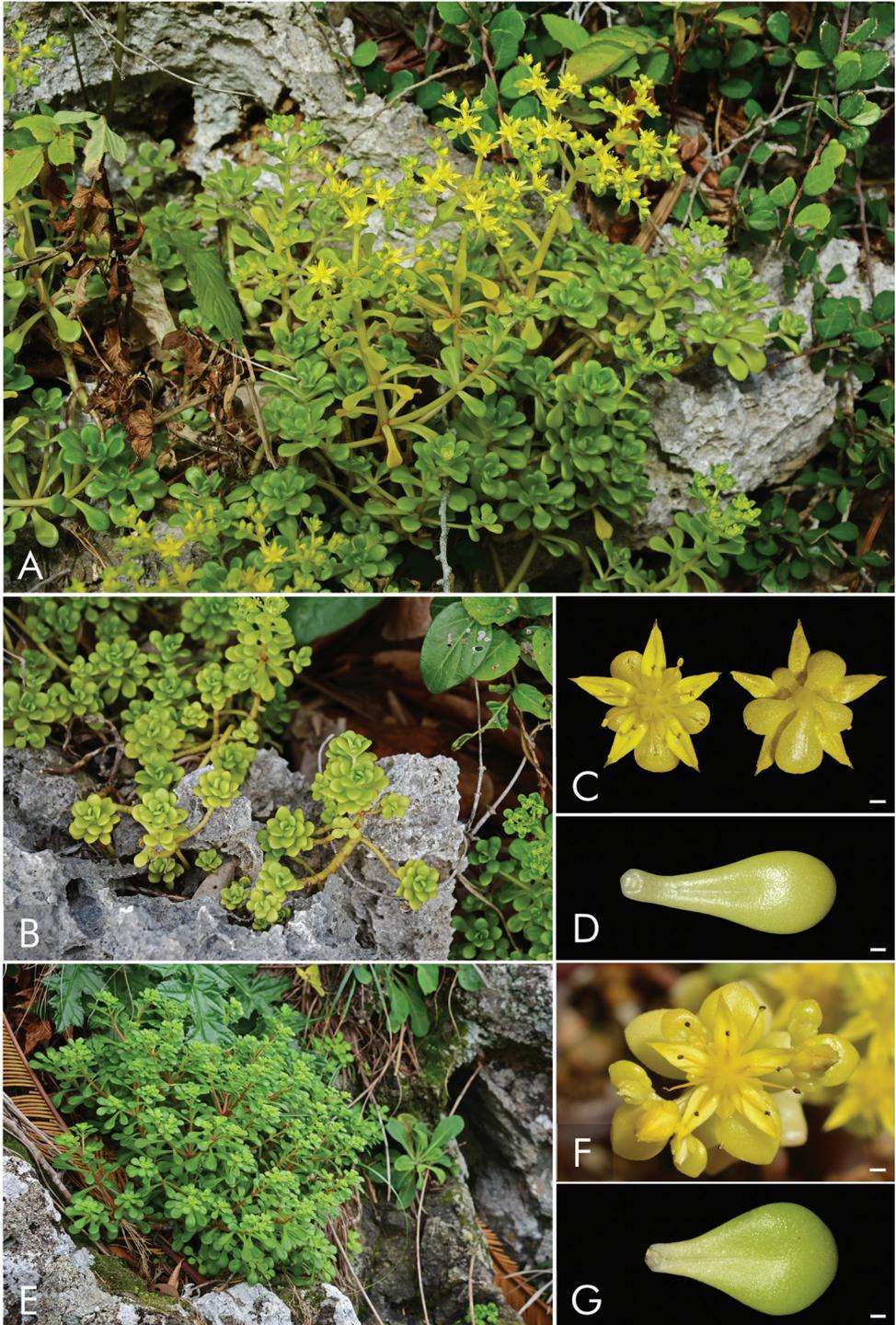


Figure 3. *Sedum formosanum* subsp. *miyakojimense* (**A–D** T. Ito 2402, 2408, Miyako-jima Island of the Ryukyus, Japan) and *S. formosanum* subsp. *formosanum* (**E** Kume-jima Island of the Ryukyus, Japan, **F, G** NewTaipei City, Taiwan). **A, B, E** habit **C, F** flower **D, G** leaf. Scale bars: 1 mm (**D–F**).

reddish or yellowish green, erect or sprawling and creeping at base, 1- or 2-trifurcate at base. Roots fibrous, sometimes adventitious at the leaf scar. Leaves alternate, evenly arranged, sessile, green or yellowish, flattish, \pm thick, spatulate to widely obovate, 1.2–3.2 cm long, 0.5–1.6 cm wide, apex rounded, base long, attenuate, margins entire. Inflorescences terminal, cymes, 1 or 2 trifurcate with 3 (rarely 4) primary axes; primary axis 2–7 cm long, ascending, 1 to several times irregularly and often unequally forking, with a flower at each fork, ultimate branches 1–2 cm long, 3–6 flowered; bracts leaf-like, smaller than cauline leaves. Flowers 5 (rarely 6)-merous, 8–12 mm wide, sessile. Sepals 5, free, yellowish green, fleshy, flattish, unequal in size, obovate to oblanceolate, 2–4 mm long, 1.5–3 mm wide, apex round or obtuse, base spurred. Petals 5, bright yellow, lanceolate, 5–6 mm long, 1.3–1.6 mm wide, apex acuminate, base slightly connate. Stamens 10, shorter than petals, 4.8–5 mm long, erect at flowering, two-whorled arrangement; anthers oblong-lanceolate, ca. 0.5 mm long, deep yellow before dehiscence. Pistils 5, 5.5–6.5 mm long; carpels 5, free, connate at the base, gibbous ventrally. Fruits star-shaped, follicle, erect, 5.5–7 mm long. Flowering in April to June.

Distribution and habitat. JAPAN: Kyushu, Kagoshima, Kumamoto; The Ryukyus, the Osumi Islands, Kami-Koshiki, Kuro-shima, Yaku-shima and Tanega-shima islands, the Tokara Islands, Akuseki, Gaja, Nakano-shima, Kodakara, Kuchino-shima, and Takara islands, the Amami Islands, Amami-oshima, Kakeroma, Kikai, Okierabu, Tokuno-shima, Uke, Yoro and Yoron islands, the Okinawa Islands, Aka, Geruma, Ie, Iheya, Izena, Kume, Okinawa, Sesoko, Tokashiki and Tonaki islands, the Yaeyama Islands, Ishigaki, Iriomote, Kuro-shima and Yonaguni islands. TAIWAN: New Taipei, Keelung, Ilan, Hualien, Lienchiang, Taitung (Lanyu and Green Islands) and Pingtung. THE PHILIPPINES: Batanes, Batan Island.

Coastal and rarely inland rocky slopes, xeric, saline, and exposed to direct sunlight.

Additional specimens examined. JAPAN. Kyushu, Kagoshima: Ichikikushikino City, 30 June 1957, *S. Hatusima 20967 (KAG)*, Minamisatsuma City, 23 May 1962, *M. Furuse 325 (KAG)*, Minamisatsuma City, 9 Nov. 1984, *S. Sako 8865 (KAG)*, Minamisatsuma City, 14 Nov. 1987, *S. Hatusima 43027 (KAG)*, Ichikikushikino City, 26 June 2003, *K. Maruno s.n. (KAG)*, Minamisatsuma City, 23 June 2013, *G. Kokubugata, Y. Saito, T. Ito 16768 (TNS)*, Kimotsuki Country, Minamiosumi Town, 30 July 1949, *S. Hatusima 13352 (KAG)*, Kimotsuki Country, Minamiosumi Town, 13 June 1957, *S. Hatusima 20891 (KAG)*, Kimotsuki Country, Minamiosumi Town, 22 June 2013, *G. Kokubugata & T. Ito 16764 (TNS)*, Kimotsuki Country, Sata Village, 26 Aug. 1910, *Y. Nakano s.n. (TNS)*, Kimotsuki Country, Sata Village, 9 Aug. 1929, *H. Asuyama s.n. (TNS)*, Kimotsuki Country, Sata Town, 28 Mar. 1958, *S. Okuyama & H. Utsumi 17098 (TNS)*, Kumamoto: Amakusa Country, Reihoku Town, 13 Jan. 1956, *R. Moran 5395 (TNS)*, The Ryukyus, the Osumi Islands, Kagoshima: Kami-Koshiki Island, Satsuma Country, Kamikoshiki Village, 26 Mar. 1930, *K. Naohara s.n. (TNS)*, Kuro-shima Island, Kagoshima Country, Mishima Village, 12 June 1981, *K. Maruno s.n. (KAG)*, Kagoshima Country, Mishima Village, 26 May 1994, *T. Shiuchi 4900 (KAG)*, Tanega-shima Island, Nishinoomote City, 25 Feb. 2013, *G. Kokubugata, M. Yokota, K. Kaburagi 15604 (TNS)*, The Ryukyus, the Tokara Islands, Kagoshima: Akuseki Island, Kagoshima Country, Toshima Village, 18 Oct. 1980, *R. Yanagida s.n.*

(KAG), Kagoshima Country, Toshima Village, 9 Sep. 1983, *Y. Hukushima s.n.* (KAG), Kagoshima Country, Toshima Village, 15 Oct. 1993, *T. Shiuchi 2800* (KAG), Gaja Island, Kagoshima Country, Toshima Village, 21 Aug. 1958, *S. Sako & K. Kawanabe 2244* (KAG), Nakano-shima Island, Kagoshima Country, Toshima Village, 18 Aug. 1958, *S. Sako & K. Kawanabe 1938* (KAG), Takara Island, Kagoshima Country, Toshima Village, 25 Aug. 1910, *S. Kawagoe s.n.* (TNS), Kagoshima Country, Toshima Village, 11 Feb. 1952, *S. Hatusima s.n.* (KAG), Kagoshima Country, Toshima Village, 14 May 1993, *T. Shiuchi 1314* (KAG), The Ryukyus, the Amami Islands, Kagoshima: Amami-oshima Island, Amami City, 28 Apr. 2012, *G. Kokubugata 16712* (TNS), Amami City, 26 Aug. 2014, *G. Kokubugata & H. Umemoto 18178* (TNS), Amami City, 12 Jan. 2016, *G. Kokubugata & M. Tabata 19011* (TNS), Naze City, 23 May 1975, *J. Haginiwa JH006639* (TNS), Naze City, 23 May 1975, *J. Haginiwa JH032447* (TNS), Naze City, 23 Nov. 1977, *A. Yamamoto, T. Nakaike & M. Ishizuka 490* (TNS), Oshima Country, Setouchi Town, 18 July 1919, *S. Kawagoe s.n.* (KAG), Oshima Country, Setouchi Town, 6 Aug. 1956, *S. Ouchiyama 49* (KAG), Oshima Country, Setouchi Town, 24–28 July 1975, *Y. Miyagi & S. Hatusima 40407* (RYU), Oshima Country, Tatsugo Town, 27 Apr. 2012, *G. Kokubugata 16722* (TNS), Kakeroma Island, Oshima Country, Setouchi Town, 11 Jan. 2016, *G. Kokubugata, M. Tabata 18978* (TNS), Kikai Island, Oshima Country, Kikai Town, 17 May 1975, *K. Yoshinaga 178* (KAG), Okierabu Island, Oshima Country, China Town, 4 June 1967, *M. Furuse s.n.* (KAG), Oshima Country, China Town, date unknown 1969, *K. Kasuga s.n.* (KAG), Oshima Country, China Town, 7 Nov. 1971, *J. Haginiwa JH006572* (TNS), Tokunoshima Island, Oshima Country, Amagi Town, 4 May 2014, *G. Kokubugata & H. Umemoto 17613* (TNS), Oshima Country, Tokunoshima Town, 3 May 2014, *G. Kokubugata & H. Umemoto 17556* (TNS), Uke Island, Oshima Country, Setouchi Town, 23 Mar. 2019, *E. Suzuki s.n.* (KAG), Yoro Island, Oshima Country, Setouchi Town, 22 May 2018, *E. Suzuki s.n.* (KAG), Oshima Country, Setouchi Town, 22 May 2018, *E. Suzuki s.n.* (KAG), Yoron Island, Oshima Country, Yoron Town, 21 Aug. 1921, *K. Uyehara s.n.* (KAG), Oshima Country, Yoron Town, 16 Aug. 1961, *G. Ikeda s.n.* (KAG), Oshima Country, Yoron Town, 16 Aug. 1961, *G. Ikeda s.n.* (KAG), Oshima Country, Yoron Town, 24 Dec. 1971, *J. Haginiwa JH006509* (TNS), Oshima Country, Yoron Town, 24 Dec. 1971, *J. Haginiwa JH006571* (TNS), The Ryukyus, the Okinawa Islands, Okinawa: Aka Island, Shimajiri Country, Zamami Village, 23–26 May 1974, *Y. Miyagi & T. Kabashima 4865* (RYU), Geruma Island, Shimajiri Country, Zamami Village, 9–12 Aug. 1977, *Y. Miyagi 7906* (RYU), Ie Island, Kunigami Country, Ie Village, 4–5 May. 1974, *S. Hatusima & Y. Miyagi 37591* (RYU), Kunigami Country, Ie Village, 16 Sep. 2014, *G. Kokubugata, M. Yokota et al. 18248* (TNS), Iheya Island, Shimajiri Country, Iheya Village, 25 Dec. 1958, *Y. Niiro s. n.* (RYU), Shimajiri Country, Iheya Village, 26 May 2008, *G. Kokubugata 10726* (TNS), Izena Island, Shimajiri Country, Izena Village, 22 July 1973, *S. Hatusima 34901* (RYU), Shimajiri Country, Izena Village, 1 June 2015, *T. Yamada TYD263-1* (TNS), Kume Island, Shimajiri Country, Kumejima Town, 1 June 2010, *G. Kokubugata, M. Yokota & K. Nakamura 12755* (TNS), Okinawa Island, Itoman City, Aug. 1966, *Y. Miyagi 3636* (RYU), Itoman City, Aug. 1967, *Y. Miyagi 5654* (RYU), Itoman City, 7 May 2001, *G. Kokubugata & C.I.*

Peng 289 (TNS), Onna Village, 18 May 1980, *Y. Miyagi* 9080 (RYU), Kunigami Country, Kunigami Village, May 1974, *S. Itoman* 63 (RYU), Kunigami Country, Motobu Town, 3 May 1974, *S. Hatusima* & *Y. Miyagi* 37633 (RYU), Nakagami Country, Kitanakagusuku Village, 30 Apr. 1955, *S. Hatusima* 17462 (KAG), Nakagami Country, Kitanakagusuku Village, 30 Apr. 1955, *S. Hatusima* 17498 (KAG), Shimajiri Country, Miwa Village, 23 May 1954, *S. Nakamine* 68 (RYU), Shimajiri Country, Miwa Village, 23 May 1954, *S. Nakamine* 68 (TNS), Sesoko Island, Kunigami Country, Motobu Town, 19 Aug. 1974, *Y. Miyagi* 4202 (RYU), Tokashiki Island, Shimajiri Country, Tokashiki Village, 5 Mar. 1973, *Y. Miyagi* & *S. Oyadomari* 1152 (RYU), Tonaki Island, Shimajiri Country, Tonaki Village, 10 Mar. 1973, *S. Hatusima* 34404A (RYU), Shimajiri Country, Tonaki Village, 17 Dec. 2010, *G. Kokubugata* & *M. Yokota* 13049 (TNS), The Ryukyus, the Yaeyama Islands, Okinawa: Ishigaki Island, Ishigaki City, 27 Mar. 2009, *G. Kokubugata*, *M. Yokota* & *K. Nakamura* 11775 (TNS), Kuroshima Island, Yaeyama Country, Taketomi Town, 4 Nov. 1974, *Y. Niiro* & *Y. Miyagi* 6103 (RYU), Yonaguni Island, Yaeyama Country, Yonaguni Town, 26–30 Oct. 1959, *S. Hatusima* 24587 (KAG), Yaeyama Country, Yonaguni Town, 29 Sep. –3 Oct. 1973, *S. Hatusima*, *Y. Miyagi* & *E. Tanaka* s.n. (TNS), Yaeyama Country, Yonaguni Town, 1 Nov. 1988, *R. Minagawa* s.n. (TNS), Yaeyama Country, Yonaguni Town, 8 Dec. 2014, *G. Kokubugata*, *M. Yokota* et al. 18586 (TNS), Yaeyama Country, Yonaguni Town, 7 Dec. 2014, *G. Kokubugata*, *M. Yokota* et al. 18548 (TNS), Yaeyama Country, Yonaguni Town, 24 Nov. 2015, *T. Yamada* TYD371 (TNS), TAIWAN. Hualien: Hualien City, 13 Dec. 1993, *T. C. Huang* 15022 (TAI), Xiulin Township 24 May 1993, *S. F. Huang*, *K. C. Yang* & *J. M. Hu* 5097 (TAI), Ilan: Su'ao Township, 18 Apr. 1987, *S. F. Huang*, *C. F. Hsieh*, *Y. F. Lin* et al. 3722 (TAI), Su'ao Township, 18 Apr. 1987, *W. S. Tang* 1795 (TAI), Su'ao Township, 21 May 1987, *W. S. Tang* 1803 (TAI), Su'ao Township, 18 Apr. 1987, *W. S. Tang* 1785 (TAI), Su'ao Township, 21 May 1987, *W. S. Tang* 1802 (TAI), Su'ao Township, 7 May 1993, *S. F. Huang* 5075 (TAI), Su'ao Township, 21 May 1987, *W. S. Tang* 1802 (TAI), Su'ao Township, 6 May 1993, *S. F. Huang* 5049 (TAI), Kueishan Island, Toucheng Township, 31 May 1970, *C. C. Hsu* 7237 (TAI), Toucheng Township, 3 July 1932, *G. Masamune* & *S. Suzuki* s. n. (TAI), Keelung: Keelung City, 11 Oct. 2004, *S. W. Chung* 7657 (TAIF), Keelung City, 7 June 2005, *S. W. Chung* 7774 (TAIF), Keelung City, 6 June 2005, *P. F. Lu* 9825 (TAIF), Keelung City, 23 May 2010, *P. F. Lu* 20381 (TAIF), Keelung City, 12 July 2011, *P. F. Lu* 22356 (TAIF), Keelung City, 22 July 1918, *M. Eizi* 907 (TAI), Keelung City, 4 June 1932, *K. Mori* s. n. (TAI), Keelung City, 31 May 1930, *S. Sasaki* 4687 (TAI), Keelung City, 26 May 1939, *G. Masamune* 1907 (TAI), Keelung City, 1 May 1937, *H. Simada* 1218 (TAI), Keelung City, 3 June 1978, *C. M. Kou* 9805 (TAI), Keelung City, 27 Apr. 1983, *C. L. Chang* 91 (TAI), Keelung City, 1 May 1937, *H. Simada* 1218 (TAI), Keelung City, date unknown, *M. L. Weng* 66 (TAI), Pengchia Island, Keelung City, 4 Aug. 1992, *T. C. Huang* 15753 (TAI), Lienchiang: Nangan Township, 29 June 1999, *S. H. Su* s. n. (TAI), New Taipei: New Taipei City, 30 Apr. 2005, *P. F. Lu* 9571 (TAIF), New Taipei City, 14 Aug. 2008, *Y. F. Chang* s. n. (TAIF), New Taipei City, 6 June 1987, *W. S. Tang* 1808 (TAI), New Taipei City, 6 June 1987, *W. S. Tang* 1808 (TAI), New Taipei City, 23 Apr. 1929, *Y. Kudo*, *S. Suzuki* & *K. Mori* 398 (TAI), New Taipei City, 23 Sep.

1931, *T. Tanaka s. n. (TAI)*, New Taipei City, 3 May 1986, *W. S. Tang 1757 (TAI)*, New Taipei City, 20 Apr. 1932, *T. Tanaka & Y. Simada 10963 (TAI)*, New Taipei City, 20 Apr. 1932, *T. Tanaka & Y. Simada s. n. (TAI)*, New Taipei City, 12 Apr. 1979, *S. H. Lin 692 (TAI)*, New Taipei City, 12 Apr. 1979, *C. M. Kuo 10939 (TAI)*, New Taipei City, 26 May 1985, *J. C. Wang 3349 (TAI)*, New Taipei City, 12 Apr. 1979, *H. N. Yang 2551 (TAI)*, New Taipei City, 27 May 1987, *W. S. Tang 1806 (TAI)*, New Taipei City, 27 May 1987, *W. S. Tang 1806 (TAI)*, New Taipei City, 20 Apr. 2002, *S. F. Cheng, S. K. Yu s. n. (TAI)*, New Taipei City, 31 May 2001, *Y. J. Lai, W. H. Wu et al. 745 (TAI)*, New Taipei City, 13 May 1988, *S. F. Huang 4297 (TAI)*, New Taipei City, 7 June 1930, *S. Sasaki 4744 (TAI)*, New Taipei City, 16 Apr. 1961, *T. C. Huang 2280 (TAI)*, New Taipei City, 7 June 1989, *W. S. Tang & C. F. Hsieh 1864 (TAI)*, New Taipei City, 30 May 1985, *T. Y. Yang 2020 (TAI)*, Pingtung: Hengchun Township, June 1912, *T. Kawakami & S. Sasaki s. n. (TAI)*, Taitung: Lanyu Island, Lanyu Township, 13 Jan. 1995, *T. P. Pan, C.-H. Horng et al. s. n. (TAIF)*, Lanyu Township, 19 Mar. 1943, *T. Hosokawa 9896 (TAI)*, Lanyu Township, 17 Apr. 1992, *S. F. Huang & Y. C. Hsu 4735 (TAI)*, Lanyu Township, 29 Apr. 1983, *T. C. Huang, Yang, Kao et al. 9440 (TAI)*, Lanyu Township, 19 Feb. 1986, *T. C. Huang, S. F. Huang, K. C. Yang et al. 10535 (TAI)*, Lanyu Township, 17 Aug. 1958, *T. I. Chuang & C. C. Hsu 2384 (TAI)*, Lanyu Township, 18 Apr. 1932, *T. Sata 1286 (TAI)*, Lanyu Township, 0 May 1924, *S. Sasaki s. n. (TAI)*, Lanyu Township, 18 Apr. 1932, *T. Sata s. n. (TAI)*, Lanyu Township, 2 Apr. 1985, *S. F. Huang 2742 (TAI)*, Lanyu Township, 6 Apr. 1983, *T. C. Huang et al. 9205 (TAI)*, Lanyu Township, 6 Apr. 1983, *T. C. Huang et al. 9179 (TAI)*, Green Island, Lyudao Township, 4 Mar. 1931, *T. Tanaka 10373 (TAI)*, THE PHILIPPINES. The Batan Islands, Batanes: Batan Island, 9 Nov. 1964, *S. Hatusima & M. Sato 28624 (KAG)*.

***Sedum formosanum* N.E. Brown., subsp. *miyakojimense* Takuro Ito, Yokota & Kokub., subsp. nov.**

urn:lsid:ipni.org:names:77209704-1

Figs 3A–D, 4

Type. JAPAN. The Ryukyus: Miyako Islands, Miyako-jima Island, Gusukube, 5 April 2015, *Takuro Ito 2402* (holotype: TNS)

Diagnosis. *Sedum formosanum* subsp. *miyakojimense* differs from its close relative *S. formosanum* subsp. *formosanum* in being perennial, polycarpic, and having lateral branches arising from the leaf axils.

Description. Perennial herb, fleshy, glabrous. First year stem stout, erect, partly woody, 1–5 lateral branches in the leaf axils, 3–10 cm tall, with lax rosettes; rosettes 2.5–6 cm wide with 7–15 leaves. Flowering stems fleshy, 10–20 cm tall, base ca. 5 mm broad, yellowish green, erect or sprawling and creeping at base. Roots fibrous, sometimes adventitious at the leaf scar. Leaves alternate, occasionally verticillate, sessile, green or yellowish, flattish, ± thick, spatulate to oblanceolate, 1.1–3.1 cm long, 0.3–1.0 cm wide, apex rounded, base long, attenuate, margins entire. Inflorescences terminal, cymes, basically trifurcate with 3 primary axes, sometimes with 2, 4, or 5 primary axes; primary axis



Figure 4. *Sedum formosanum* subsp. *miyakojimense*. **A** flower **B** sepal **C** leaf, adaxial **D** leaf, abaxial **E** habit. Scale bars: 1 mm (**A–D**); 1 cm (**E**). Line drawings by Naomi Kizaki.

2–8 cm long, ascending, 1 to several times irregularly and often unequally forking, with a flower at each fork, ultimate branches 1–2 cm long, 3–7 flowered; bracts leaf-like, smaller than cauline leaves. Flowers 5 (rarely 6)-merous, 7–11 mm wide, sessile. Sepals 5, free, yellowish green, fleshy, flattish, unequal in size, obovate to oblanceolate, 1.8–4.5 mm long, 1.2–3.3 mm wide, apex round or obtuse, base spurred. Petals 5, bright yellow, lanceolate, 4.6–6 mm long, 1.3–1.6 mm wide, apex acuminate, base slightly connate. Stamens 10, shorter than petals, 4.2–5 mm long, erect at flowering, two-whorled arrangement; anthers oblong-lanceolate, ca. 0.5 mm long, deep yellow before dehiscence. Pistils 5, 5.2–6.3 mm long; carpels 5, free, connate at the base, gibbous ventrally. Fruits star-shaped, follicle, erect, 5.3–6.8 mm long. Flowering in April to June.

Taxonomic note. This new subspecies is classified in the sect. *Sedum* because of its adaxially gibbous carpels (Fu and Ohba 2001) (Fig. 3).

Etymology. The epithet refers to the Japanese name of the type locality.

Distribution and habitat. Endemic to the southeastern portion of Miyako-jima Island (The Ryukyus), on sunny, coastal limestone.

Additional specimens examined. JAPAN. The Ryukyus: the Miyakojima Islands, Miyako-jima Island, Gusukube, 5 April 2015, *Takuro Ito 2403, 2408* (isotype: TNS).

Conservation. IUCN Red list category: Critically Endangered (CR). The distribution of *Sedum formosanum* subsp. *miyakojimense* is restricted to only one location ca. 0.15 km² in Miyako-jima Island, the Ryukyu Islands. The population of the species contains fewer than 200 mature individuals. The plant occurs on limestone rocks scattered in a private golf course, therefore, it is not formally protected. In the future, the population could become threatened, given ongoing land development for tourism in the Ryukyus. Because of the small population size (≤ 250 mature individuals) and small area of occupancy (≤ 10 km²), *S. formosanum* subsp. *miyakojimense* is classified as CR (IUCN 2019).

Japanese common name. Miyako-hama-mannen-gusa (nov.).

Possible biogeographical history of *S. formosanum* subsp. *miyakojimense*

The Ryukyu Islands, including Miyako-jima Island, experienced extensive land configuration changes throughout the Neogene and the Quaternary as a result of tectonic movements and sea level fluctuations induced by climatic oscillations (Kimura 2002; Osozawa et al. 2011; Furukawa and Fujitani 2014). Miyako-jima Island was likely originally located at the eastern margin of the continent, based on evidence of deposits derived from the continent during the late Miocene to Pliocene (Osozawa et al. 2011). The highest point on Miyako-jima Island is only 100 m above sea level; therefore, the entire island was likely submerged in the past under higher sea levels. Furthermore, the mud-dominant Shimajiri Group is mostly overlaid by the Ryukyu Group, which is composed of Pleistocene reef-complex deposits (Shokita et al. 2006). Although some endemic freshwater and terrestrial organisms, such as the Miyako toad (*Bufo gargarizans miyakonis* Okada) and the potamid crab (*Geothelphusa miyakoensis* Shokita, Naruse & Fujii) are reported from Miyako-jima Island (Shokita et al. 2006). Oshiro and Nohara

(2000) suggested that the island likely reconnected to the Yaeyama Islands, located in the southern Ryukyus, during the last glacial period. However, these endemic species and their close relatives are not distributed in the Yaeyama Islands, and it is highly unlikely that they experienced long-range dispersal. Therefore, if these islands were connected during the last glacial period, it is unlikely that migration occurred from the Yaeyama Islands via a land bridge. Interestingly, the Shimajiri Group is partly exposed to the surface on the eastern portion of Miyako-jima Island (Shokita et al. 2006). This suggests that some areas of the island may have remained above water during sea level fluctuations, and freshwater species such as *G. miyakoensis*, freshwater red alga (*Thorea gaudichaudii* C. Agardh), and oriental weatherfish (*Misgurnus anguillicaudatus* Cantor) are only distributed in this area (Shokita et al. 2002, 2006). Collectively, this suggests that some organisms may have survived in isolation as relict populations, and further implies that the island may not have been entirely submerged in the past or, potentially, the existence of an ancient landmass adjacent to the island after its division from the continent (Shokita et al. 2006; Furukawa and Fujitani 2014). Previous molecular dating of East Asian *Sedum* species reported that *S. formosanum* diverged from the endemic Taiwanese species during the Pleistocene 1.41 Ma (0.79–2.25 Ma) (Ito et al. 2017b). Thus, it is reasonable to assume that *S. formosanum* subsp. *miyakojimense* may have diverged during the Pleistocene and has long since been genetically isolated from other species. Furthermore, *S. formosanum* subsp. *miyakojimense* is distributed in a restricted area on the eastern part of the island, in a similar location as the aforementioned endemic freshwater organisms. The discovery of a new endemic plant taxon, *S. formosanum* subsp. *miyakojimense*, on Miyako-jima Island is biogeographically important because it may imply that portions of the island remained above water over long time periods.

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From *Polyalthia* to *Polyalthiopsis* (Annonaceae): transfer of species enlarges a previously monotypic genus

Bine Xue¹, Hong-Bo Ding², Gang Yao³, Yun-Yun Shao⁴,
Xiao-Jing Fan⁵, Yun-Hong Tan^{2,6}

1 College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, Guangdong, China **2** Southeast Asia Biodiversity Research Institute & Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China **3** College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China **4** Guangdong Provincial Key Laboratory of Digital Botanical Garden, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China **5** South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, Guangdong, China **6** Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China

Corresponding author: Yun-Hong Tan (tyh@xtbg.org.cn)

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Abstract

The genus *Polyalthiopsis* Chaowasku (Annonaceae) was a poorly known monotypic genus from Vietnam that was recently segregated from the highly polyphyletic genus *Polyalthia* s.l. The sister clade relationship between *Polyalthiopsis* and *Miliusa* was not well established in previous study. The phylogenetic position of two *Polyalthia* spp. from China, *P. chinensis* S.K.Wu ex P.T.Li and *P. verrucipes* C.Y.Wu ex P.T.Li, remains unresolved and is shown here to be phylogenetically affiliated with *Polyalthiopsis*. Phylogenetic analyses of six chloroplast regions (*matK*, *ndbE*, *psbA-trnH*, *rbcL*, *trnL-F* and *yef1*; ca.7.3 kb, 60 accessions) unambiguously placed *Polyalthia chinensis* and *P. verrucipes* in the same clade with *Polyalthiopsis floribunda* (PP = 1, MP BS = 97%); the entire clade is sister to *Miliusa* with weak to strong support (PP = 1, MP BS = 54%). *Polyalthia chinensis* and *P. verrucipes* share several diagnostic characters with *Polyalthiopsis floribunda*, including the raised midrib on the upper surface of the leaf *in vivo*, conspicuous foliar glands when dried, petiole with transverse striations when dried and axillary inflorescences. The two species differ from *Polyalthiopsis floribunda* in having fewer flowers per inflorescence, longer linear petals and two ovules

per carpel. On the basis of the combined molecular phylogenetic and morphological data, we propose two new combinations, *Polyalthiopsis chinensis* (S.K.Wu ex P.T.Li) B.Xue & Y.H.Tan and *Polyalthiopsis verrucipes* (C.Y.Wu ex P.T.Li) B.Xue & Y.H.Tan. The protologue of *Polyalthia verrucipes* did not include a description of the flowers, which we provide here. An updated description for the genus *Polyalthiopsis* and a key to species in the genus *Polyalthiopsis* is also provided.

Keywords

Annonaceae, molecular phylogeny, morphology, *Polyalthia chinensis*, *Polyalthia verrucipes*, *Polyalthiopsis*

Introduction

The genus *Polyalthia* Blume has historically been the source of considerable taxonomic confusion in Annonaceae due to its highly polyphyletic status (van Setten and Koek-Noorman 1992; Doyle and Le Thomas 1994; Doyle et al. 2000). Recent molecular phylogenetic studies have clarified generic circumscriptions and resulted in the segregation of disparate elements from the previously highly polyphyletic genus *Polyalthia* s.l., including removal of species now placed in several new genera—*Maasia* Mols & al. (Mols et al. 2008), *Huberantha* Chaowasku (Chaowasku et al. 2012 [as “*Hubera*”], Chaowasku et al. 2015); *Wuodendron* B.Xue, Y.H.Tan & T.Chaowasku (Xue et al. 2018) and *Polyalthiopsis* Chaowasku (Chaowasku et al. 2018); the transfer of species to *Fenerivia* Diels (Saunders et al. 2011), *Marsyopetalum* Scheff. (Xue et al. 2011), *Monoon* Miq. (Xue et al. 2012), *Goniothalamus* (Blume) Hook. f. & Thomson (Tang et al. 2013), *Meiogyne* Miq. (Xue et al. 2014) and *Wangia* X.Guo & R.M.K.Saunders (Xue et al. 2016). The circumscription of *Polyalthia* s.str. was consequently redefined (Xue et al. 2012).

Amongst the newly segregated genera, *Polyalthiopsis* Chaowasku is a poorly known monotypic genus from Vietnam (Chaowasku et al. 2018). The type species *Polyalthiopsis floribunda* is known from only two field collections (*Poilane 10052*, P, A, BO, CMUB, HN, K, L, P; and *Chaowasku 128*, CMUB). The species was first collected in 1924 and described under the name *Polyalthia floribunda* Jovet-Ast (Jovet-Ast 1940). I.M. Turner (2016) subsequently transferred the name to *Huberantha*. Ninety years after the first collection, Chaowasku collected this species again in 2014 and was able to sequence it for a phylogenetic study. It was shown not to be congeneric with *Huberantha* and was instead retrieved (without statistical support) as sister to *Miliusa*, leading Chaowasku et al. (2018) to erect a monotypic genus, *Polyalthiopsis* Chaowasku, to accommodate it. With only one *Polyalthiopsis* species and limited DNA regions used in the phylogenetic study, the sister relationship between *Polyalthiopsis* and *Miliusa* was not well established. It is also difficult to identify important diagnostic characters for *Polyalthiopsis* with only one flowering collection and a single monocarp available for taxonomic comparison.

Several species names remain unresolved in *Polyalthia* and await assignment to specific genera (Xue 2013; Xue et al. 2012), including the Chinese endemics

Polyalthia chinensis S.K.Wu ex P.T.Li and *P. verrucipes* C.Y.Wu ex P.T.Li. As with *P. floribunda*, these two species are represented by very few collections and lack adequate flowering and fruiting descriptions.

The name *Polyalthia chinensis* was published in 1976, based on a flowering collection (*Qinghai-Xizang Exped. 74-4451*, KUN & PE) from Mêdog, Xizang, China, in 1974 (Li 1976; Li and Gilbert 2011). A second sterile specimen was subsequently collected in 1983 (*B. S. Li & S. Z. Cheng 2668*, PE). Until now, the species was only represented by these two collections.

The name *Polyalthia verrucipes* was published in 1976, based on a fruiting collection (*C.W. Wang 76321*, IBSC, NAS, A, PE) from Menghai, Yunnan, China, in 1936 (Li 1976; Li and Gilbert 2011). A second collection with immature fruits was collected in 1957 (*Sino-Russia Exped. 9527*, KUN & PE). Although Hou and Li (2007) cited further collections (*S. K. Wu 1345, 1375*, KUN; *X. L. Hou 112*, CANT, IBSC; and *T. X. Sun 200037*, CANT), we failed to locate those specimens in the cited herbaria.

The relationship between these two species has been controversial. Both species are represented by very few collections, with *P. chinensis* lacking fruiting specimens and *P. verrucipes* lacking flowering specimens, rendering morphological comparison problematic. Based on the foliar glands and leaf venation, Hou and Li (2007) regarded the name *P. chinensis* as synonymous with *P. verrucipes*, although this treatment was rejected by Li and Gilbert (2011) in the *Flora of China* without explanation. Li and Gilbert (2011) included identical floral descriptions in their treatment of *P. verrucipes* and *P. chinensis*, but with no clear indication of the source of this information, casting some doubt over the floral description of *P. verrucipes*.

With limited morphological characters, especially the lack of flowers in *P. verrucipes* and the limited material available, the relationship between *P. chinensis* and *P. verrucipes* and their taxonomic placement has never been resolved. We therefore, carried out several field explorations to search for these two species. This resulted in new collections of *Polyalthia verrucipes*, including flowers, enabling clarification of the relationship between *P. chinensis* and *P. verrucipes*, as well as their phylogenetic position. As a consequence, we were able to enlarge the poorly known genus *Polyalthiopsis*, supplementing available descriptions and providing better support for its sister relationship.

Phylogenetic analysis

Taxon and DNA region sampling

Two accessions of *Polyalthia chinensis* (*B. S. Li & S. Z. Cheng 2668*, PE; and *Qinghai-Xizang Exped. 74-4451*, KUN), as well as two accessions of *Polyalthia verrucipes* (*Sino-Russia Exped. 9527*, PE; and *Y.H. Tan MH1603*, IBSC) were sampled and integrated with data of 56 Annonaceae accessions from previous datasets (Chaowasku et al. 2018; Guo et al. 2014; Xue et al. 2016, 2018). The final dataset comprised a total of 60 accessions of Annonaceae representing all major clades in the family, including 44 acces-

sions representing 29 genera from subfam. Malmeoideae, 12 accessions representing 11 genera from subfam. Annonoideae, three species from subfam. Ambavioideae and one species of *Anaxagorea* A. Saint.-Hilaire. (subfam. Anaxagoreoideae). For Miliuseae, representatives of all currently accepted genera were included.

Six chloroplast DNA regions (*matK*, *ndhF*, *rbcL*, *psbA-trnH* and *trnL-F* and *yefl*) were sequenced for the above-mentioned four collections of *Polyalthia chinensis* and *P. verrucipes*. The samples, localities and GenBank accession numbers are listed in Appendix I.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from herbarium materials using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). A single amplification protocol was used for amplification of the chloroplast regions: template denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec; primer annealing at 50 °C for 1 min; and primer extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. The primers used to amplify the *psbA-trnH* intergenic spacer were psbAF (Sang et al. 1997) and trnH2 (Tate and Simpson 2003); other primers are the same as those used by Thomas et al. (2012). PCR products were visualised using agarose gel electrophoresis. Successful amplifications were purified and sequenced on an Applied Biosystems 3730xl DNA Analyzer at Sangon Biotech (Shanghai) Co. Ltd., Guangzhou, China.

Alignment and phylogenetic analyses

Sequences were assembled and edited using Geneious ver. 5.4.3 (Drummond et al. 2010) and pre-aligned with the MAFFT (Katoh et al. 2002) plugin in Geneious using the automatic algorithm selection and default settings and, subsequently, manually checked and optimised. An inversion of 15 positions in *psbA-trnH* was identified and reverse complemented in the alignment, following a strategy previously applied by Pirie et al. (2006), to retain substitution information in the fragments.

Maximum parsimony (MP) analyses of the seven combined regions were conducted using PAUP ver. 4.0b10 (Swofford 2003). All characters were weighted equally and gaps treated as missing data. The most parsimonious trees were obtained with heuristic searches of 1,000 replicates of random stepwise sequence addition, tree bisection-reconnection (TBR) branch swapping with no limit to the number of trees saved. Bootstrap support (BS) was calculated following Müller (2005), with 10,000 simple stepwise addition replicates with TBR branch swapping and no more than 10 trees saved per replicate.

Bayesian analysis was performed using NSF Extreme Science & Engineering Discovery Environment (XSEDE) application of MrBayes ver. 3.2.2 (Huelsenbeck and

Ronquist 2001; Ronquist and Huelsenbeck 2003) provided by the CIPRES Science Gateway (Miller et al. 2010). PartitionFinder2 was used to test the dataset for partitions (model of evolution: mrbayes; model of selection: AICc; scheme search: greedy) (Guindon et al. 2010; Lanfear et al. 2012, 2016). The best partition scheme suggested six partitions, based on DNA region identity with GTR+G chosen for *matK*, *psbA-trnH*, *trnL-F* and *ycf1* regions and GTR+I+G selected for the *ndhF* and *rbcL* regions. Two independent Metropolis-coupled Markov Chain Monte Carlo (MCMC) analyses were run. Each search used three incrementally heated and one cold Markov chain and was run for 10 million generations and sampled every 1,000th generation. The temperature parameter was set to 0.08. The mean branch length prior was set from the default mean (0.1) to 0.01 (brlenspr = unconstrained: exponential (100.0)) to reduce the likelihood of stochastic entrapment in local tree length optima (Brown et al. 2010). Convergence was assessed using the standard deviation of split frequencies, with values < 0.01 interpreted as indicating good convergence. Tracer ver. 1.6 (Rambaut et al. 2014) was used to determine whether the parameter samples were drawn from a stationary, unimodal distribution and whether adequate effective sample sizes (ESS) for each parameter (ESS > 200) were reached. The first 25% of samples (2,500 trees) were discarded as burn-in and the post-burn-in samples summarised as a 50% majority-rule consensus tree.

Morphological studies

Comparative morphological data were obtained from specimens deposited in KUN, IBSC and PE herbaria and from published literature. Field surveys were carried out in Menghai County, Yunnan Province, with voucher specimens deposited in HITBC and IBSC.

Results

The concatenated alignment of the 60-terminal dataset consisted of 7,334 characters. The MP heuristic search retrieved four equally most parsimonious trees of 3,519 steps (consistency index, CI = 0.664; retention index, RI = 0.709).

The MP and Bayesian analyses resulted in similar topologies. The 50% majority-rule consensus tree resulting from the Bayesian analyses under the six partitioned model is shown as Fig. 1. The results are consistent with previous phylogenetic analyses of the family, with the backbone of the tribe Miliuseae unresolved as in previous studies.

Polyalthia chinensis and *P. verrucipes* are not retrieved in the same clade as *Polyalthia johnsonii*, but were strongly supported as members of the same clade as *Polyalthiopsis floribunda* (PP [posterior probability] = 1, MP BS = 97%), with the entire clade sister to *Miliusa* with weak to strong support (PP = 1, MP BS = 54%).

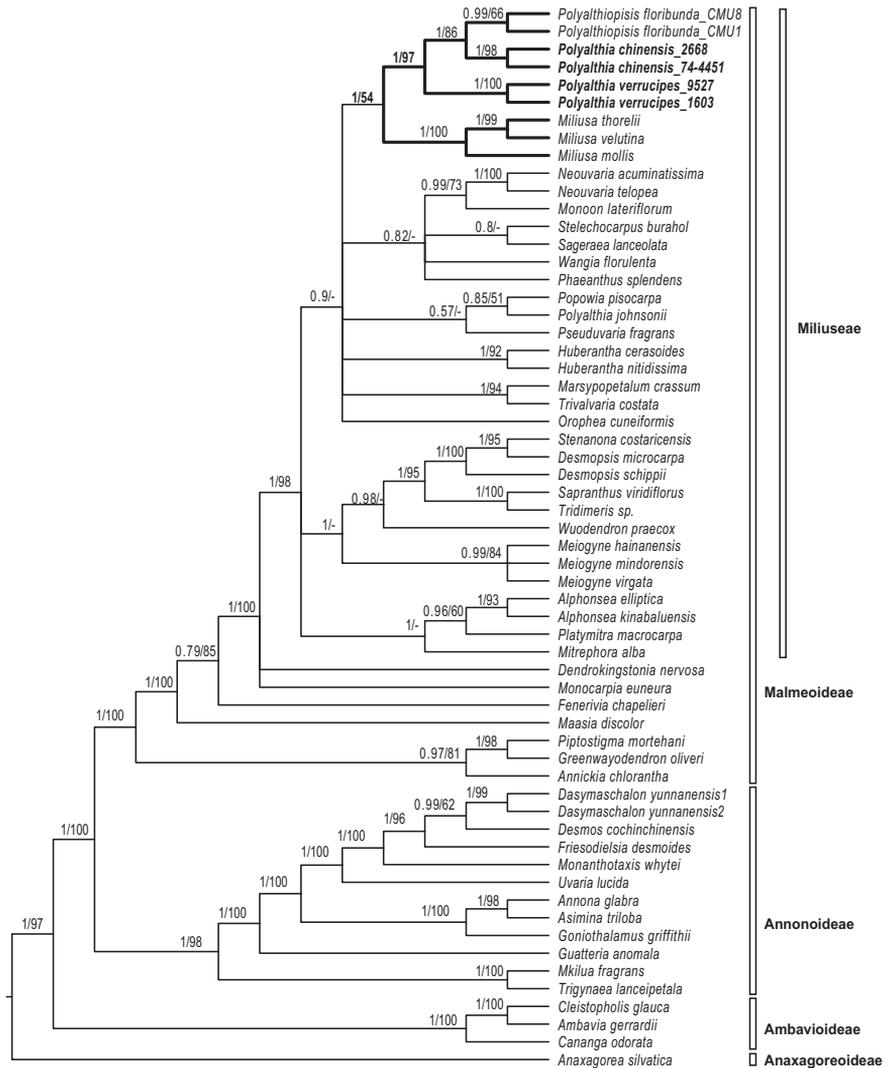


Figure 1. Bayesian 50% majority-rule consensus tree under partitioned models (cpDNA data: *matK*, *ndhF*, *psbA-trnH*, *rbcL* and *trnL-F*; 60 taxa) showing Annonaceae subfams. Anaxagoreoideae, Ambavioideae and Annonoideae. Numbers at the nodes indicate Bayesian posterior probabilities and maximum parsimony bootstrap values (> 50%) in that order.

Discussion

Polyalthia chinensis was regarded as a synonym of *P. verrucipes* by Hou and Li (2007). The phylogenetic positions of these two species are quite distinct, with the following relationship: (*Polyalthia verrucipes*, (*Polyalthia chinensis*, *Polyalthiopsis floribunda*)) (Fig. 1). Our field collection of the flowers of *Polyalthia verrucipes* provides further evidence for the distinction between these two species.

Although these two species resemble each other vegetatively (Fig. 2A, C), they differ in the number of flowers per inflorescence, the length and thickness of the pedicel and the colour of the petals. The inflorescences of *Polyalthia chinensis* have 1–2 flowers (Fig. 2A, C), whereas those of *Polyalthia verrucipes* comprise a solitary flower (Figs 2D, 3F, G). The pedicel of *P. chinensis* is slender and ca. 7 mm long, whereas that of *P. verrucipes* is stout and shorter than 2 mm. The petals of *P. chinensis* are green (Li 1976), whereas those of *P. verrucipes* are white (Fig. 3F–H). The leaf also differs slightly, with the leaf lamina of *P. chinensis* (2.5–3.8 cm) narrower than that of *P. verrucipes* (2.5–5 cm) and slightly thinner. The morphological data are therefore congruent with the phylogenetic topology and our phylogenetic and morphological analyses support the hypothesis that both species are not conspecific, as suggested by Li and Gilbert (2011).

The monotypic genus *Polyalthiopsis* Chaowasku was published in 2018, based on *Polyalthia floribunda* collected in Vietnam (Chaowasku et al. 2018). It was reconstructed as the sister group of *Miliusa*, but without statistical support. *Polyalthiopsis*, *Huberantha* and *Miliusa* have previously been retrieved as an unsupported to weakly supported clade in Chaowasku et al. (2018). Although Chaowasku et al. (2018) mentioned that a more comprehensive phylogenetic study, using the whole plastome sequence data, demonstrates the same topology with strong support, the result has yet to be published. *Polyalthiopsis* is also retrieved as sister to *Miliusa* in this study, with weak support in the maximum parsimony analysis (MP BS = 54%), but strong support in the Bayesian analysis (PP = 1). This sister relationship was also well supported in Xue et al. (2020) (ML BS = 86%, suppl. material 1: fig. S1). The relationship between *Huberantha* and the *Polyalthiopsis*-*Miliusa* collective clade is, however, not retrieved in this study. The long-recognised sister relationship between *Miliusa* and *Huberantha* in previous studies (Mols et al. 2008; Saunders et al. 2011; Xue et al. 2011, 2012; Chaowasku et al. 2012, 2014; Chatrou et al. 2012; Guo et al. 2017) can be redefined here following the inclusion of *Polyalthiopsis*.

Polyalthiopsis Chaowasku is easily distinguished from most of the other genera in the tribe Miliuseae by its raised midrib on the adaxial leaf surface. When dry, such an adaxial leaf midrib appears slightly sunken. The raised midrib on the adaxial leaf surface is rarely observed in the Annonaceae, but is known from *Artabotrys* (Sinclair 1955; Turner 2012), *Crematosperma* (Pirie 2005), *Cyathocalyx* (Surveswaran et al. 2010), *Isolona* (Couvreur 2009), *Monodora* (Couvreur 2009), *Mezzettia* (van der Heijden and Kessler 1990), *Pseudephedranthus* (Erkens et al. 2017) and *Stelechocarpus* (Chaowasku et al. 2013; van Heusden 1995). Another distinct feature of *Polyalthiopsis floribunda* is the dried petiole with multiple transverse striations (Chaowasku et al. 2018). Not many Annonaceae species have this pronounced drying artifact. One more distinct feature is the obvious foliar glands on the leaf surface when dried (obvious in fig. 2C in Chaowasku et al. 2018). Foliar glands are also observed in *Wuodendron* B.Xue, Y.H.Tan & Chaowasku in Miliuseae (Xue et al. 2018).

Based on one species with only two collections, the genus is not well described and compared and, hence, it is difficult to identify important diagnostic characters.



Figure 2. Morphological comparison between *Polyalthia chinensis* and *P. verrucipes* **A** type specimen of *P. chinensis* (Qinghai-Xizang Exped. 74-4451, PE) **B** inflorescence of *P. chinensis* (Qinghai-Xizang Exped. 74-4451, PE) **C** type specimen of *P. verrucipes* (C. W. Wang 76321, A) **D** inflorescence of *P. verrucipes* (B. Xue & H.B. Ding 311, IBSC) **E** adaxial leaf surface of *P. chinensis* (Qinghai-Xizang Exped. 74-4451, KUN) **F** abaxial leaf surface of *P. chinensis* (Qinghai-Xizang Exped. 74-4451, KUN) **G** adaxial leaf surface of *P. verrucipes* (Yunnan Exped. 9527, KUN) **H** abaxial leaf surface of *P. verrucipes* (Yunnan Exped. 9527, KUN).

Polyalthia chinensis and *P. verrucipes* are retrieved in the same clade as *Polyalthia floribunda* in the molecular phylogeny (Fig. 1). Sterile material of these three species is very similar. The leaves are elliptic with a cuneate base and acute to acuminate apex, with brochidodromous venation and reticulate tertiary veins. The leaf midrib in all three species is furthermore raised adaxially *in vivo* (Fig. 3C; raised midrib still visible in the specimen of *P. chinensis*), with multiple transverse striations on the dried petiole (Figs 3E, 4C) and obvious foliar glands on dried leaf surface (Figs 2E–H, 3D, 4B). Although all three species have axillary inflorescences, the number of flowers per inflorescence differs: *P. chinensis* has one to two flower(s) per inflorescence (Fig. 2A, B), *Polyalthia verrucipes* has only one flower per inflorescence (Figs 2D, 3F–H), while *Polyalthia floribunda* has 1–5 flower(s) per inflorescence. The shape of the petal also differs: the petals of *Polyalthia chinensis* and *P. verrucipes* are linear (Figs 2B, D, 3F–H, 4D, E),

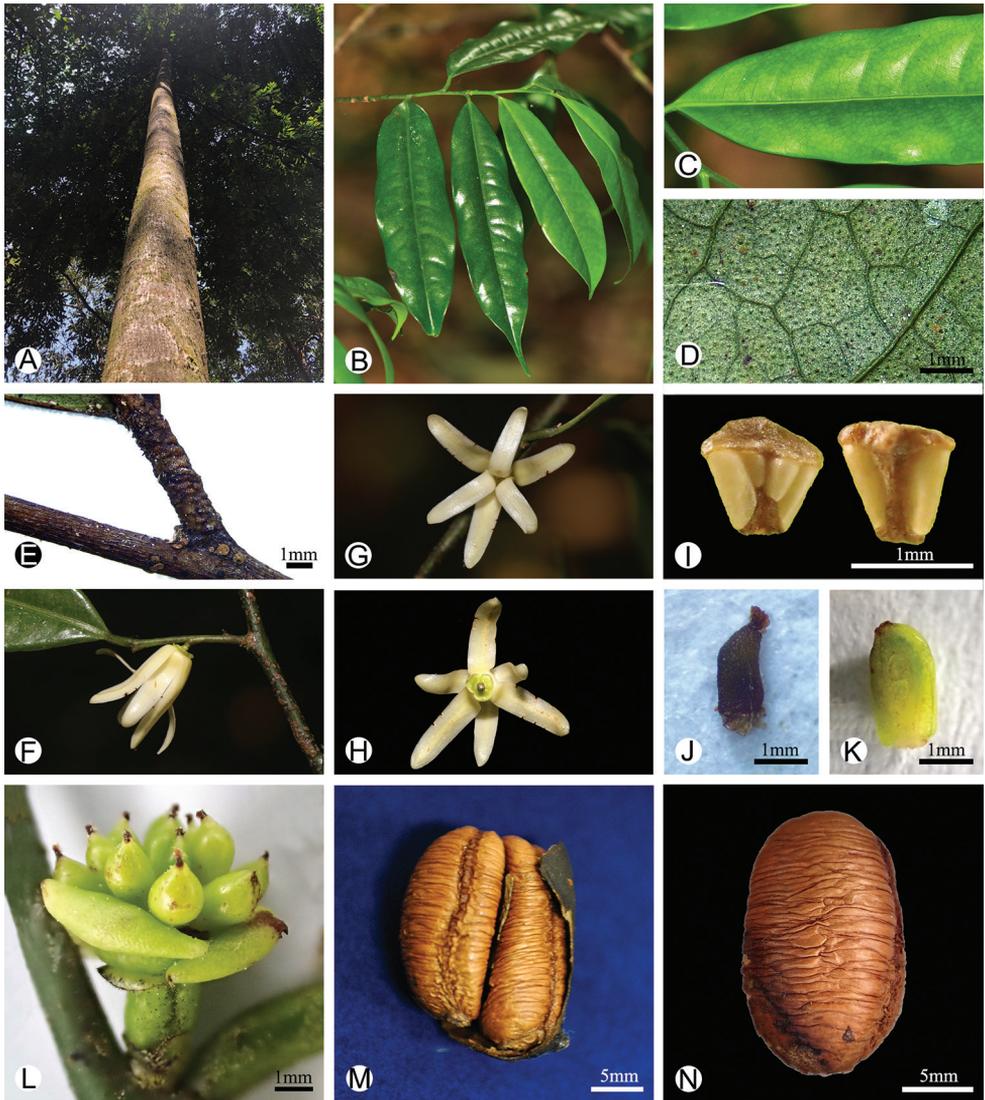


Figure 3. Morphology of *Polyalthiopsis verrucipes* comb. nov. **A** trunk, showing greyish bark **B** a branch, showing the leaf lamina **C** adaxial leaf surface, showing the raised midrib **D** close-up of the abaxial surface of dried leaf, showing glands **E** petiole with transverse striations when dried **F** lateral view of the flower **G** top view of the flower **H** bottom view of the flower **I** adaxial and abaxial view of the stamen **J** carpel **K** longitudinal section of a developing carpel, showing two developing ovules **L** developing young fruits **M** single dried monocarp, showing the two seeds 01187409 (C. W. Wang 76321, PE) **N** cylindrical seed, showing longitudinal groove around circumference (C. W. Wang 76321, PE). – Photos: **A, C–E, I–N**, by Bine Xue; **B**, by Yun-Hong Tan; **G–H** by Hong-Bo Ding.

while those of *Polyalthiopsis floribunda* are elliptic-ovate (Chaowasku et al. 2018). The carpel characters of *Polyalthia chinensis* and *P. verrucipes* also differ greatly from those of *Polyalthiopsis floribunda*: the former two species have two ovules per ovary and hence

two seeds in each monocarp (Fig. 3K, M), whereas *Polyalthiopsis floribunda* has only one ovule per ovary (Jovet-Ast 1940; Chaowasku et al. 2018).

In conclusion, *Polyalthia chinensis*, *P. verrucipes* and *Polyalthiopsis floribunda* share axillary inflorescences, a raised midrib on the adaxial leaf surface (Fig. 3C), petioles with transverse striations when dry (Fig. 3E) and foliar glands on dried leaf surface (Figs 2E–H, 3D, 4B). These characters render the three species distinctive from other species in the tribe and are thus diagnostic for the enlarged genus *Polyalthiopsis*.

The present phylogenetic study shows that *Polyalthia chinensis* is strongly supported as sister to *Polyalthiopsis floribunda* (PP = 1, MP BS = 86%). The collective clade is strongly supported as sister to *Polyalthia verrucipes* (PP = 1, MP BS = 97%). The whole clade (comprising the three species) is weakly to strongly supported (PP = 1, MP BS = 54%) as sister to *Miliusa*. The morphological and molecular phylogenetic data therefore support the transfer of *Polyalthia verrucipes* and *P. chinensis* to *Polyalthiopsis* and the new nomenclatural combinations are proposed here.

As *Polyalthia verrucipes* was published based on fruiting material only (Li 1976), with the newly collected flowers, an updated description is presented. It is noteworthy that the floral description of *P. verrucipes*, published by Li and Gilbert (2011), does not correspond with the material we collected in the field, but is instead similar to that of *P. chinensis*.

As more species were included in the genus *Polyalthiopsis*, an updated description and a key to the three species is also provided.

Taxonomy

Polyalthiopsis Chaowasku, *Ann. Bot. Fennici* 55: 130. 2018.

Figs 2–5

Chinese name: you ye mu shu (疣叶木属)

Type species. *Polyalthiopsis floribunda* (Jovet-Ast) Chaowasku – *Polyalthia floribunda* Jovet-Ast, *Notul. Syst.* 9: 75. 1940. – *Huberantha floribunda* (Jovet-Ast) I.M. Turner, *Webbia* 71: 229. 2016. – Type: Vietnam. Phanrang Prov., Tra Ca, 10 March 1924, *Poilane 10052* (holotype P [barcode no. P00411080]; isotypes A [barcode no. A00351290], BO?, CMUB, HN, K [barcode no. K000608178], L [barcode no. L3728819], P [barcode no. P00411081; P00411082]), in flower.

Description. Medium-sized to large trees. Young twigs glabrous. Leaves petiolate, blade elliptic, with glandular dots observable when dry, base cuneate, apex acute to bluntly (caudate-)acuminate; petiole with transverse striations when dry; upper surface of midrib raised in living plants, becoming slightly sunken when dry, lower surface of midrib raised; secondary veins rather faint in living plants, becoming slightly raised on both sides when dry, leaf venation brochidodromous; tertiary veins reticulate. Flower(s) in 1- to 5-flowered inflorescences, bisexual, pedicellate; inflorescences axillary, peduncle inconspicuous, bracts present. Sepals broadly ovate-triangular. Petals membranous-papyraceous to leathery. Outer petals elliptic-ovate or linear-lanceolate. Inner petals (narrowly) elliptic-ovate or linear-lanceolate. Stamens numerous per flow-

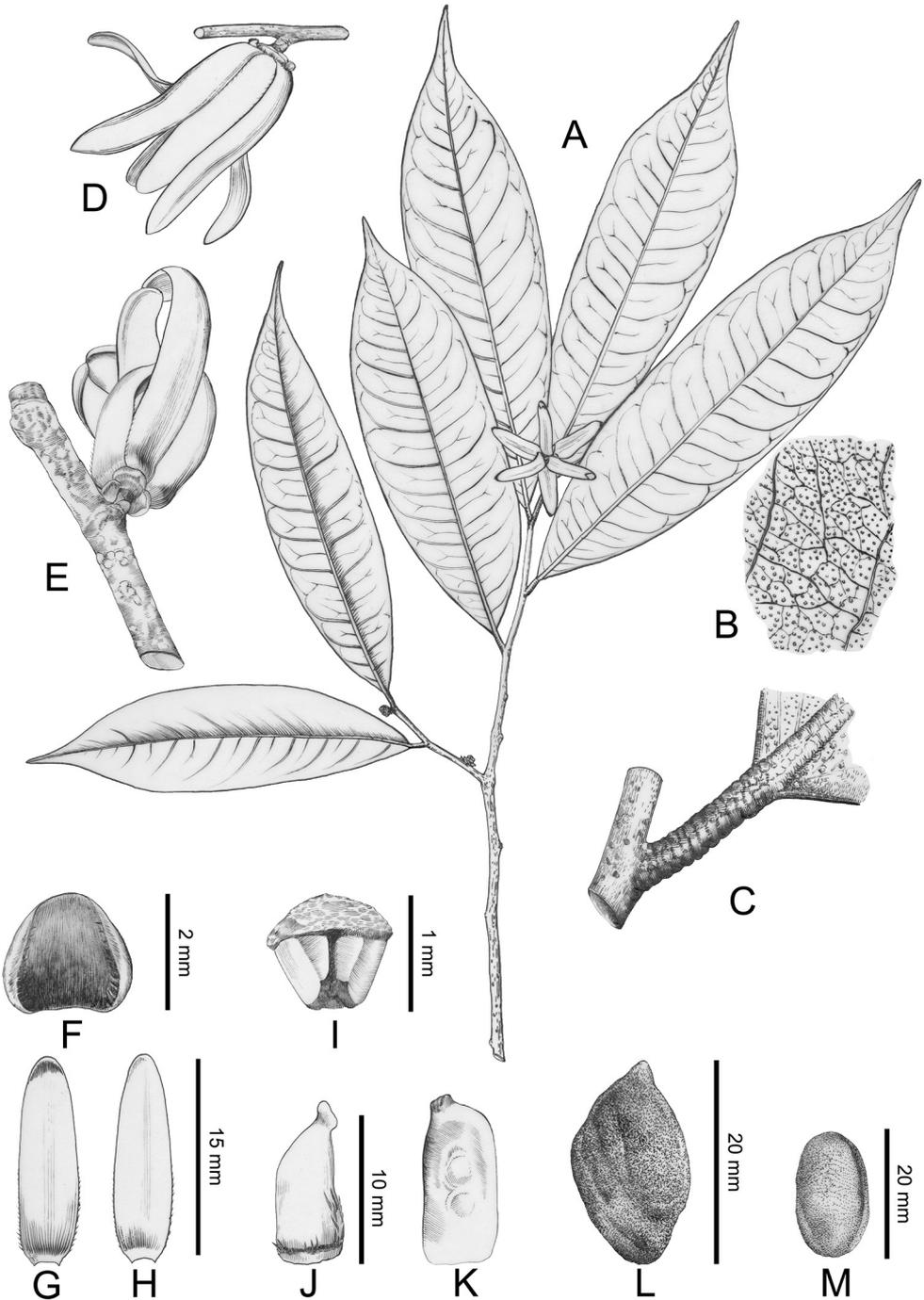


Figure 4. *Polyalthiopsis verrucipes* comb. nov. **A** flowering branch **B** close-up of adaxial surface of leaf, showing glands **C** close-up of leaf petiole, showing the transverse striations on dried petiole **D** lateral view of the flower **E** adaxial view of the flower **F** sepal **G** outer petal **H** inner petal **I** stamen **J** carpel **K** longitudinal section of the developing carpel, showing two lateral ovules **L** a dried monocarp **M** a seed. Drawn by Ding-Han Cui. (**A–K** from *B. Xue & H. B. Ding* XB311, IBSC; **L, M** from *C. W. Wang* 76321, PE)

er, connective truncate, covering thecae. Carpels numerous per flower; ovaries with 1 or more line(s) of hairs; stigma terete; ovule(s) 1 or 2 per ovary, sub-basal or lateral. Monocarps oblong to rhomboidal or cylindrical, stipitate, glabrous. Seed(s) 1 or 2 per monocarp, cylindrical, surface smooth, raphe broadly sunken and partially slightly raised in middle, endosperm ruminations lamelliform.

Distribution. Three species, known from Xizang, Yunnan Provinces of China and Thừa Thiên-Hu, Ninh Thuận Provinces of Vietnam (Fig. 5).

***Polyalthiopsis chinensis* (S.K.Wu ex P.T.Li) B.Xue & Y.H.Tan, comb. nov.**

urn:lsid:ipni.org:names:77209705-1

Figs 2, 5

Chinese name: xi zang you ye mu (西藏疣叶木)

Polyalthia chinensis S.K.Wu & P.T.Li in *Acta Phytotax. Sin.* 14 (1): 108, t. 4. 1976.
Basionym

Type. China. Xizang: Mêdog, 20 August 1974, *Qinghai-Xizang Exped. 74-4451* (holotype, PE! [barcode no., PE01187290]; isotypes, PE! [[barcode no., PE01187291, PE01187292, PE01187293], KUN! [barcode no., KUN0677650]).

Distribution and habitat. Known from Mêdog in Xingzang Province (Fig. 5), growing in rain forests, at low to medium elevations (800–1000 m a.s.l.).

Phenology. Flowering in August.

Additional specimens examined. *B. S. Li & S. Z. Cheng 2668* (PE).

Preliminary IUCN conservation status. DD (IUCN 2012). This species is only represented by two collections in Mêdog in Xizang Province. As Mêdog is not well explored, we tentatively recommend the conservation status as Data Deficient.

***Polyalthiopsis verrucipes* (C.Y.Wu ex P.T.Li) B.Xue & Y.H.Tan, comb. nov.**

urn:lsid:ipni.org:names:77209706-1

Figs 2–5

Chinese name: you ye mu (疣叶木)

Polyalthia verrucipes C.Y.Wu ex P.T.Li in *Acta Phytotax. Sin.* 14 (1): 110. 1976.
Basionym

Type. Lectotype (designated here). China. Yunnan: Menghai, July 1936, *C. W. Wang 76321* (IBSC! [barcode no. IBSC0003386]; isolectotypes, A [barcode no. A00039580, photo!], IBSC! [barcode no. IBSC0003386], PE! [barcode no. PE01187287, PE01187470], NAS[barcode no. NAS00321991, photo!]).

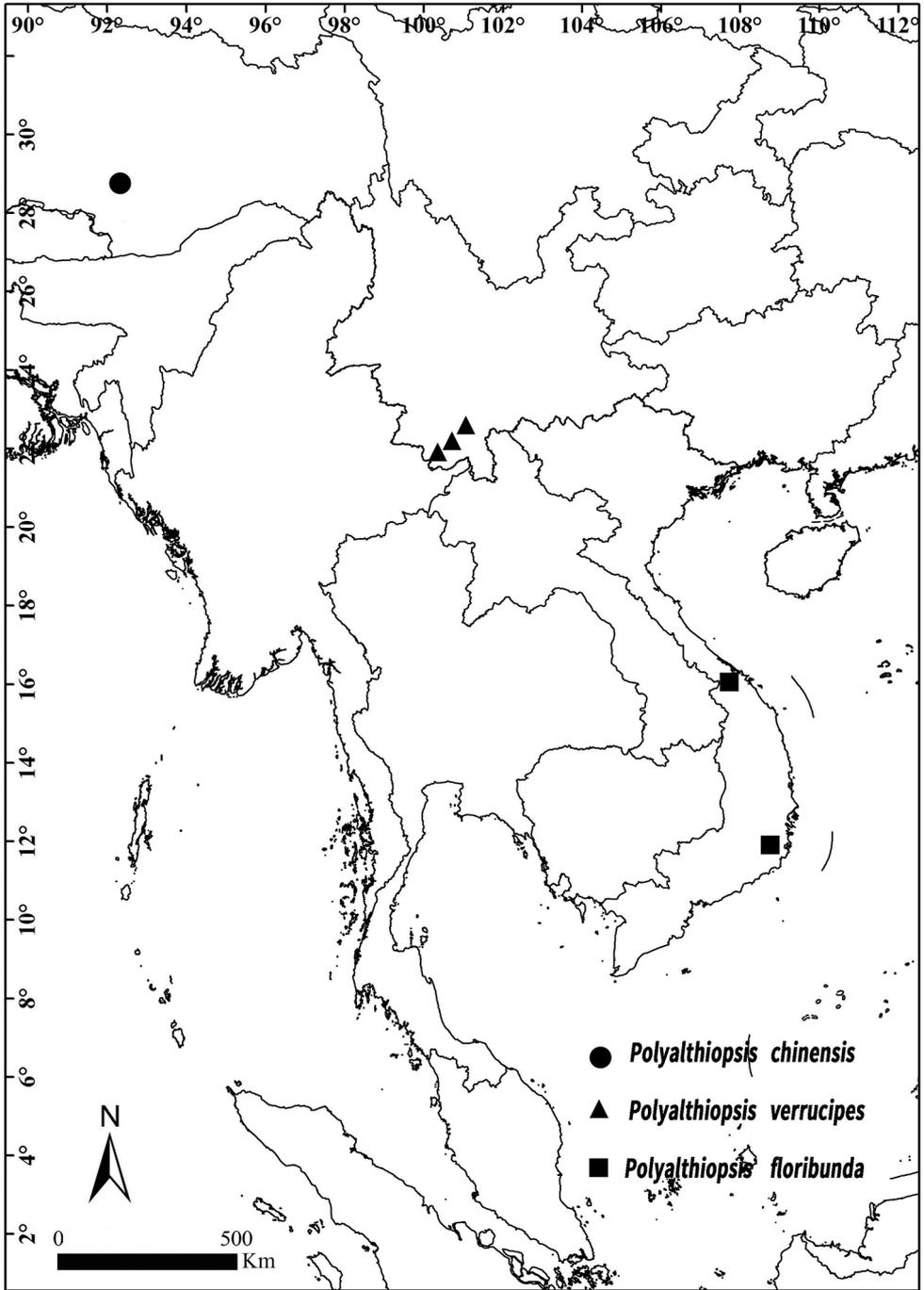


Figure 5. Distribution of *Polyalthiopsis chinensis*, *P. floribunda* and *P. verrucipes*.

Description. Trees to 15 m tall (Fig. 3A). Branches greyish-black, glabrous. Petiole 3–7 mm long, 1–2 mm in diameter, glabrous, with transverse striations when dry (Figs 3E, 4C); leaf laminas oblong to oblong-lanceolate, 10–17 × 2.5–5 cm, base broadly cuneate or obtuse, apex acuminate (Figs 3B, 4A), both surfaces glabrous, thinly leathery, densely verrucate with foliar glands when dry (Figs 2G, H, 3D, 4B); upper surface of midrib raised when fresh (Fig. 3C), becoming flat or slightly sunken when dry (Fig. 2G), lower surface of midrib raised; secondary veins 15–18 on each side of midrib, delicate and prominent on both surfaces; tertiary veins reticulate. Inflorescences axillary, with solitary flower (Figs 2D, 3F, H, 4A, D, E). Pedicel 1–2 mm long, hispid, with one ovate bracteole at top, 2–3 ovate bracteoles at base (Fig. 3F, L). Sepals ovate, 2 × 2 mm, slightly reflexed, ciliate (Fig. 4F). Petals 6, valvate, free, in 2 whorls; white, linear, both whorls subequal, ca. 16 × 3–5 mm, thinly leathery, glabrous, slightly ciliate (Figs 3F–H, 4G, H). Stamens 40–50 per flower, ca. 1 mm long (Figs 3I, 4I); connective truncate. Carpels 12–16 per flower, oblong, glabrous; stigma ovoid, puberulent; ovary with 1 or 2 line(s) of hairs (Figs 3J, 4J); ovules 2 per ovary, lateral (Figs 3K, 4K). Fruiting pedicel becoming longer and thicker, 5–7 mm long, ca. 3 mm in diameter; monocarp stipes 7–9 mm long; monocarps oblong to rhomboid, ca. 2 cm long, 1 cm in diameter (Figs 3M, 4M). Seeds 2 per monocarp, yellowish, semi-ellipsoid to ellipsoid, ca. 18 cm long, 8 mm in diameter, endosperm rumination lamelliform (Figs 3M, N, 4M).

Distribution and habitat. Known from several localities in Yunnan Province (Fig. 5): Hei-long-tan and Manxi in Meng-hai County and Kun-man in Meng-yang Town, Jinghong, growing in rain forests, at medium elevations (1300–1800 m a.s.l.).

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

Additional specimens examined. China. Yunnan: Kun-man, Xiao-meng-yang, 27 April 1957, *Yunnan Exped. 9527* (IBSC, KUN, PE); Man-xi, Menghai, 16 March 2016, *Y.H. Tan MH1603* (HITBC, IBSC); 5 March 2019, *B. Xue & H.B. Ding 311, 312, 313* (HITBC, IBSC, KUN).

Preliminary IUCN conservation status. CR A2ac, C2(a)(i) (IUCN 2012). *Polyalthiopsis verrucipes* was assessed as EN A2c by the China Plant Specialist Group (2004). Prior to this study, *P. verrucipes* was only represented in herbaria by two collections from Yunnan, China (two localities, both of which have subsequently been severely deforested). Our field survey in 2016 identified one population with dozens of individuals of dbh ca. 10 cm and dozens of young treelets in Manxi village, Menghai County. We made a second visit to the location in 2019 and found only a few individuals with dbh larger than 10 cm and few treelets. Herbicide had been used in that location. The bark at the bottoms of the tree trunks was damaged. The local farmers appear to clear the forests in this way for tea plantation and it is anticipated that the trees with damaged bark could not survive. We hope additional undocumented subpopulations will be found and protected, although further field investigation is needed to better understand the current status of populations. At present, we recommend that this species be regarded as critically endangered (CR) based on current IUCN Red List Categories and Criteria (IUCN 2012).

Key to *Polyalthiopsis*

- 1a Inflorescences 1–5-flowered; petals elliptic-ovate; ovule 1 per carpel; distributed in Vietnam.....*P. floribunda*
- 1b Inflorescences 1–2-flowered; petals linear; ovules 2 per carpel; distributed in China **2**
- 2a Inflorescences 1–2-flowered; pedicel to 5–7 mm long; flowers green; distributed in Xizang, China.....*P. chinensis*
- 2b Inflorescences with a single flower; pedicel 1–2 mm long; flowers white; distributed in Yunnan, China.....*P. verrucipes*

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Appendix I

Voucher information and GenBank accession numbers for samples used in this study (–, missing data; *, newly generated sequences). Voucher data are given for accessions for which DNA sequences were newly obtained, using the following format: species, origin, voucher and Genbank accession numbers for *matK*, *ndhF*, *rbcL*, *psbA-trnH* and *trnL-F* and *ycf1*. For DNA sequences published in previous studies, voucher information is available from GenBank.

Alphonsea elliptica Hook. f. & Thomson, AY518807, JQ690401, JQ690402, –, AY319078, JQ690403; *Alphonsea kinabaluensis* J. Sinclair, AY518811, –, –, AY318968, AY319080, –; *Ambavia gerrardii* (Baill.) Le Thomas, AY220435, AY218168, –, –, AY220411 (intron)AY220358 (spacer), –; *Anaxagorea silvatica* R. E. Fr., AY743477, EF179280, –, AY743439, AY743458, –; *Annickia chlorantha* (Oliv.) Setten & Maas, AY841393, AY841401, –, AY841594, AY841671, –; *Annona glabra* L., DQ125050, EF179281, –, AY841596, AY841673, –; *Asimina triloba* (L.) Dunal, AY743479, EF179287, –, AY743441, AY743460, –; *Cananga odorata* (Lam.) Hook. f. & Thomson, AY841394, AY841403, –, AY841602, AY841680, –; *Cleistopholis glauca* Pierre ex Engl. & Diels, AY841395, AY841404, –, AY841603, AY841681, –; *Dasy-maschalon yunnanense* (Hu) Bân 1, JQ768560, JQ768598, JQ768639, JQ768680, JQ768720, –; *Dendrokingstonia nervosa* (Hook. f. & Thomson) Rauschert, KJ418392, KJ418386, KJ418400, KJ418382, KJ418407, –; *Desmopsis microcarpa* R. E. Fr., AY518804, JX544771, AY841461, AY319059, AY319173, JX544758; *Desmopsis schippii* Standl., AY518805, JQ723786, –, AY319060, AY319174, –;

Desmos cochinchinensis Lour., JQ768568, JQ768604, –, JQ768688, JQ768728, –; *Dasymaschalon yunnanense* (Hu) Bàn 2, KF680919, KF680919, –, –, –, –; *Fenerivia chapelieri* (Baill.) R. M. K. Saunders, JF810375, JQ723788, –, JF810387, JF810399, –; *Friesodielsia desmoides* (Craib) Steenis, JQ768577, JQ768612, –, JQ768696, JQ768738, –; *Goniothalamus griffithii* Hook. f. & Thomson, AY743484, EF179296, –, AY743446, AY743465, –; *Greenwayodendron oliveri* (Engl.) Verdc., AY743489, AY841408, AY841465, AY743451, AY743470, –; *Guatteria anomala* R. E. Fr., AY740913, EF179298, –, AY740962, AY741011, –; *Hubera cerasoides* (Roxb.) Chaowasku, AY518854, JQ723810, KF709055, AY319017, AY319131, JQ723950; *Hubera nitidissima* (Dunal) Chaowasku, KF682110, KF682116, KF709056, KF682103, KF682105, –; *Maasia discolor* (Diels) Mols P. J. A. Kessler & Rogstad, AY518872, AY841416, AY841500, AY319021, AY841584, –; *Marsyopetalum crassum* (R. Parker) B. Xue & R. M. K. Saunders, HQ286571, JQ723792, KF709057, HQ286577, HQ286583, JQ723929; *Meiogyne hainanensis* (Merr.) Bàn, JQ723773, –, –, JQ723860, JQ723913, JQ723936; *Meiogyne mindorensis* (Merr.) Heusden, JQ723776, JQ723800, –, JQ723863, JQ723916, JQ723939; *Meiogyne virgata* (Blume) Miq., AY518798, JQ723805, JX544784, AY318982, AY319094, JQ723945; *Miliusa mollis* Pierre, AY518851, JQ690503, JQ690504, –, AY319102, JQ690505; *Miliusa thorelii* Finet & Gagnep., AY518846, JQ690519, JQ690520, –, AY319104, JQ690521; *Miliusa velutina* (Dunal) Hook. f. & Thomson, AY518847, JQ690536, JQ690537, AY318993, AY319105, JQ690538; *Mitrephora alba* Ridl., AY518855, JQ723807, KF709058, AY318994, AY319106, JQ723947; *Mkilua fragrans* Verdc., DQ125060, EF179303, –, AY841634, AY841712, –; *Monanthotaxis whytei* (Stapf) Verdc., EF179278, EF179304, –, AY841635, AY841713, –; *Mono-carpia euneura* Miq., AY518865, AY841412, AY841477, AY318998, AY319111, –; *Monoon lateriflorum* (Blume) B. Xue & R. M. K. Saunders, JQ723783, JQ723811, KF709060, JQ723870, JQ723923, JQ723951; *Neo-uvaria parallelivenia* (Boerl.) H. Okada & K. Ueda, AY518794, –, –, AY319000, AY319113, –; *Neo-uvaria telopea* Chaowasku, JX544751, JX544778, JX544791, JX544755, JX544783, JX544766; *Orophea cuneiformis* King, KF682112, KF682119, –, –, KF682107, –; *Phaeanthus splendens* Miq., AY518864, JX544777, JX544790, JX544754, AY319126, JX544765; *Piptostigma mortehani* De Wild., AY743492, AY841415, –, AY743454, AY743473, –; *Platymitra macrocarpa* Boerl., AY518812, JQ723809, KF709062, AY319013, AY319127, JQ723949; *Polyalthia johnsonii* (F. Muell.) B. Xue & R. M. K. Saunders, JQ723767, JQ723791, KF709063, JQ723854, JQ723907, JQ723928; *Polyalthiopsis chinensis* (S.K.Wu ex P.T.Li) B.Xue [= *Polyalthia chinensis* S.K.Wu & P.T.Li, *Polyalthia chinensis_74-4451*], China, Xizang Province, *Qinghai-Xizang Exped. 74-4451* (KUN), MT239203*, –, –, –, MT239213*, –; *Polyalthiopsis chinensis* (S.K.Wu ex P.T.Li) B.Xue [= *Polyalthia chinensis* S.K.Wu & P.T.Li, *Polyalthia chinensis_2668*], China, Xizang Province, *B. S. Li & S. Z. Cheng 2668* (PE), MT239201*, –, –, –, MT239211*, –; *Polyalthiopsis verrucipes* (C.Y.Wu ex P.T.Li) B.Xue [= *Polyalthia verrucipes* C.Y.Wu ex P.T.Li, *Polyalthia verrucipes_1603*], China, Yunnan Province, *Y.H. Tan MH1603* (IBSC), MT239202*, MT239205*, MT239207*, MT239209*,

MT239212*, MT239215*; *Polyalthiopsis verrucipes* (C.Y.Wu ex P.T.Li) B.Xue [= *Polyalthia verrucipes* C.Y.Wu ex P.T.Li, *Polyalthia verrucipes_9527*], China, Yunnan Province, *Sino-Russia Exped. 9527* (PE), MT239200*, MT239204*, MT239206*, MT239208*, MT239210*, MT239214*; *Polyalthiopsis floribunda* (Jovet-Ast) Chaowasku, *Chaowasku 168* (CMUB), MG264583, MG264588, MG264570, MG264580, MG264575, –; *Polyalthiopsis floribunda* (Jovet-Ast) Chaowasku, *Chaowasku 128* (CMUB), MG264585, MG264590, MG264572, –, MG264577, –; *Popowia pisocarpa* (Blume) Endl., AY518862, JQ723812, KF709065, AY319044, AY319158, JQ723953; *Pseuduvaria fragrans* Y. C. F. Su, JQ723784, JQ723813, XXXXXXXX, JQ723871, JQ723924, JQ723954; *Sageraea lanceolata* Miq., AY518799, JX544774, JX544787, AY319050, AY319164, JX544762; *Sapranthus viridiflorus* G. E. Schatz, AY743493, AY841422, AY841515, AY319051, AY319165, JQ723955; *Stelechocarpus burahol* (Blume) Hook. f. & Thomson, AY518803, JQ723814, KF709067, AY319053, AY319167, JQ723956; *Stenanona costaricensis* R. E. Fr., AY518801, JX544772, AY841516, AY319069, AY319183, JX544759; *Tridimeris* sp., JX544750, JX544773, JX544786, JX544753, JX544782, JX544761; *Trigynaea lanceipetala* D. M. Johnson & N. A. Murray, AY743487, EF179309, –, AY743449, AY743468, –; *Trivalvaria costata* (Hook. f. & Thomson) I. M. Turner, HQ286574, JQ723815, XXXXXXXX, HQ286580, HQ286586, JQ723957; *Uvaria lucida* Benth. subsp. *virens* (N. E. Br.) Verdc., AY238966, EF179310, –, AY238957, EF179319, –; *Wangia florulenta* (C. Y. Wu ex P. T. Li) Bine Xue, KX495154, KX495158, KX495156, KX495162, KX495160; *Wuodendron praecox* (Hook. f. & Thomson) B.Xue, Y.H.Tan & X.L.Hou, MF687367, MF687369, MF687371, MF687373, MF687375, MF687377.

Athyrium bipinnatum K.Hori (Athyriaceae), a new cornopteroid fern from Japan

Kiyotaka Hori¹

¹ *The Kochi Prefectural Makino Botanical Garden, Kochi, Japan*

Corresponding author: Kiyotaka Hori (khori@makino.or.jp)

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Abstract

I describe *Athyrium bipinnatum* **sp. nov.** and discuss morphological differences between closely related species. The new species is endemic to Japan, occurring on the islands of Honshu, Kyushu, and Shikoku. Based on the criteria of the International union for conservation of nature and natural resources, this new species is here considered endangered.

Keywords

Athyrium, *Cornopteris*, Japan, new species

Introduction

Cornopteris Nakai is a small Asian (Himalayas, East and Southeast Asia) genus of terrestrial ferns that used to be recognized by many pteridologists (e.g., Nakai 1930; Ito 1939; Ching 1945; Holttum 1958; Tagawa 1959; Kato 1977, 1979), on the basis of fleshy stipes, corniculate leaf axes, and exindusiate sori.

Regarding its phylogenetic relationships, Ching (1945) regarded it to be “a little offshoot of the exindusiate *Diplazium*.” Based on the presence of J-shaped sori in some species of *Cornopteris*, as well as on stipe features and spinulose midribs of the laminae, Kato (1977) concluded that *Cornopteris* was actually more closely related to *Athyrium* than to *Diplazium*. Serizawa (1981) agreed with Kato’s conclusion and subsumed *Cornopteris* in *Athyrium*. To corroborate his taxonomic decision of lumping the two genera, Serizawa (1981) also highlighted the existence of natural hybrids between them.

These are: *Athyrium* × *cornopteroides* Sa.Kurata (*Cornopteris opaca* (D.Don) Tagawa × *Athyrium kuratae* Seriz.), *Athyrium* × *glabrescens* Seriz. (*Cornopteris decurrentialata* (Hooker) Nakai × *A. kuratae*), and *Athyrium* × *petiolatum* Sa.Kurata (*C. opaca* × *Athyrium yakusimense* Tagawa). Sano et al. (2000) showed monophyly of the genus *Cornopteris* based on *rbcL* gene phylogeny, but Adjie et al. (2008) showed *A. distentifolium* placed in the same clade of *Cornopteris* in *rbcL* phylogeny. Therefore, *Cornopteris* spp. have been classified in *Athyrium* (Athyraceae) based on DNA phylogenies (Rothfels et al. 2012, PPG I 2016, Ebihara 2017). However, Moran et al. (2019) distinguished the genus *Cornopteris* from *Athyrium* based on plastid DNA phylogeny. In the present study, *Cornopteris* spp. are treated as members of *Athyrium*.

Cornopteroid species of the genus *Athyrium* are mainly distributed in the Himalayas, in East and Southeast Asia. The exact number of species is unknown. Kato (1979) recognized nine species of cornopteroid ferns, whereas Zhaorong and Kato (2013) recognized 16 species only in China.

The following is a brief history of the classification of one *Athyrium* species, namely *Athyrium* × *christensenianum* (Koidz.) Seriz., which is partly the focus of the present study. *Athyrium* × *christensenianum* was first described by Koidzumi (1924) as *Diplazium christensenianum* Koidz., from Jeju (Quelpaert)-Island, South Korea (Fig. 1). Kato (1979) synonymized *Cornopteris hakonensis* Nakai, from Hakone, Japan (Nakai 1930, Fig. 2) under *Cornopteris christensenianum*. Subsequently, Serizawa (1981) transferred it to *Athyrium* and created the nothospecific name *A.* × *christensenianum*. The hybrid origin of this species has been pointed out by many authors (Kurita 1964, Hirabayashi 1970, Kato 1979, Park and Kato 2003). According to these authors (Kurita 1964, Hirabayashi 1970, Kato 1979, Park and Kato 2003), this is a triploid “species” that has resulted from a cross between diploid sexual *Athyrium crenuloserrulatum* Makino and tetraploid sexual *Athyrium decurrentialatum* (Hook.) Copel. It remains unclear whether *A.* × *christensenianum* is merely a sterile hybrid or an independent fertile species. Kato (1979) categorized it as being of a “hybrid origin species” between *A. crenuloserrulatum* Makino and *A. decurrentialatum* because it has intermediate morphological characteristics between these two species such as serrated segments, subcartilaginous margins of the blade, and elliptical sori. On the other hand, Serizawa (1981) treated this “species” as a sterile “hybrid.” The present study agrees with the treatment of *A.* × *christensenianum* by Serizawa (1981).

Recent studies have reported complicated relationships in the *A.* × *christensenianum* complex. First, Hori and Murakami (2019) reported a tetraploid sexual cytotype of *A.* × *christensenianum* (as the independent species *A. christensenianum*). Subsequently, Hori (2019) reported that tetraploid sexual *A.* × *christensenianum* had one allele of *A. crenuloserrulatum* and one of *A. decurrentialatum*, each in the biparental inherited nuclear DNA marker of the gene *AK1*. He also found that triploid *A.* × *christensenianum* had two alleles of *A. crenuloserrulatum* and one allele of *A. decurrentialatum*. Therefore, he suggested two hypotheses: (1) tetraploid sexual *A.* × *christensenianum* originated from the hybridization of diploid sexual *A. crenuloserrulatum* with an ancestral or extinct diploid *A. decurrentialatum*; and (2) triploid *A.* × *christensenianum* originated from the hybridization of diploid sexual *A. crenuloserrulatum* and tetra-

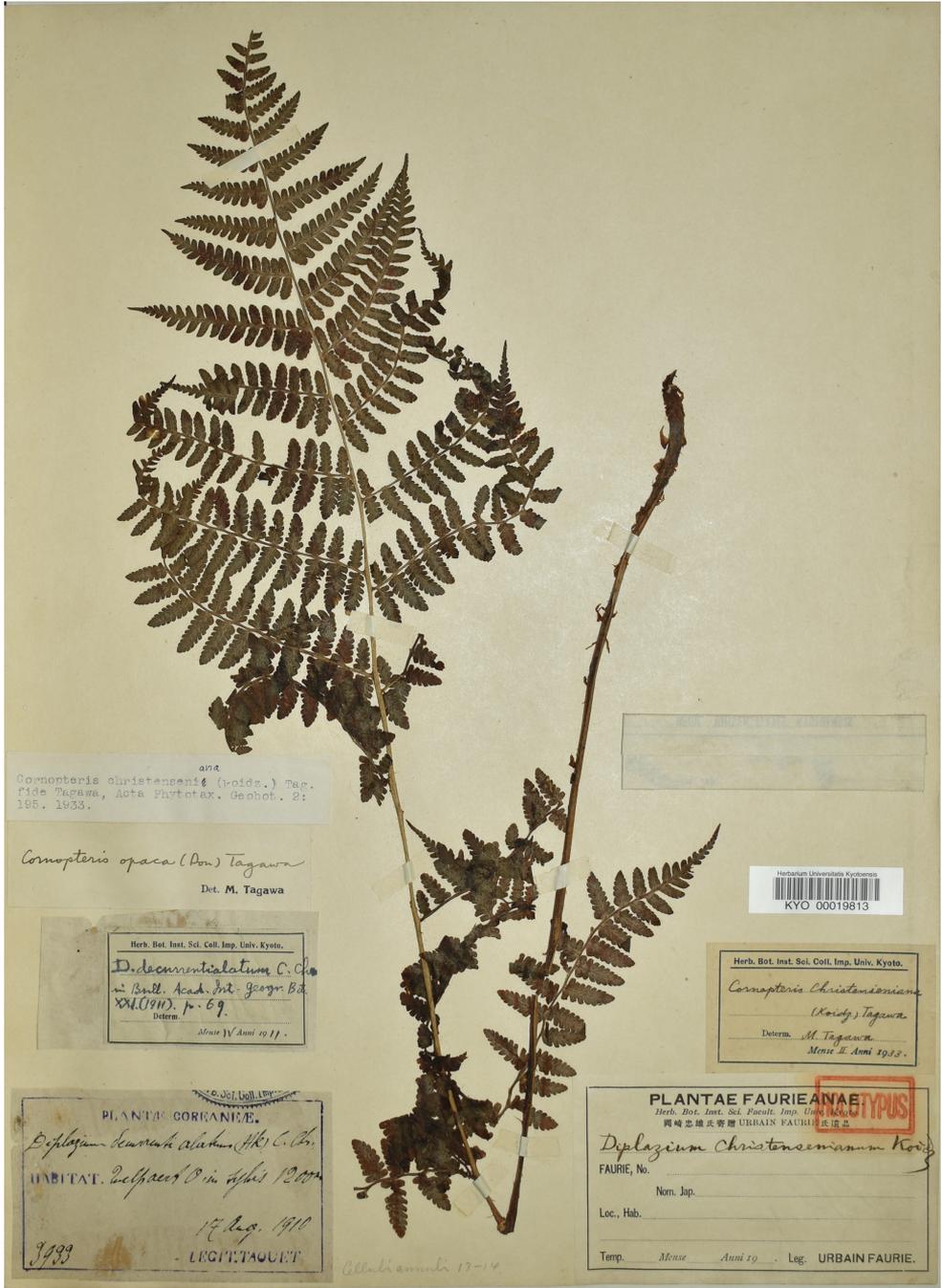


Figure 1. Holotype of *Diplazium* × *christensenianum* Koidz.

ploid sexual *A.* × *christensenianum* (Fig. 3). In the present study, the unclear taxonomy of *A.* × *christensenianum* was clarified by describing tetraploid sexual *A.* × *christensenianum* as a new species: *Athyrium bipinnatum* from Japan (Fig. 3).



Figure 2. Holotype of *Cornopteris hakonensis* Nakai.

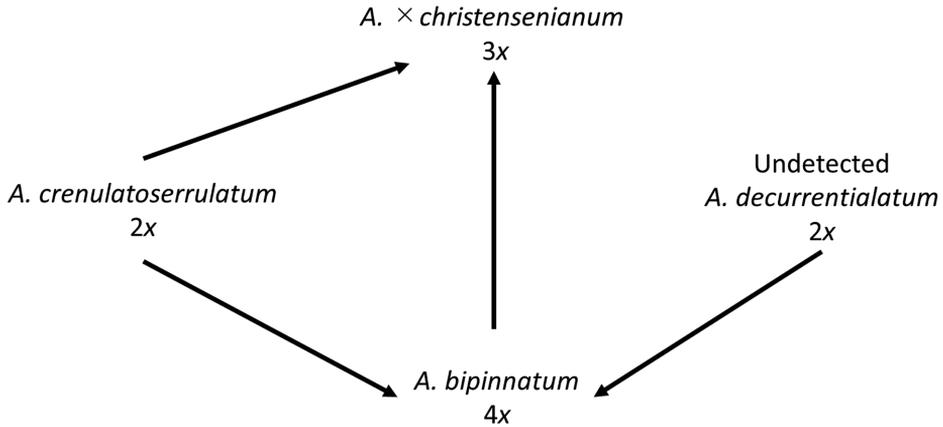


Figure 3. Relationships of *Athyrium bipinnatum* and its related species.

Materials and methods

First, the morphological characteristics of a tetraploid sexual specimen of *Athyrium bipinnatum* (Hori 2974) and the sexual specimens (Hori 2975, 2976) described by Hori (2019) were examined. Two type specimens of *A. christensenianum*, deposited at KYO (as *Diplazium christensenianum*; Fig. 1) and TI (as *Cornopteris hakonensis*; Fig. 2), were also examined. Furthermore, based on morphological characteristics, more specimens of *A. bipinnatum* were identified at MAK and MBK, and from online images at PE herbarium (PE: <http://pe.ibcas.ac.cn/en/>), Taiwan Forestry Research Institute herbarium (TAIF: <http://taif.tfri.gov.tw/search.php>), Collection Database of Specimens and Materials (TNS: <http://db.kahaku.go.jp/webmuseum/>), and from those in JSTOR Global Plants (<https://plants.jstor.org/>) and the Global Biodiversity Information Facility (GBIF: <https://www.gbif.org>). Additional samples were also collected in the field and cultivated samples were taken from Koishikawa Botanical Garden of Tokyo University. For the conservation assessment, the area of occupancy (AOO) and extent of occurrence (EOO) were estimated using GeoCAT (Bachman et al. 2011), with the default settings for grid size applied. The morphology of scales and spores was observed using a KEYENCE VHX-D500.

Taxonomic treatment

Athyrium bipinnatum K.Hori, sp. nov.

urn:lsid:ipni.org:names:77209707-1

Figure 4

Diagnosis. *A. bipinnatum* is similar to *A. x christensenianum* (Koidzumi 1924, Nakai 1930, Kato 1979, Serizawa 1981) as it has serrated pinnae and exindusiate sori. However, *A. bipinnatum* has more short stipes (10–20 cm long), smaller blades (20–40 cm

× 14–20 cm), 2-pinnate pinnae in the middle of the blades, and fronds with dark green adaxial surfaces. In contrast, *A.* × *christensenianum* has longer stipes (25–40 cm long), larger blades (30–60 cm × 25–40 cm), 2-pinnate pinnatifid pinnae in the middle of the blades, and fronds with light green adaxial surfaces.

Type. JAPAN. Shikoku: Kochi prefecture, Ochi town, Mt. Yokogura, 33°32'11"N, 133°12'33"E, alt. 664 m, planted coniferous forest containing *Cryptomeria japonica* (Thunb. ex L.f.) D. Don, on soil, 29 Jun 2019, *K. Hori* 3277 (holotype: MAK466762; isotype: MBK).

Description. *Terrestrial, summer green fern. Rhizomes* creeping, occasionally 2-branched, stramineous, 8–15 × 0.8–1.5 cm, closely set with roots and persistent, densely clothed by old stipe bases, glabrous; *fronds* 1–3 per rhizome; *stipes* stramineous or slightly purple-red, 7–20 × 0.3–0.8 cm, sparsely clothed with stramineous to dark brown scales at the base (3–5 × 1–1.5 mm), lanceolate; *blades* dark green adaxially, 3-pinnatifid at the base, 2-pinnate in the middle to upper section, pinnatifid at the apex, 20–40 × 14–20 cm, deltoid; *rachises* stramineous or slightly purple-red, dark brown, abaxially densely pilose, several projections 0.1 mm long on the adaxial surface at the bases of costae; *pinnae* 7–11 pairs, ascending, lanceolate, opposite from the base to the middle, alternate in the apex section of the blade, petioled (1–3 mm), sessile near the apex, closely spaced or overlapping, lowest pinnae slightly reduced, second lowest pair usually largest, 6–16 × 1.5–3 cm; *pinnules*, alternate on the basal and middle sections of the blade, opposite on the apex of the blade, 10–20 pairs on the basal and middle sections of the blade, 8–10 pairs on the apex of the blade, reduced distally, ovate to lanceolate, shallowly serrate to lobed, margin subcartilaginous, vein-free, close to or reaching to the margin, 3–8 pairs in the middle lobe; *the most basicopic pinnules on the lowest pinnae* clearly short, independent from the costa, 0.6–1 × 0.3–0.5 cm; *sori* tend to appear on the abaxial surface of the middle part of blades, short linear- or oblong-shaped, single, 1.5–5 mm long, on the apex or middle of veinlets, 1–5 per ultimate segment, exindusiate, rather persistent; *spores* regular shaped, 64 regular-shaped spores per sporangium, fertile, ovoid, wrinkled, 42–54 μm × 29–35 μm, brown.

Etymology. *Athyrium bipinnatum* is named as a new species with bipinnate blades, which distinguish it from *A.* × *christensenianum*. This new species could be one of the parents of *A.* × *christensenianum* (Hori 2019).

Specimens examined. Japan. Honshu: Wakayama pref., Ito county, Kouya town, Mt. Kouyasann, Okunoin, alt. 800 m 7 Aug 1954, coll. *M. Tagawa* (NMNH 01529356, image!); *loc. cit.*, ca. 800 m alt., 7 Aug 1954, coll. *S.K.* (MAK139!); *loc. cit.*, on soil under planted coniferous forest containing *C. japonica*, alt. 800 m, 19 Aug 2019, *K. Hori* 3324 (MAK); Mie pref., Taki county, Miyagawa village, Doukuradani, alt. 1000 m, 6 Aug 1962, coll. *Y. Higuchi* (TNS471359, image!); Nara pref., Tenkawa village, Mt. Gyojagaeridake, 15 Jul 1954, coll. *Iwastuki* (MAK 26788!); *loc. cit.*, Dorokawa, Mitarai valley, on soil under planted coniferous forest containing *C. japonica*, alt. 840 m, 20 Aug 2019, *K. Hori* 3326 (MAK). **Shikoku:** Kochi pref., Ochi town, Mt. Yokogurayama, 24 May 1956, *Iwastuki* 1603 (PE, NMNH, MNHN, images! MAK! TI!); *loc. cit.*, on soil under planted coniferous forest containing *C. japonica*, alt. 800 m, 30 May

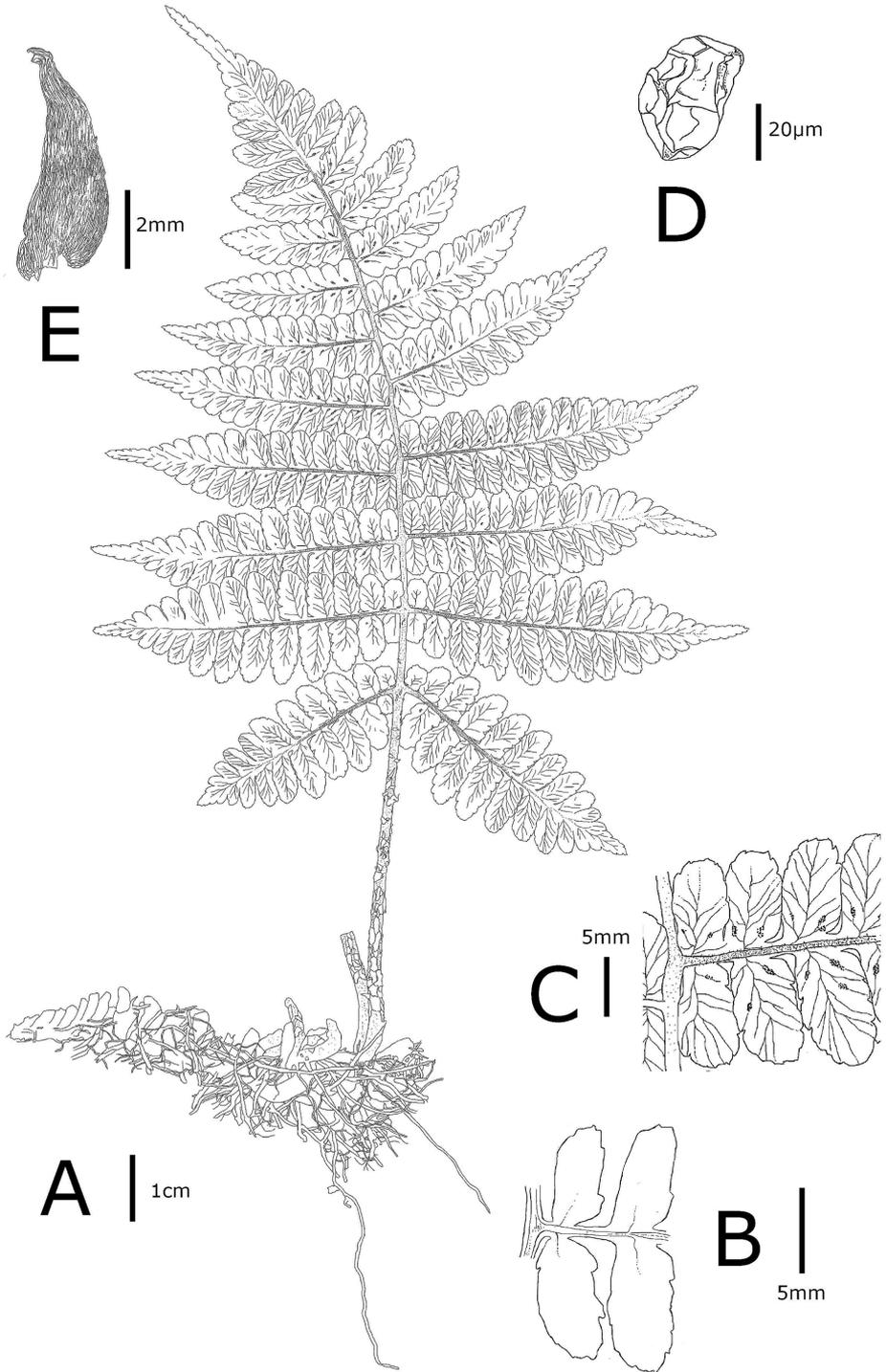


Figure 4. *Athyrium bipinnatum* K.Hori. **A** Habit **B** detail of adaxial pinnule **C** detail of abaxial pinnule **D** lower stipe scale **E** spore. **A–E** from the holotype (MAK466762) (illustration by K. Hori).

2019, *K. Hori* 2974, 2975, 2976 (MAK, MBK); *loc. cit.*, Aki county, Umaji village, Yanase, 19 June 1983, *Y. Koukami* M83-163 (MBK); *loc. cit.*, Muroto city, Kiragawa town, Nishinogawa river, 14 July 1976, *Y. Kazuoki* 6614 (MBK). **Kyushu:** Fukuoka pref., Maebara town, Mt. Haganeyama, cultivated at Koishikawa Botanical Garden of Tokyo University, 17 June 2019, *K. Hori* 3268 (MAK); *loc. cit.*, Kaho county, Chikuho town, Mt. Toishiyama, 17 July 1966, *S. Tsutsui* 2655 (TNS, image!); Oita pref., Takeda city, Kuju town, Mt. Kurodake, alt. 1140, 31 Aug 1997, *S. Tsuji* TJ-4346 (MAK).

Key to *A. bipinnatum* and closely related species in Japan

- 1 Blades 2-pinnate pinnatifid in the middle section, yellowish green or light green adaxially **2**
- Blades 2-pinnate or 1-pinnate pinnatifid in the middle section, dark green adaxially..... **3**
- 2 Blades yellowish green adaxially, pinnule in the middle section of blade deeply serrated on margin, spores regular ***A. crenuloserrulatum***
- Blades light green adaxially, pinnule in the middle section of blade shallowly serrated on margin, spores abortive ***A. × christensenianum***
- 3 Blades 2-pinnate, pinnae straight, sori often single..... ***A. bipinnatum***
- Blades 1-pinnate pinnatifid, pinnae curved to the apex, sori often lobed.....
..... ***A. decurrentialatum***

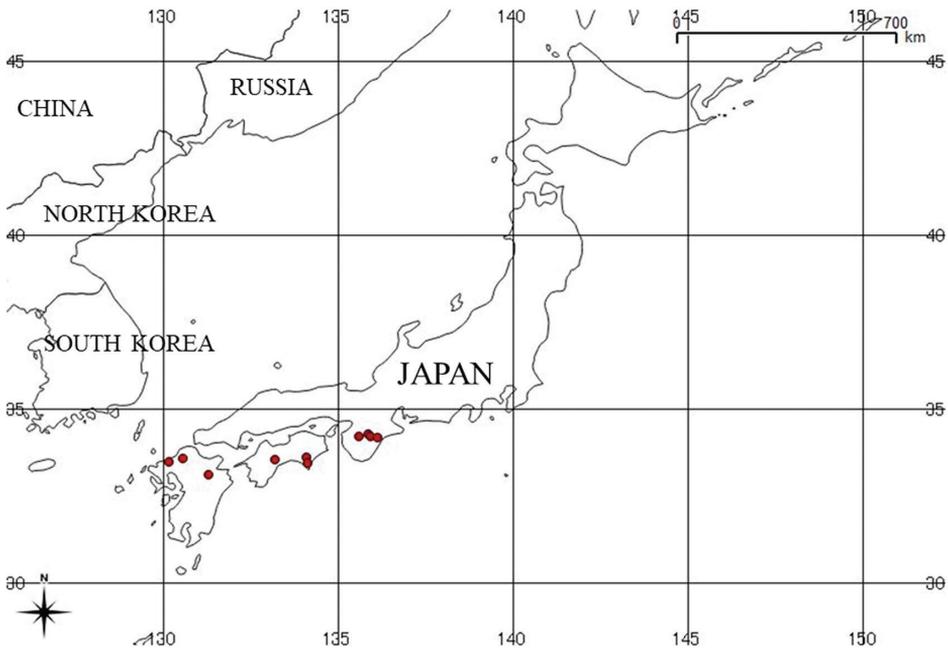


Figure 5. Map showing the known distribution of *Athyrium bipinnatum* in Japan.

Distribution and ecology. *Athyrium bipinnatum* is known from the western part of Honshu, Shikoku, and Kyushu in Japan (Fig. 5). It was observed to grow on soil under planted coniferous forest containing *Cryptomeria japonica* at ca. 600–1100 m alt. This species is endemic to Japan.

Conservation status. *IUCN Red List Category.* Based on estimates from GeoCAT, the EOO of *A. bipinnatum* was 53,068 km²; however, this estimate should be smaller because this EOO contains an area of sea around Japan. Wild deer are known to eat *A. bipinnatum* (pers. obs.), so individual numbers within populations are currently decreasing and population reduction is occurring now. The known AOO of *A. bipinnatum* is 44 km². Based on the IUCN criteria (IUCN 2001, 2012), *A. bipinnatum* falls into the Endangered (EN) category. A formal evaluation of endangerment can be summarized by the following IUCN hierarchical alphanumeric coding system of criteria and subcriteria: EN A1abc+A2+C1+C2a(i).

Discussion

Athyrium × *christensenianum* was first described by Koidzumi (1924) as *Diplazium christensenianum* Koidz., from Jeju (Quelpaert)-Island, South Korea (Fig. 1). Subsequently, Nakai (1930) described *Cornopteris hakonensis* Nakai, from Hakone, Japan (Fig. 2). I examined type material of both taxa, and found that they represent the same hybrid in having large size of stipes, blades, 2-pinnate pinnatifid pinnae in the middle of the blades. *Athyrium bipinnatum* is distinguished from them in having shorter stipes, smaller blades, and by its 2-pinnate pinnae in the middle of the blades.

Athyrium bipinnatum is of hybrid origin between a diploid sexual *A. crenuloserrulatum* and an extinct or undetected diploid sexual *A. decurrentialatum*, and it is one of the parents of *A. × christensenianum* (Hori 2019, Fig. 3). *Athyrium bipinnatum* is clearly smaller than *A. × christensenianum*, and its morphological characteristics, which include 2-pinnate blades, are intermediate between those of *A. crenuloserrulatum* and *A. decurrentialatum* (Figs 6, 7, Table 1). In contrast, the morphological characteristics of *A. × christensenianum*, which include 2-pinnate pinnatifid blades, are intermediate between *A. bipinnatum* and *A. crenuloserrulatum*; the large size of this plant indicates this hybrid has heterosis. *Athyrium bipinnatum* and *A. crenuloserrulatum* each have shorter stipes (10–20 cm and 20–30 cm long, respectively) and smaller blades (20–40 cm × 14–20 cm and 30–35 cm × 25–30 cm, respectively) than *A. × christensenianum* (25–40 cm long stipes and 30–60 cm × 25–40 cm blades) (Table 1).

Contrary to the findings of the present study, Park and Kato (2003) reported regular-shaped and germinative spores in their description of *A. × christensenianum*. Here, regular-shaped spores could not be found from dozens of *A. × christensenianum* herbarium specimens; however, regular-shaped spores were found from several specimens of *A. bipinnatum*, *A. crenuloserrulatum*, and *A. decurrentialatum*. Therefore, it is possible to speculate that Park and Kato (2003) perhaps confused the materials of these different species when they examined spore shape.

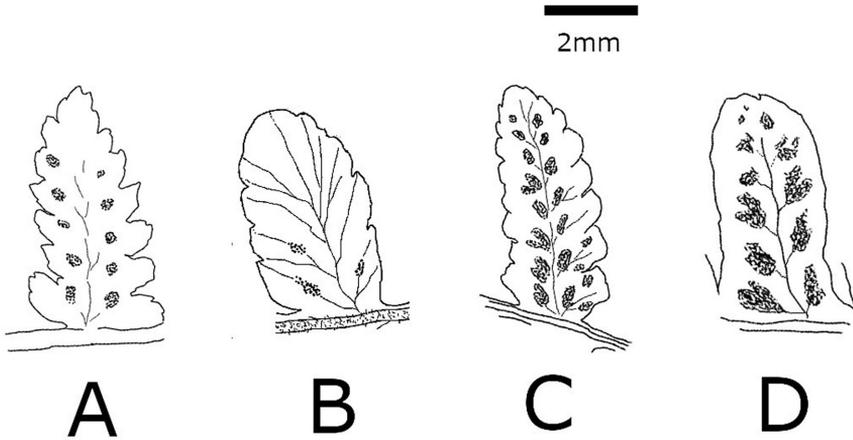


Figure 6. Abaxial surface of pinnule and sori of **A** *Athyrium crenuloserrulatum* **B** *Athyrium bipinnatum*, **C** *Athyrium* × *christensenianum* **D** *Athyrium decurrentialatum* (illustration by K. Hori).



Figure 7. Wild plants of **A** *Athyrium crenuloserrulatum* **B** *Athyrium bipinnatum* **C** *Athyrium* × *christensenianum* **D** *Athyrium decurrentialatum*.

Table 1. Morphological comparison among *A. bipinnatum* and related species.

Characters	<i>A. bipinnatum</i>	<i>A. × christensenianum</i>	<i>A. crenatoserrulatum</i>	<i>A. decurrentialatum</i>
Stipe length (cm)	7–20	25–40	20–30	20–30
Pinna size (cm)	6–16 × 1.5–3	15–30 × 5–9	10–20 × 5–8	8–15 × 3–5
Pinna stalk length (cm)	0.2–0.4	0.2–0.4	0.4–0.9	0–0.2
Blade size (cm)	15–40 × 14–20	30–60 × 25–40	30–35 × 25–30	20–35 × 15–25
Blade color adaxially	dark green	light green	yellowish green	dark green
Blade division medially	2-pinnate	2-pinnate pinnatifid	2-pinnate pinnatifid	1-pinnate pinnatifid
Pinnule division medially	shallowly serrated	deeply to shallowly serrated	deeply serrated	shallowly serrated or entire
Sori	single	sometimes lobed	single	often lobed
Spore shape	regular	irregular	regular	regular
Spore size (µm)	42–54 × 29–35	30–44 × 22–39	25–37 × 16–20	27–45 × 21–35

In summary, the previously unclear taxonomy of *A. × christensenianum* was clarified in the present study by describing tetraploid sexual “*A. × christensenianum*” as the new species *A. bipinnatum*. Importantly, the conservation status analysis reported here suggests that *A. bipinnatum* may be endangered; thus, this study has relevance to the conservation of cornopteroid ferns.

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Gnetum chinense, a new species of Gnetaceae from southwestern China

Wei-Yin Jin^{1,5}, Bing Liu¹, Shou-Zhou Zhang², Tao Wan², Chen Hou^{3,4}, Yong Yang¹

1 State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, China **2** Shenzhen Fairy Lake Botanical Garden, Shenzhen, China **3** Guangdong Provincial Key Laboratory of Silviculture, Protection and Utilization, Guangdong Academy of Forestry, Guangzhou, 510520, China **4** Guangdong Academy of Forestry, Guangzhou, 510520, China **5** Tonghua Normal University, 950 Yucui Road, Dongchang District, Tonghua City, Jilin Province 134000, China

Corresponding author: Yong Yang (ephedra@ibcas.ac.cn)

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Abstract

Gnetum chinense **sp. nov.**, a new lianoid species of Gnetaceae, is described from southwestern China. The new species is morphologically similar to *G. montanum* Markgr. in its oblong elliptic leaves and the ovoid to ellipsoid chlamydosperm, but differs from the latter by its shorter male spikes having fewer involucrell collars (7–10 vs. 13–18 in *G. montanum*). We also did a new molecular analysis using one nuclear marker (i.e. nrITS) and four chloroplast markers (i.e. *matK* gene, *rpoC1* intron, *psbB-rps12* IGS, and *trnF-trnV* IGS). The result suggests that this specific clade is sister to a large clade consisting of all other known Chinese lianoid species of *Gnetum* except *G. parvifolium* (Warb.) W.C. Cheng.

Keywords

Gnetum, China, morphology, phylogeny, taxonomy

Introduction

Gnetum L., belongs to the monotypic family Gnetaceae of gymnosperms, and contains ca. 40 extant species that are widely distributed in tropical and subtropical forests in Asia, Africa and South America (Yang et al. 2017a). This genus is evergreen, mostly

lianas, rarely trees, and possesses a set of unusual characters for gymnosperms, e.g. dicots-like broad leaves with pinnate venation, female gametophytes lacking archegonia, male and female reproductive units assembled into whorls, male spikes usually having abortive chlamydosperms and appearing to be bisexual, chlamydosperms possessing two outer envelopes, etc. (Pearson 1929; Maheshwari and Vasil 1961; Martens 1971; Gifford and Foster 1989; Friedman and Carmichael 1996). Although the earliest macrofossil of the gnetoid clade is known from the mid-Jurassic in northeastern China, the modern *Gnetum* is believed to diversify in South America and split into the New World clade and the Old World clade around the K-Pg boundary (Hou et al. 2015; Yang et al. 2017b). The Chinese clade diverged from other southeastern Asian species around 38 mya (million years ago, 95% posterior density 27–49 mya), and became more diversified after the earliest Miocene (ca. 21 mya, Hou et al. 2016).

Gnetum has a wide range of distribution in southern China (Cheng 1978; Fu et al. 1999). Cheng (1978) recognized seven species in China, while Fu et al. (1999) accepted nine species in the *Flora of China*. Both studies were based on herbarium material only. These traditional taxonomic treatments laid much emphasis on reproductive characters, but variation patterns of important reproductive characters are ambiguous because i) fruiting material is poorly represented in herbaria, and ii) it is difficult to match male and female specimens to a certain species when studying a dioecious taxon like *Gnetum*.

Molecular phylogeny was successfully applied to the delimitation of species of *Gnetum* in combination with morphological characters (Hou et al. 2016; Kim and Won 2016). Kim and Won (2016) applied a barcode method and recognized three species in Cambodia, i.e. *G. macrostachyum* Hook. f., *G. montanum* Markgr., and *G. aff. gracilipes* C.Y. Cheng. Hou et al. (2016) conducted a taxonomic revision based on molecular and morphological data, and recognized the following six lianoid species in China: *G. catasphaericum* H. Shao, *G. formosum* Markgr., *G. luofuense* C.Y. Cheng, *G. montanum* Markgr., *G. parvifolium* (Warb.) W.C. Cheng, and *G. pendulum* C.Y. Cheng.

A new *Gnetum* species was identified when we worked on a *Gnetum* genome project a few years ago. Further morphological and molecular studies on newly collected materials during field investigations in southern China allowed us to describe this species here as new to science.

Materials and methods

Plant materials, comprising silica-dried leaves and vouchers, were sampled in Yunnan and Guizhou of southern China. All vouchers were deposited in the Herbarium (PE), State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences (Table 1).

Total genomic DNA was extracted from the dried leaf materials using the CTAB method (Doyle and Doyle 1987) and purified using a QIAquick PCR Purification Kit. For phylogenetic studies, it is thought that nrITS and four chloroplast markers including *matK* gene, *rpoC1* intron, *psbB-rps12* IGS, and *trnF-trnV* IGS are highly variable

Table 1. Sequences of *Gnetum chinense* sp. nov. generated in this study and their vouchers. All vouchers have been deposited in PE.

Collection	Locality	ITS	<i>matK</i>	<i>rpoC1</i>	<i>psbB-rps12</i>	<i>trnF-trnV</i>
T. Wan MLP001	China, Yunnan: Malipo.	MT362085	MT373322	MT373311	MT373300	MT373333
T. Wan MLP002	China, Yunnan: Malipo		MT373323	MT373312	MT373301	MT373334
T. Wan MLP003	China, Yunnan: Malipo		MT373324	MT373313	MT373302	MT373335
T. Wan MLP005	China, Yunnan: Malipo		MT373325	MT373314	MT373303	MT373336
B. Liu & al. 1360	China, Yunnan: Malipo	MT362086	MT373326	MT373315	MT373304	MT373337
B. Liu & al. 1441	China, Yunnan: Malipo		MT373327	MT373316	MT373305	MT373338
B. Liu & al. 1725	China, Yunnan: Malipo		MT373328	MT373317	MT373306	MT373339
B. Liu & al. 2627	China, Yunnan: Cangyuan	MT362087	MT373329	MT373318	MT373307	MT373340
B. Liu & al. 2675	China, Yunnan: Lancang	MT362088	MT373330	MT373319	MT373308	MT373341
B. Liu & al. 3045	China, Yunnan: Jiangcheng	MT362089	MT373331	MT373320	MT373309	MT373342
C.Y. Deng 12466	China, Guizhou: Xingyi	MT362084	MT373321	MT373310	MT373299	MT373332

and suitable for delimiting species of *Gnetum* (Kim and Won 2016; Hou et al. 2016). We followed the methods described in Hou et al. (2016), and one nuclear marker (i.e. nrITS) and four chloroplast markers (i.e. *matK* gene, *rpoC1* intron, *psbB-rps12* IGS, and *trnF-trnV* IGS) were targeted. *Gnetum* sequences generated in Hou et al. (2016) were downloaded from the GeneBank (Table 2). Sanger sequencing was conducted at Majorbio, Beijing, China. The output files were assembled and edited using Sequencer ver. 4.5 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.) and DNA sequences were aligned using Clustal X ver. 2.1 (Larkin et al. 2007) and manually adjusted using BioEdit ver. 7.2.5 (Hall 1999). Sequences of the five markers were concatenated using SequenceMatrix Windows ver. 1.7.8 (Vaidya et al. 2011).

Previous studies suggested that the African species are sister to all Asian species (Won and Renner 2006; Hou et al. 2015), as a result, we chose the African *G. africanum* Welw. as the outgroup. Maximum likelihood (ML) analyses were conducted using the RAxML-HPC2 on XSEDE (8.0.0) executed in the CIPRES portal (<http://www.phylo.org/>, Stamatakis 2014). The ML bootstrap values (BS) for each node were summarized after 1,000 replicates of bootstrapping iterations. The obtained trees were viewed and edited using FigTree ver. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bayesian inference (BI) analyses were performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) on XSEDE (8.0.0) in CIPRES. The Markov Chain Monte Carlo (MCMC) algorithm was run for 3,000,000 generations with the sampling frequency 1,000. Bayesian posterior probabilities (PP) were calculated for the majority consensus tree of all sampled trees after discarding trees sampled within the burn-in (25%) phase in MrBayes v.3.2.1.

The distribution map was generated using ArcGIS 9.3 (ESRI, Redlands, CA, USA; <http://www.esri.com>). The photos were taken using digital cameras (Nikon D700 and Olympus TG-3), manually edited and created using Adobe Photoshop CS2 ver. 9.0. Phylogenetic trees were viewed and adjusted using FigTree ver. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Table 2. *Gnetum* sequences obtained from the GenBank for this study.

Species	ITS	matK	rpoC1	psbB-rps12	trnF-trnV
<i>G. catasphaericum_24</i>	KX234206	KX234250	KX234304	KX234352	KX234382
<i>G. catasphaericum_31</i>	KX234205	KX234249	KX234303	KX234351	KX234381
<i>G. catasphaericum_32</i>	KX234203	KX234247	KX234301	KX234349	KX234379
<i>G. catasphaericum_44</i>	KX234202	KX234246	KX234300	KX234348	–
<i>G. catasphaericum_33</i>	KX234204	KX234248	KX234302	KX234350	KX234380
<i>G. cuspidatum_20</i>	KX234174	KX234222	KX234274	KX234325	–
<i>G. cuspidatum_C52</i>	KP256660	KP256698	KX234273	KX234324	–
<i>G. diminutum_C101</i>	KP256664	KP256702	KX234275	–	–
<i>G. edule_C4</i>	KP256658	KP256696	KX234268	KX234322	–
<i>G. formosum_14</i>	KX234200	KX234230	KX234298	KX234346	KX234377
<i>G. formosum_21</i>	KX234201	KX234231	KX234299	KX234347	KX234378
<i>G. giganteum_37</i>	KX234209	KX234252	KX234307	KX234354	KX234384
<i>G. giganteum_41</i>	KX234213	KX234256	KX234311	KX234358	KX234388
<i>G. giganteum_8</i>	KX234211	KX234254	KX234309	KX234356	KX234386
<i>G. gnemon</i> var. <i>brunonianum_1</i>	KX234173	KX234221	KX234265	KX234319	–
<i>G. gnemon</i> var. <i>brunonianum_102</i>	–	KX385188	KX385188	KX385188	KX385188
<i>G. gnemon</i> var. <i>gnemon_2</i>	KX234170	KX234219	KX234263	KX234317	–
<i>G. gnemon</i> var. <i>gnemon_101</i>	KX234171	KX385189	KX385189	KX385189	KX385189
<i>G. gnemon</i> var. <i>griffithii_23</i>	KX234172	KX234220	KX234264	KX234318	–
<i>G. gnemonoides_C51</i>	KP256656	KP256694	KX234266	KX234320	–
<i>G. gracilipes_39</i>	KX234210	KX234253	KX234308	KX234355	KX234385
<i>G. gracilipes_42</i>	KX234214	KX234257	KX234312	KX234359	–
<i>G. hainanense_15</i>	KX234184	KX234232	KX234283	KX234333	KX234364
<i>G. hainanense_10</i>	KX234195	KX234241	KX234293	KX234341	KX234373
<i>G. hainanense_11</i>	KX234193	KX234238	KX234290	KX234338	KX234370
<i>G. hainanense_12</i>	KX234194	KX234239	KX234291	KX234339	KX234371
<i>G. hainanense_13</i>	KX234198	KX234244	KX234296	KX234344	KX234375
<i>G. hainanense_16</i>	KX234199	KX234245	KX234297	KX234345	KX234376
<i>G. hainanense_18</i>	KX234196	KX234242	KX234294	KX234342	–
<i>G. hainanense_25</i>	–	KX234240	KX234292	KX234340	KX234372
<i>G. hainanense_5</i>	KX234197	KX234243	KX234295	KX234343	KX234374
<i>G. hainanense_9</i>	KX234192	KX234237	KX234289	KX234337	KX234369
<i>G. hainanense_107</i>	KX234187	KX385193	KX385193	KX385193	KX385193
<i>G. hainanense_110</i>	KX234186	KX385194	KX385194	KX385194	KX385194
<i>G. latifolium_C15</i>	KP256661	–	KX234269	–	–
<i>G. leptostachyum_C102</i>	KP256665	KP256703	KX234271	–	–
<i>G. luofuense_19</i>	KX234190	KX234236	KX234287	KX234336	KX234367
<i>G. luofuense_27</i>	KX234189	KX234235	KX234286	–	–
<i>G. luofuense_28</i>	KX234185	KX234233	KX234284	KX234334	KX234365
<i>G. luofuense_6</i>	KX234188	KX234234	KX234285	KX234335	KX234366
<i>G. luofuense_C61</i>	KX234191	KP256710	KX234288	–	KX234368
<i>G. montanum_105</i>	–	KX385195	KX385195	KX385195	KX385195
<i>G. montanum_106</i>	–	KX385196	KX385196	KX385196	KX385196
<i>G. montanum_17</i>	KX234208	KX234251	KX234306	KX234353	KX234383
<i>G. montanum_29</i>	KX234207	–	KX234305	–	–
<i>G. neglectum_C19</i>	KP256667	KP256705	KX234270	–	–
<i>G. parvifolium_35</i>	KX234178	KX234224	KX234277	KX234327	–
<i>G. parvifolium_108</i>	KX234176	KX385190	KX385190	KX385190	KX385190
<i>G. parvifolium_109</i>	KX234177	KX385192	KX385192	KX385192	KX385192
<i>G. parvifolium_22</i>	KX234181	KX234227	KX234280	KX234330	–
<i>G. parvifolium_26</i>	KX234180	KX234226	KX234279	KX234329	–

Species	ITS	<i>matK</i>	<i>rpoC1</i>	<i>psbB-rps12</i>	<i>trnF-trnV</i>
<i>G. parvifolium_34</i>	KX234179	KX234225	KX234278	KX234328	–
<i>G. parvifolium_36</i>	KX234175	KX234223	KX234276	KX234326	–
<i>G. parvifolium_40</i>	KX234182	KX234228	KX234281	KX234331	–
<i>G. parvifolium_43</i>	KX234183	KX234229	KX234282	KX234332	–
<i>G. parvifolium_C50</i>	KP256675	KX385191	KX385191	KX385191	KX385191
<i>G. pendulum_103</i>	–	KX385197	KX385197	KX385197	KX385197
<i>G. pendulum_104</i>	–	KX385198	KX385198	KX385198	KX385198
<i>G. pendulum_38</i>	KX234212	KX234255	KX234310	KX234357	KX234387
<i>G. pendulum_4</i>	KX234217	KX234260	KX234315	KX234362	KX234391
<i>G. pendulum_45</i>	KX234216	KX234259	KX234314	KX234361	KX234390
<i>G. pendulum_46</i>	KX234215	KX234258	KX234313	KX234360	KX234389
<i>G. pendulum_47</i>	KX234218	KX234261	KX234316	KX234363	–
<i>G. raya_C11</i>	KP256657	–	KX234267	KX234321	–
<i>G. tenuifolium_C18</i>	KP256662	KP256700	KX234272	KX234323	–
<i>G. africanum_C41</i>	KP256642	KP256681	KX234262	–	–

Results

Phylogeny

The ML tree (Fig. 1), in general, was better resolved than the BI tree (Fig. 2). All Chinese lianoid taxa of *Gnetum* included in this study formed a strongly supported monophyletic group (BS: 100%; PP: 1.00). Ten samples of *G. parvifolium* constituted a sister group (BS: 100%; PP: 1.00) to a clade consisting of the rest of the lianoid congeners from China included in this study. The analyses revealed the 11 newly collected specimens as a monophyletic group (BS: 100%; PP: 1.00) sister to a clade composed of *G. formosum*, *G. catasphaericum*, *G. luofuense*, *G. montanum* and *G. pendulum*. Delineations between *G. montanum* and *G. pendulum* were not resolved. The two samples of *G. formosum* formed a weakly supported group (BS: 81%; PP < 0.70), which was followed by a split between the strongly supported *G. catasphaericum* (BS: 98%; PP: 0.99) and a large clade containing a subclade of *Gnetum montanum*, *G. pendulum*, *G. giganteum* H. Shao, and *G. gracilipes* (BS: 98%; PP: 1.00), and another one of *G. hainanense* C.Y. Cheng ex L.K. Fu et al. and *G. luofuense* (BS: 98%; PP: 0.81). Delineations between the two species were not resolved.

Taxonomy

Gnetum chinense Y. Yang, Bing Liu & S.Z. Zhang, sp. nov.

urn:lsid:ipni.org:names:77209708-1

Figs 3, 4

Type. CHINA. Yunnan: Cang-yuan County, on the way from Ban-hong to Ban-lao Prefecture, forest margin, male cones, March 31st, 2015, B. Liu, Y. Yang & T.W. Xiao 2627 (PE, holotype).

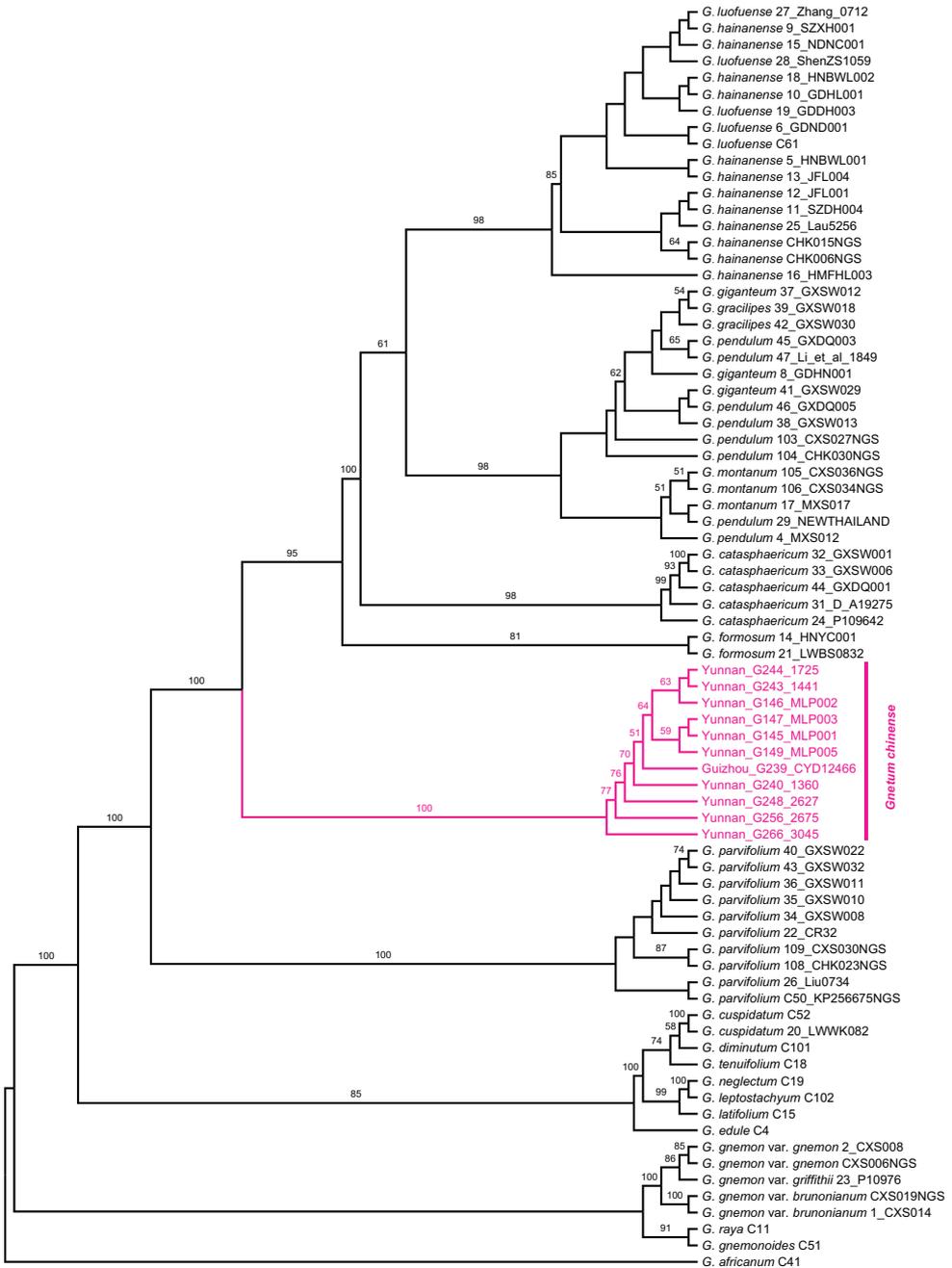


Figure 1. Maximum likelihood tree based on nuclear ribosomal ITS and chloroplast *matK*, *rpoC1*, *psbB-rps12*, and *trnF-trnV*, showing the robust species clade of *Gnetum chinense* sp. nov. Bootstrap values are displayed when they are greater than 50%.

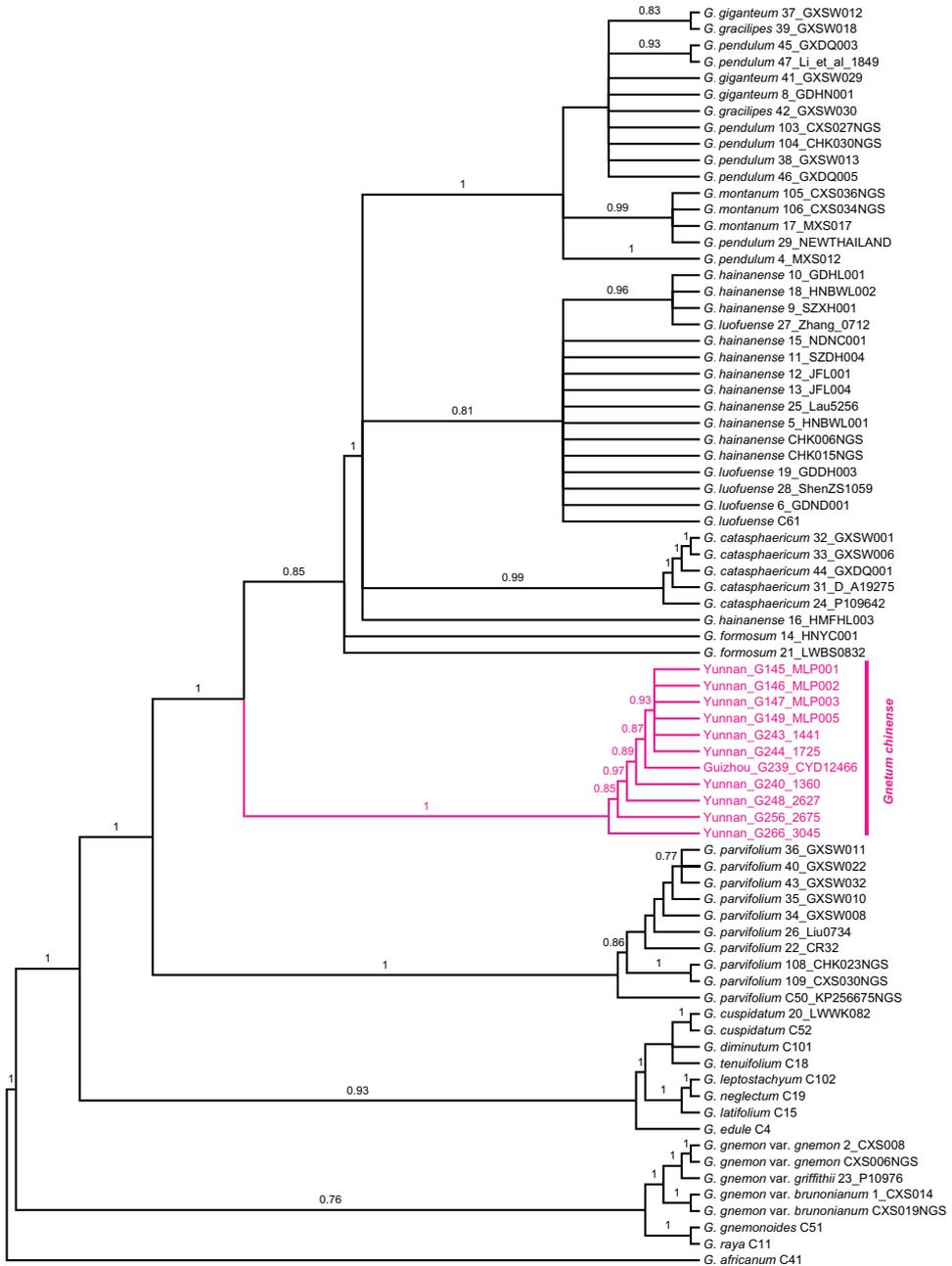


Figure 2. Bayesian inference tree based on nuclear ribosomal ITS and chloroplast *matK*, *rpoC1*, *psbB-rps12*, and *trnF-trnV*, showing the robust species clade of *Gnetum chinense* sp. nov. Posterior probabilities are shown when they are greater than 0.70.

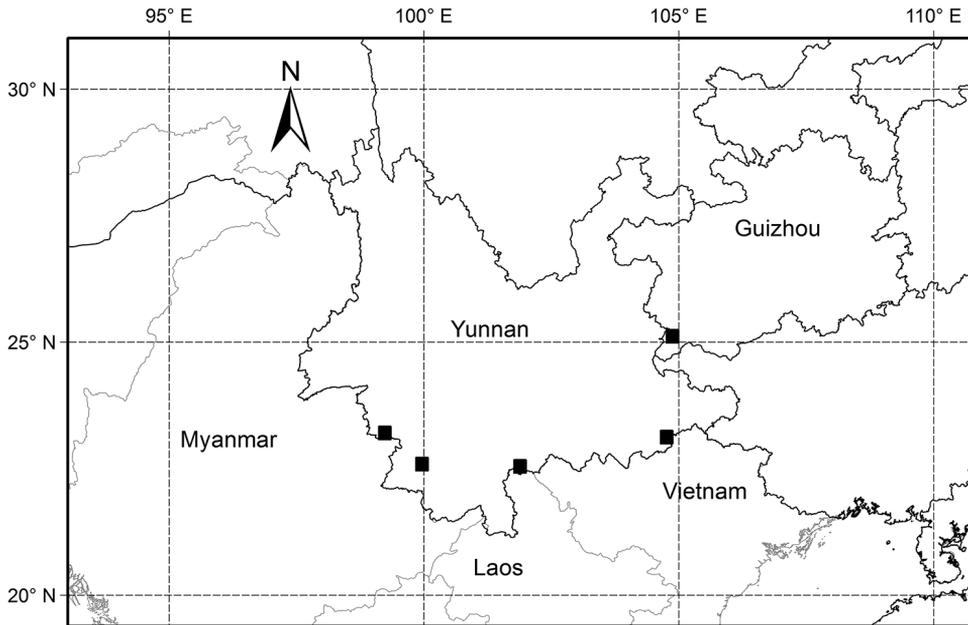


Figure 3. Map displaying the distribution of *Gnetum chinense* sp. nov. (black squares).

Diagnosis. This species is similar to *G. montanum* in its oblong elliptic leaves and sessile chlamydosperm, but differs from the latter by its shorter male cones (1–1.5 cm long in the new species vs. 2–3 cm in *G. montanum*) having fewer involucre collars (7–10 in the new species vs. 13–18 in *G. montanum*), nearly sessile or extremely shortly stiped chlamydosperms (vs. markedly stiped, stipes 3–5 mm long in *G. montanum*).

Description. Lianas; twigs terete, dichasially branched having swollen nodes. Leaves opposite (Fig. 4a), oblong to elliptic, 11–16 cm long, 4–8 cm wide, base rounded to acute, apex acute to acuminate, pinnately veined, midvein impressed adaxially and elevated abaxially, lateral veins 6–8 (Fig. 4a), more or less elevated on both sides, petioles 1–1.2 cm long, grooved adaxially. Male reproductive shoots terminal, dichasial, branched once or twice (Fig. 4a). Male cones pedunculate, peduncles 2–10 mm long; cylindrical, ca. 10–15 mm long, 4 mm in diam., involucre collars 8–10 (Fig. 4b). Chlamydosperms ellipsoid to subglobose, ca. 2.2 cm long, 1.4 cm in diam., apex obtuse, base contracted into an extremely short stalk or sessile, green when young, and orange when mature (Fig. 4c).

Distribution. In Yunnan and Guizhou provinces of China (Fig. 3).

Habitats. In evergreen tropical and subtropical forests.

Etymology. The specific epithet '*chinense*' is derived from China.

Phenology. Blooming male cones and mature chlamydosperms were found in late May and early November, respectively.

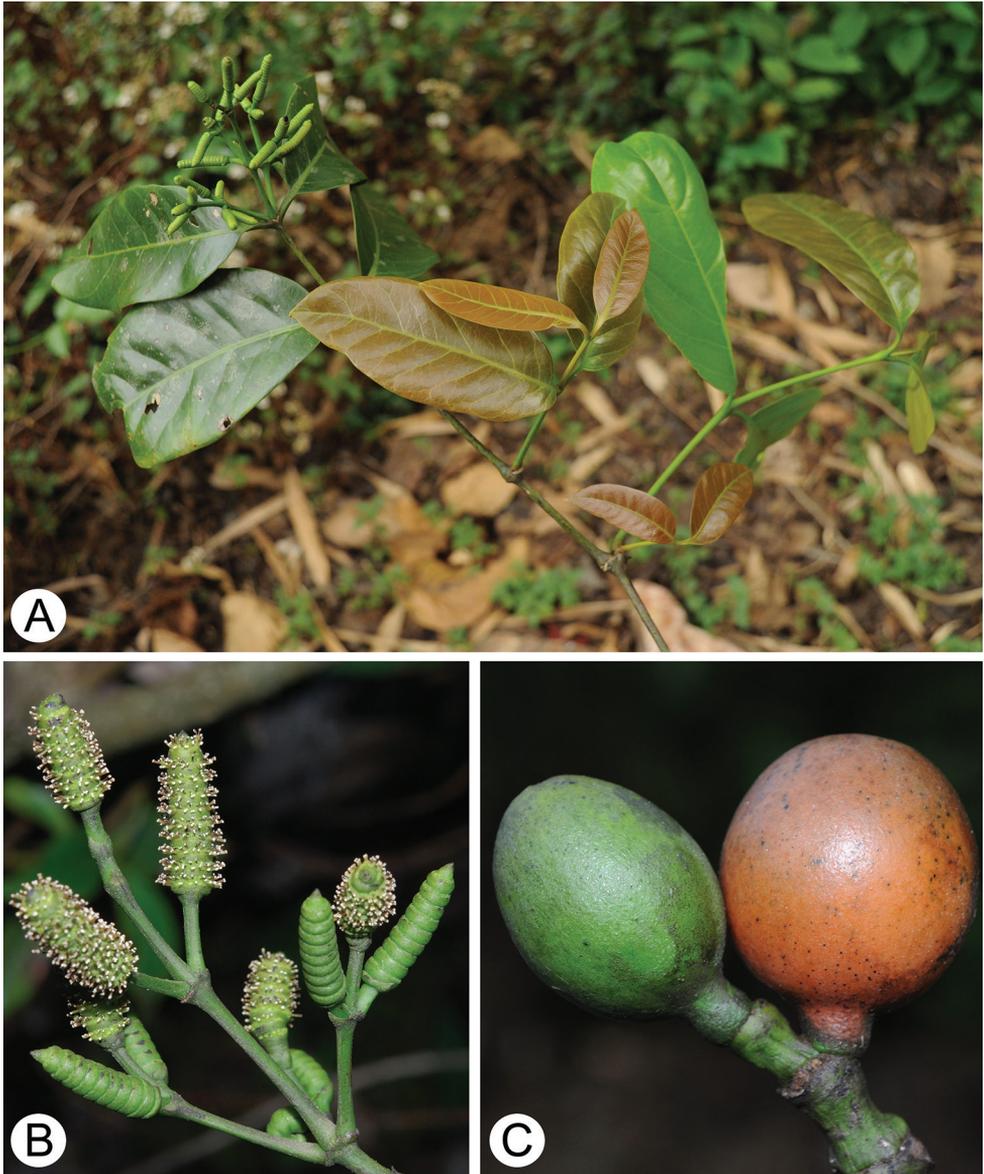


Figure 4. *Gnetum chinense* sp. nov. **A** branch bearing male cones **B** male cones **C** female cone portion displaying chlamydo sperm morphology.

Conservation. *Gnetum chinense* is common in evergreen forests in Yunnan and Guizhou. We consider this species to be of Least Concern (LC) under the IUCN Red List Categories and Criteria ver. 3.1 second edition (IUCN 2012).

Specimens examined. **China.** Yunnan: Lan-cang Lahuzu Autonomous County, from Shang-yun to Xi-meng, Apr. 2, 2015, *B. Liu, Y. Yang & T.W. Xiao* 2675

Table 3. A morphological comparison between *Gnetum chinense* and other Chinese lianoid species.

Species	Leaf blade shape	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (mm)	Male spike length (cm)	Male spike involucral collars	Chlamydosperm shape	Chlamydosperm length (cm)	Chlamydosperm stipe length (mm)
<i>Gnetum catasphaericum</i> H. Shao	Ovate to oblong ovate	7–12	4–6.5	6–10	ca. 2	10–16	Oblong, subglobose	1.8–2.2	2–6
<i>G. chinense</i> sp. nov.	Oblong to elliptic	11–16	4–8	10–12	1–1.5	8–10	Ellipsoid to subglobose	ca. 2.2	Subsessile
<i>G. formosum</i> Markgr.	Elliptic to narrowly oblong	11–14	4–7	9–10	?	?	Narrowly oblong, fusiform	2–2.5	Sessile
<i>G. luofuense</i> C.Y. Cheng	Elliptic to oblong ovate	4.5–16	3–8.5	8–13	2–3	12–15	Broadly ellipsoid to cylindrical	1.8–2.5	2–5
<i>G. montanum</i> Markgr.	Elliptic to oblong	10–28	4.5–13	9–26	2–3.5	16–25	Cylindrical ovoid, cylindrical	1.6–2	3–5
<i>G. parvifolium</i> (Warb.) W.C. Cheng	Elliptic to narrowly oblong	4–11	2–4	5–7	0.8–1.5	9–11	ellipsoid	1.3–1.8	Sessile
<i>G. pendulum</i> C.Y. Cheng	Narrowly elliptic to oblong ovate	10–18	4–8.5	8–15	1–1.5	12–15	Elongate ellipsoid	3–4	10–30

Data were collected from Hou et al. (2016) and this study.

(PE); Jiang-cheng County, Qu-shui Prefecture, Apr. 18, 2015, *B. Liu* 3045 (PE); Ma-li-po County, March 15–17, 2015, *T. Wan* MLP001, MLP002, MLP003, MLP005 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, May 27, 2011, *B. Liu* 1360 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, Sept. 24, 2011, *B. Liu* 1441 (PE); Ma-li-po County, Xia-jin-chang Prefecture, Li-jia-wan, Nov. 2, 2012, *B. Liu* 1725 (PE). Guizhou: unknown collection date, *C.Y. Deng* CYD12466 (PE).

Discussion

Phylogenies based on molecular data have clearly resolved major lineages of *Gnetum*, including a South American clade, an African clade, and several Asian clades (Won and Renner 2003, 2005a, 2005b, 2006; Kim and Won 2016; Hou et al. 2015, 2016). Taxonomy of the Asian *Gnetum* is rather complicated because plants of the genus are usually dioecious woody climbers, and there are few taxonomic characters, so it is difficult to identify species without diagnostic reproductive characters (Kim and Won 2016).

Phylogenetic methods were successfully applied to discover and delimit species of Asian *Gnetum* (Kim and Won 2016; Hou et al. 2016). Our phylogenetic study found a new specific clade that was not recognized in previous studies; this clade is well resolved (Figs 1, 2; BS: 100%; PP: 1.00).

We did a morphological comparison between our new species and those known lianoid species from China (Table 3), and found that the specimens of this new specific clade are similar to *G. montanum* in the shape of leaves and chlamydosperms, and to *G. parvifolium* in the length of the male spikes and number of involucrel collars, but differ from *G. montanum* by their shorter male cones having fewer involucrel whorls, and from *G. parvifolium* by their larger leaves 11–16 cm long and bigger chlamydosperms ca. 2.2 cm long (vs. smaller leaves ca. 4–11 cm long, smaller chlamydosperms 1.3–1.8 cm long).

A few morphological details of the new species are taxonomically important but not known to us, e.g. shape and the number of sterile ovules in male spike. As a result, further field investigations are encouraged.

Acknowledgements

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Heterostemma cucphuongense (Apocynaceae, Asclepiadoideae), a new species from Vietnam

The Bach Tran^{1,2}, Le Ngoc Han¹, Do Van Hai^{1,2}, Bui Hong Quang^{1,2},
Nguyen Thi Thanh Huong^{1,2}, Bui Thu Ha³, Tran Van Hai², Michele Rodda⁴

1 Department of Botany, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam **2** Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam **3** Hanoi National University of Education, 136, Xuan Thuy Street-Cau Giay District, Hanoi, Vietnam **4** Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, Singapore, 259569, Singapore

Corresponding author: Michele Rodda (rodda.michele@gmail.com)

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Abstract

Heterostemma cucphuongense (Apocynaceae, Asclepiadoideae), a new species from Vietnam is described, illustrated and compared with the similar species *Heterostemma succosum* Kerr. *Heterostemma cucphuongense* differs from *H. succosum* by the morphology of the rachis of the inflorescence, the margins of the corolla lobes and the colour of the adaxial surface of the corolla.

Keywords

Ceropegieae, Cuc Phuong National Park

Introduction

The genus *Heterostemma* Wight and Arn. comprises approximately 30 to 40 species and is widely distributed from India and China to Australia and the Western Pacific Islands (Li et al. 1995; Swarupnandan et al. 1989; Forster 1992). The number of species has increased in recent years and three new species were published in 2019 alone. These are: *Heterostemma barikiana* P.Agnihotri et al. from India, Myanmar and

Thailand, (Agnihotri et al. 2019), *H. ficooides* A.Kidyoo (Kidyoo 2019) from Thailand and *Heterostemma trilobatum* A.Kidyoo & Thaithong also from Thailand (Kidyoo and Thaithong 2019). However, this third species is indistinguishable from *H. barikiana*. Furthermore, the specimen *C. Maknoi* & *P. Srisanga* 2258 (QBG) was cited under both *H. barikiana* and *H. trilobatum* and, therefore, *H. trilobatum* should be considered as a synonym of *H. barikiana*.

For Vietnam, extensive literature is available on *Heterostemma* (Costantin 1912; Ho 1993; Li et al. 1995; Tran 2005; Rodda 2016), as well as a recent revision (Tran 2017 [in Vietnamese]), where seven species have been recorded for the country.

While conducting fieldwork in Cuc Phuong National Park, Nho Quan district, Ninh Binh Province in Vietnam, an unidentified species of *Heterostemma* was collected. From the relevant literature (Costantin 1912; Swarupanandan et al. 1989; Ho 1993; Li et al. 1995; Tran 2005, 2017; Tran and Kim 2010; Thaithong et al. 2018; Agnihotri et al. 2019; Kidyoo 2019; Thammarong et al. 2019), as well as an examination of specimens in the herbaria BK, BKF, BM, HN, HNU, HNPM, IBK, IBSC, K, KUN, KYO, P, SING, TI, TO, TUT and VNM (acronyms according to Thiers 2020), we have confirmed that it is a new species. Here, we describe and illustrate this new species as *H. cucphuongense* T.B. Tran and Rodda. We also provide a key to the species of *Heterostemma* that are now known to occur in Vietnam.

Taxonomy

Heterostemma cucphuongense T.B. Tran & Rodda, sp. nov.

urn:lsid:ipni.org:names:77209709-1

Fig. 1

Diagnostic characters. This new species is similar to *H. succosum* Kerr, as both species have shortly pedunculate inflorescences, on which the flowers open in gradual succession (with generally a single flower open) and have relatively large rotate flowers (generally > 14 mm diam.). They are separated by the presence of a distinct rachis that develops in the inflorescence (which is absent in *H. succosum*); the margins of the corolla lobes are revolute (vs. flat in *H. succosum*), the pedicels are shorter (5–10 mm, vs. 15–30 mm in *H. succosum*) and by the colour of the adaxial surface of the corolla (red with white-yellow spots vs. yellow-orange with reddish-brown spots in *H. succosum*).

Type. Vietnam. Ninh Binh province, Nho Quan district, Cuc Phuong commune, Cuc Phuong National Park, 5 June 2019, *Le Ngoc Han et al.* VB 809 (HN, holotype; HN, isotype).

Description. *Liane*, at least 1 m in length. **Stem and branches** pubescent, longitudinally grooved, 1.5–2 mm diam.; internodes 2.5–10 cm long. **Leaves: petiole** 5–25 × 0.6–2 mm, glabrous or sparsely pubescent; **lamina** herbaceous, ovate to oblong, 4.5–8.5 × 2–4.5 cm, glabrous above, pubescent below, apex acuminate, base rounded to obtuse, venation pinnate with 3–4 basal secondary veins and 3–6 secondary veins departing from main vein, anastomosing near the edge of the lamina; basal colleter

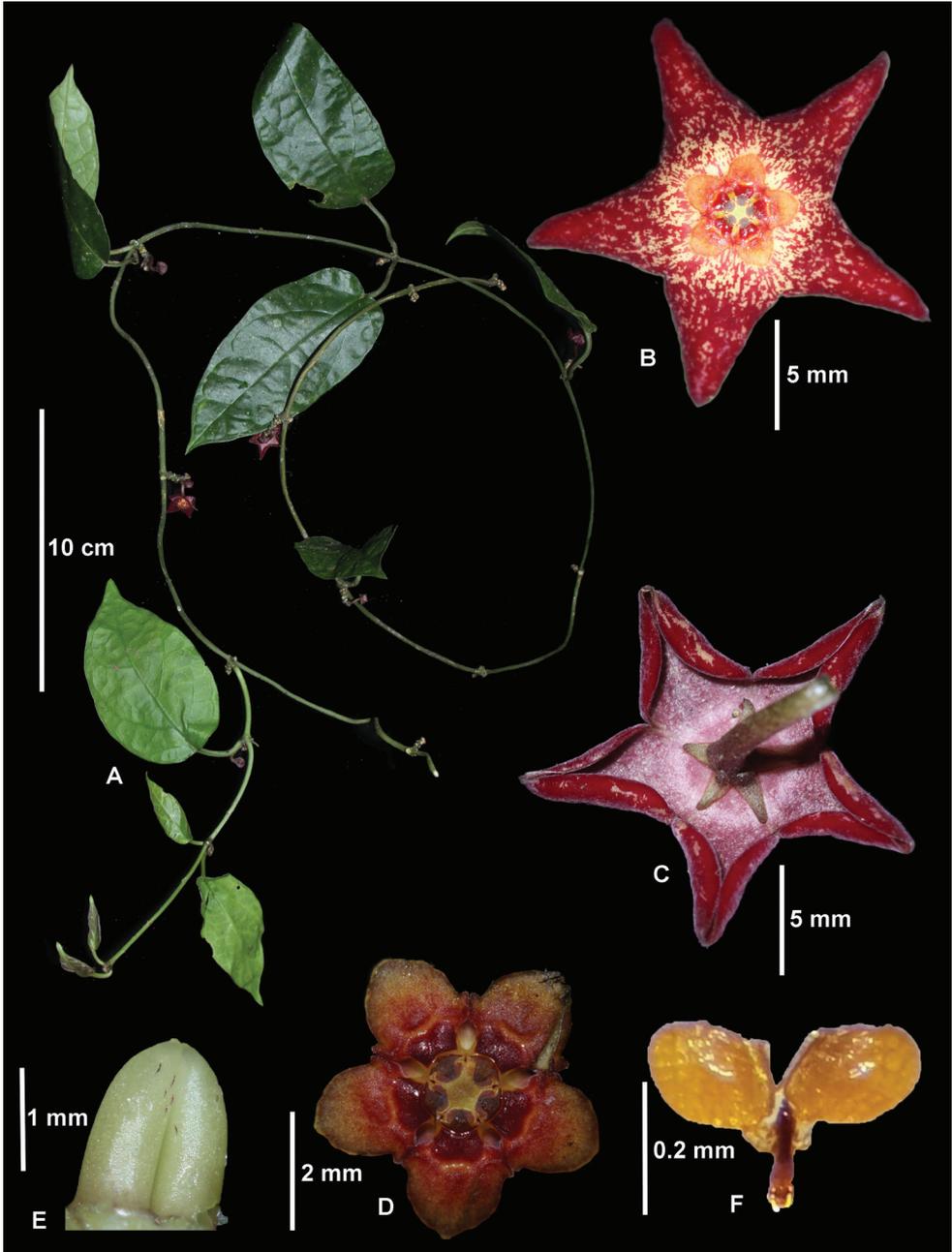


Figure 1. *Heterostemma cucphuongense* T.B. Tran & Rodda **A** flowering branch **B** flower, view from above (adaxial) **C** flower, from underneath **D** corona, from above **E** ovary **F** pollinarium. (Photographs by N.Q. Dat, T.B. Tran, Thuong V.A., photo edit by M. Rodda)

6–8 at base of lamina; **Inflorescences** 1(–8) flowered cymes; **peduncle** (0)3–5.5 × 2–2.5 mm, older peduncles developing a rachis to 7 mm long. **Pedicel** 5–10 × 1–1.35 mm, brown-red, glabrous or sparsely pubescent. **Flower** 1.4–1.9 cm diam. **Flower**

buds 5-angled, just before anthesis 0.9–1 cm diam. **Sepals** deltate, 1.5–2.2 × 0.8–1.2 mm, brownish-green, sparsely pubescent on both surfaces; with colleters at sinus. **Corolla** rotate, 1.4–1.9 cm diam., red with fine white-yellow spots (becoming more concentrated towards the centre) and glabrous or sparsely pubescent adaxially; bright purple-red and glabrous abaxially; tube 3.4–4.2 × 6.8–8.4 mm; lobes deltate, 5.5–6.7 × 4.5–5.2 mm, margins recurved. **Corona** staminal, 4.9–5.2 mm diam., ca. 0.9 mm high, upper surface orange with red centre, lower surface dull red and shiny, glabrous or sparsely pubescent; lobes broadly ovate, 1.58–1.66 × 1.10–1.14 mm, with 2 lateral ledges at the junction between adjoining lobes, inner apex acute, outer apex rounded. **Pollinarium**: pollinia erect, broad elliptic, yellow, 0.2–0.21 × ± 0.16 mm; corpusculum linear lanceolate, brown, ca. 0.18 × 0.04 mm; caudicles ± 0.05 × 0.04 mm; crests translucent, ± 0.12 × 0.02 mm. **Ovary** ca. 1.42 × 1.18 mm, greenish-white, sparsely pubescent. **Fruits and seeds** not observed.

Etymology. The species is named after the type locality, Cuc Phuong National Park, in Ninh Binh Province, northern Vietnam.

Distribution and ecology. *Heterostemma cucphuongense* was only collected once, near one of the main trails in the Cuc Phuong National Park. It was found in primary evergreen forest on soils derived from degraded limestone. It was collected in flower in June.

Conservation status. *Heterostemma cucphuongense* is endemic to the Cuc Phuong National Park. Since it is known from a single collection, its conservation status is Data Deficient (DD; IUCN 2012).

Notes. *Heterostemma cucphuongense* is similar to *H. succosum* Kerr (1939), a species found in Thailand and Laos. Both have shortly pedunculate inflorescences, in which the flowers generally open one at a time and are relatively large and rotate (mostly > 14 mm diam.). The two species can be easily separated because, in *H. cucphuongense*, the inflorescences form a rachis to 7 mm long with age while the inflorescences of *H. succosum* do not develop any rachis. Furthermore, the pedicels of *H. cucphuongense* are 5–10 mm long, while *H. succosum* has pedicels 15–30 mm long. Further distinguishing characters (that, however, are less obvious in dried material) are the margins of the corolla lobes that are recurved in *H. cucphuongense* (vs. flat in *H. succosum*). The two species also differ in that the colour of the adaxial surface of the corolla is red with white-yellow spots in *H. cucphuongense* (vs. yellow-orange with reddish-brown spots in *H. succosum*). These and additional diagnostic characters separating the two species are listed in Table 1.

Table 1. Morphological differences between *Heterostemma cucphuongense* and *H. succosum*.

Characters	<i>H. succosum</i>	<i>H. cucphuongense</i>
Length of petiole (cm)	2.5–7	0.5–2.5
Shape of leaf blade	Elliptic	Ovate to oblong
Length of peduncle (mm)	4–6	(0)3–5.5
Rachis	Absent	Present, to 7 mm long
Length of pedicel (mm)	15–30	5–10
Colour of adaxial surface of corolla	Yellow-orange with reddish-brown spots	Red with white-yellow spots
Length of corolla tube (mm)	4–8	3.4–4.2
Margins of corolla lobes	Not recurved	Recurved
Corona colour	Brownish-red	Orange with a darker red centre

Key to the species of *Heterostemma* in Vietnam

- 1 Mature stems developing a corky bark with age.....*H. suberosum*
 – Mature stems not becoming covered with a corky bark 2
 2 Corolla diam. > 6 times corona diam., corona pubescent*H. xuansonense*
 – Corolla diam. < 4 times corona diam., corona glabrous..... 3
 3 Peduncle < 5.5 mm long, stout, 2–3 mm thick 4
 – Peduncle > 6 mm long, slender, 1–1.5 mm thick 5
 4 Pedicels 5–10 mm long..... *H. cucphuongense*
 – Pedicels 20–32 mm long..... *H. oblongifolium*
 5 Corona lobes shorter than corolla tube..... 6
 – Corona lobes as long as or longer than corolla tube..... 7
 6 Corona lobes spreading on surface of corolla, almost flat, outer apex simple
 *H. brownii*
 – Corona lobes raised from the corolla surface, outer apex trilobed....*H. acuminatum*
 7 Peduncle 7–25 mm long, corolla < 8 mm diam..... *H. piperifolium*
 – Peduncle 25–60 mm long, corolla > 10 mm diam..... *H. grandiflorum*

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