

Tectaria danangensis (Tectariaceae), a new fern species from Vietnam

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Abstract

A new fern species, *Tectaria danangensis* (Tectariaceae) from Vietnam, which had long been misreported as *T. crenata*, is described and illustrated. The new species resembles *T. poilanei*, a species long neglected in the fern flora of Indochina, in the frond shape and sori arrangement, but differs by its irregularly 2-rowed sori (versus regularly 2-rowed, distantly and evenly arranged) between lateral veins of pinnae, fronds being more or less dimorphic (versus monomorphic) and basal pinnae each with a base-joined (versus free) lobe. Phylogenetic analyses of five plastid regions (*atpB*, *ndhF* + *ndhF-trnN*, *rbcL*, *rps16-matK* + *matK* and *trnL-F*) suggested *T. danangensis* has a close affinity to *T. harlandii*. *Tectaria danangensis* appears to be an intermediate species between *T. harlandii* and *T. poilanei*.

Keywords

Indochina, morphology, molecular phylogeny, taxonomy, *Tectaria crenata*

Introduction

The fern species *Tectaria crenata* Cav. represents a morphologically distinctive group in the genus *Tectaria* Cav. (Tectariaceae). It was originally described on the basis of plants from the Mariana Islands and is characterised by the 1-pinnate fronds and indusiate sori in regular rows parallel to lateral veins of pinnae (Copeland 1907). Tardieu-Blot

and Christensen (1941) recorded *T. crenata* in the flora of Indochina and cited five collections from Vietnam. However, *T. crenata* was reported by Holttum (1991) only from western Malesia, Philippines and southern Pacific Islands, but not in Indochina (including Cambodia, Laos, southern Myanmar, Thailand and Vietnam). The distribution of *T. crenata* in Vietnam remains uncertain (Hassler 2004–2021).

During recent years, we examined herbarium specimens of *Tectaria* from Asia in many herbaria and did not find any specimens of *T. crenata* from Indochina (excluding Peninsular Thailand) with typical morphology of this species as those in Malesia and Pacific Islands. The specimens from Vietnam, cited as *T. crenata* by Tardieu-Blot and Christensen (1941), turned out to represent an undescribed species which is reported here as *T. danangensis*. To test the relationships of *T. danangensis* with other species, we also conducted phylogenetic analyses of sequences of five plastid regions (*atpB*, *ndhF* + *ndhF-trnN*, *rbcL*, *rps16-matK* + *matK* and *trnL-F*).

Methods

For morphological comparisons, we studied herbarium specimens from Indochina in Herbaria BM, BO, CDBI, E, HN, HNU, IBSC, K, KUN, L, P, PE, SING and TAIF. We also conducted field observations of *Tectaria* species in Vietnam focusing on the variations of frond dimorphism, the shape and number of lateral pinnae, venation, sori arrangement and the presence or absence of indusia.

To infer the phylogenetic position of *T. danangensis*, we assembled a sequence matrix containing five plastid regions (*atpB*, *ndhF* + *ndhF-trnN*, *rbcL*, *rps16-matK* + *matK* and *trnL-F*) of 61 specimens (Appendix 1). The sampling was based on previous phylogenetic studies of *Tectaria* by Ding et al. (2014), Zhang et al. (2017) and Dong et al. (2018). *Tectaria crenata* was revealed to be a non-monophyletic species, but its sampled specimens from western Malesia to the Solomon Islands were resolved in a strongly supported clade with *T. decurrens* (C. Presl) Copel. and *T. sulitii* Copel. (Dong et al., in press). One of the analysed specimens, *Chen et al. SITW11094* (BSIP, IBSC, TNM), was used here to represent *T. crenata*. Except for one specimen of *T. danangensis* (i.e. *Dong 4909*) which was newly sequenced and analysed in this study, other specimens were analysed in previous studies and their corresponding sequences are available in GenBank. The methods to obtain and align the five cpDNA sequences for *Dong 4909* have been as described in Ding et al. (2014).

We analysed the matrix using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). The MP analysis was conducted in PAUP* version 4.0d100 (Swofford 2002), with all characters weighted equally and gaps treated as missing data. One thousand heuristic replicated searches were carried out using random stepwise addition with branch swapping by tree bisection-reconnection (TBR), saving 100 trees per replicate. Bootstrap values (BS) were calculated with 1000 heuristic bootstrap replicates, one random sequence addition and TBR swapping. For BI and ML analyses, we used the software jModelTest (Posada 2008) to determine the best-

fitting substitution models for the concatenated sequences and the results suggested GTR+G+I as the best-fitting model. The BI analysis was conducted with MrBayes 3.2.6 (Ronquist et al. 2012), using 10 million generations with one tree sampled every 1,000 generations; four runs with four chains were performed in parallel. The first 25% trees were discarded as burn-in. The ML analysis was conducted using raxmlGUI 2.0 (Edler et al. 2020). A thorough tree search for the best ML tree was performed. The ML bootstrap analysis was performed with 1000 replications. The analysed sequence matrix and resulting trees are available in Dryad Digital Repository (<https://doi.org/10.5061/dryad.51c59zw9t>).

Results

Morphological comparisons showed that the specimens recorded as *T. crenata* by Tardieu-Blot and Christensen (1941) represent an undescribed species which is recognised as *T. danangensis*. This new species superficially resembles *T. crenata* in the 1-pinnate fronds and entire pinnae, but distinctly differs in sori features, such as being borne on anastomosing veins (versus terminal on free veins included in areoles) and in irregular two rows (versus regularly 2-rowed, distantly and evenly arranged) between lateral veins of pinnae/segments (Fig. 1A and B). Based on herbarium specimens and recent collections, we found that *T. danangensis* is quite variable in the frond dimorphism, with fertile fronds contracted to different extents compared with sterile ones and its sori are in irregular two rows between lateral veins, close or distant to each other. A few specimens of *T. danangensis* with less contracted fertile fronds are similar to those of *T. poilanei* Tardieu, but differ mainly in their irregular 2-rowed sori (versus regularly 2-rowed) between lateral veins, upper pinnae mostly being adnate (versus pointed) to rachis and basal pinnae each having a basisopic base-joined (versus free) lobe (Figs 1 and 2). We detected a total of 25 herbarium collections of *T. danangensis* containing fertile fronds, of which seven collections bore evidently abortive sporangia.

Our phylogenetic analyses of cpDNA sequences with all three methods (BI, ML or MP) consistently resolved *T. danangensis* in Clade IV-8 of *Tectaria* (Fig. 3). Based on the current sampling, two specimens of *T. danangensis* and an unidentified specimen (*Zhang et al.* 8817, for which we had no chance to examine the morphology) formed a strongly support sister relationship with *T. harlandii* clade including *T. × hongkongensis* S.Y. Dong and a *T. harlandii*-like specimen (PP = 1.0, MLBS = 94% and MPBL = 90%). In contrast, *T. crenata* and allied species were resolved in a different clade (IV-9, Fig. 3). Though *T. danangensis* was suggested as having a close affinity to *T. harlandii* (Hook.) C.M. Kuo, these two species are morphologically strikingly different in sori features. Specifically, *T. danangensis* has discrete sori, whereas *T. harlandii* has nearly acrostichoid sori. A comparison of morphological characters amongst *T. danangensis*, *T. poilanei* and *T. harlandii* is listed in Table 1.

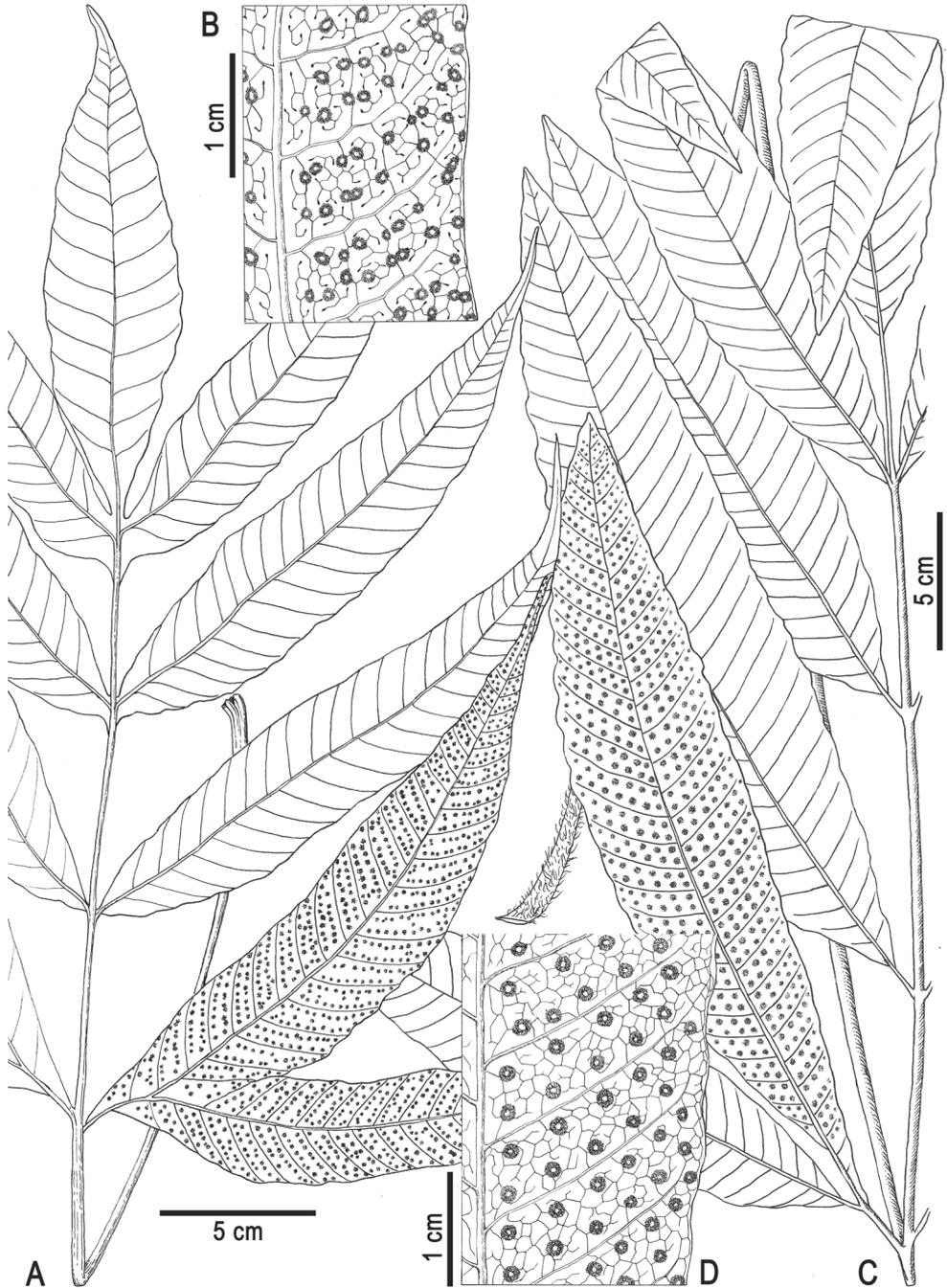


Figure 1. Morphological comparison between *Tectaria danangensis* (A, B) and *T. poilanei* (C, D) A, C habit B, D detail of a pinna showing venation and sori arrangement. Drawn by Shu-Han Li, with A and B based on *Dong 4909* (holotype, IBSC) and C and D on *Poilane 24074* (holotype, P).

Table 1. Morphological differences amongst *T. danangensis*, *T. fissa*, *T. harlandii* and *T. poilanei*.

	<i>T. harlandii</i>	<i>T. danangensis</i>	<i>T. poilanei</i>	<i>T. fissa</i>
Fronde dimorphism	Strongly dimorphic	Mostly semi-dimorphic	Monomorphic	Monomorphic
Number of lateral pinnae/segments	1–3 pairs	2–5(6) pairs	3–4 pairs	1–5 pairs
Upper pinnae/segments	Adnate to rachis, mostly connate with terminal segment at base	Adnate to rachis, connate with terminal segment or not	Free, shortly petiolulate or sessile	Adnate to rachis, connate with terminal segment or not
Lobes on basal pinnae	Absent	Present; their bases connate to basal pinnae	Present; their bases cuneate, sessile or shortly petiolulate	Mostly present; their bases cuneate or connate
Wingless petioles of basal pinnae	Absent	Almost absent, 0–0.5 cm long	1.2–2 cm long	0–1.5 cm long
Transverse veins between lateral veins of pinnae	Distinct on sterile fronds, absent on fertile fronds	Variable, mostly indistinct	Absent	Distinct
Sori	Nearly acrostichoid, with sporangia running along veins between lateral veins	Round; irregularly in 2 rows between lateral veins, close or distant	Round; regularly in 2 rows between lateral veins, uniformly distant	Round; irregularly in 4–6 rows between lateral veins, close to each other
Indusia	Absent	Present	Present	Present

Discussion

Tardieu-Blot and Christensen (1941) overlooked the sori differences between *T. danangensis* and *T. crenata* and misidentified the former as the latter in Vietnam. Though having similar shape and dissection of fronds to *T. danangensis*, *T. crenata* and its allied species in Clade IV-9 (Fig. 3) (including *T. decurrens*, *T. pleiosora* (Alderw.) C. Chr. and *T. repanda* (Willd.) Holttum) differ from *T. danangensis* in their characteristic sori which are large and regularly 2-rowed between lateral veins, with each sorus being terminal on a single veinlet in an areole (Tagawa and Iwatsuki 1988: 372; Holttum 1991: 80). Such sori features are stable in these species and can be considered as a synapomorphy for Clade IV-9. In contrast, for species in Clade IV-8, the sori are never in regular two rows between lateral veins nor terminal on free veinlets included in areoles. Instead, their sori are relatively small, scattered between lateral veins and mostly borne on anastomosing veins in most species clustered in Clade IV-8, except for *T. danangensis*, *T. harlandii* and *T. × hongkongensis*. *Tectaria danangensis* has a unique arrangement of sori which are in irregular two rows between lateral veins (Fig. 1A and B); while in *T. harlandii* and *T. × hongkongensis*, the sori are nearly acrostichoid, with sporangia running along veinlets between lateral veins, as shown in Zhao and Dong (2016: Fig. 2C).

By examining specimens of all *Tectaria* species with 1-pinnate, pinnae-entire fronds recorded in Indochina and nearby regions (Tardieu-Blot and Christensen 1941; Tagawa and Iwatsuki 1988; Xing et al. 2013; Fraser-Jenkins et al. 2018), we found that some specimens of *T. danangensis* look very like those of *T. fissa* (Kunze) Holttum, a species frequently occurring in western Malesia but not in Indochina (Holttum 1991; Lindsay and Middleton 2012 onwards). A detailed comparison (Table 1) showed that

T. danangensis differs from *T. fissa* and other species having 1-pinnate fronds by its venation lacking distinct transverse veins between lateral veins and its sori being generally in only two rows (versus 4–6 rows) between lateral veins.

Tectaria danangensis appears to be an intermediate species between *T. harlandii* and *T. poilanei*; the latter (*T. poilanei*) has long been neglected in literature accounting for the fern flora of Indochina (e.g. Tardieu-Blot and Christensen 1941; Tagawa and Iwatsuki 1988; Phan 2010; Lindsay and Middleton 2012 onwards). According to herbarium specimens examined, *T. danangensis* is not rare in Vietnam; it has been collected from 1837 to 2014 across nearly all the country and, morphologically, is quite variable in the frond dimorphism and sori distribution between lateral veins. As shown in Table 1, some characters in *T. danangensis*, such as frond dimorphism, attachment pattern of pinnae to rachis, venation and sori distribution, exhibit intermediate states of those between *T. harlandii* and *T. poilanei*. Notably, *T. poilanei* is quite stable in pinnae features (i.e. the broad-lanceolate shape, lower pinnae consistently being petiolulate and basal pinnae each bearing a free basiscopic lobe), venation lacking transverse veins between lateral veins and regularly 2-rowed well-spaced sori (Fig. 1C and D); this species is currently represented, so far as we know, by its type specimen from southern Vietnam (Tardieu-Blot 1940) and a few collections from Thailand extend its distribution (e.g. *Beusekom & Smitinand 2193* (L) from Chantaburi, *Hansen & Smitinand 12644* (K, L) from Mae Hong Son and *Maxwell 04-156* (L) and *Hansen et al. 10886* (K, L) from Chiang Mai). Based on its variable morphology and frequently abortive sporangia, we hypothesised that *T. danangensis* possibly involved hybridisation with other species. Further studies, especially chromosome number and reproductive mode, are needed to better determine the origin of *T. danangensis* and its relationships with other *Tectaria* species.

Taxonomic treatment

Tectaria danangensis S.Y. Dong, sp. nov.

urn:lsid:ipni.org:names:77296979-1

Figs 1A, B, 2

Type. Vietnam. On the border between Da Nang and Thua Thien Hue Prov.: Bach Ma National Park, 107°51'37"E, 16°17'59"N, 680 m elev., 02 Dec 2017, S.Y. Dong 4909 (holotype: IBSC!, designated here; isotypes: HNU!, IBSC!).

Diagnosis. *Tectaria danangensis* is similar to *T. poilanei* Tardieu, but differs in its irregularly 2-rowed sori (versus regularly 2-rowed, well-spaced and evenly arranged) between lateral veins, fronds more or less being dimorphic (versus monomorphic) and basal pinnae each having a base-joined (versus free) lobe.

Description. **Rhizome** short, erect or decumbent. **Fronds** more or less dimorphic, with fertile fronds slightly contracted; stipe reddish-brown, 3–4 mm in diameter, 30–50 cm long, bearing scales only at base; scales lanceolate, ca. 8–10 × 1–1.5 mm,



Figure 2. Herbarium specimens of *Tectaria danangensis*, showing contracted fertile fronds (**A, C**) as compared with sterile fronds (**B, D**) **A, B** Dong 4909 (type, IBSC) **C, D** Cadiere 165 (P).

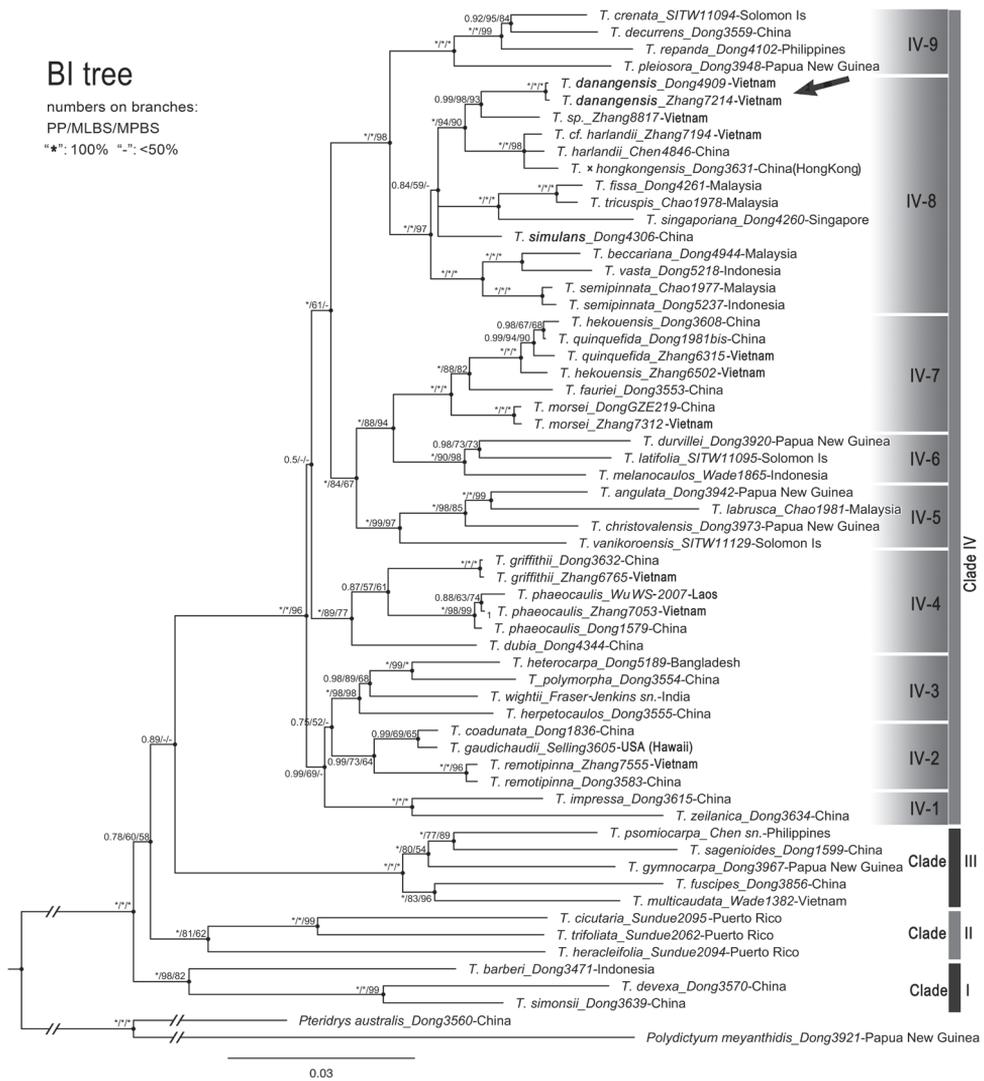


Figure 3. Bayesian consensus tree of *Tectaria*, based on combined plastid regions of *atpB*, *ndbF* + *ndhF-trnN*, *rbcL*, *rps16-matK* + *matK* and *trnL-F*. The position of the new species, *T. danangensis*, is indicated by an arrow.

reddish-brown; lamina nearly round or oblong, 35–55 × 25–35 cm, imparipinnate or terminated by tri-lobed segments, having 2–4(6) pairs of lateral pinnae, pinnae and segments entire at margin, herbaceous in texture, hairless; basal pinnae forked, 14–27(33) × 1.7–5 cm, petiolules 0–5 mm long, acroscopic base cuneate, basicopic base round, apex caudate-acuminate, having a basicopic lobe, the basicopic lobes 7–24 × 1–3.5 cm; suprabasal pinnae linear, 14–30 × 1–4.5 cm, sessile, base cuneate, apex acuminate or caudate; upper pinnae similar to suprabasal pinnae in size and shape, but mostly adnate to rachis. **Veins** fully anastomosing, with most areoles having

included free or forked veinlets, transverse veins between lateral veins mostly indistinct. **Sori** round, borne on anastomosing veins, generally in two rows between lateral veins of pinnae (more or less with additional sori present beyond two rows), 8–11 each row in broad pinnae or 4–6 in obviously contracted fertile pinnae, well-spaced or adjacent. **Indusia** round-reniform, mostly curled and almost covered by sporangia when mature.

Distribution and habitat. Vietnam (Da Nang, Lam Dong, Quang Binh, Quang Nam, Quang Tri, Thanh Hoa, and Thua Thien Hue); terrestrial in broadleaved evergreen forest, occurring in slopes of valley or along mountain ridge, elev. 200–1400 m, locally common.

Additional specimens examined (paratypes). **Vietnam. Da Nang:** Ba Na Mountain, Hoa Vang District, *Sallet s.n.* (P); without locality, *Gaudichaud s.n.* (P). **Lam Dong:** Da Lat, *Wu et al. WP1447* (HN). **Quang Binh:** Phong Nha – Ke Bang National Park, *Nguyen NT39, NT69 & NT102* (HNU); without locality, *Phan s.n.* (HNU). **Quang Nam:** without locality, *Poilane 29484 & 31661* (P). **Quang Tri:** Huong Hoa District, *Averyanov et al. CPC2906 & CPC2907* (HNU); Dakrong District, *Phan et al. HLF6122* (HNU); Dakrong Nature Reserve, *Lu 19232* (TAIF); “Mai-lanh”, *Poilane 1189* (P, PE, SING). **Thanh Hoa:** Phu luc, *Lecomte & Finet 1338* (P). **Thua Thien Hue:** A Luoi District, *Averyanov et al. HAL7289, HAL7342, HAL7423, HAL7622, HAL7738* (HNU); Nam Dong District, *Averyanov et al. HAL6940* (HNU); “Tua Luu”, *Cadiere 165* (P). **Southern Vietnam** (Annam, with localities’ names unreadable): *Eberhardt 373* (P).

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Appendix I

Accessions used for phylogenetic analyses in this study. Information are arranged in this order: species name, voucher specimen (collector, number, and herbarium), place of origin, and GenBank numbers for *rbcL*, *atpB*, *rps16-matK* + *matK*, *ndhF* + *ndhF-trnN*, and *trnL-F* spacer (NA indicates data absent).

Polydictyum menyanthidis (C.Presl) Copel., *Dong 3921* (IBSC, LAE), Papua New Guinea (Lae), MF623752/MF623680/MF623704/MF623728/MF623776. *Pteridrys australis* Ching, *Dong 3560* (IBSC), China (Yunnan), KJ196892/KJ196486/KJ196796/KJ196678/KJ196522. *Tectaria angulata* (Willd.) Copel., *Dong 3942* (IBSC, LAE), Papua New Guinea (Kimbe), MF623756/MF623756/MF623708/MF623732/MF623779. *Tectaria barberi* (Hook.) Copel., *Dong 3471* (IBSC), Indonesia (Kalimantan), KJ196846/KJ196445/KJ196584/KJ196778/KJ196628. *Tectaria beccariana* (Ces.) C.Chr., *Dong 4944* (IBSC), Indonesia (Java), OK104174/OK480073/OK480115/OK480153/OK480194. *Tectaria cf. harlandii* (Hook.) C.M.Kuo, *Zhang et al. 7194* (CDBI), Vietnam (Ha Tinh), NA/NA/KY937237/NA/KY937513. *Tectaria christovalensis* (C.Chr.) Alston, *Dong 3973* (IBSC), Papua New Guinea (Kimbe), OK104179/OK480081/OK480121/OK480161/OK480202. *Tectaria cicutaria* (L.) Copel., *Sundue 2095* (VT), Puerto Rico, KJ196905/KJ196408/KJ196621/KJ196729/KJ196696. *Tectaria coadunata* (J.Sm.) C.Chr., *Dong 1836* (IBSC), China (Yunnan), KJ196878/KJ196451/KJ196531/KJ196779/KJ196661. *Tectaria crenata* Cav., *Chen et al. SITW11094* (BSIP, IBSC, TNM), Solomon Islands (Temotu), OK104204/OK480084/OK480124/OK480164/OK480205. *Tectaria danangensis* S.Y.Dong, *Dong 4909* (HNU, IBSC), Vietnam (Da Nang), OM671282/OM671283/OM671284/OM671285/OM671286; *Zhang et al. 7214* (CDBI), Vietnam (Quang Binh), KY937368/NA/KY937261/NA/KY937550. *Tectaria decurrens* (C.Presl) Copel., *Dong 3559* (IBSC), China (Yunnan), KJ196870/KJ196471/KJ196535/KJ196741/KJ196674. *Tectaria devexa* (Kunze) Copel., *Dong 3570* (IBSC), China (Yunnan), KJ196883/KJ196456/KJ196787/KJ196668/KJ196605. *Tectaria dubia* (C.B.Clarke & Baker) Ching, *Dong 4344* (IBSC), China (Yunnan), MF623762/MF623690/MF623714/MF623738/MF623783. *Tectaria durvillei* (Bory) Holttum, *Dong 3920* (IBSC, LAE), Papua New Guinea (Lae), MF623763/MF623691/MF623715/MF623739/MF623784. *Tectaria fauriei* Tagawa, *Dong 3553* (IBSC), China (Yunnan), KJ196887/KJ196480/KJ196526/KJ196792/KJ196671. *Tectaria fissa* (Kunze) Holttum, *Dong 4261* (IBSC), Malaysia (Selangor), MF623766/MF623694/MF623718/MF623742/MF623787. *Tectaria fusciipes* (Bedd.) C.Chr., *Dong 3856* (IBSC), China (Hainan), MF623767/MF623695/MF623719/MF623743/MF623788. *Tectaria gaudichaudii* Maxon, *Selling 3605* (H), USA (Hawaii), KF887176/NA/NA/NA/NA. *Tectaria griffithii* (Baker) C.Chr., *Dong 3632* (IBSC), China (Guangxi), KJ196872/KJ196473/KJ196578/KJ196775/KJ196651; *Zhang et al. 6765* (CDBI), Vietnam (Bac Kan), KY937337/NA/KY937229/NA/KY937501. *Tectaria gymnocarpa* Copel., *Dong 3967* (IBSC), Papua New Guinea

(Kimbe), MF623765/MF623693/MF623717/MF623741/MF623786. *Tectaria barlandii* (Hook.) C.M.Kuo, *Chen et al. 4846* (IBSC), China (Guangdong), KJ196839/KJ196432/KJ196612/KJ196758/KJ196718. *Tectaria hekousis* Ching & Chu H.Wang, *Dong 3608* (IBSC), China (Yunnan), KJ196894/KJ196487/KJ196520/KJ196799/NA; *Zhang et al. 6502* (CDBI), Vietnam (Hanoi), KY937340/NA/KY937238/NA/KY937514. *Tectaria heracleifolia* (Willd.) Underw., *Sundue 2094* (VT), Puerto Rico, KJ196904/KJ196407/KJ196597/KJ196728/KJ196695. *Tectaria herpetocaulos* Holttum, *Dong 3555* (IBSC), China (Yunnan), KJ196884/KJ196482/KJ196570/KJ196789/KJ196669. *Tectaria heterocarpa* (Bedd.) C.V.Morton, *Dong 5189* (IBSC), Bangladesh (Sylhet), MW795598/MW795606/MW795612/MW795620/MW795628. *Tectaria* × *hongkongensis* S.Y.Dong, *Dong 3631* (IBSC), China (Hong Kong), KJ196886/KJ196484/KJ196568/KJ196783/KJ196666. *Tectaria impressa* (Fée) Holttum, *Dong 3615* (IBSC), China (Yunnan), KJ196841/KJ196420/KJ196536/KJ196772/KJ196626. *Tectaria labrusca* (Hook.) Copel., *Chao 1981* (TAIF), Malaysia (Sarawak), KJ196818/KJ196499/KJ196600/KJ196745/KJ196692. *Tectaria latifolia* (G.Forst.) Copel., *Chen et al. SITW11095* (BSIP, IBSC, TAIF, TNM), Solomon Islands (Temotu), OK104190/OK480096/OK480135/OK480174/OK480217. *Tectaria melanocaulos* (Blume) Copel., *Chen Wade1865* (TAIF), Indonesia (Java), KJ196832/KJ196422/KJ196562/KJ196735/KJ196709. *Tectaria morsei* (Baker) S.Y.Dong, *Dong GZE219* (IBSC), China (Guizhou), KJ196893/KJ196418/KJ196521/KJ196798/KF561675; *Zhang et al. 7312* (CDBI), Vietnam (Quang Binh), KU605205/NA/KU605139/NA/KU605117. *Tectaria multicaudata* (C.B.Clarke) Ching, *Chen Wade1382* (TAIF), Vietnam (Bu Gia Map National Park), KJ196834/KJ196425/KJ196558/KJ196756/KJ196713. *Tectaria phaeocaulis* (Rosenst.) C.Chr., *Dong 1579* (IBSC), China (Hainan), KJ196879/KJ196453/KJ196546/KJ196780/KJ196662; *Zhang et al. 7053* (CDBI), Vietnam (Thanh Hoa), KU605201/NA/KU605142/NA/KU605119; *Wu WS-2007* (KUN, MO), Laos, NA/NA/NA/NA/KY937532. *Tectaria pleiosora* (Alderw.) C.Chr., *Dong 3948* (IBSC, LAE), Papua New Guinea (Kimbe), MF623759/MF623687/MF623711/MF623735/MF623793. *Tectaria polymorpha* (Hook.) Copel., *Dong 3554* (IBSC), China (Yunnan), J196889/KJ196477/KJ196524/KJ196794/KJ196657. *Tectaria psomiocarpa* S.Y.Dong, *Chen s.n.* (TAIF), Philippines (Luzon), J196822/KJ196502/KJ196595/KJ196723/KJ196698. *Tectaria quinquefida* (Baker) Ching, *Dong 1981bis* (IBSC), China (Yunnan), KJ196885/KJ196483/KJ196528/KJ196890/KJ396622; *Zhang et al. 6315* (CDBI), Vietnam (Hoa Binh), KY937358/NA/KY937250/NA/KY937537. *Tectaria remotipinna* Ching & Chu H.Wang, *Dong 3583* (IBSC), China (Yunnan), KJ196851/KJ196450/KJ196574/KJ196781/KJ196663; *Zhang et al. 7555* (CDBI), Vietnam (Quang Tri), KY937325/NA/NA/NA/KY937482. *Tectaria repanda* (Willd.) Holttum, *Dong 4102* (IBSC), Philippines (Palawan), OK104195/OK480104/OK480144/OK480183/OK480228. *Tectaria sagenioides* (Mett.) Christenh., *Dong 1599* (IBSC), China (Hainan), KJ196896/KJ196436/KJ196550/KJ196760/KJ196625. *Tectaria semipinnata* (Roxb.) C.V.Morton, *Chao 1977* (TAIF), Malaysia, KJ196817/KJ196498/KJ196601/KJ196744/KJ196691; *Dong 5237* (IBSC),

Indonesia (Sumatra), NA/OK480106/OK480146/OK480185/OK480230. *Tectaria simonsii* (Baker) Ching, *Dong 3639* (IBSC), China (Guangxi), KJ196837/KJ196430/KJ196555/KJ196730/KJ196717. *Tectaria simulans* Ching, *Dong 4306* (IBSC), China (Yunnan), OK104197/OK480108/OK480148/OK480187/OK480231. *Tectaria singaporiana* (Hook. & Grev.) Copel., *Dong 4260* (IBSC), Singapore, MF623771/MF623699/MF623723/MF623747/MF623791. *Tectaria* sp., Zhang et al. 8817 (CDBI), Vietnam (Khanh Hoa), NA/NA/KY937269/NA/KY937557. *Tectaria tricuspis* (Bedd.) Copel., *Chao 1978* (TAIF), Malaysia (Kuala Lumpur), KJ196820/KJ196501/KJ196598/KJ196847/KJ196694. *Tectaria trifoliata* (L.) Cav., *Sundue 2062* (VT), Puerto Rico, KJ196901/KJ196409/KJ196565/KJ196848/NA. *Tectaria vanikoroensis* S.Y.Dong & C.W.Chen (ined.), *Chen et al. SITW11129* (BSIP, IBSC, TNM), Solomon Islands (Temotu), OK104200/OK480111/NA/OK480190/OK480234. *Tectaria vasta* (Blume) Copel., *Dong 5218* (IBSC), Indonesia (Sumatra), NA/OK480112/OK480151/OK480191/NA. *Tectaria wightii* (C.B.Clarke) Ching, *Fraser-Jenkins s.n.* (TAIF), India (Kerala), KJ196906/KJ196416/KJ196561/KJ196732/KJ196710. *Tectaria zeilanica* (Houtt.) Sledge, *Dong 3634* (IBSC), China (Yunnan), KJ196862/KJ196442/KJ196540/KJ196768/KJ196637.

Primula longipilosa (Primulaceae), a new species from Yunnan, China

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Abstract

Primula longipilosa from SW Yunnan, China, is described as a species new to science and illustrated. The systematic placement of this new species is also discussed based on an nrITS molecular tree. It is morphologically most similar to *P. mollis*, but differs from the latter in its racemose inflorescence, green calyx tube, pink to pink rose corolla, stamens at 1/3 length above the base of the corolla tube and appanate globose capsule.

Keywords

Morphological characteristics, nrITS, phylogenetic analysis, *Primula mollis*

Introduction

Primula L. is one of the largest genera of Primulaceae, including ca. 500 species mostly indigenous to the north temperate zone. There are ca. 300 species of 24 sections in China, mostly distributed in western Sichuan, eastern Xizang, and northwestern Yunnan (Hu 1990; Hu and Kelso 1996). Section *Cortusoides* Balf. f. (39: 140, 1913) of the genus *Primula* comprises ca. 20 species, mainly distributed in Eastern Himalaya

and Hengdian Mountain in China. The species of this section can be distinguished by a set of morphological characters: Perennial herb, plants always with multicellular hairs; Leaves entire, shallowly undulate or palmately divided, base cordate or rounded, with long stipe; Inflorescences umbellate in 1–10 whorls, rarely racemose; flowers usually heteromorphic; Calyx narrowly campanulate to tubular, shorter than corolla tube, with many longitudinal veins; Corolla pink to violet; Capsule split into several pieces.

As one of the hotspots of biodiversity in China, Yunnan Province has ca. 130 species of *Primula* distributed all over its range (Fang 2003). The number is still increasing with new taxa constantly being reported in this province over the past two decades (Rankin et al. 2002; Gong and Fang 2003; Xue and Zhang 2004; Shui and Chen 2006; Li and Hu 2009; Hu and Hao 2011; Yang et al. 2017; Wu et al. 2019; Ma et al. 2021).

During the National Survey of Traditional Chinese Medicine Resources field survey in Gengma County, Yunnan province, we discovered one flowering population of *Primula* with distinct long white soft multicellular hairs, racemose inflorescences, and pink corolla. After further morphological studies and molecular phylogenetic analysis, we confirmed that it represents a species new to science, which is described and illustrated here.

Materials and methods

Morphological analysis

The morphological description of the new species was based on examining the type specimens (KUN) collected from the type locality and corresponding photos taken in the field. We referred to the keys to sections and species in Flora Reipublicae Popularis Sinicae (Hu 1990) and Flora of China (Hu and Kelso 1996). The comparison with morphologically similar species (*Primula mollis*) was based on studies of the descriptions and illustrations in the protologue (Hooker 1854), Flora Reipublicae Popularis Sinicae (Hu 1990), and Flora of China (Hu and Kelso 1996).

Taxon sampling and outgroup selection

The phylogenetic analysis was mainly based on the recently published framework of *Primula* (Xu et al. 2016); we performed nuclear nrITS sequencing of *P. longipilosa* and remained all the taxa of subgen. *Auganthus* (Link) Wendelbo (11: 34, 1961) and subgen. *Carolinella* (Hemsl.) Wendelbo (11: 36, 1961), added a few species available on GenBank to make our analysis focus on the sect. *Cortusoides* and sect. *Malvacea* Balf. f. (39: 145, 1913). *Androsace sublanata* Hand.-Mazz. was used as the outgroup to keep consistent with the former framework. All the sequences downloaded from GenBank (www.ncbi.nlm.nih.gov/Genbank) were marked with the accession number in the phylogenetic tree.

DNA extraction, sequencing, and phylogenetic analysis

For the molecular phylogenetic analysis, DNA sequences were newly generated with the protocols described by Wang et al. (2013) for the nuclear ribosomal internal transcribed spacer (nrITS). The GenBank accession number of the new sequence is OM436005. The nrITS dataset was analyzed for phylogenetic tree reconstruction with Bayesian Inference and Maximum Parsimony as described by Wang et al. (2013); SYM + I + G substitution model was selected using jModelTest2 2.1.6 (Darriba et al. 2012) for Bayesian inference (BI) analysis.

Results

Phylogenetic reconstruction

As the Bayesian and Maximum Parsimony analysis generated similar results, only the Jackknife 50% majority-rule consensus tree is presented here (Fig. 1). The phylogenetic analysis showed that the sequence of the new species nested within the subgen. *Auganthus*, forming a clade with the sect. *Malvacea*, sect. *Pycnoloba* Balf. f. (39: 144,

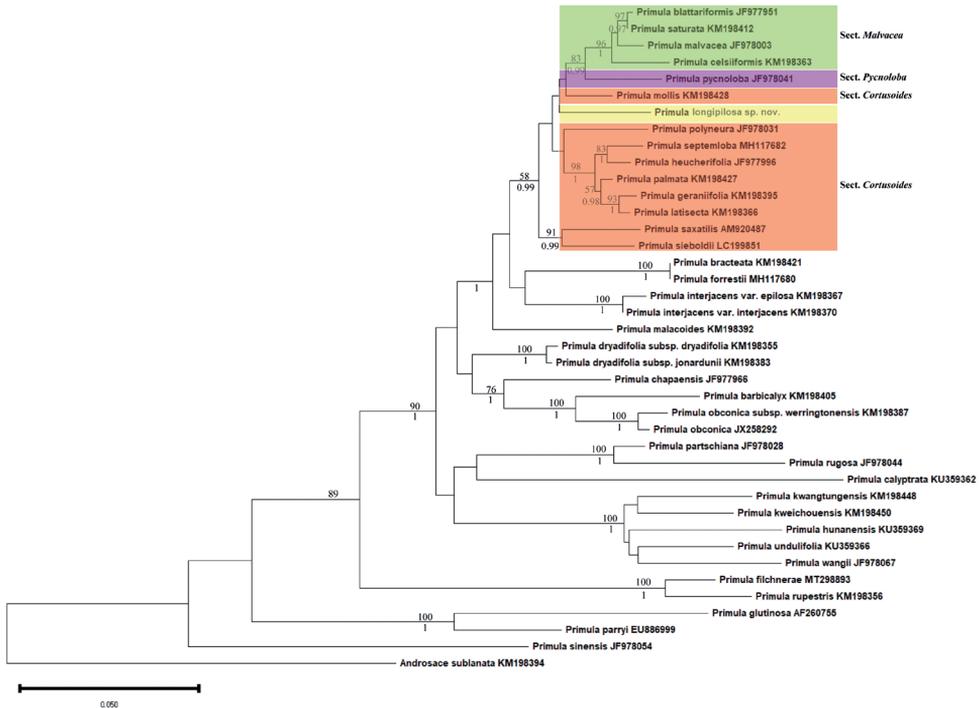


Figure 1. Jackknife 50% majority-rule tree of selected species from *Primula* (Primulaceae) based on the nrITS region, focusing on the subgen. *Auganthus* and the subgen. *Carolinella*. The MP jackknife support (JK) values are shown above the branches, and the BI posterior probabilities (PP) are given below the branches.

1913) and *P. mollis* of the sect. *Cortusoides* with low support value (JK < 50; BI < 0.9). However, if we collapse all the low support nodes, the relationships between the new species with these three sections will remain unresolved.

Taxonomic treatment

Primula longipilosa Ze H. Wang & H. Peng, sp. nov.

urn:lsid:ipni.org:names:77296980-1

Figs 2, 3

Type. China, Yunnan Province, Gengma County, Gengma Town, new Aiguo Village. 23°39.91'N, 99°32.44'E, alt. 1384 m, 31 July 2020, Gengma TCM Resources Survey Exped. 5309260482 (holotype: KUN!, isotypes: KUN!).

Diagnosis. *Primula longipilosa* is most similar to *P. mollis* in the long soft multicellular hairs all over the plant, the shape of their leaves and corolla. But it differs from the latter mainly in its racemose inflorescence, green calyx tube, pink to pink rose corolla, stamens of the pin flowers at 1/3 length above the base of corolla tube, and applanate globose capsule. The main morphological differences between *P. longipilosa* and *P. mollis* are summarized in Table 1.

Description. Perennial herbs with several robust fibrous roots. The whole plant is covered with long white soft multicellular hairs. Stem extremely shortened, inconspicuous. Leaves all rising from the root, forming a rosette; petiole 5–20 cm, clothed with long spreading soft multicellular hairs, slightly sheathing at the base; leaf blade cordate to broad cordate, 3.5–19 cm long, 4–16 cm wide, covered with white soft multicellular hairs on both sides; apex obtuse, base cordate or deeply cordate, margin undulate; basal veins usually 3–5, lateral veins 5–6, all raised abaxially and further branched to form reticulate fine veins. Scapes 7–17 cm long, 2–3 rising from the middle of the rosette leaves, shorter than or almost equal to the leaves, densely covered with long soft multicellular hairs, each scape has 7–25 flowers arranged in a racemose inflorescence, or sometimes several nearby flowers grow close to each other to form an umbel in some part of the inflorescence; bracts narrowly lanceolate, 0.5–1 cm long, with long soft multicellular hairs. Pedicel 1.5–2.5 cm, extended to 4 cm in fruit, densely covered with long soft multicellular hairs. Flowers heterostylous. Calyx narrowly campanulate, green, 6–10 mm, covered with long soft multicellular hairs abaxially, parted to the middle; lobes triangulate to ovate-triangulate; veins 3–5. Corolla pink to pink rose, with long soft multicellular hairs outside; tube 1–1.2 cm long; limb ca. 1.5–2 cm in diameter; lobes obcordate, ca. 9 mm, with several rays sending out from the mouth, which is prominent especially at the base, bifid at the apex; pin flowers: stamens at ca 3 mm above the base of corolla tube, their style ca 8 mm long; thrum flowers: stamens at 2/3 length of corolla tube, ca. 1 cm above the base of corolla tube, their style ca 2 mm. Ovary applanate globose, stigma a depressed globose disc. Capsule applanate globose, ca. 5 mm in diameter, hidden by the persistent calyx, 5-toothed split.

Distribution and habitat. *Primula longipilosa* is currently encountered and seen growing on the moist mountain slopes along the valley forest margin near the new Aiguo Village, Gengma County, Yunnan Province, China.

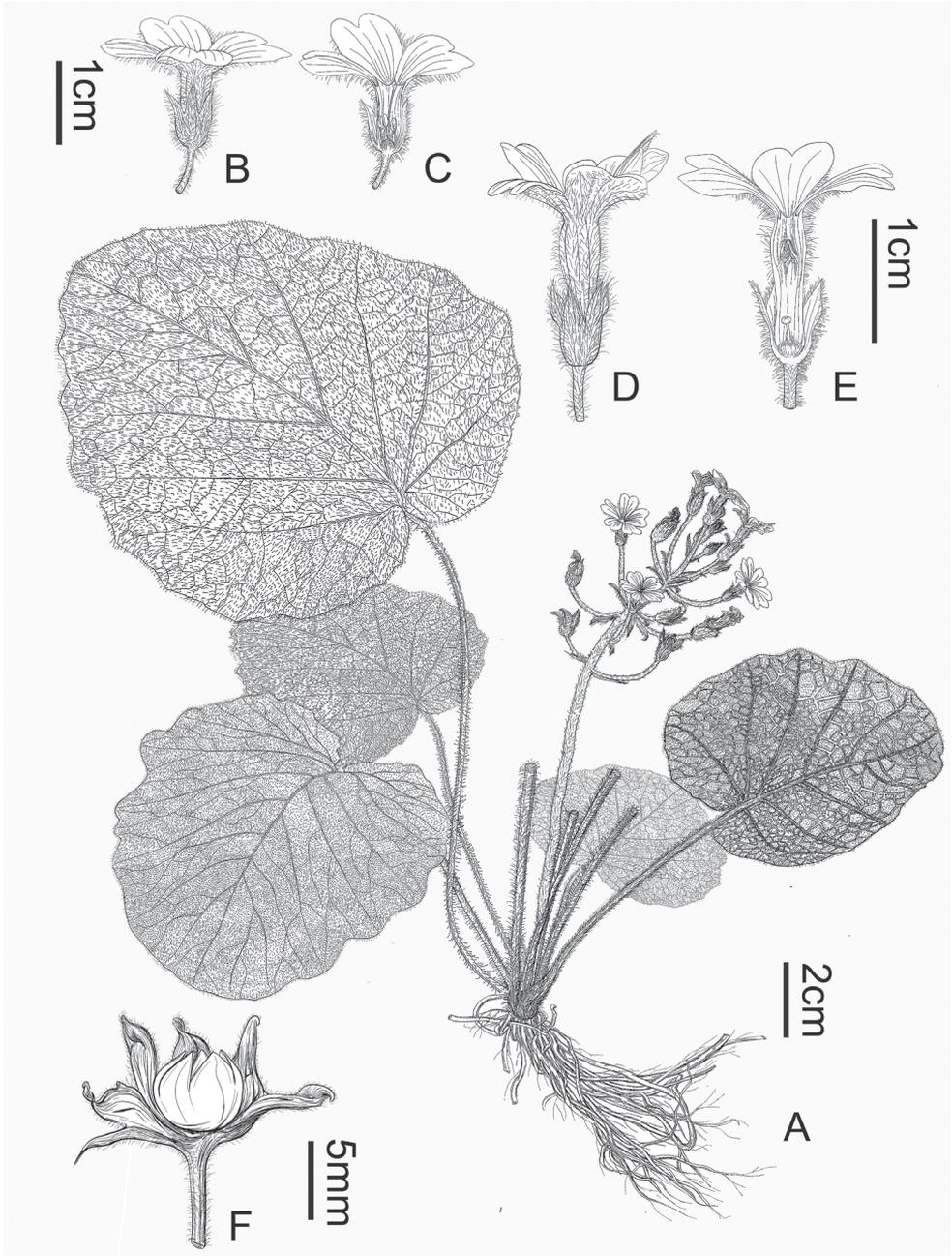


Figure 2. *Primula longipilosa* sp. nov. **A** habit **B–C** pin flowers **D–E** thrum flowers **F** capsule with dissected calyx. Drew by Dr. Yuan Luo.

Phenology. Flowering and fruiting from July to August.

Etymology. The specific epithet refers to the impressive long spreading white soft multicellular hairs on the whole plant.

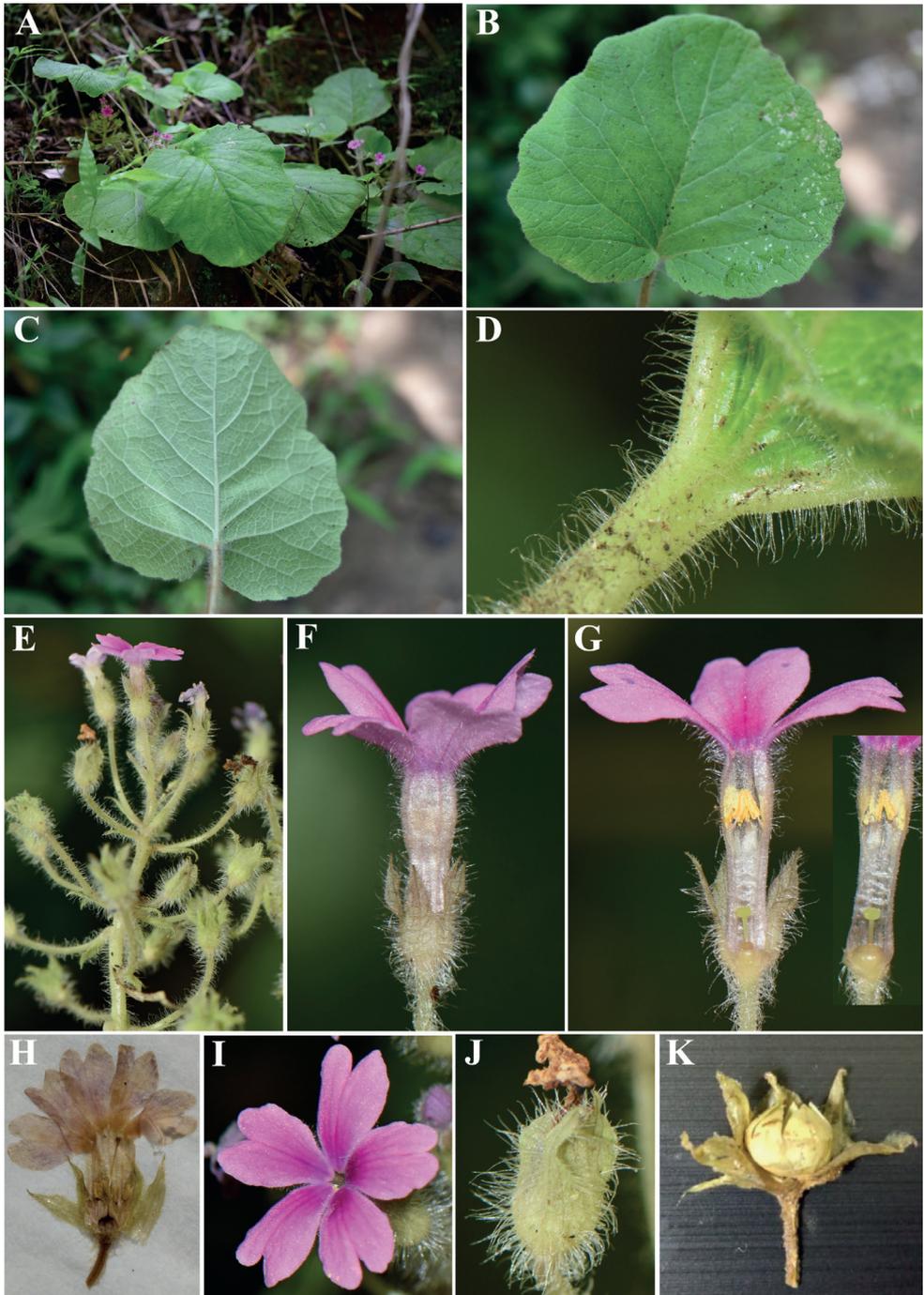


Figure 3. *Primula longipilosa* sp. nov. **A** habit **B** upper face of leaves **C** lower face of leaves **D** hairs on the petiole **E** racemose inflorescence **F–G** thrum flowers **H** pin flower **I** front side of corolla **J** calyx in late-flowering **K** capsule with dissected calyx. Photographed by Li Chen.

Table 1. Comparison of the morphological characters between *Primula longipilosa* and *P. mollis*.

Character	<i>P. longipilosa</i>	<i>P. mollis</i>
Leaf blade	margin undulate	margin sinuate-lobulate and denticulate-crenulate
Inflorescence	racemose, shorter than or almost equal to the leaves	umbellate, umbels 3–10, superimposed, significantly longer than leaves
Pedicel	covered with dense hairs	covered with sparse hairs
Calyx	tube green	tube deep red
Corolla	pink to pink rose	deep red
Stamens of Pin flowers	stamens at 1/3 length above the base of corolla tube	stamens at the middle of the corolla tube
Capsule	applanate globose	Ellipsoid

Vernacular name. Simplified Chinese: 长毛报春; Chinese Pinyin: Chángmáo Bào chūn.

Threat status. Currently, the authors have discovered only one population of *Primula longipilosa* with ca. 30 individuals from the type locality. Some plants grow very close to the path to face a stronger strength from human activities. However, as the authors conducted no detailed field survey for this new species in the adjacent districts, whether some other populations exist remains unknown. Considering its localized distribution in SW Yunnan, its status should nevertheless be of concern and addressed by further investigations.

Relationship with related species. According to the keys of *Primula* in Flora Reipublicae Popularis Sinicae (Hu 1990) and Flora of China (Hu and Kelso 1996), *Primula longipilosa* should be ascribed to the sect. *Cortusoides* by a combination of the following morphological characters: long soft multicellular hairs all over the plant; leaves with long stipe, base cordate or deeply cordate, margin undulate; Flowers heterostylous; Calyx narrowly campanulate, veins 3–5; Corolla pink to pink rose.

The molecular phylogenetic study also showed that *P. longipilosa* had a close relationship with the species of the sect. *Cortusoides*. It was most closely related to *P. mollis* of this section in terms of the long soft multicellular hairs all over the plant, the shape of their leaves and corolla, and also their distribution area. As there are some discrepancies or feature omissions in the description of *P. mollis* in different flora, we referred to its protologue and color illustration for the morphological comparison between them. The detailed morphological comparison between both species is shown in Table 1.

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Vaccinium bangliangense, a new species of Ericaceae from limestone areas in Guangxi, China

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Abstract

Vaccinium bangliangense, a new species from limestone areas in Guangxi, China, is described and illustrated. It is morphologically most similar to *V. pseudotonkinense* and *V. sciaphilum* in having small and dense obovate leaf blades with a retuse apex, hairy young branches and calyx and campanulate corollas, but can be distinguished from them by the distance of basal gland from petiole, the length of peduncle, pedicel and filaments, the indumentum of calyx tube and corolla and the existence of apical glands on calyx lobes. A table to distinguish the new species from other morphologically similar *Vaccinium* species, as well as colour plates of comparison of key characters, is also provided.

Keywords

limestone flora, morphology, new species, south-western Guangxi, *Vaccinium*

Introduction

The genus *Vaccinium* L. contains 450–500 species distributed worldwide (Fang 1991; Fang and Stevens 2005; Vander Kloet and Dickinson 2009). Currently, there are 98 species of *Vaccinium* known from China (Fang 1986; Fang and Stevens 2005; Tong and

Xia 2015; Tong et al. 2018, 2020, 2021a, b, 2022). As one of the most biodiverse regions of China, Guangxi has 27 species and two varieties of the genus *Vaccinium*, including four endemic species, viz. *V. damingshanense* Y.H. Tong & N.H. Xia, *V. napoense* Y.H. Tong & N.H. Xia, *V. crassivenium* Sleumer and *V. cuspidifolium* C.Y. Wu & R.C. Fang (Qin and Liu 2010; Tang 2011; Huang et al. 2015; Tong and Xia 2015; Tong et al. 2018, 2020).

During fieldwork in Bangliang Gibbon National Nature Reserve of Guangxi in June 2021, we discovered a special flowering plant of *Vaccinium* never recorded from Guangxi with the characteristics of inflorescence being shortly racemose, axillary or borne on leafless old stems, peduncle being very short or 4–5 mm long and corolla being broadly campanulate, yellowish-green or tinged reddish. After consulting Flora of China (Fang and Stevens 2005) and other relevant literature (Dop 1930; Pham 1999; Nguyen 2005; Newman et al. 2007; Qin and Liu 2010; Tang 2011; Tong and Xia 2015; Watthana 2015; Tong et al. 2020), as well as comparisons amongst this unknown species and its morphologically most similar species, based on herbarium specimens including types, we confirmed that this species is new to science, which is described and illustrated below.

Materials and methods

Field surveys have been conducted in flowering and fruiting phases at the type locality. Measurements and assessments of morphological characters were based on the living plants in the wild and the specimens gathered from the type locality. Type specimens were deposited in the herbaria of South China Botanical Garden (IBSC) and Guangxi Institute of Botany (IBK). The comparisons amongst this unknown species, *V. sciaphilum* C.Y. Wu and *V. pseudotonkinense* Sleumer were based on the descriptions from protologues and the examination of herbarium specimens or photos of specimens (including types) at IBK, IBSC, KUN and P (Sleumer 1941; Fang and Wu 1987). The habitat information and threatened factors were recorded during field surveys. The assessment of threatened status of the new species is based on the IUCN Red List of Threatened Species Categories and Criteria and Guidelines for using the IUCN Red List Categories and Criteria (IUCN 2012; IUCN Standards and Petitions Committee 2022).

Taxonomic treatment

Vaccinium bangliangense Y.S. Huang & Y.H. Tong, sp. nov.

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Figs 1, 2A–C, 3A, B, 4

Diagnosis. *Vaccinium bangliangense* Y.S. Huang & Y.H. Tong belongs to *V.* section *Conchophyllum* Sleumer (1941) and is morphologically similar to *V. pseudotonkinense* Sleumer and *V. sciaphilum* C.Y. Wu in having small and dense obovate leaf blades with a retuse apex, hairy young branches and calyx and campanulate corollas, but can be distinguished from the former by basal glands on leaf blade margin at 0.3–0.8 mm (vs.

Table 1. A morphological comparison of key characters of *Vaccinium bangliangense*, *V. sciaphilum* and *V. pseudotonkinense*.

Character	<i>V. bangliangense</i>	<i>V. pseudotonkinense</i>	<i>V. sciaphilum</i>
Distance of basal gland from petiole	0.3–0.8 mm	2.6–4 mm	0.8–1.7 mm
Inflorescence	2-flowered or solitary, peduncle very short or 3–5 mm long	solitary, sometimes 2-flowered, peduncle very short	solitary, sometimes 2-flowered, peduncle very short
Pedicel	5–7 mm long, glabrous	ca. 4 mm long, glabrous	ca. 3 mm long, densely pubescent
Calyx tube	glabrous or sparsely villous	glabrous or sparsely pubescent	densely hispid
Calyx lobes	glabrous or sparsely pubescent abaxially, margin ciliate, apex with a gland	glabrous or sparsely pubescent abaxially, margin ciliate and glandular, apex without a gland	densely hispid abaxially, margin ciliate, apex without a gland
Corolla lobes	pubescent abaxially at apex	glabrous	glabrous
Filaments	densely villous, ca. 2 mm long	sparsely pilose, ca. 1 mm long	glabrous, ca. 1 mm long
Ratio of anther thecae and tubule	1:2	1:2	1:1

2.6–4 mm, Fig. 3C) distance from petiole, inflorescence with very short peduncle or up to 5 mm long (vs. very short, Fig. 2E), calyx lobes with ciliate margin and a gland at apex (vs. with ciliate and glandular margin and without a gland at apex, Fig. 3D) and, from the latter, by inflorescence with very short peduncle or up to 5 mm long (vs. very short, Fig. 2G), longer (5–7 mm vs. ca. 3 mm) and glabrous (vs. densely pubescent, Fig. 2G) pedicel, glabrous or sparsely villous (vs. densely hispid) calyx tube, glabrous or sparsely pubescent (vs. densely hispid, Fig. 3F) calyx lobes with a gland at apex (vs. without a gland at apex, Fig. 2I) and densely villous (vs. glabrous) filaments. A detailed morphological comparison amongst the three species is summarised in Table 1.

Type. CHINA. Guangxi Zhuang Autonomous Region: Baise City, Jingxi City, Renzhuang Town, Bangliang protection station, campsite of Huitun, 850 m a.s.l., 2 June 2021, *S. Y. Nong & P. Yang ZYA00199* (holotype: IBSC!; isotypes: IBK!, IBSC!).

Description. Small evergreen shrubs, 10–20 cm tall, usually epilithic, sometimes epiphytic on tree trunks. Stem ascending, with long creeping rhizomes. Roots with bead-like swellings 1–3 cm in diam. Young branches brownish-red, grey when older, densely white villous, glabrescent. Leaves dense; petiole 1–2 mm long, red, adaxially sparsely pubescent, glabrescent, abaxially glabrous; blades obovate or broadly obovate, 1–1.7 × 0.6–1.2 cm, leathery, adaxially near margin sparsely villous when young, glabrescent, abaxially sparsely brown glandular hispidulous, base cuneate, basal gland 1 per side, at 0.3–0.8 mm distance from petiole, margin entire, revolute, apex obtuse, slightly retuse; mid-vein impressed adaxially, raised abaxially; lateral veins 2–4 pairs, flat and inconspicuous or impressed adaxially, raised abaxially. Inflorescence shortly racemose, 2-flowered or solitary, axillary or borne on leafless old stems; peduncle very short or 3–5 mm long, base with several bracts, glabrous or sparsely white villous on distal part; bracts ovate, 0.5–1 mm long, ca. 0.5 mm wide, margin ciliate, apex glandular; pedicel 5–7 mm long, glabrous, thickening towards the apex, articulate with the calyx tube; bracteoles 2, adnate to 1–3 mm

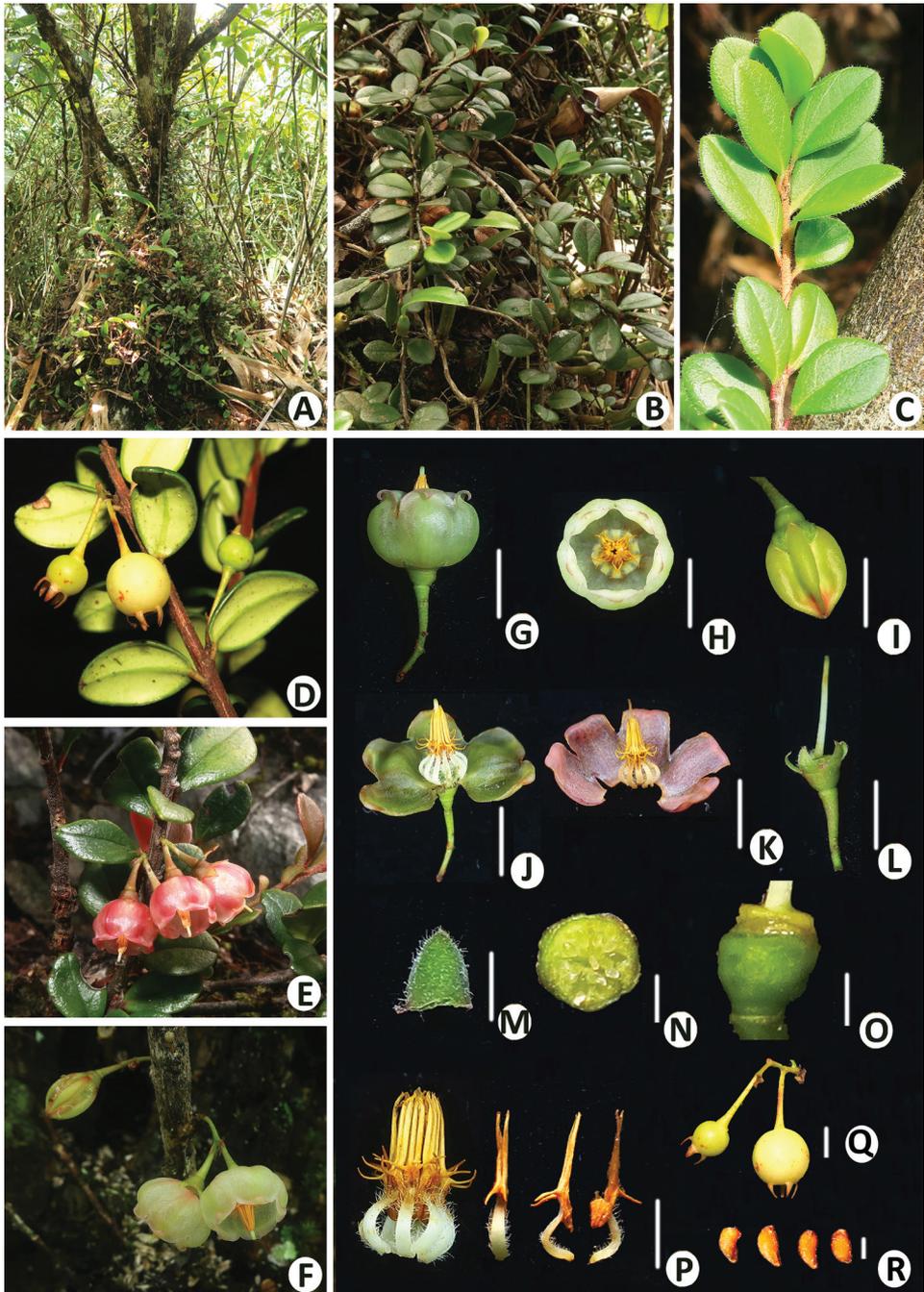


Figure 1. *Vaccinium bangliangense* **A** habitat **B** habit **C** young branch, showing the leaves with a sparsely villous margin **D** fruiting branch **E** flowering branch with tinged reddish flowers **F** inflorescence borne on leafless old stem with yellowish-green flowers **G** flower (lateral view) **H** flower (vertical view) **I** flower bud **J, K** flowers with opened corolla **L** flower with corolla and stamens removed **M** bracteole **N** transection of ovary **O** calyx tube and disc **P** androecium and adaxial (left), lateral (middle) and abaxial (right) view of a stamen **Q** infructescence **R** seeds. Scale bars: 5 mm (**G–I, P, Q**); 1 mm (**M–O, R**).

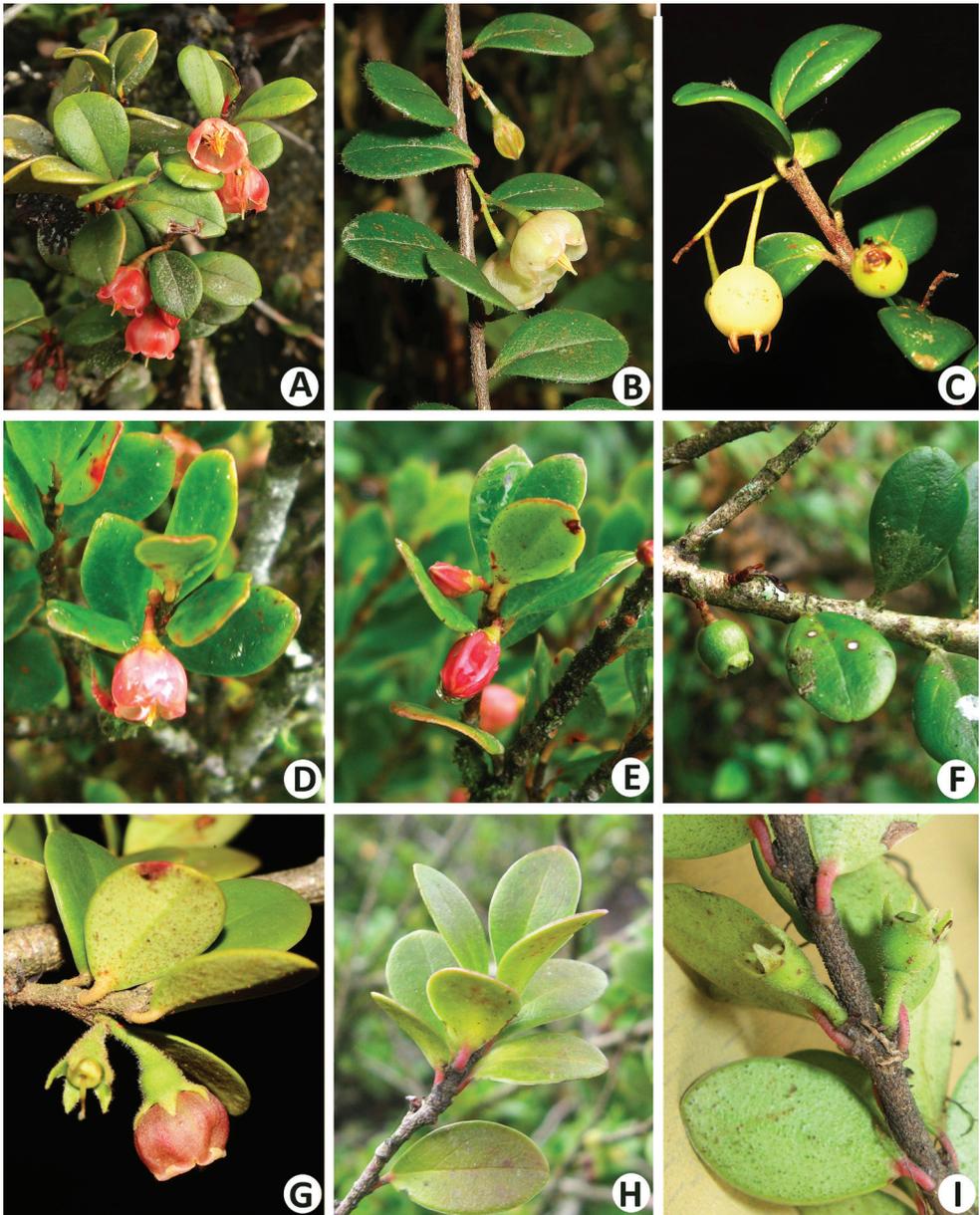


Figure 2. **A–C** *Vaccinium bangliangense* **A** flowering branch with tinged reddish flowers **B** flowering branch with yellowish-green flowers **C** fruiting branch **D–F** *V. pseudotonkinense* **D** flowering branch with opened flower **E** flowering branch with unopened flower **F** fruiting branch **G–I** *V. sciaphilum* **G** flowering branch **H** leafy branch **I** fruiting branch. All by Yi-Hua Tong, except **G** by Xin-Xin Zhu.

above the pedice base, triangular-ovate, ca. 1 × 0.5 mm, margin ciliolate, apex glandular; calyx tube green or purple green, obconical, ca. 1.5 × 2 mm, glabrous or sparsely villous; calyx limb divided nearly to the base; lobes 5, triangular-ovate, ca. 2 × 1.5 mm, both surfaces glabrous or sparsely pubescent abaxially, margin ciliolate, apex glandular; corolla

yellowish-green or tinged reddish, broadly campanulate, ca. 9×5 mm, both surfaces glabrous, 5-lobed; lobes triangular-ovate, apical part reflexed, apex acute, pubescent abaxially, glabrous adaxially; stamens 10, 5.5–6 mm long; filaments white, tinged reddish in reddish flowers, flat, incurved, ca. 2 mm long, densely villous; anthers yellow, 4–5 mm long, thecae ca. 1.5 mm long, tubules 3–3.5 mm long, with 2 spurs at the base abaxially, spurs ca. 1.3 mm long, interlocked (that is: the spurs on antesealous stamens extending laterally outside of antepetalous anthers and strongly overlapping with spurs of next antesealous stamens, those on antepetalous anthers strongly hooked outward below spurs of antesealous stamens); disc yellowish, annular, glabrous; style greenish, tinged reddish in reddish flowers, cylindrical, ca. 6.5 mm long, glabrous, stigma truncate; ovary pseudo-10-locular, each locule with several ovules. Berry globose, ca. 8 mm in diam., glabrous, greenish when young, white when mature, fruiting calyx persistent, narrowly triangular-ovate and slightly inflexed; seeds reniform, ventrally compressed, 1.5–2 mm long, testa brownish, cells elongated, with thickened anticlinal walls.

Phenology. *Vaccinium bangliangense* was observed flowering from May to June and fruiting from August to October (and up to January of the following year in indoor cultivated plants).

Etymology. The specific epithet is derived from the type locality, Bangliang Gibbon National Nature Reserve of Guangxi, China. The Chinese name is given as “邦亮越橘 (pinyin: bāng liàng yuè jú)”.

Distribution and habitat. Thus far, *Vaccinium bangliangense* was found only in Bangliang Gibbon National Nature Reserve of Guangxi, China. It usually grows on rocks of limestone hillside or peak at an elevation from 850–900 m, sometimes on the trunks of *Pistacia weinmanniifolia* J. Poisson ex Franchet (Anacardiaceae). The slope direction is to the south and the slope gradient is ca. 30° . The tree layer is up to 8 m tall with a canopy cover of 70% and the shrub and herb layer covers are 85% and 20%, respectively. The associated species include *Quercus phillyreoides* A. Gray (Fagaceae), *Sinosideroxylon pedunculatum* (Hemsl.) H. Chuang var. *pubifolium* H. Chuang (Sapotaceae), *Ardisia pseudocrispa* Pit. (Primulaceae), *Schefflera pesavis* R. Vig. (Araliaceae), *Tetradium calcicola* (Chun ex C.C. Huang) T.G. Hartley (Rutaceae), *Sageretia camelliifolia* Y.L. Chen & P. K. Chou (Rhamnaceae), *Paraboea swinhoei* (Hance) B.L. Burtt (Gesneriaceae), *Bonia amplexicaulis* (L.C. Chia et al.) N.H. Xia (Poaceae), *Bulbophyllum andersonii* (Hook. f.) J.J. Smith (Orchidaceae) etc.

Conservation status. *Vaccinium bangliangense* has only been found in Bangliang Gibbon National Nature Reserve of Guangxi, China. As a new species, more subpopulations of *V. bangliangense* could probably be found in similar habitats of surrounding limestone areas in the future. However, wild surveys have been conducted for more than ten years in the area where the new species was found. Only two subpopulations were found in the protected region with a total of fifteen individuals and seven of these are mature. Based on the current data, its population size is very small, and the area of occupancy (AOO) is restricted. According to Guidelines for Using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022), the conservation status of *V. bangliangense* should be assessed as Critically Endangered (CR), based on criteria D of (IUCN 2012).

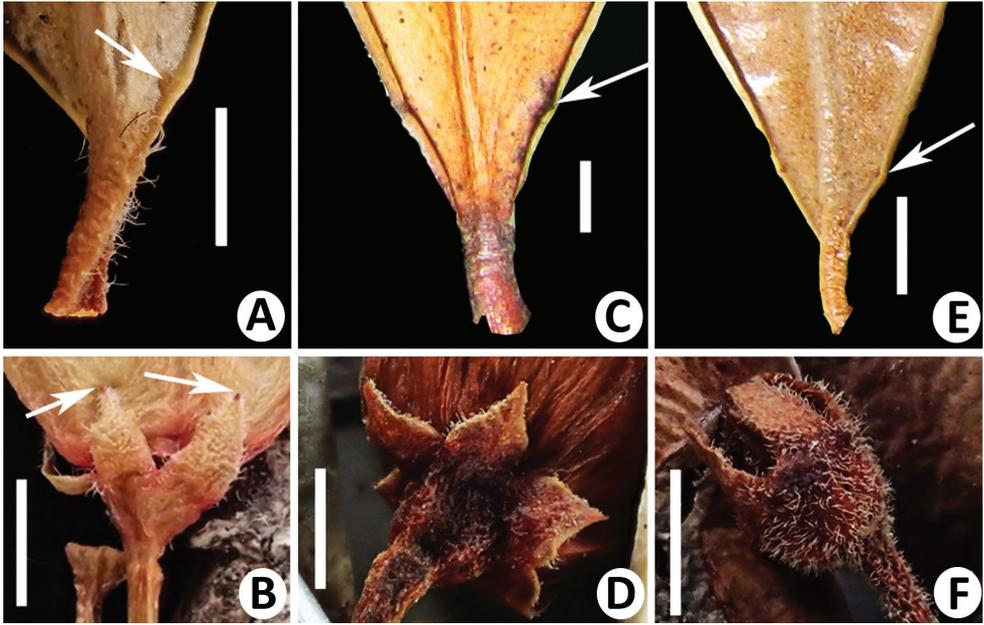


Figure 3. **A, B** *Vaccinium bangliangense* **A** basal gland **B** calyx, arrows showing apical glands **C, D** *V. pseudotonkinense* **C** basal gland **D** calyx, showing lobes with a ciliate and glandular margin **E, F** *V. sciaphilum* **E** basal gland **F** calyx. Scale bars: 2 mm.

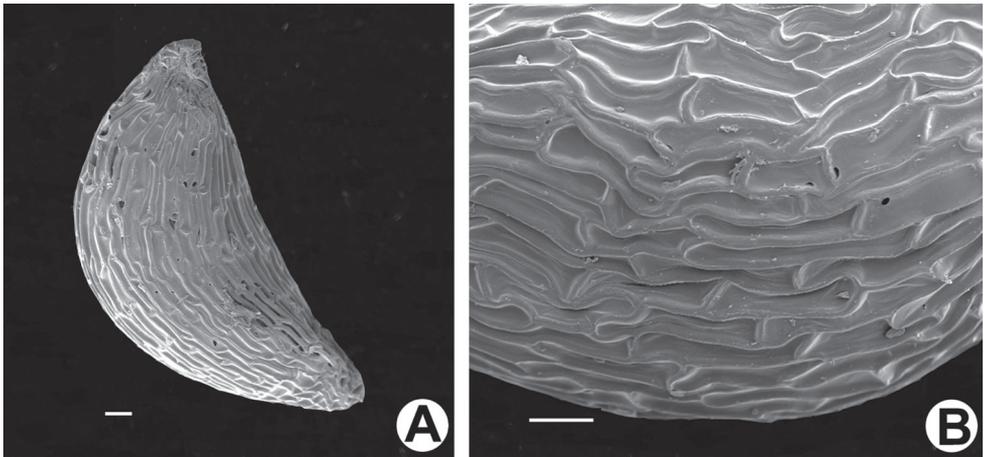


Figure 4. *Vaccinium bangliangense* **A** SEM micrograph of seed **B** elongated cells of testa. Scale bars: 100 μ m.

Additional specimens examined (paratypes). CHINA. Guangxi Zhuang Autonomous Region: Baise City, Jingxi City, Renzhuang Town, Bang Liang protection station, 900 m a.s.l., 2 June 2021, *S.Y. Nong & P. Yang* NSY2021060201 (IBK); Guilin City, cultivated in Botany Garden of Guilin, collected from the same locality as above, 16 January 2022, *Y.S. Huang* 2022011601 (IBK).

Discussion

In Guangxi, another species of *V.* sect. *Conchophyllum*, namely *V. triflorum* Rehder, is also somewhat similar to this new species in the small and dense leaves, short racemes and campanulate corollas, but can be readily distinguished by its thickly leathery and elliptic or obovate-elliptic leaf blades with a strongly rugose adaxial surface. The two species also have allopatric distribution in Guangxi: *V. triflorum* is distributed in Huanjiang County, north Guangxi, while *V. bangliangense* occurs in Jingxi County, southwest Guangxi.

Two kinds of flower colour of *Vaccinium bangliangense* were observed in the wild: the plants growing in shaded habitat always bear inflorescences with yellowish-green flowers and longer peduncles (Figs 1F, 2B), while inflorescences with tinged reddish flowers and shorter peduncles (Figs 1E, 2A) are normally found in sunlit habitat. In addition, the leaf blades of plants growing in shady habitat are thinner and with sparsely white villous margin (Fig. 2B), while those of plants growing in sunlit habitat are thicker and with less hairy or glabrous margins (Fig. 2A). It is speculated that these variations on flower colour, peduncle length and texture and indumentum of leaf blades may be caused by different light intensity.

Vaccinium bangliangense is a small shrub with a beautiful tree form and thus an excellent species for landscaping. It is adaptive to limestone areas and has important application value in limestone mountain greening. The first author once inserted one branch of this species into clear water for more than 3 months and surprisingly found that it grew new roots, which indicates that this plant is relatively easy to be cultivated.

Acknowledgements

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Rediscovery of *Gasteranthus extinctus* L.E.Skog & L.P.Kvist (Gesneriaceae) at multiple sites in western Ecuador

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Abstract

We report the rediscovery of the Critically Endangered cloud forest herb *Gasteranthus extinctus*, not seen since 1985. In 2019 and 2021, *G. extinctus* was recorded at five sites in the western foothills of the Ecuadorian Andes, 4–25 km from the type locality at the celebrated Centinela ridge. We describe

* These authors contributed equally to this work.

the species' distribution, abundance, habitat and conservation status and offer recommendations for further research and conservation efforts focused on *G. extinctus* and the small, disjunct forest remnants it occupies.

Keywords

Andes, Centinela, Chocó, cloud forest, endemic, extinction, iNaturalist, tropical forest

Introduction

Extensive deforestation in western Ecuador during the 20th century resulted in an alarming loss of habitat and the presumed extinction of a number of plant species with small geographic ranges (Dodson and Gentry 1991). *Gasteranthus extinctus* L.E.Skog & L.P.Kvist (Gesneriaceae) is a low terrestrial herb with uniformly bright orange flowers (Skog and Kvist 2000) and one of the 26 species of the genus currently known to occur in western Ecuador (Kvist et al. 2004). At the time of its description in 2000 (Skog and Kvist 2000), the only known records were four collections made between 1977 and 1985 in cloud forests at Centinela (Fig. 1 and Appendix 1, site 1), “an Andean foothill ridge... isolated from the main Andean range farther east by a broad, flat valley about 15 km wide” (Dodson and Gentry 1991: 277). Visited repeatedly by plant collectors in the 1970s and 1980s, Centinela became a celebrated site because of the dozens of apparently undescribed and endemic species in its flora (Gentry 1986a, 1986b, 1989; Gentry and Dodson 1987; Dodson and Gentry 1991; Dodson and Gentry 1993). These same publications reported that Centinela's forests had been completely destroyed and converted to farmland by the year 1990 and posited that a large number of its putative endemics had become extinct.

This hypothesis was amplified by E. O. Wilson's (1992) coining of the phrase ‘Centinelan extinction’ to describe geographically localised species that are driven to extinction by habitat destruction before they can be discovered or described. These reports prompted Skog and Kvist (2000) to give *G. extinctus* its dramatic specific epithet. They noted in the species description that “all four collections come from... [a] forest [that] has been totally cleared, likely causing the extinction of this species” (Skog and Kvist 2000: 67).

Around the time of the description, however, scientists began reporting that a large number of plant species once thought endemic to Centinela had been found at other sites (Pitman et al. 2000). Four years after describing *G. extinctus*, Kvist et al. (2004) themselves noted that five of the six *Gasteranthus* species considered Centinela endemics by Dodson and Gentry (1991) had been found elsewhere, leaving *G. extinctus* as the only remaining Gesneriaceae believed to be endemic to Centinela. During the same period, botanists who visited Centinela reported that small stands of forest still remained in the region (e.g. W. Alverson, pers. comm. to N. Pitman). Together, these lines of evidence supported the competing hypothesis that *G. extinctus* was potentially still extant, both at Centinela and elsewhere (Scheffers et al. 2011; Watson and Davis 2017).

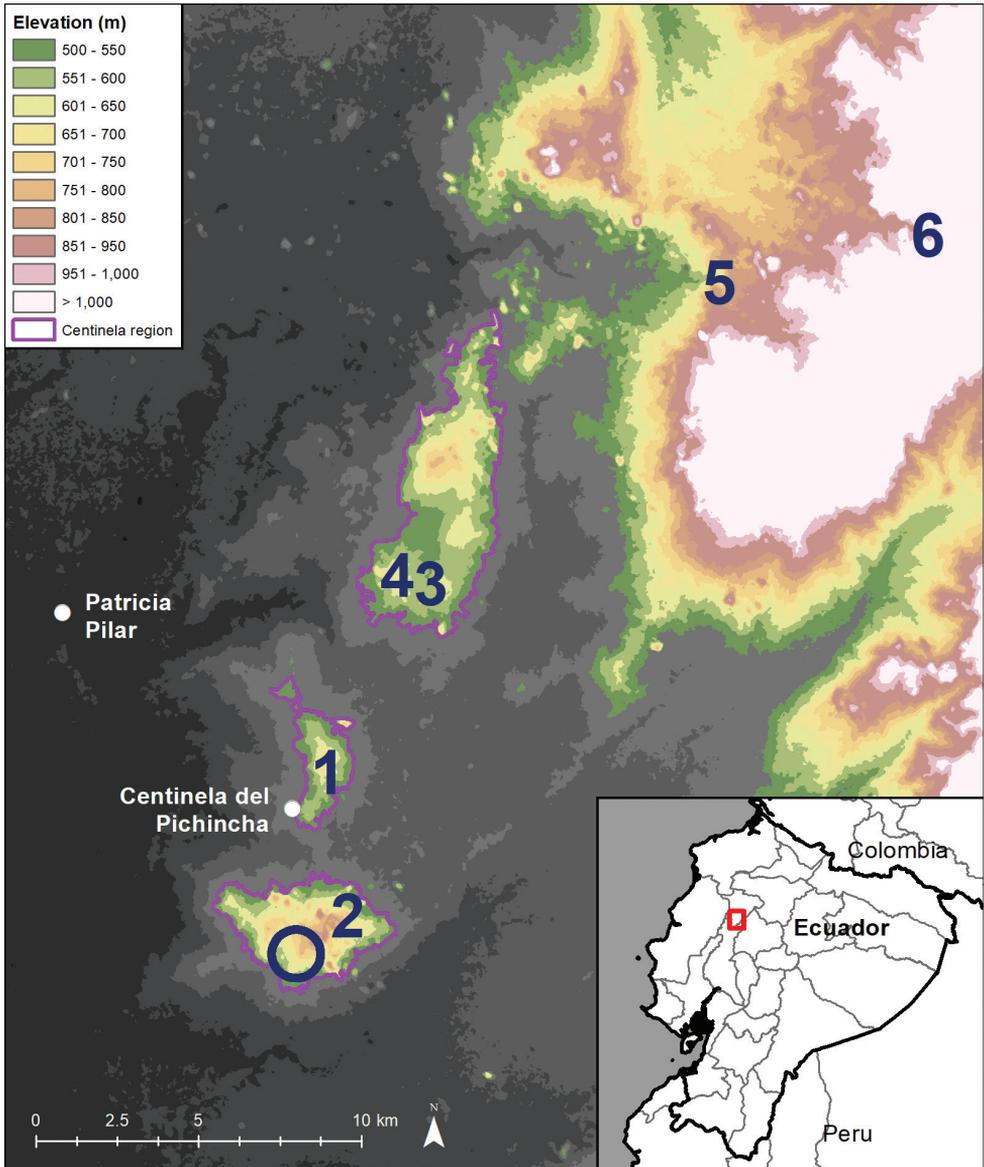


Figure 1. Map of the Centinela Region and documented localities of *Gasteranthus extinctus*. Numbers indicate the approximate locations of all populations confirmed to date, including the original collection locality (1) and the four sites where we observed the species in 2021 (2–5). Site 6 is from iNaturalist occurrence records. The hollow circle indicates a site where we searched for, but did not find *G. extinctus*. See Appendix 1 for a detailed description of each site. Inset: the placement of the Centinela Region in the Santo Domingo de los Tsáchilas Province (grey lines) in western Ecuador.

At least one previous targeted search failed to locate new populations. For three days in 2009, J. L. Clark searched a lowland site 7 km WNW of Centinela (the Río Palenque Science Center) and surrounding areas for *G. extinctus*. That search did not locate any extant forest fragments outside of Río Palenque.

Methods

In June–November 2021, we searched for *Gasteranthus extinctus* in three large Ecuadorian herbaria (QCNE, QCA, GUAY) and in GBIF (<https://www.gbif.org>) data from Ecuador, Colombia and Peru (DOIs: <https://doi.org/10.15468/dl.x7j8cj>, <https://doi.org/10.15468/dl.3anwv6> and <https://doi.org/10.15468/dl.ajrxp3>, respectively). Those searches revealed no records beyond those mentioned in the species protologue.

On 13–15 November 2021, we visited the Centinela Region to search for *G. extinctus* and other putative Centinela endemics (see Appendix 1 for notes on geographic names). Over three days of fieldwork, our 10-person team travelled the extensive network of rural roads by truck, searching for remnants of primary forest above 500 m. We observed > 20 such remnants (Fig. 2), most of them strips of forest along ravines or small (< 5 ha) patches on slopes too steep for the most common land uses in the region: dairy farming or plantations (mostly banana, balsa, *Gmelina arborea* and cacao). We also confirmed the existence of one remnant of well-preserved forest measuring at least 50 ha and large enough to maintain a population of the Ecuadorian mantled howler monkey (*Alouatta palliata aequatorialis* [Festa, 1903]). We were told another large remnant with howler monkeys exists in the northern part of the Centinela Region, south of Bellavista. These landscape observations will be reported elsewhere in greater detail.

Results and discussion

We spent 2–6 hours searching each of four remnant patches of forest in the Centinela Region and encountered *G. extinctus* (Fig. 3) at three of them (Fig. 1 and Appendix 1, sites 2–4). During the same dates, we also recorded *G. extinctus* at one site close to, but outside of, the Centinela Region, on the main flanks of the Andes (Fig. 1 and Appendix 1, site 5).

Identification of the plants was straightforward. All five diagnostic characters mentioned in the original description were evident in the observed individuals (Fig. 3): “1) urceolate [with a protruding pouch], relatively large corollas (2.8–4 cm long); 2) inflorescences with relatively short peduncles (maximally 4 cm long); 3) few flowers (2–4) per cyme; 4) a conspicuous pilose-villous indumentum on stems, inflorescences and calyces; and 5) fairly small, elliptic leaves (maximally 11 cm long)” (Skog and Kvist 2000: 67). Plants observed in the field closely resembled the original line drawing (Skog and Kvist 2000: fig. 26). The two *Gasteranthus* species mentioned by Skog and Kvist (2000)

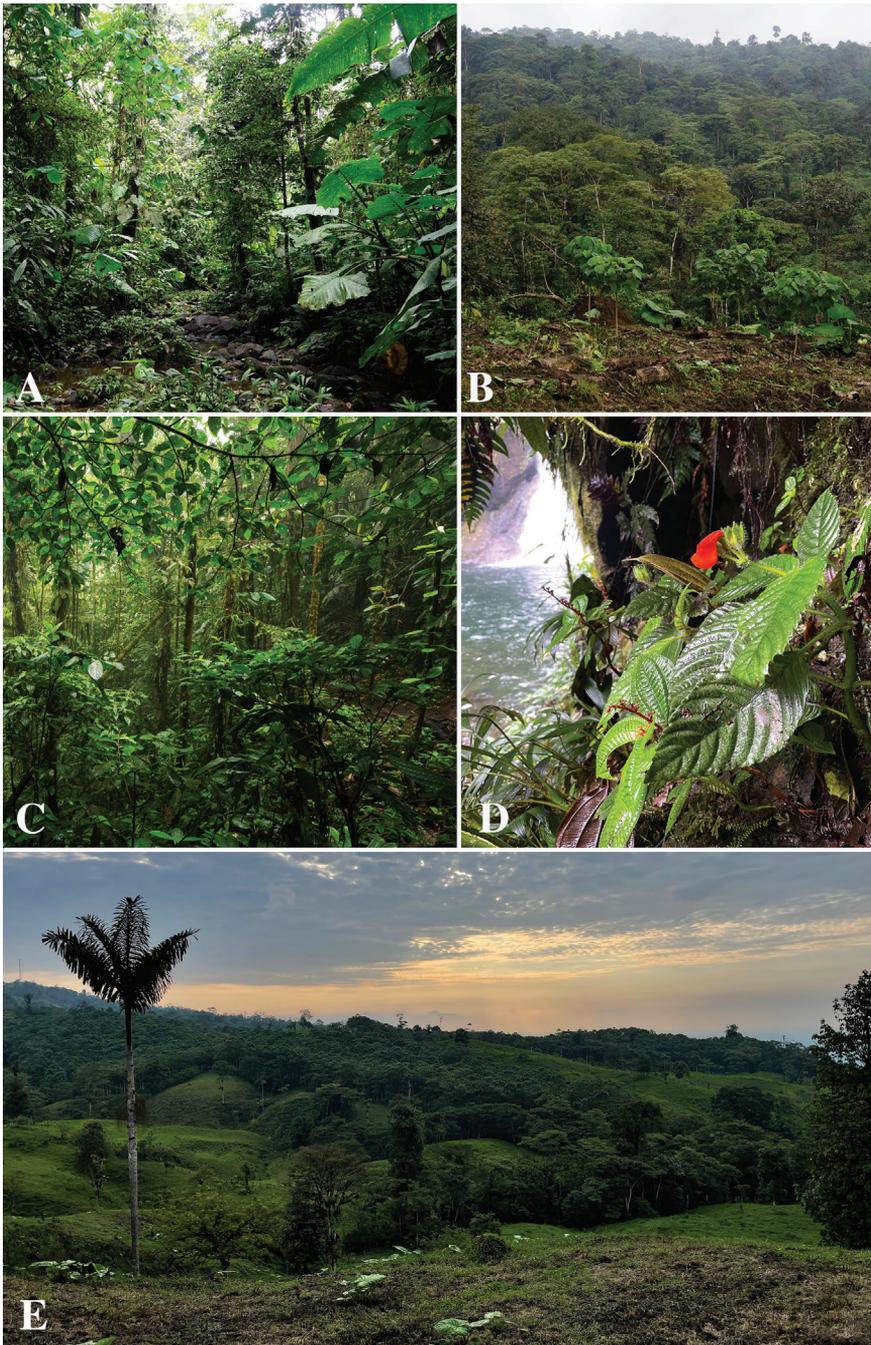


Figure 2. Field photographs of *Gasteranthus extinctus* habitats in the Centinela Region of western Ecuador **A** small stream where *Thomas Couvreur et al. 1502* was collected **B** recently planted *Gmelina arborea* plantation and forest **C** cloud forest understorey **D** *D. White et al. 830* river-side at Bosque y Cascadas Las Rocas **E** steep hills covered in a mosaic of cattle pasture, tree plantations and forest remnants. Photographs **A, B** by T.L.P. Couvreur **C** by R. Fortier **D** by D. White **E** by N. Pitman.

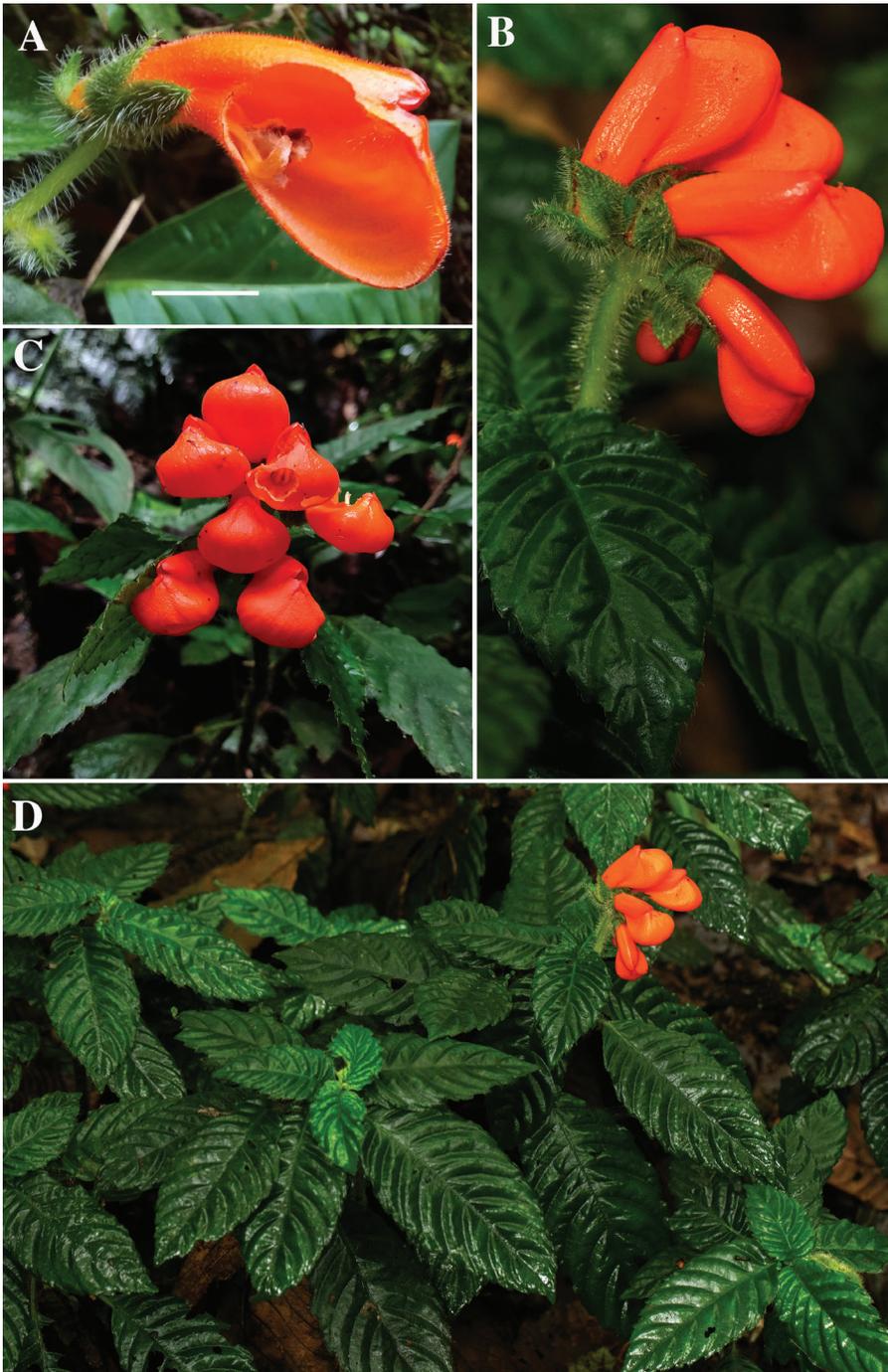


Figure 3. Field images of *Gasteranthus extinctus* **A** herbivore-damaged corolla showing inflorescence branches and calyx with conspicuous pilose-villous indument **B** relatively (< 4 cm) short peduncles **C** relatively fewer flowers (2–6) per inflorescence and pouched or hypocyrtoid corollas **D** elliptic leaves. Photographs **A** by X. Cornejo **B, D** by R. Fortier **C** by N. Zapata. Scale bar: 1 cm.

as most closely resembling *G. extinctus* (*G. calcaratus* Kunth (Wieler) and *G. mutabilis* L.E.Skog & L.P.Kvist) were easily eliminated as possibilities, due to our plants' conspicuous indumentum and pouched (or hypocyrtoid) urceolate corollas, respectively.

Field images of the plants were immediately shared with J.L. Clark, who was not part of the field team, but who is a taxonomic expert of Gesneriaceae with extensive knowledge of the flora of western Ecuador. By comparing the field images with the species description, an image of the holotype specimen (*C.H. Dodson 11595*, QCNE) and a field image taken of the species by C. Dodson in the 1980s, he confirmed that the plants were *G. extinctus*.

These populations were vouchered via five herbarium specimens under five different collector series (Appendix 1). The majority of these sheets will be deposited at four Ecuadorian herbaria (GUAY, QCA, QCNE and QUSF); duplicates will be deposited at herbaria outside of Ecuador (likely F, SEL, US, WAG and P). For all specimens, 1–3 leaves were stored in silica gel desiccant for genetic analysis; these were deposited at QUSF. Permits for herbarium voucher and DNA collection are listed in the acknowledgements section of this paper.

Field photographs will be linked with herbarium specimen databases and made available on GBIF. We have also posted three field photographs of *G. extinctus* on the community science platform iNaturalist (<https://www.inaturalist.org>; Appendix 1).

Additional records

After completing our fieldwork, we observed on iNaturalist three records predating our field work that showed flowering plants we recognised as *G. extinctus*. Identified as *Gasteranthus* sp., the three records were made on a single day (30 November 2019) by three iNaturalist users and two show the same plant. We were not able to determine the precise locality or localities of these records from the iNaturalist records alone. We contacted the users, three university students at Ecuador's Armed Forces University (ESPE) and learned that all three records were made at the Cascadas de Cristal Private Conservation Area near our site 5 (Fig. 1 and Appendix 1, site 6).

Habitat, abundance and phenology

Gasteranthus extinctus was neither rare nor common at the sites where we observed it. It was, however, conspicuous due to its large and brightly coloured orange flowers and relatively easy to find. At two of the sites where it occurred, we sighted *G. extinctus* within the first 10 minutes of exploration. In some places, the species occurred as isolated individuals and in others as small clumps (i.e. 10 individuals in an area of 4 m²). Nowhere we visited was *G. extinctus* the dominant species in the understorey, but at some sites (and at some smaller areas within the sites), it appeared to be the most common terrestrial Gesneriaceae.

The populations of *G. extinctus* which we observed suggest a very broad environmental tolerance. We found individuals inside well-preserved forest and in cattle

pastures just outside of forest; in deeply shaded understorey and in sunny open conditions; on soil with few to no rocks, on soil mixed with small rocks and on vertical rock walls near waterfalls; close to streams and far from them; and from 520 to 990 m elevation. The most commonly observed habitats were steep to vertical walls of damp soil along ravines, within 10 m of streams or rivers, inside relatively well-preserved forest (Fig. 2A–D).

In the places *G. extinctus* occurred, it was accompanied by a number of terrestrial aroids, ferns (*Diplazium*, *Danaea*, *Asplenium*, *Dennstaedtia*, *Tectaria*) and other Gesneriaceae (most conspicuously *Gasteranthus corallinus* (Fritsch) Wiehler). In habitats on steeper slopes, some of the common and conspicuous trees we recorded around *G. extinctus* populations were *Carapa megistocarpa* A.H. Gentry & Dodson, *Talisia equatoriensis* Acev.-Rodr., *Faramea* sp., *Quararibea* sp., *Swartzia decidua* Torke & Á.J.Pérez, *Eschweilera rimbachii* Standl., *Eschweilera awaensis* S.A.Mori & Cornejo, *Browneopsis macrofoliolata* Klitg., *Socratea rostrata* Burret and *Wettinia quinaria* (O.F.Cooke & Doyle) Burret. In habitats on less rugged topography, other conspicuous woody elements included *E. rimbachii*, *Carpotroche platyptera* Pittier, *Bauhinia pichinchensis* Wunderlin and numerous species in the genera *Inga*, *Matisia*, *Faramea* and *Jacaratia*. Common epiphytes included the orchids *Sobralia valida* Rolfe, *Platystele acutilingua* Kapuler & Hascall, *Scaphosepalum* sp., *Lepanthes* sp. and the bromeliads *Guzmania wittmackii* (André) André ex Mez and *Guzmania rhonhofiana* Harms. Field photographs of several other plant species that co-occur with *G. extinctus* at Centinela are accessible at <https://www.inaturalist.org/projects/flora-of-centinela>.

The original description notes that flowering specimens were collected in July, August and October (Skog and Kvist 2000). All new records reported here were flowering in November. We did not observe fruits, whose phenology and specific description remain unknown to science, but which are presumed to be laterally compressed semi-fleshy capsules like all other *Gasteranthus*.

Conservation status

Gasteranthus extinctus is currently considered Critically Endangered, both globally (Clark et al. 2004) and in Ecuador (León-Yáñez et al. 2011). The newly-discovered populations necessitate a reassessment of the species' threat status. We analysed our six unique occurrences with the R package ConR (Dauby et al. 2017) and identified two subpopulations and four locations, based on a 10-km radius for equal impact. We estimated an area of occupancy (AOO) of 106 km² and an extent of occurrence (EOO) of 20 km² (grid resolution = 2 km). However, the species' habitat is severely fragmented within this area. These values would place *G. extinctus* in the Endangered (EN) category (AOO < 500 km², EOO < 5000 km², locations < 5). The massive scale of the habitat loss since its discovery and the lack of formal protection means that the 'B' criterion applies, resulting in a new proposed assessment of EN B1(a,b(ii,iii,iv)) +B2(a,b(ii,iii,iv)).

However, our field observations offer a measure of optimism regarding the plant's conservation prospects. Most importantly, it appears that significant populations may occur within private conservation areas (Appendix 1, sites 5–6). Others could potentially occur in the Murocomba Protection Forest; this requires confirmation. The species' broad habitat tolerance, preference for ravines and ability to grow on sheer rock walls mean that there is a relatively large amount of high-quality habitat in the Centinela Region and on the nearby flanks of the Andes that is unlikely to disappear even with continued deforestation.

We did not collect live specimens of *G. extinctus*. Given its broad environmental tolerances, however, the species appears to be an excellent candidate for *ex situ* conservation. Observations of other streamside species of Gesneriaceae, native to western Ecuador (Ertelt 2013), suggest that *G. extinctus* likely possesses root-shoot vegetative propagation and might be easily propagated *ex situ*. However, the plant's striking appearance also puts it at risk of unsustainable harvesting and trafficking of live specimens by commercial plant collectors (Lavorgna et al. 2018).

Conclusion

A short burst of targeted fieldwork demonstrated that *Gasteranthus extinctus*, long considered both extinct and endemic to the Centinela Region, is in fact neither. The ease with which it was located at four sites in three days suggest that the species has a larger population and geographic distribution than previously recognised. Likewise, its broad tolerance of environmental conditions suggests relatively high frequency even in a massively modified landscape like this one. This implies a global population size in the thousands, at least several dozen individuals of which would appear to grow inside a formally-protected area. These field observations suggest that *G. extinctus*, while still meriting globally Endangered status, might be capable of persisting *in situ* even if the largest forest fragments in the region are not conserved.

Our work with *G. extinctus* underlines the urgency of targeted fieldwork to assess the conservation status of the dwindling forest fragments throughout western Ecuador and of the range-restricted plant species that depend on them. On the research front, what is needed is a comprehensive update of Dodson and Gentry's (1991) survey of biological extinction in western Ecuador, backed by satellite imagery analysis, field surveys of remnant forests and field, herbarium and modelling work to understand the current status and distribution of endemic species. That research should also include newly-available tools to characterise extinction risk and effective population size, such as metabolomic analyses (Wetzel and Whitehead 2020) and population genomics (Wang et al. 2016). We also call on researchers to update Ecuador's *Red List of Endemic Plants*, a vital resource for Ecuador's large endemic flora, last published a decade ago (Valencia et al. 2000; León-Yáñez et al. 2011).

On the conservation front, it is now clear that published reports of the total destruction of Centinela's world-famous cloud forests were premature. Significant

remnants of relatively healthy, intact forest persist in the Centinela Region. None of these remnants is formally protected and all of them are vulnerable to conversion to pasture or plantations in the near future. Especially in the southernmost, highest-elevation portions of the Centinela Range, a concerted campaign of land protection and habitat restoration has the potential to protect a contiguous, > 100-ha block of Centinela cloud forest. Success will require coordinated efforts by local landowners, Ecuadorian government agencies, conservation NGOs and other stakeholders to ensure the long-term persistence of these remnants and the *G. extinctus* populations they harbour.

Acknowledgements

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Appendix I

A list of sites with documented occurrences of *G. extinctus* and notes on toponymy at the study site in western Ecuador. For each site, we provide a description of the locality, notes on *G. extinctus* abundance and habitat preferences, herbarium vouchers and iNaturalist occurrences. Coordinates are not provided due to the species' global conservation status of Critically Endangered. Approximate localities are given in Fig. 1. Leaf samples for genetic analysis are stored in the QUSF Herbarium reference collection in the custody of GR-T.

Site 1. Original collections (1977–1985)

Dates: 11 August 1977, 17 August 1978, 4 October 1981, 7 July 1985

Elevation: 600 m

Site description: The exact localities of the earliest collections of *G. extinctus* are not known. The type specimen label describes the location as: “Los Ríos or Pichincha: Montañas de Ila, cloud forest along ridge line near La Centinela, Km 12, on road from Patricia Pilar to Flor de Mayo, 600 m.” We believe that these collections were made on the ridge above and continuing north from the community of Centinela del Pichincha, Santo Domingo de los Tsáchilas Province, which is 12 km from Patricia Pilar (Fig. 1). We do not know where on the ridge the collections were made, nor if they were made at one or multiple locations. We did not explore this ridge during the 2021 fieldwork.

Vouchers: *Calaway H. Dodson 11595* (holotype), *Calaway H. Dodson & T. A. Dodson 6809*, *Calaway H. Dodson & T. A. Dodson 15867*, *Calaway H. Dodson, T.A. Dodson & Alvin Embree 7117*

Site 2.

Date: 13 November 2021

Elevation: 659 m

Distance from Site 1: 4.5 km

Site description: This site was located near the top of the road to the Corporación Nacional de Telecomunicaciones antennas at the highest part of the Centinela Range, in a neighbourhood known to local landowners as Bijagual. We searched a fragment of forest along a high ridgeline directly east of the road and did not find *Gasteranthus extinctus* there. We then descended a steep meadow to the south of that fragment until reaching a small creek running west to east. The creek was bordered by a thin strip of old and secondary forest, with cattle pasture to either side. We found three flowering individuals of *G. extinctus* in the pasture – two to the south and one to the north – and one flowering individual in the dark understorey of the creek forest.

Vouchers: No herbarium voucher was collected due to the limited number of individuals; a silica-dried leaf sample is stored at QUSF; <https://www.inaturalist.org/observations/101229701>, <https://www.inaturalist.org/observations/103371741>

Site 3.

Date: 13 November 2021

Elevation: 520 m

Distance from Site 1: 6.6 km

Site description: This fragment of relatively well-preserved forest is close to the hamlet of San Pedro de Pambil and mainly surrounded by *Gmelina arborea* plantations. Two close, but distinct populations were seen at this site: the first with several individuals (10–15) alongside a stream and the second higher up the slope with fewer individuals (3–4).

Vouchers: *Thomas Couvreur et al. 1502* (to be deposited at P, QCA, SEL [078639], WAG, US)

Site 4.

Date: 14 November 2021

Elevation: 620 m

Distance from Site 1: 6.5 km

Site description: This site, which is ~ 1 km from Site 3, is surrounded by a narrow strip of secondary forest that transitions into a *Gmelina arborea* plantation. The fragment of relatively well-preserved forest is characterised by numerous steep ravines and small streams. *G. extinctus* was seen growing on walls of bare soil above the streambeds and also on flatter parts of the secondary forest.

Vouchers: *Nigel Pitman et al. 11201*, *Nigel Pitman et al. 11202*, *Riley Fortier et al. 210* (to be deposited at F, GUAY, QCNE, SEL, US), <https://www.inaturalist.org/observations/101761531>

Site 5. Bosque y Cascadas Las Rocas

Date: 15 November 2021

Elevation: 665 m

Distance from Site 1: 19.3 km

Site description: Bosque y Cascadas Las Rocas Private Conservation Area is a privately owned Reserve that protects a primary, humid cloud forest remnant along the Bolo River, near the town of Polanco. We observed *G. extinctus* growing in several clustered populations. One population was adjacent to the main trail, growing on a steep embankment in rich soil with no visible rocks. The other population was growing on almost bare rock next to a waterfall, where it likely receives mist from the falls for most of the day. Another individual was observed on a cliff face adjacent to a tall waterfall.

Vouchers: *Dawson White et al. 830* (to be deposited at QCNE, US), <https://www.inaturalist.org/observations/103420808>, <https://www.inaturalist.org/observations/103420715>

Site 6. Cascadas de Cristal

Date: 30 November 2019

Elevation: 990 m (estimated from imagery)

Distance from Site 1: 24.8 km

Site description: Cascadas de Cristal is a privately owned Reserve with a protected forest remnant near the town of Los Ángeles, above the Río Otongo. Observations of *G. extinctus* at this site were made by Josselyn Lizbeth Chacón Ibarra, María Gabriela Sánchez Nicolalde and Marianela Frias in November 2019. Various plants were observed in the forest understorey along the trail to the waterfalls.

Vouchers: <https://www.inaturalist.org/observations/36478271>, <https://www.inaturalist.org/observations/36473651>, <https://www.inaturalist.org/observations/36415279>

Notes on the toponymy of Centinela and Montañas de Ila:

During our visit, we learned that neither of the two names historically used by plant collectors to describe this region are commonly used by local residents. The name ‘Centinela’ is locally used to refer to the small settlement of Centinela del Pichincha, a 12-km drive east of Patricia Pilar, which Dodson and Gentry appear to have used as a base from which to climb up to what they called ‘Centinela Ridge’ or ‘La Centinela’ (see Site 1 above and in Fig. 1). The name ‘Montañas de Ila’ was not familiar to anyone we spoke with during our fieldwork; residents typically referred to individual settlements and neighbourhoods. Until a more precise name can be determined for the broader mountain range, we use ‘the Centinela Region’ to describe the ~ 40-km² landscape above 500 m elevation (the polygons outlined in purple in Fig. 1).

Taxonomic revision of the peculiar genus *Xylopodia* (Loasaceae) with a new species from Argentina and Bolivia demonstrating an atypical trans-Andean disjunction

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Abstract

Loasaceae subfam. Loasoideae are a nearly exclusively American plant group with a center of diversity in Peru. Numerous new taxa have been described over the past decades; one of the most striking discoveries was that of the narrowly endemic *Xylopodia* with the single species *Xylopodia klaprothioides* in Peru, Dpto. Cajamarca in 1997. Surprisingly, field studies in the past years have resulted in the discovery of material clearly belonging to the same genus in both Bolivia and northern Argentina, approximately 1500 km SE of the next known population of *Xylopodia* in Contumazá, Peru. A closer examination shows that Argentinian and Bolivian material belongs to a single species, clearly different from *Xylopodia klaprothioides*. We here describe *Xylopodia laurensis* and the entire genus is revised. Both species are illustrated, all aspects of their biology and ecology are portrayed and their threat status is discussed.

Keywords

Andes, Argentina, Bolivia, endemism, Loasoideae, taxonomy, *Xylopodia*

Introduction

Loasaceae are a largely Neotropical family with a center of diversity in the Central Andes of Peru and neighboring Ecuador. This is particularly true for the most diverse subfamily Loasoideae (>200 spp.) and its most speciose genus, *Nasa* Weigend (>100 spp., Weigend 2004a; Henning et al. 2019). Loasaceae of southern South America are relatively well understood with several recent compilations and systematic studies available (Weigend 2007; Weigend et al. 2008; Weigend and Ackermann 2015; Acuña-Castillo et al. 2017). Most recently, a national treatment for the flora of Argentina was completed (Acuña-Castillo et al. 2021). Phylogenetic studies have largely resolved the major lineages of Loasaceae (Weigend et al. 2004; Acuña-Castillo et al. 2017; Acuña-Castillo et al. 2019) including many of the recently discovered taxa. One of the most striking discoveries of the past decades was the narrowly endemic genus *Xylopodia* Weigend, a peculiar subshrub with a thick, persistent, woody rhizome (xylopodium) and short-lived erect flowering branches. The taxon was discovered in two populations, restricted to northern Peru near the town of Contumazá (Dpto. Cajamarca, Prov. Contumazá), a region long known for its rich and diverse flora (Figs 1, 4C). Based on the characteristic morphology (especially tetramerous flowers and petals with adaxial lamellae) the plant was recognized as a close ally of widespread *Klaprothia* Kunth as member of the tribe Klaprothieae (Weigend 2006). Phylogenetic studies confirmed a close and well supported relationship between these two genera with Acuña-Castillo et al. (2019) dating the split between *Xylopodia klaprothioides* Weigend and its sister group (*Klaprothia* + *Plakothira* J. Florence) to the mid-Oligocene (ca. 28 Ma). Although definitively part of Loasoideae, the relationships of Klaprothieae to other Loasoideae are still uncertain, although low support has been retrieved for a relationship with African *Kissenia* R. Br. ex Endl. (Acuña-Castillo et al. 2019). Since its discovery in 1997, *Xylopodia klaprothioides* (Fig. 2) has been considered a rare paleoendemic, that survived only in a single spot on the western Andean slope in northern Peru, part of the so-called Amotape-Huancabamba-Zone, a region of extraordinary species-richness including many old relic species, disjuncts and massive recent radiations (Weigend 2002, 2004b; Weigend et al. 2005; Mutke and Weigend 2017). However, recent field studies by one of us (CM) have led to the discovery of plants that can be clearly assigned to the genus *Xylopodia* in Argentina, Prov. Jujuy, approximately 2,500 km from the closest known population in northern Peru. Also, field studies in Bolivia yielded additional sightings and collections of *Xylopodia* from both the Dptos. of La Paz (as far back as 2017 <http://legacy.tropicos.org/Image/100494035>) and Chuquisaca (in 2019) (Fig. 1). High-quality photographs of the living plants permitted a close comparison of the material from Peru, Argentina, and Bolivia, leading to the conclusion that the southern material can be assigned to a single species, distinct from,

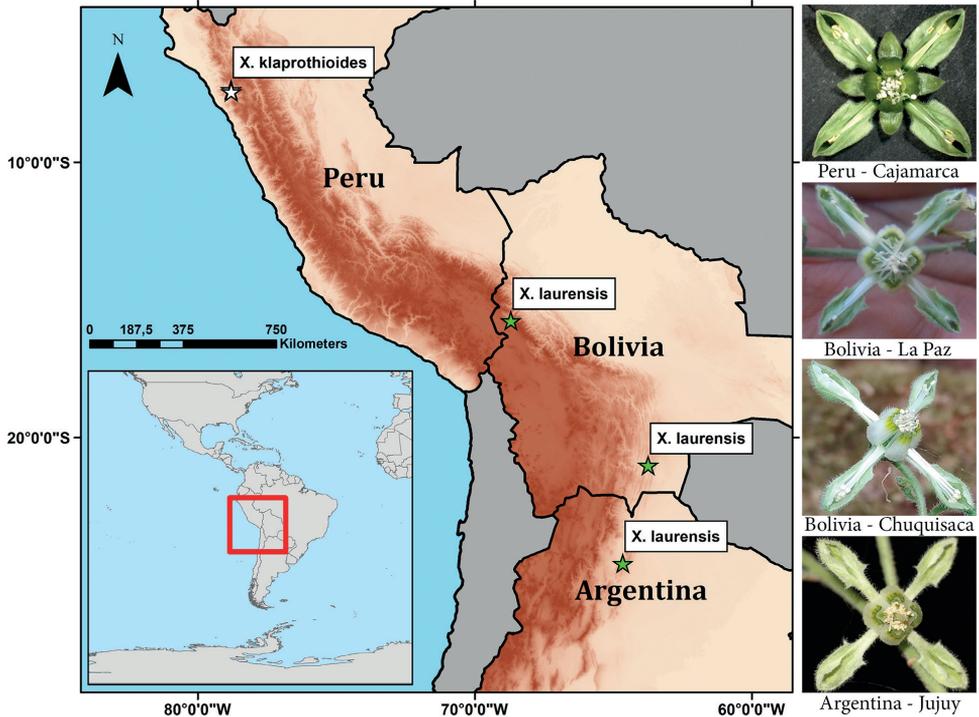


Figure 1. Known distribution of *Xylopodia klaprothioides* and *X. laurensis* (photo credit for La Paz: A.F. Fuentes).

but similar to Peruvian *Xylopodia klaprothioides*. Based on these findings, we describe a new species of *Xylopodia*. Its ecology, habitat, and distribution are discussed and its conservation status assessed. Lankester Composite Dissection Plates (LCDP) have been prepared to compare and document the most important morphological traits of both known species of this poorly-known and poorly-collected genus.

Materials and methods

Field studies were carried out in 2019 and 2021 in Chuquisaca, Bolivia and in 2021 in Jujuy, Argentina. Photographs of the plant habit and macro-morphological characters were taken in the field with a Canon EOS 7D. Morphological observations and measurements of the new species were obtained from living and dry specimens from Argentina and Bolivia. Micro-morphological characters were observed and photographed with a Leica EZ4 stereomicroscope. Argentinian and Bolivian specimens are deposited in SI and USZ respectively. The comparison with Peruvian *X. klaprothioides* was carried out using photographs and specimens from Bolivia, Argentina and Peru including the living type collection (Weigend *et al.* 97/450 – F, M, USM) of *X. klaprothioides* at Bonn University Botanic Gardens. Likewise, we studied previous *X. klaprothioides* collections,

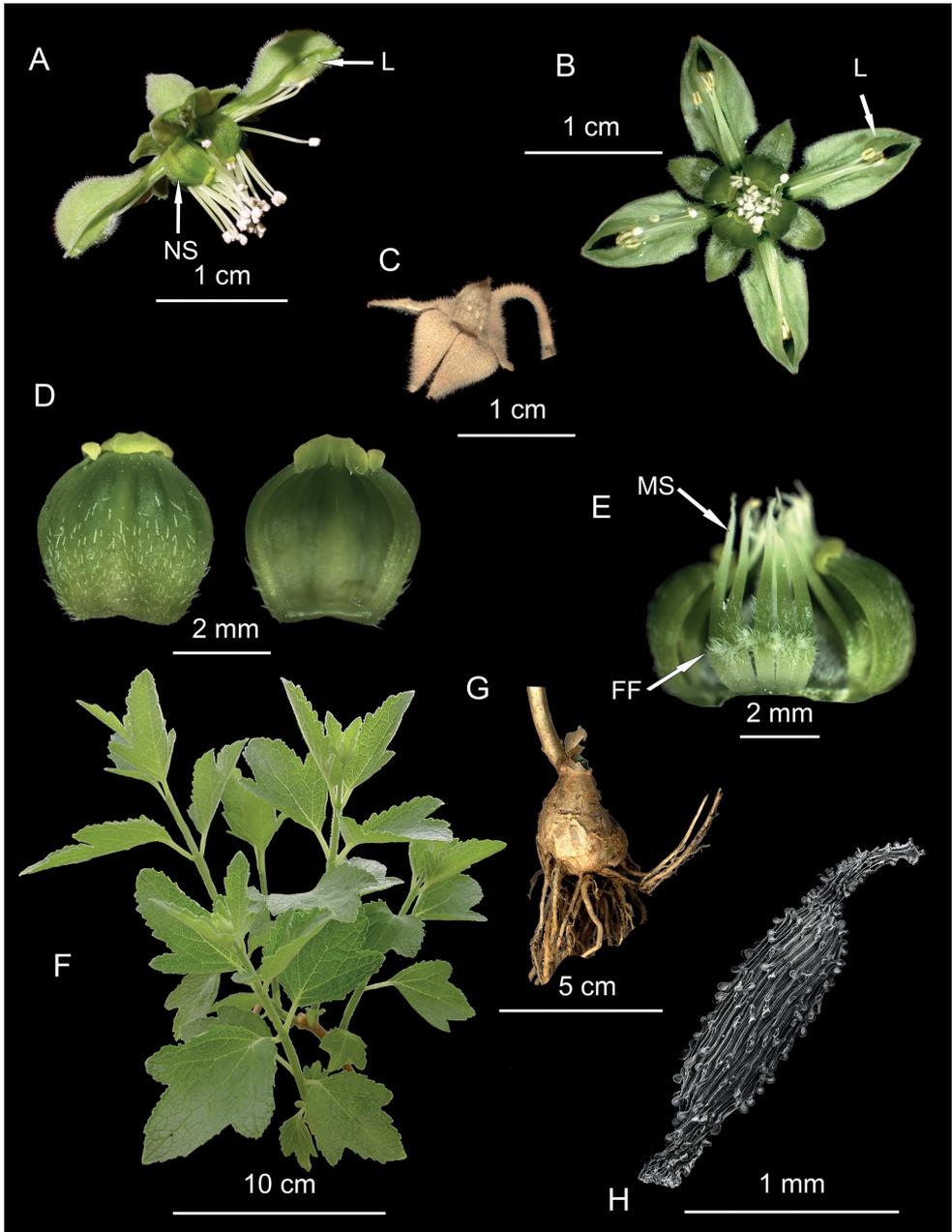


Figure 2. *Xylopodia klaprothioides* **A** flower, lateral view, showing the lamellae (L) and nectar scales (NS) **B** flower, frontal view, showing the lamellae (L) **C** mature fruit with slightly opened apical valves **D** nectar scale in dorsal (left) and ventral (right) views **E** nectar scale complex with one scale removed to reveal the internal monomorphic staminodes (MS) and the inconspicuous filament flanges (FF) **F** sterile branches with mature leaves **G** xylopodium of a 2-year-old plant **H** seed. All from Weigend et al. 971450.

now deposited in B, F, HUT, M and USM (all made by MW, TH and their field companions since 1997). The listed herbarium acronyms follow the Index Herbariorum abbreviations (Thiers 2021). The conservation status was assessed using GeoCAT (Bachmann et al. 2011). The photographic plates showing different parts of living plants, are adapted from the LCDP-technique developed by Pupulin and Bogarín (2004). The colored SEM images for figure 5 were prepared by H.J. Ensikat (Bonn) in the course of studies on the biomineralization of trichomes in Loasaceae. For methodological details see Ensikat et al. (2017). The distribution map was prepared using ArcGIS.

Taxonomic treatment

Xylopodia Weigend, *Taxon* 55: 467. 2006.

Type species. *Xylopodia klaprothioides* Weigend.

Description. Shrubs with erect branches from horizontal xylopodia; aerial vegetative organs densely hairy, with scabrid and glochidiate trichomes. Leaves simple, opposite, estipulate, petiolate; lamina with 1–3 lobes on each side, margin serrate. Inflorescences terminal dichasia, each flower with 2 bracts. Flowers tetramerous, deflexed. Sepals triangular. Petals cymbiform, greenish-white to green, aestivation valvate. Nectar scales 4, antesepalous, white and green or green and yellow, formed by 4–6 fused staminodes. Free staminodes 4–5, opposite and internal relative to the nectar scale. Fertile stamens arranged in 4 antepetalous groups. Ovary semisuperior with 4 placentae. Fruits semisuperior capsules, subglobose, straight, opening with 4 apical valves. Seeds narrowly ovoidal, testa papillose-reticulate. $2N = 24$.

Etymology. This name makes reference to the well-developed xylopodia (ligneous rhizomes) found in the type species of the genus.

Distribution. So far, *Xylopodia* is known in a few widely separated, disjunct localities in strongly seasonal habitats of the tropical Andes in NW Peru, NW and S Bolivia and NW Argentina, from low (850–900 m) to moderate (2,900 m) elevations.

Identification remarks. Plants of this genus can be recognized from all other Loasaceae by the diagnostic combination of the presence of underground xylopodia, opposite, lobed leaves, tetramerous flowers, petals with longitudinal lamellae and well developed nectar scales.

Xylopodia klaprothioides Weigend, *Taxon* 55: 467. 2006.

Figs 2, 5

Type. PERU. Cajamarca: Prov. Contumazá: Road from Contumazá to Chilete, first road bend after highest point pass, 2900 m, (07°19'48"S, 078°48'38.5"W) 5. Feb. - 2. Abr. 1997, M. Weigend, N. Dostert & K. Drießle 97/450 (**holotype**: M! mounted on two sheets, barcodes: M-0274954 & M-0274955; **isotypes**: F!, USM!).

Description. Shrub with erect or leaning shoots up to ca. 300 cm tall from a horizontal xylopodium (= ligneous rhizome), up to 30 × 5 cm, with several aerial branches arising from it; aerial vegetative organs densely hairy, with white scabrid and glochidiate trichomes, ca. 1 mm long. Leaves opposite, 8–20 × 65–15 cm, lamina with 1–3 lobes on each side, margin serrate, apices acute to rounded, base cuneate, petiole 1–3 cm long. Inflorescences terminal dichasia; peduncle ca. 7–20 cm long; basal bracts 1.5 × 0.5 cm, 3-lobed, margin denticulate, base cuneate; distal bracts 0.5 × 0.1 cm, lanceolate, subentire. Flowers tetramerous, pedicels 2–4 mm long, deflexed. Sepals broadly triangular, ca. 3 × 2 mm, margins entire, erect in bud, spreading during anthesis, and pubescent on both sides, pubescence similar to that of vegetative organs. Petals 10–15 × 3–5 mm, pale to deep green, unguiculate, with a short claw (< 1/4 of petal length), cucullate in the distal half, with two membranous longitudinal lamellae with densely ciliate margin, aestivation valvate, margin with a tooth on each side near the base where the limb starts, with scabrid-glochidiate trichomes abaxially, adaxially glabrous. Nectar scales 4, antesepalous, 3.5–4 × 2.5–3 mm, cucullate, formed by 5–6 fused staminodes, mostly green, pubescent in lower half, virtually uniform in color, apex yellowish green, slightly recurved. Free staminodes 4–5 per scale, opposite and internal to it, monomorphic, lower halves densely pubescent, green, ca. 4 mm long, with an inconspicuous, pubescent, knee-like filament flange below the middle, apex filiform, twisted randomly or reflexed. Fertile stamens arranged in 4 antepetalous groups of ca. 7–15 per petal, free, filament ca. 6–8 mm long, anthers white, with two ovate thecae. Ovary half-superior, broadly ovate to conical, placentae 4; style to 7 mm long, lower half pubescent, stigma with four ribs tapering towards the apex. Fruits subglobose capsules, with a conical apex, opening with 4 apical valves. Seeds numerous, narrowly ovoidal, testa papillose-reticulate up to ca. 2 × 0.4 mm.

Etymology. The species name refers to the clear affinity of this taxon to the genus *Klaprothia* Kunth.

Distribution. *Xylopodia klaprothioides* is restricted to a small area of the Pacific slope of the Cordillera Occidental in Andean NW Peru. It is a very narrowly endemic species, known from only two localities some 8 km apart (in a direct line) in Dpto. Cajamarca Prov. Contumazá, at elevations from ca. 2,500–3,000 m (Fig. 1).

Ecology. *Xylopodia klaprothioides* occurs in seasonally dry Andean scrub habitats, mostly around hedges and gullies, often on rocky soil dominated by xeric adapted shrubs, small trees and other vegetation including *Cylindropuntia* (Engelm.) F.M. Knuth and terrestrial bromeliads (Fig. 4C). Near the town of Contumazá, it grows sympatrically with another shrubby Loasaceae, *Nasa macrothyrsa* (Urb. & Gilg) Weigend. These localities are surrounded by a mosaic of fields, fallows and small patches of seasonally dry montane forest according to the Ministerio del Ambiente del Perú (2015, 2018). These habitats often show signs of human alteration. Rodríguez-Rodríguez and Weigend (2006) mention that this species is found in the Mesoandina Ecological Region. *Xylopodia klaprothioides* is deciduous and the plants shed their leaves at the beginning of flowering in May/June.

Phenology. Flowering and fruiting of this species have been recorded at the very end of the rainy season and the start of the dry season in May and June; thus most plants are leafless during the peak of the flowering period.

Additional specimens examined. PERÚ: Dpto. Cajamarca, Prov. Contumazá, Road from Contumazá to Chilete. First road bend after highest point of pass, 2900 m, (07°19'48"S, 078°48'38.5"W) 17. Jun. 1998, *M. Weigend et al.* 98/536 (F, HUT, M, USM); same locality, 09. May 2003, *M. Weigend et al.* 7601 (B, F, HUT, M, USM); Dpto Cajamarca, Prov. Contumazá, road Contumazá to Cascas, after tunnel, ca. 2400–2600 m, (07°24'14"S, 078°47'53"W) 10. May 2003, *M. Weigend et al.* 7617 (B, F, HUT, M, USM).

Notes. See *Xylopodia laurensis*.

Conservation status. *Xylopodia klaprothioides* seems to be restricted to only a few populations near Contumazá in northern Peru and has been considered as globally endangered (Rodríguez and Weigend 2006). Using the data from the only two known populations, the estimated EOO (Extent Of Occurrence) of the species is just 0.081 km², resulting in a conservation status assessment of “critically endangered” (CR) according to the IUCN categories and criteria (2012) and guidelines (2019). The AOO (Area Of Occupancy) for the species is also extremely small (8 km² when applying a reasonable grid cell size of 2 × 2 km in GeoCat) and also assigns *X. klaprothioides* a “critically endangered” (CR) status according to the IUCN. We thus would update the threatened assessment of this species to CR under the IUCN categories and criteria B2ab(iii); C2a(i), The criterion B2 was selected because its AOO is < 10 km² (8 km²). The criterion “a” was selected because it presents a very fragmented distribution. The criterion “b(iii)” was selected because there is a projected decline in the area, extent and quality of habitat. The region around Contumazá represents a mosaic of different habitats including small patches of natural habitat surrounded by agricultural land. The criterion C2a(i) was selected because we observed fewer than 50 individuals per population in the two known localities.

***Xylopodia laurensis* C.M. Martín & C.A. Zanotti, sp. nov.**

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Fig. 3

Diagnosis. *Xylopodia laurensis* is closely related to *Xylopodia klaprothioides* Weigend, but the former is readily distinguishable by the sepals reflexed in anthesis, petals greenish white with a long claw (ca. 1/3 of petal length); back of nectar scale white, apically striped green and yellowish; free staminodes are dimorphic, ligulate, the central pair with a conspicuous, papillose, scale-like filament flange below the middle.

Type. Argentina: Prov. Jujuy, Dpto. San Pedro, San Juan de Dios, Estancia Las Lauras, camino al “Chorro”, a tres metros del arroyo Chico, 24°33'49.8"S, 64°39'05.8"W, 925 m, 22 Aug 2021, *C. M. Martín* 2887 (holotype SI)

Description. Shrub with erect shoots up to 130 cm tall from a short horizontal xylopodium (= ligneous rhizome), ca. 5 × 3 cm, sometimes divided into 3–4 branches distally; aerial vegetative organs densely hairy, with white scabrid and glochidiate trichomes, ca. 0.5 mm long. Leaves opposite, 14–22 × 13–9 cm, lamina with 1–2 lobes in each side, margin serrate, apices acute to rounded, base cuneate, petiole 1.5–3 cm long. Inflorescences terminal dichasia; peduncle ca. 6 cm long; basal bracts 3 × 2 cm,

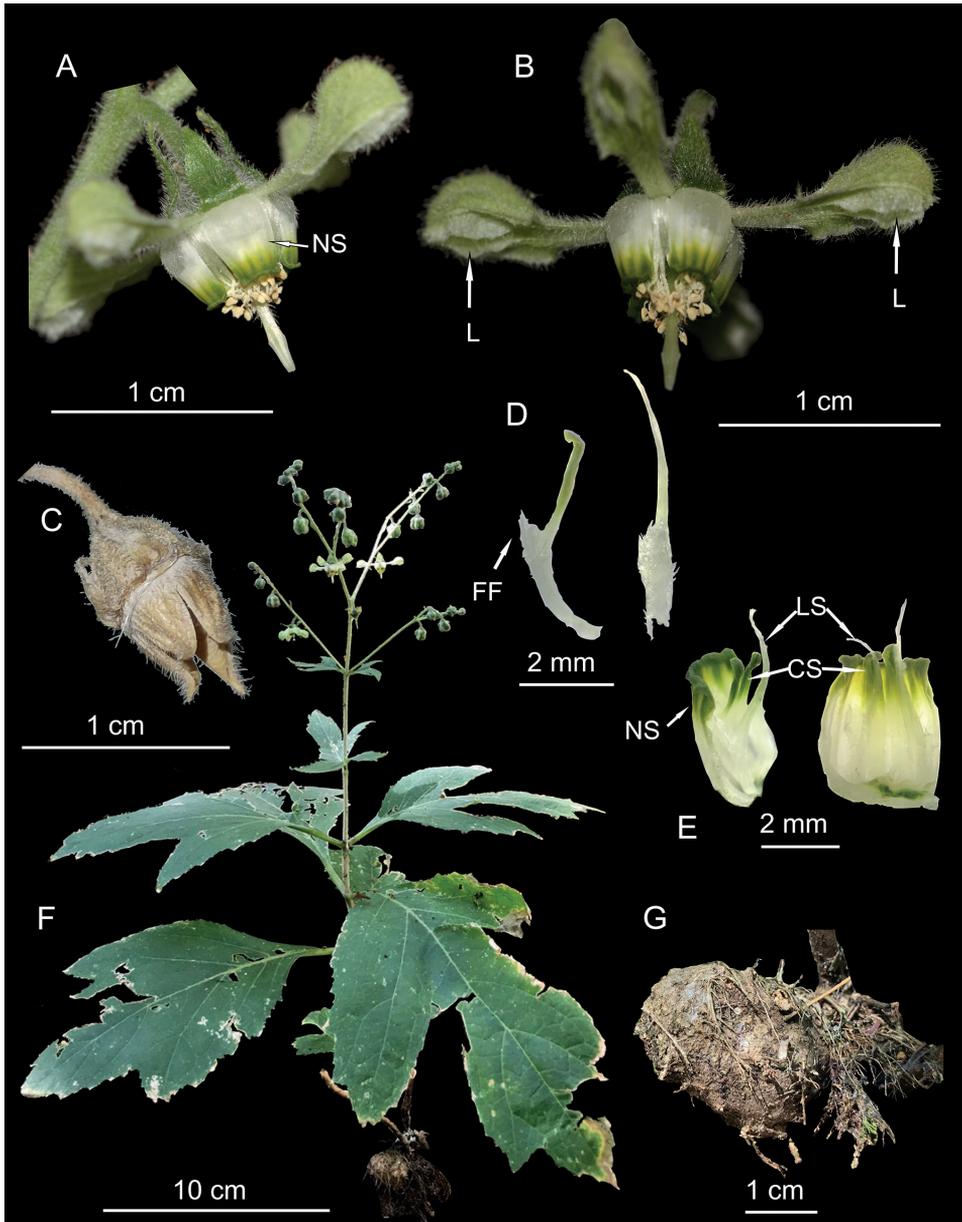


Figure 3. *Xylopodia laurensis*. **A** flower, lateral view, showing the nectar scales (NS) **B** flower, showing the lamellae (L) **C** mature fruit, showing the four apical valves **D** details of the central staminode (left), showing the well-developed filament flange (FF), lateral staminode (right) **E** staminodes within the nectar scale, showing the lateral staminodes (LS) and the central ones (CS) **F** plant **G** xylopodium. All from Martín 2887, except C. (Catari 2510).

3-lobed, margin denticulate, base cuneate; distal bracts 0.8–1.8 × 0.3–0.5 cm, lanceolate, margin entire. Flowers tetramerous, pedicels 4–6 mm long, deflexed. Sepals broadly triangular, ca. 5 × 3 mm, margins entire, erect in bud, reflexed during anthesis,

and pubescent on both sides, pubescence similar to that of vegetative organ. Petals 7 × 3 mm, greenish white, unguiculate, with a long claw (ca. 1/3 of petal length), cucullate in the distal half, with two membranous longitudinal lamellae with densely ciliate margin, aestivation valvate, margin with a tooth on each side near the middle, where the limb starts, dorsal veins three, with scabrid-glochidiate trichomes abaxially, mostly glabrous adaxially. Nectar scales 4, antesealous, 4 × 3 mm, cucullate, formed apparently by 6 fused staminodes, mostly white, pubescent in lower half, striped green and yellowish in distal third, apex green, slightly recurved. Free staminodes 4 per scale, opposite and internal to it, dimorphic, their lower halves partly fused, densely pubescent, with lacinate margins, central staminodes green, 4 mm long, with a conspicuous, papillose, scale-like filament flange below the middle, apex reflexed, spatulate the lateral ones yellowish-white, 5.3 mm long, with a filiform apex. Fertile stamens arranged in 4 antepetalous groups of ca. 7 per petal, free, filament ca. 6 mm long, base pubescent, anthers white, with two obovate thecae. Ovary half-superior, broadly ovate to conical; style to 5 mm long, lower half pubescent, stigma with four ribs tapering towards the apex. Fruits subglobose capsules, with a conical, slightly asymmetrical apex, opening with 4 apical valves.

Etymology. The name of the species refers to the locality of the type collection in Argentina (Estancia Las Lauras, Jujuy, Argentina).

Distribution. *Xylopodia laurensis* grows on the eastern slope of the Andes from Bolivia (Dptos. La Paz and Chuquisaca) to northern Argentina (Prov. Jujuy) at elevations from 850–900 m asl (Jujuy and Chuquisaca) to 2360 m asl (La Paz) (Fig. 1). The known distribution thereby covers a latitudinal range of ca. 1000 km in a straight line between the northernmost locality in Dpto. La Paz and the southernmost population from Prov. Jujuy. The distributional range is even larger (~1300 km) when following the curve of the eastern slope of the Andes along a suitable elevation with appropriate climatic conditions and respective available habitats. *X. laurensis* can hence be considered a rather widespread taxon, albeit with very specific saxicolous habitat requirements, and this contrasts markedly with the very narrow endemism of its sister taxon, *X. klaprothioides*. Narrow endemism as found in *X. klaprothioides* is a common pattern reported for many plant groups whose elements reach into the Amotape-Huancabamba zone (e.g. Gentianaceae – Struwe et al. 2009; Boraginaceae – Weigend et al. 2010; Grossulariaceae, Urticaceae – Mutke et al. 2014; Solanaceae – Deanna et al. 2018; Arecaceae – Escobar et al. 2018; Lentibulariaceae – Casper et al. 2020; Henning et al. 2021) and particularly common in the Loasaceae (Henning and Weigend 2009; Henning et al. 2011, 2019; Mutke et al. 2014).

Ecology. *Xylopodia laurensis* occurs in the understory of seasonally dry (scrub) forest in shallow rocky soil and in soil pockets on rock-outcrops across its range (Fig. 4). In Argentina, it grows in xerophilous and deciduous forests (Fig. 4A) at 925 m a. s. l. corresponding to the Chaco Occidental phytogeographic district sensu Cabrera 1994. Typical components are *Schinopsis lorentzii* (Griseb.) Engl., *Libidibia paraguariensis* (D. Parodi) G.P. Lewis, *Handroanthus impetiginosus* (Mart. ex DC.) Mattos, *Ceiba chodatii* (Hassl.) Ravenna, *Aspidosperma quebracho-blanco* Schltld. and *Athyana weinmanniifolia* (Griseb.) Radlk. In Chuquisaca, *Xylopodia laurensis* forms small



Figure 4. Habitats of *Xylopodia*. **A** deciduous forest near Jujuy, Argentina, habitat of *X. laurensis* **B** *X. laurensis* habitat in a dry creek near Chuquisaca, Bolivia **C** rocky slopes with xerophilous vegetation near the town of Contumazá, Peru, habitat of narrowly endemic *X. klaprothioides*.

colonies on rocky outcrops and the margins of temporary streams (torrenteras, Fig. 4B) in the Boliviano-Tucumano Seasonal Dry Forests (Pilcomayo-Alto Parapeti Sector of the Boliviano-Tucumano Biogeographic Province sensu Navarro and Maldonado 2002 and Navarro and Ferreira 2009). The most common species here are *Schinopsis lorentzii*, *Anadenanthera colubrina* (Vell.) Brenan, *Ceiba chodatii*, *Saccellium lanceolatum* Bonpl., *Piptadenia boliviana* Benth., *Ruprechtia apetala* Wedd. and *Libidibia paraguariensis*. These rock outcrop habitats are poorly studied in Bolivia and several new species from other plant groups still await description (JC unpublished data). In La Paz, the new species is found in the Yungueño Montane semi-deciduous forest. This area has been more profoundly influenced by human activities. The original forests have been replaced by scrub and secondary forests (these correspond to the Cuenca Alta del Beni Sector of the Yungueña Peruviana-Boliviana Biogeographic Province sensu Navarro and Maldonado 2002 and Navarro and Ferreira 2009).

Phenology. The flowering and fruiting of this species have been recorded in the dry season, from late August to mid-September in Jujuy and from April to August in Chuquisaca. In Chuquisaca, plants lose their foliage by August and produce new leaves when the rainy season starts in October to November.

Additional specimens examined. BOLIVIA: Dpto. Chuquisaca. Prov. Luis Calvo, Municipio Villa Vaca Guzmán, A 5.5 km al Norte de la Comunidad Ivoca y 3 km al Este de la Quebrada Angoba. Estancia del Sr. Jhony Labras. 850 m, 11. Sep. 2019, *J.C. Catari* & *Z. Pérez 2501* (USZ); same locality, 17. Oct. 2021, *J.C. Catari 2510* (USZ). Photographs examined: Bolivia: Dpto. La Paz, Prov. Larecaja, Municipio Sorata, Cueva de San Pedro, s.d., autor: A.F. Fuentes (available in <http://legacy.tropicos.org/Image/100494035>)

Notes. *Xylopodia laurensis* is a close ally of *Xylopodia klapprothioides*. Vegetatively, it mainly differs in the much smaller woody rhizome, which easily reaches 30 cm and more in length in mature *X. klapprothioides*. Aerial stem and leaf morphology appear to be similar, although the leaves of *X. klapprothioides* are usually smaller and not as deeply lobed (Figs 2F, 3F). The major differences, however, are found in the details of floral morphology, where *X. laurensis* differs in its reflexed (versus spreading) sepals during anthesis (Figs 2A–B, 3A–B), floral scale coloration (white and yellowish-green pattern apically versus uniformly green with yellowish scale neck, Figs 2A–B, D, 3A–B, E) and the morphology of the staminodes (Figs 2E, 3D). The central staminodes are particularly distinctive and their enlarged filament flanges resemble those of *Kissenia capensis* R.Br. ex Harv. (Urban and Gilg 1900).

There is a disjunction of ca. 1500 km between the populations of *X. klapprothioides* in northern Peru and *X. laurensis* in Bolivia (Fig. 1). The recent discovery of this genus demonstrates that it is very difficult to locate in the field – mostly because it has few if any leaves when in flower, and the green flowers are hardly differentiated from the surrounding vegetation, rendering visual recognition of even flowering plants a genuine challenge. If found when sterile, it could be confused with some other oppositely leaved subshrubs or shrubs (high magnification lenses are necessary to see the distinctive scabrid-glochidiate trichomes, Fig. 5). Likewise, the species, even if widely distributed, could be very localized in distribution. One of us (JC) explored an area

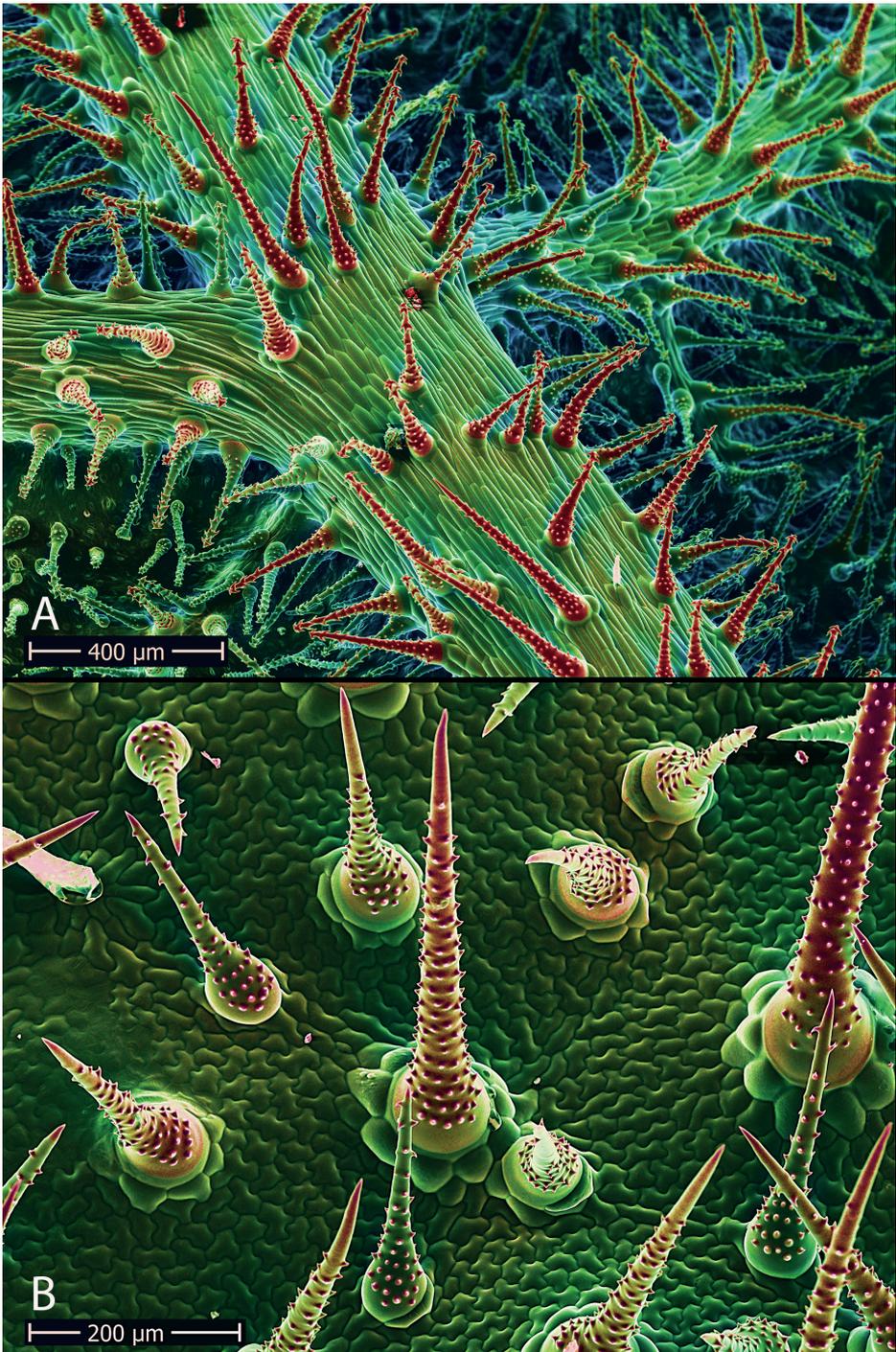


Figure 5. False-color BSE-image of the trichome cover of the leaves of *X. klaprothoides*. Mineralized areas are highlighted in red, non-mineralized areas in green **A** abaxial surface densely covered with unicellular, glochidiate and a few scabrid trichomes **B** adaxial surface densely set with scabrid hairs. (Credit: H.J. Ensikat, Bonn).

of ca. 10 ha of suitable habitat near the collection locality in Chuquisaca and was able to locate only two small colonies distributed in an area of < 1000 m². It is therefore conceivable that *Xylopodia* is also present in similar vegetation in the intervening area between the known ranges of these two species, for example in the poorly explored Río Mantaro and Río Apurímac systems in Peru. Future explorations may turn up additional localities for this species. However, as it now stands, it appears to represent a highly unusual disjunction shown by few other plant taxa restricted to the seasonally dry forests of northwestern Peru and the eastern Andean slope in Bolivia and Argentina, corresponding to what Prado and Gibbs (1993) consider as the Pleistocene arc of tropical seasonal dry forests.

Preliminary conservation status. As mentioned above, the actual abundance of *Xylopodia* is very difficult to assess, both globally and locally. Both species share a very nondescript appearance, even during flowering and especially after the shedding of leaves during the dry season. Hence, there are only very few collections. However, unlike the type species, *X. laurensis* is evidently more widespread and likely present in suitable habitats in between the known populations (Fig. 1). The estimated EOO (Extent Of Occurrence) is > 72.000 km², resulting in a conservation status assessment of “least concern” (LC) according to the IUCN categories and criteria (2012) and guidelines (2019). Conversely, given the fact that all collections and observations made so far only report very small populations or single individuals, the AOO (Area Of Occupancy) for the species is extremely small (<0.03 km² when applying the smallest possible grid cell of 100 × 100 m in GeoCat) presumably reflecting the narrowness of the ecological niche of *Xylopodia*. The latter value would consider *X. laurensis* as “critically endangered” (CR) according to the IUCN. These contradictory results show how difficult even a tentative assessment of a species’ conservation status can be, if the data are too limited due to either collection gaps in certain regions or taxonomic groups or real rarity of the taxon in question. At the moment, we cannot give a satisfactory answer on the threat status of *X. laurensis*. It might be rare and under immediate threat or maybe it has been just under collected. For the time being its conservation status must hence be categorized as “data deficient” (DD).

Key to the Species of *Xylopodia*

- 1 Sepals reflexed at anthesis, petals greenish white, claw long (ca. 1/3 of length of petals); back of nectar scale white, apically striped green and yellowish, apex green; free staminodes dimorphic, with their lower halves partly fused, the central pair abruptly expanded basally into unusually large, scale-like, abaxial filament flanges ca. ½ from base *X. laurensis*
- Sepals spreading at anthesis, petals pale to deep green, claw short (< 1/4 of length of petals); back of nectar scale uniformly green, apex yellowish-green; free staminodes monomorphic, free, with small and inconspicuous “knee shaped” abaxial filament flanges ca. ¼ from base..... *X. klaprothioides*

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Plagiothecium talbotii, a new species from the Aleutian Islands (Alaska, U.S.A.)

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Abstract

Plagiothecium talbotii **sp. nov.** is described from Attu Island, Alaska, U.S.A. The newly-described species is not similar in appearance to any Northern Hemisphere species; only the habit is similar to *P. platyphyllum*. However, it not only occupies a different habitat than that species, but genetically and morphologically, it is clearly distinct from it. The results of DNA sequencing, a detailed description of the morphological features, illustrations, ecological preferences and currently known geographical distribution of *P. talbotii* are presented. The most important distinguishing morphological features of this species are: the size of the plant; dimensions and symmetry of the leaves; dimensions of cells and their areolation; entire leaf apex; and long decurrencies with some inflated cells. Additionally, we propose to place *P. talbotii* in section *Plagiothecium*, which is confirmed by genetic analysis and morphological features.

Keywords

Attu Island, Bryophyta, Plagiotheciaceae, S. S. Talbot, taxonomy

Introduction

Herbarium collections are unquestionably a rich and very important source of data on the ecology and distribution of plants. Revisions based on herbarium material are the primary source for taxonomic research. Now, having a number of methods at our

disposal (e.g. genetic analyses, mathematical modelling, SEM), integrative taxonomy sheds new light not only on the status or distribution of the taxa studied, but also on the relationships between them and not only at the species level (e.g. Huttunen et al. 2018; Guerra et al. 2019; Ignatov et al. 2020; Plášek and Ochyra 2020; Vigalondo et al. 2020; Wolski and Nowicka-Krawczyk 2020; Wolski et al. 2020; Melamed et al. 2021).

Despite the fact that the Northern Hemisphere is relatively well studied botanically, there are still many areas that are blank spots for this part of the world. The same is true for bryophytes as for vascular plants, but even more so. Many species, genera and even families require basic research related to their variability, distribution, ecology or taxonomic status (Anderson et al. 1990; Ignatov et al. 2006; Hodgetts 2015; Suzuki 2016; Hodgetts and Lockhart 2020; Wolski et al. 2021a, b).

The genus *Plagiothecium* Schimp. which currently has been divided by Wynns et al. (2018) into nine sections (*Leptophyllum* Jedl.; *Lycambium* Jedl.; *Ortholimnobia* (Dixon) J.T. Wynns; *Orthophyllum* Jedl.; *Plagiothecium*; *Pseudo-Neckera* (Kindb.) J.T. Wynns; *Rectithecium* (Hedenäs & Huttunen) J.T. Wynns; *Saviczia* (Abramova & I.I. Abramov) Z. Iwats.; *Struckia* (Müll. Hal.) J.T. Wynns) over the past decades may give the impression of being well-researched. However, the genus was described as fairly species-poor and represented in the Northern Hemisphere by only a dozen taxa (e.g. Ireland 1969, 1986; Iwatsuki 1970; Lewinsky 1974; Smith 2001). The understanding of *Plagiothecium* has changed relatively recently, when DNA-based research proved that many of the taxa have been too broadly circumscribed (Zuo et al. 2011; Wynns et al. 2018; Ignatova et al. 2019; Wolski and Nowicka-Krawczyk 2020) and that the bryoflora of North America, Europe and Asia is much richer in *Plagiothecium* than previously assumed (e.g. Wynns et al. 2018; Ignatova et al. 2019; Wolski, Jukoninè 2019; Wolski 2020a, b; Müller and Wynns 2020; Wolski and Nowicka-Krawczyk 2020; Wolski et al. 2021b).

However, despite extensive research now being carried out, the number of Northern Hemisphere species still seems to be underestimated. Alaska, including the Aleutian Islands, are a “blank spot” in our knowledge of mosses, including the genus *Plagiothecium*. As a result of the taxonomic revision of *Plagiothecium* from the Aleutians, we recently described a new species, *Plagiothecium schofieldii* (Wolski et al. 2021a). Examination of additional material resulted in yet another undescribed species being discovered.

Materials and methods

Taxonomic analyses

Specimens from the Missouri Botanical Garden (MO), the University of British Columbia (UBC) and The New York Botanical Garden (NY) were analysed during the revision of *Plagiothecium* from the Aleutian Islands. A single specimen (MO 5925637) was selected for DNA analysis, with the appropriate consent from the herbarium curator.

DNA isolation, amplification and sequencing

Total DNA was extracted using the GeneMATRIX Plant & Fungi DNA Purification Kit (Eurx, Gdansk, Poland) following the manufacturer's protocol. Three equal samples of 20 mg of dry tissue from the leafy stems of bryophytes were homogenised in the lysis buffer, delivered by the purification kit, using a hand-held stainless steel homogeniser (Schlüter Biologie, Eutin, Germany). DNA extracts were quantified with a BioDrop DUO Spectrophotometer (BioDrop Ltd, Cambridge, U.K.) and the sample with high quality DNA (1.7–1.9 OD₂₆₀/OD₂₈₀) was selected for further analysis.

The molecular research was based on nuclear and chloroplast DNA markers: ITS (from the 3' end of the nuclear spacer ITS1, through the 5.8S rDNA, to the 5' end of the ITS2 spacer); and *rpl16* cpDNA gene encoding ribosomal protein L16. Markers were selected, based on Wynns et al. (2018), Wolski and Nowicka-Krawczyk (2020) and Wolski et al. (2021) from *Plagiothecium*-focused studies.

All markers were amplified by PCR in a few replicates to obtain high quality amplicons for sequencing. PCR was performed using primers and reaction conditions as described in Wolski et al. (2021).

PCR products were visualised on an agarose gel (1.5%, 90V, 40 minutes) stained with GelRED fluorescent dye (Biotum, Fremont, CA, U.S.A.) and two replicates of each marker per sample were chosen for sequencing. Amplicons from the PCR reaction were cleaned using Syngen Gel/PCR Mini Kit (Syngen Biotech, Wrocław, Poland) according to the manufacturer's protocol. Samples were sequenced with Sanger sequencing, using primers from amplification by SEQme s.r.o. company (Dobris, Czech Republic). The obtained sequences were assembled in Geneious 11.1.5 (Biomatters Aps, Aarhus, Denmark) (<http://www.geneious.com>). The sequences were submitted to the NCBI GenBank database (www.ncbi.nlm.nih.gov) under the accession numbers OM337522 for ITS and OM311940 for *rpl16*.

Phylogenetic analyses

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

Phylogenetic calculations were performed using Maximum Likelihood analysis (ML) in the IQ-TREE web server (Trifinopoulos et al. 2016) (<http://iqtree.cibiv.univie.ac.at/>) with the ultrafast bootstrap (UFBoot) pseudolikelihood algorithm (Hoang et al. 2018) and 10000 replicates; and Bayesian Inference (BI) in MrBayes 3.2.2 (Ronquist et al. 2012)

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

Taxon	Collection	Locality	ITS	rpl16
<i>Isopterygiopsis pulchella</i>	UC barcode 1947397	USA: CA	KY550336	KY514042
<i>P. angusticellum</i>	Wolski 5	Poland	MN077501	MN311136
<i>P. angusticellum</i>	Wolski 22	Poland	MN077507	MN311142
<i>P. angusticellum</i>	Wolski 23	Poland	MN077508	MN311143
<i>P. angusticellum</i>	Wolski 25	Poland	MN077510	MN311145
<i>P. angusticellum</i>	Wolski 26	Poland	MN077511	MN311146
<i>P. angusticellum</i>	Wolski 29	Poland	MN077513	MN311148
<i>P. brasiliense</i>	E barcode 00387968	Brazil	KY550266	KY513971
<i>P. cavifolium</i>	CP:J.T. Wynns 1885	Denmark: Sjælland	KF882225	KF882325
<i>P. cavifolium</i>	CP:J.T. Wynns 2960	Germany: Schauinsland, Hochschwarzwald	KF882226	KF882326
<i>P. conostegium</i>	NY:S.P. Churchill et al. 19839	Bolivia	KY550271	KY513976
<i>P. conostegium</i>	NY barcode 00845279	Guatemala	KY550318	KY514024
<i>P. conostegium</i>	S-B53327	Mexico	KY550272	KY513977
<i>P. denticulatum</i>	CP:J.T. Wynns 2081	Denmark: Sjælland, Sorø Kommune	KF882229	KF882329
<i>P. denticulatum</i>	BONN:O.M. Afonina s.n.	Russia: Far East, Chukotka	KY550275	KY513980
<i>P. denticulatum</i>	C:R.R. Ireland 23098	Canada: ON	KY550276	KY513981
<i>P. denticulatum</i> var. <i>bullulæ</i>	UC barcode 1798690	USA: NV	KY550278	KY513983
<i>P. denticulatum</i> var. <i>bullulæ</i>	UC barcode 1947417	USA: CA	KY550277	KY513982
<i>P. denticulatum</i> var. <i>obtusifolium</i>	CP:J.T. Wynns 2842	Germany: Hochschwarzwald, Schauinsland	KF882230	KF882330
<i>P. denticulatum</i> var. <i>obtusifolium</i>	UC barcode 1724036	USA: WA	KY550279	KY513984
<i>P. denticulatum</i> fo. <i>pungens</i>	DUKE barcode 0150010	USA: Alaska, Simenof Island	KY550280	KY513985
<i>P. japonicum</i>	DUKE barcode 0172241	USA: Alaska, Simenof Island	KY550291	KY513996
<i>P. lamprostachys</i>	S-B54613	Australia: VIC	KY550284	KY513989
<i>P. lamprostachys</i>	DUKE barcode 0156846	Australia: VIC	KY550285	KY513990
<i>P. lamprostachys</i>	S:H. Streimann 47719	Australia: NSW	KY550282	KY513987
<i>P. longisetum</i>	Wolski 12	Poland	MN077502	MN311137
<i>P. longisetum</i>	Wolski 14	Poland	MN077503	MN311138
<i>P. longisetum</i>	Wolski 15	Poland	MN077504	MN311139
<i>P. longisetum</i>	Wolski 19	Poland	MN077506	MN311141
<i>P. membranosulum</i>	BONN:J.-P. Frahm7756	Democratic Republic of the Congo	KY550310	KY514015
<i>P. membranosulum</i>	S-B78514	South Africa	KY550303	KY514008
<i>P. membranosulum</i>	DUKE barcode 0016754	South Africa	KY550304	KY514009
<i>P. nemorale</i>	CP:J.T. Wynns 3044	Germany: Farnberg, Schwarzwald-Baar	KF882239	KF882339
<i>P. nemorale</i>	CP: J.T. Wynns 2684	Germany: Mooswald, Kaiserstuhl	KF882237	KF882337
<i>P. nemorale</i>	Mishler 3835	Iran: Sisangan National Park, Manzadaran Prov.	KF882238	KF882338
<i>P. ovalifolium</i>	DUKE barcode 0188886	Chile	KY550314	KY514019
<i>P. platyphyllum</i>	C:J. Lewinsky et al. s. n.	Finland: Haluna, Nilsia, Savonia borealis	KF882241	KF882341
<i>P. ruthi</i>	CP: J.T. Wynns 1997	Denmark: Sjælland, Lyngby Aamose	KF882242	KF882342
<i>P. talbotii</i>	W.B. Schofield, S.S. Talbot 120206, MO 5925637 (dupl. UBC B193528)	USA: Alaska, Aleutian Island	OM337522	OM311940

Table 2. Summary of partitions for ITS-*rp16* matrix (1526 bp) evolutionary model selection and phylogenetic interference using PartitionFinder2.

	ITS1	5.8S gDNA	ITS2	<i>rp16</i> intron	<i>rp16</i> codon
ML	F81	JC	HKY +I	TN+I	F81
BI	F81	JC	HKY	GTR	F81

where two parallel Markov Chain Monte Carlo (MCMC) runs for four million generations each, with trees sampled every 1000 generations. The average standard deviation of split frequencies in both cases remained below 0.01 for the last 1000 generations and posterior probabilities were estimated from the 50% majority-rule consensus tree after elimination of the first 25% of samples as burn-in. The alignment and tree files were submitted to the figshare online database (<https://doi.org/10.6084/m9.figshare.18586082.v1>).

Haplotype network analysis was performed using Median Joining Network in PopART v. 1.7 with gap coding as a single event, irrespective of length and haplotype geographic distribution (Leigh and Bryant 2015).

Results and discussion

Phylogenetic analyses using the concatenated ITS-*rp16* matrix placed this Alaskan specimen within the sister branch of the sect. *Plagiothecium* clade with high support from Bayesian Inference (PP = 0.99), but slightly lower support from Maximum Likelihood (BS = 87) (Fig. 1). Although the topology of the tree shows clear distinction of the examined material, the closest representative with maximum support from BI to *P. talbotii* is the specimen BONN: *O.M. Afonina s.n.* described as *P. denticulatum*.

The haplotype network (Fig. 2) also confirms a closer relationship of *P. talbotii* with sect. *Plagiothecium* than with representatives from sect. *Orthophyllum*; however, the position of *P. talbotii* is isolated. The lowest number of mutational steps to *P. talbotii* as mentioned earlier is the specimen BONN: *O.M. Afonina s.n.* of *P. denticulatum*. This material was collected from the Far East of Russia – from Chukotka (Table 1), a geographic region adjacent to Alaska.

Although DNA analysis places the tested specimen as a sister clade to the clade represented by taxa from section *Plagiothecium*, it is morphologically different from most of the representatives of this section.

The shape of the decurrent angular cells is one of the most important features that divide the taxa of this genus into individual sections (Wynns et al. 2018; Wolski et al. 2021a, b). Almost all taxa from sect. *Plagiothecium* form distinct auricles, but not *P. talbotii*. The alar regions in this species are long and composed of sometimes inflated cells, a feature common to both *P. talbotii* and *P. platyphyllum* (Nyholm 1965; Lewinsky 1974; Smith 2001).

Having leaves shrunk in a dry condition and symmetrical make *P. talbotii* resemble, amongst the Northern Hemisphere of *Plagiothecium*, the previously-mentioned *P. platyphyllum* (Lewinsky 1974; Smith 2001). However, *P. talbotii* is distinguished from *P. platyphyllum* by the size of the leaves; lack of serration near leaf apex; lack of a group of

eroded, thin-walled cells (nematocysts) and rhizoids near the leaf apex; dimensions of laminar cells; and habitat occupied by the species (Nyholm 1965; Lewinsky 1974; Smith 2001).

Amongst the Northern Hemisphere species belonging to sect. *Plagiothecium* at present, there is only one species characterised by a symmetrical leaf: *P. schofieldii*. However, for example, the size and appearance of the turf; the lack of shrinkage and leaf shape, i.e. their concavity and serration and the shape of the decurrent angular cells differentiates this species from *P. talbotii* (Wolski et al. 2021b).

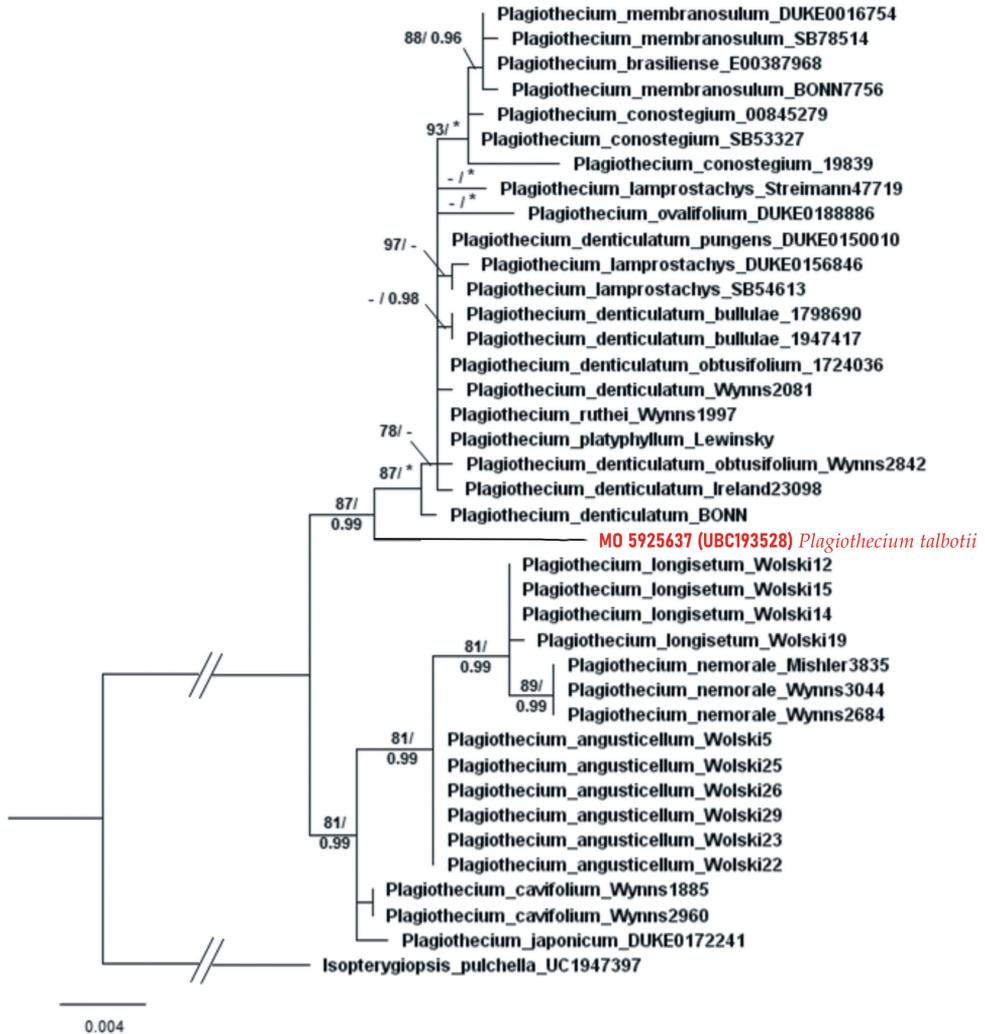


Figure 1. Phylogenetic tree of *Plagiothecium* taxa (sect. *Plagiothecium* [upper] and *Orthophyllum* [lower clade] with *Isopterygiopsis pulchella* as the outgroup, based on concatenated nuclear (ITS1-5.8S-ITS2) and chloroplast (*rpl16*) DNA markers (total 1526 bp). The tree presents the position of the studied specimen from Alaska amongst *Plagiothecium*. Numbers on branches indicate bootstrap values from ML followed by posterior probabilities from BI analysis. An asterisk (*) indicates 100 (ML) and 1.00 (BI), while a minus sign (-) indicates values below 75 (ML) and 0.95 (BI). The topology of the tree was based on ML analysis.

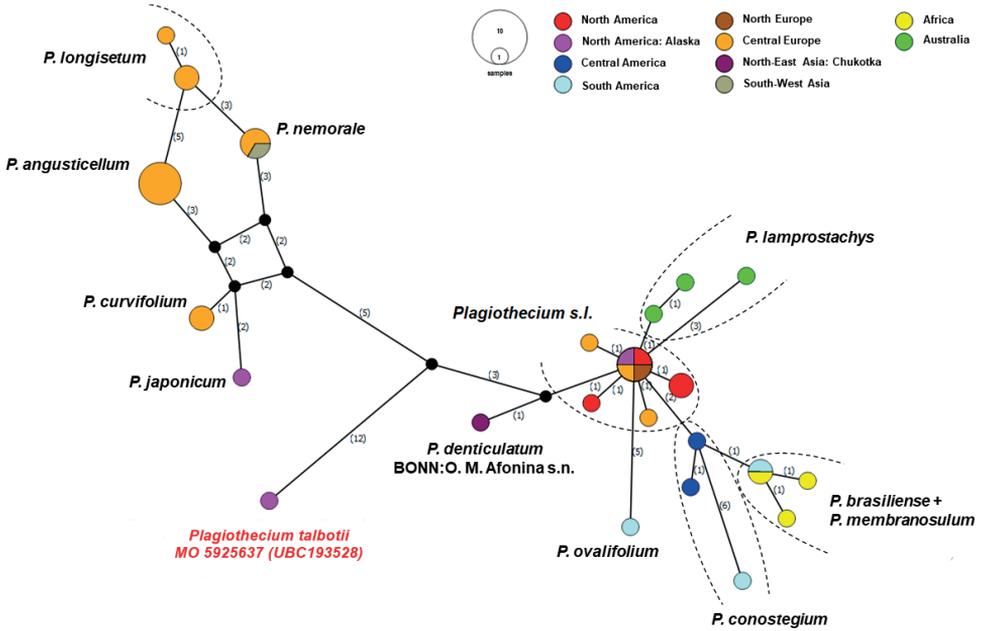


Figure 2. Median-joining haplotype network of *Plagiothecium* [bottom-right] and *Orthophyllum* sections [top-left] of *Plagiothecium* constructed in PopART. Haplotypes are represented by circles with colours indicating geographic distribution. Numbers on branches indicate the mutational steps.

Other species of this section are characterised by asymmetrical to slightly asymmetrical leaves and a completely different set of features when compared with *P. talbotii* (e.g. Lewinsky 1974; Smith 2001; Wolski et al. 2021b).

Taking into account the above facts, we believe that this species belongs to *Plagiothecium* sect. *Plagiothecium*. Thus, we consider that all the above morphological data, supported by molecular studies, warrant the recognition of the Aleutian samples as a new species.

Taxonomy

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

Type. U.S.A. Alaska, Attu Island, Lake Elwood area, under tall herbs on slope, 52°51'N, 173°10'E, 14 Sep 2002, *W.B. Schofield* and *S.S. Talbot* 120206, **holotype** MO 5925637, **isotype** UBC B193528.

Description. Plants large, dark green, without metallic luster, forming loose mats. Stems erect, julaceous in the lower part, more complanate above, 3.0–5.0 cm long (Fig. 3), very thick, in cross-section rounded, with a diameter of 500–700 μm, the central strand very well developed, epidermal cells 16–43 (M 25) × 12–39 (M 25) μm, the parenchyma thin-walled, 25–50 (M 36) × 18–60 (M 37) μm; leaves large, concave, symmetrical, ovate, imbricate, in wet condition, rather closely arranged on the stem,

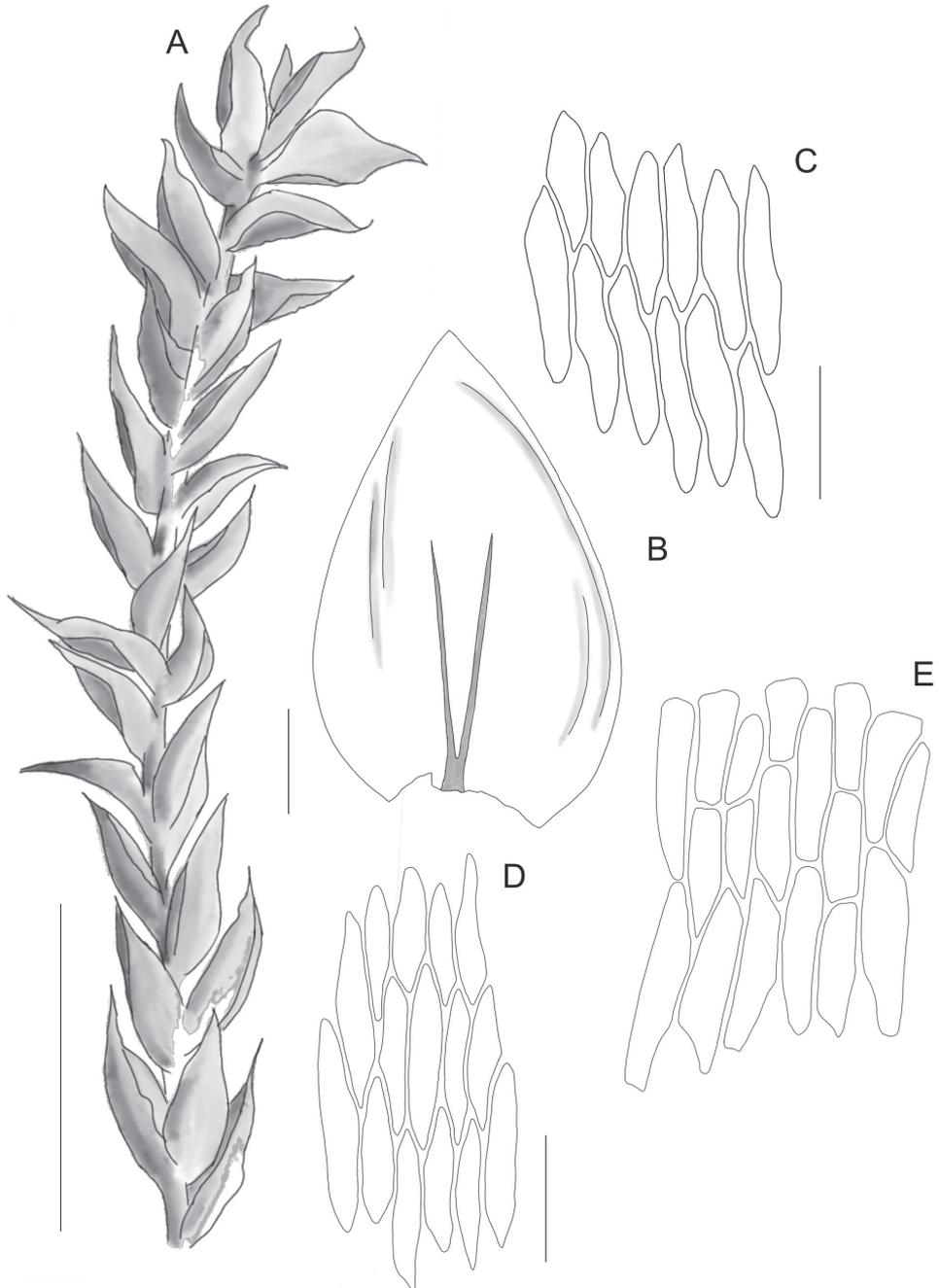


Figure 3. The most important taxonomic features of *Plagiothecium talbotii*. Stem (A) in dry condition; leaves from the middle of the stem (B), dimensions of cells from the apex (C), the middle (D) and basal part of the leaf (E). Drawing by G.J. Wolski from the holotype (*W.B. Schofield* and *S.S. Talbot* 120206, MO 5925637 [dupl. UBC B193528]). Scale bars: 1 cm (A); 1000 µm (B); 100 µm (C–E).

shrunk and sticking out when dry, those leaves from the middle of the stem 3.8–5.0 (M 4.4) mm long and the width measured at the widest point 1.9–3.1 mm (M 2.4); the apex obtuse and apiculate, entire, not denticulate; costae two, thick, strong and very large, extending usually more than ½ of the leaf length, reaching 1.0–3.0 mm (M 2.0); laminal cells rather symmetrical, in unregulated transverse rows, the length and width very variable, but dependent on location: 83–137 (M 101) × 17–22 (M 19) µm at apex, 100–175 (M 139) × 18–32 (M 24) µm at mid-leaf and 88–197 (M 132) × 22–35 (M 28) µm towards insertion, cell areolation loose; decurrencies very long, 700–1000 µm, composed of 3–4 rows of rectangular, at least some gently inflated cells, 90–216 (M 143) × 24–34 (M 28) µm. Sporophytes unknown so far.

Etymology. The present species is named in honour of Stephen S. Talbot who spent decades studying the northern regions of North America, including the Aleutian Islands and who, with Wilfred B. Schofield on 14 September 2002, collected the specimen (No. 120206), chosen here as the holotype of *Plagiothecium talbotii*.

Distribution and ecology. *Plagiothecium talbotii* so far has only been recorded from Attu Island in Alaska. In this area, it has been recorded in a non-forested area, on a slope, under tall herbs.

Acknowledgements

We thank the late Dr. Judy Harpel for the opportunity to revise the Aleutian *Plagiothecium* species. The research was funded from a grant *Genetic study on variability of selected taxa of the genus Plagiothecium* NCN “Miniatura 4” – DEC-2020/04/X/NZ8/00420.

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Relationships among *Calibrachoa*, *Fabiana* and *Petunia* (Petunieae tribe, Solanaceae) and a new generic placement of Argentinean endemic *Petunia patagonica*

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Abstract

Calibrachoa Cerv., *Fabiana* Ruiz & Pav., and *Petunia* Juss. form a clade within tribe Petunieae (Solanaceae). Phylogenetic studies of Petunieae, either as part of a family-wide analysis or focusing on the genera *Calibrachoa* and *Petunia*, have either left *Fabiana* unsampled or included only a single species. These studies have found conflicting relationships among the three genera with all three possible topologies obtained. *Petunia patagonica* (Speg.) Millán, originally described in the genus *Nierembergia* Ruiz & Pav., is morphologically distinct within *Petunia* and geographically disjunct from other members of the genus. For the first time, in this study we include multiple species of *Fabiana*, *Calibrachoa*, and *Petunia*, including *P. patagonica*. Using three chloroplast DNA regions and the nuclear gene GBSSI, or “waxy,” our results provide strong support for a sister group relationship between *Calibrachoa* and *Fabiana* and for the placement of *P. patagonica* within *Fabiana*. Since there is already a species *Fabiana patagonica* Speg., we provide the new name *Fabiana australis* Alaria nom. nov. to replace *Petunia patagonica*.

Keywords

Fabiana australis, Patagonia, *Petunia patagonica*, Petunieae, phylogeny

Introduction

Solanaceae Juss. are one of the most important families among Angiosperms not only for their fundamental contribution to the human diet but also for their diversity and ecological functions in many ecosystems worldwide, especially in the Neotropics (Olmstead 2013). Solanaceae are a family of approximately 90 genera and ca. 2700–2800 species (Olmstead and Bohs 2007; Olmstead et al. 2008, but see Knapp et al. 2004 for a higher estimate). Molecular phylogenetic studies in Solanaceae, initially based on chloroplast DNA sequence data (Olmstead and Palmer 1992; Spooner et al. 1993; Olmstead and Sweere 1994; Olmstead et al. 1999; Gemeinholzer and Wink 2001; Santiago-Valentin and Olmstead 2003; Clarkson et al. 2004; Bohs 2005; Levin et al. 2005, 2006; Weese and Bohs 2007; Olmstead et al. 2008), and more recently using a combination of chloroplast and nuclear loci and with nearly all genera and over 1,000 species sampled, have produced a robust dated molecular phylogeny (Goldberg et al. 2010; Särkinen et al. 2013; Ng and Smith 2015; Dupin et al. 2016).

Molecular phylogenetic studies unraveled relationships that were not consistent with traditional classification of the family and split up tribe Nicotianeae and resurrected tribe Petunieae to include most of the former Nicotianeae, excluding *Nicotiana* L., but including *Brunfelsia* L. (Olmstead et al. 1999, 2008; Olmstead and Bohs 2007). These results showed that the genera *Petunia* Juss., *Calibrachoa* Cerv., and *Fabiana* Ruiz & Pav. form a strongly supported clade and provided weak evidence suggesting that *Fabiana* is sister to *Calibrachoa* and that together they are sister to *Petunia*. The tribe Petunieae has been subject to several phylogenetic studies, with most of the focus on *Petunia* and *Calibrachoa* (Ando et al. 2005; Kulcheski et al. 2006; Stehmann et al. 2009; Fregonezi et al. 2012, 2013; Reck-Kortmann et al. 2014, 2015; Mäder and Freitas 2019). Few of these studies have included representatives of *Fabiana*, and when they do, relationships among the three genera sometimes do not agree (e.g., Särkinen et al. 2013; Reck-Kortmann et al. 2015; Mäder and Freitas 2019). Also, the rare species *Petunia patagonica* (Speg.) Millán, which is morphologically distinct and geographically disjunct from other *Petunia* species, has an uncertain placement in Petunieae (Stehmann and Greppi 2013; Reck-Kortmann et al. 2015).

Fabiana is endemic to South America, distributed in southern Peru, Bolivia, Chile, and Argentina, with 15 species of shrubs adapted to the high Andean deserts of Puna, Prepuna, Monte, and Patagonia, growing from sea level to 4900 m elevation in sandy, rocky soils of very low fertility, low organic matter and variable salt content (Barboza and Hunziker 1993; Alaria and Peralta 2013; Alaria 2015). *Fabiana* species have characteristic morphological adaptations: leaflessness or small leaves, photosynthetic and resinous stems, and flat or cushion growth habits (Alaria 2015), and they play an important ecological role as codominant shrubs in some plant communities. *Petunia patagonica* is restricted to the Patagonian region of Argentina, and its identity is controversial; it was first described in *Nierembergia* Ruiz & Pav. by Spegazzini (1897) and subsequently transferred to *Petunia* by Millán (1941).

In this study we explore the relationships among these three genera using sequences of the plastid *trnS-trnG*, *trnL-trnF* and *psbA-trnH* regions and partial sequences of the nuclear GBSSI or “waxy” gene, providing the first evidence of relationships among species of *Fabiana* and resolving the systematic position of the enigmatic species *Petunia patagonica*.

Materials and methods

Taxon sampling

Leaf samples were obtained from fresh material collected in the natural habitat of species or from cultivated plants, and preserved in silica gel. Data, including collecting site, voucher, and herbarium where the voucher has been deposited, are indicated in Appendix 1 for specimens of tribe Petunieae: *Bouchetia* Dunal (1 species), *Brunfelsia* (1), *Calibrachoa* (4), *Fabiana* (9), *Nierembergia* (1), and *Petunia* (8, including *P. patagonica*), and outgroup taxa in the genera *Benthamiella* Speg. (1 species), *Nicotiana* (3), *Pantacantha* Speg. (1), and *Solanum* L. (1).

Additional specimens were analyzed for morphological traits, mainly species of *Calibrachoa*, *Fabiana*, *Nierembergia*, and *Petunia* in the Argentinean herbaria: BAB, CORD, CTES, LP, LIL, MEN, MERL, and SI, as well as in herbaria in Bolivia: LPB and HSB; Chile: LS; Perú: USM; and England: K (all acronyms are in accordance to *Index Herbariorum*; <http://sweetgum.nybg.org/science/ih/>). Specimens of *Petunia patagonica* were examined and are cited after the species description in the Discussion.

DNA amplification and sequencing

DNA extraction was performed using the Qiagen DNeasy Plant Mini Kit (<http://www.qiagen.com>). Three regions of chloroplast DNA were amplified. For the *trnL-trnF* region, the primers and amplification conditions of Taberlet et al. (1991) were used. This region was included in the phylogenetic analysis of Olmstead et al. (2008), where it provided essential characters for their classification of Solanaceae. Two other plastid fragments were selected for being more variable molecular markers in *Calibrachoa* (Fregonezi et al. 2012) and *Petunia* (Lorenz-Lemke et al. 2006; Kulcheski et al. 2006): the *trnS-trnG* region and the *psbA-trnH* region. For the *trnS-trnG* region, the primers and amplification conditions of Hamilton (1999) were used, and for the *psbA-trnH* region, the primers and amplification conditions of Sang et al. (1997) were used. The nuclear gene GBSSI or “waxy”, which has a single copy in tomato (*Solanum lycopersicum* L.) and diploid potato varieties (*Solanum tuberosum* L.) (van der Leij et al. 1991), was selected because it is an informative marker for phylogenetic analyses in Solanaceae (Peralta and Spooner 2001; Walsh and Hoot 2001; Levin and Miller 2005; Yuan et al. 2006; Spooner et al. 2008; Levin et al. 2011; Särkinen et al. 2013).

The first waxy sequences of *Fabiana* specimens were obtained with primers initially designed for *Solanum lycopersicum* (Peralta and Spooner 2001), but new Petunieae-specific primers were generated: WAXY 5' GTGGGTACTGAGGTTGGTCCTT and WAXY 3' GGGCTCACTGTAACCACCCTAT, improving amplification of representative samples. Tomato and potato specimens were also amplified for waxy as controls for the expected fragment length, using a known molecular mass marker (Lambda/EcoRI-HindIII Marker). Purified PCR products were sequenced using standard Sanger sequencing methods at the Biogenomics Unit of the Institute of Biotechnology of the National Institute of Agricultural Technology (INTA Castelar), Buenos Aires, Argentina.

Editing and aligning of chloroplast and nuclear sequences

The sequences were manually edited with PROcessor of SEquences (PROSEQ) version 2.9 (Filatov 2002). Alignment was performed in BioEdit version 5.0.6. (Hall 2004), first using ClustalW and then adjusted by manual alignment. Waxy alignments were made by comparing sequences with the gene in *Solanum tuberosum* (GenBank accession X83220). Chloroplast fragments were aligned with *Petunia axillaris* sequences for *trnS-trnG* (JF918370), *trnL-trnF* (AY098702), and *psbA-trnH* (DQ225610).

Phylogenetic analysis

We created three datasets for phylogenetic analyses. One dataset consisted of the three plastid loci for 21 species. A second dataset comprising 20 samples consisted of the nuclear waxy sequences. The third dataset comprising 18 samples consisted of the three plastid loci and the nuclear waxy gene concatenated for each species for which sequences for waxy and at least two of the three plastid loci were available. For each locus, we compared nucleotide substitution models using the Akaike Information Criterion from analyses in jModeltest 2.1.4 (Guindon and Gascuel 2003; Darrriba et al. 2012) and chose an appropriate model within the 95% confidence interval. Phylogenetic analyses were performed using two different inference methods, maximum likelihood (ML) and Bayesian. In analyses with a concatenated sequence dataset, each locus was treated as a separate partition, and the GTR + gamma model of nucleotide substitution was used for each partition. In analyses with just waxy, the HKY + gamma model was chosen. We performed ML analyses using GARLI 2.0 (Zwickl 2006). We executed four replicates of each full search, and used a generation threshold for termination of 20,000 and score threshold for termination of 0.001. Default settings were used for all other parameters. We additionally performed bootstrap searches using a generation threshold for termination of 10,000. For concatenated datasets, 500 bootstrap replicates were done, and for the waxy dataset, 1,000 bootstrap replicates were done. Bayesian analyses were done in MrBayes 3.2.1 (Ronquist et al. 2012) with two runs with four chains each. For concatenated datasets, analyses were run for 10,000,000 generations, sampling every 1,000 generations. For the waxy dataset, analyses were

run for 5,000,000 generations with a sampling frequency of 500 generations. We determined that convergence was attained when the average standard deviation of split frequencies was <0.05 and the estimated sample size of parameters was >200 in Tracer 1.5 (Rambaut and Drummond 2009). Majority-rule consensus trees were constructed after discarding the initial 25% of samples as burn-in.

Results

The analyses of concatenated chloroplast sequences (Fig. 1) confirmed monophyly of the genera *Calibrachoa*, *Fabiana*, and *Petunia*, with the exclusion of *P. patagonica* (see below) and the phylogenetic relationships among the three genera was the same found by Olmstead et al. (2008) with *Petunia* sister to *Fabiana* and *Calibrachoa*, and the last two sister to each other. With limited sampling in the rest of tribe Petunieae and outgroups, *Nierembergia* is weakly supported as sister to *Nicotiana*. The analysis also strongly supports the placement of *Petunia patagonica* within *Fabiana*.

Waxy amplifications always showed a single band for a fragment of similar size to the one in potato and tomato controls. Although waxy gene copy number is unknown in *Fabiana*, *Calibrachoa*, and *Petunia*, it is expected to be a single copy and orthologous in the analyzed taxa, as has been demonstrated in other diploid Solanaceae species (van der Leij et al. 1991). The waxy phylogeny of 20 taxa (Fig. 2) is consistent with the chloroplast DNA analyses in recovering the monophyly of *Calibrachoa*, *Fabiana*, and *Petunia*, and the same relationships among them. Similarly, *Petunia patagonica* is included within *Fabiana*. With strong support, *Nierembergia* is resolved as sister to *Bouchetia*.

The results of the analyses of the concatenated sequences of chloroplast loci and waxy combined also recovered the same major relationships (Fig. 3). Monophyly of *Fabiana* with *Petunia patagonica* nested within it is strongly supported (posterior probability = 1.0). In these analyses, *P. patagonica* is weakly supported as sister to *Fabiana imbricata* Ruiz and Pav. Additionally, *Fabiana* is resolved as sister to *Calibrachoa* with strong support, and *Petunia* is sister to the clade comprising *Fabiana* and *Calibrachoa*. *Nierembergia* forms a clade with the remaining representatives of Petunieae with strong support.

Discussion

Sequences of three chloroplast markers and the nuclear gene waxy were informative for the inference of phylogenetic relationships among *Calibrachoa*, *Fabiana*, and *Petunia* in tribe Petunieae. Both the analyses of the combined chloroplast regions and the nuclear gene waxy corroborated *Calibrachoa* as sister to *Fabiana* with strong support, as obtained by Olmstead et al. (2008). A few studies of Petunieae with a single species of *Fabiana* sampled obtained results with *Fabiana* either sister to *Petunia* plus *Calibrachoa* (Särkinen et al. 2013) or *Petunia* (Reck-Kortmann et al. 2015; Mäder and Freitas 2019), in contrast to our results. All of our analyses also found *Petunia patagonica* to

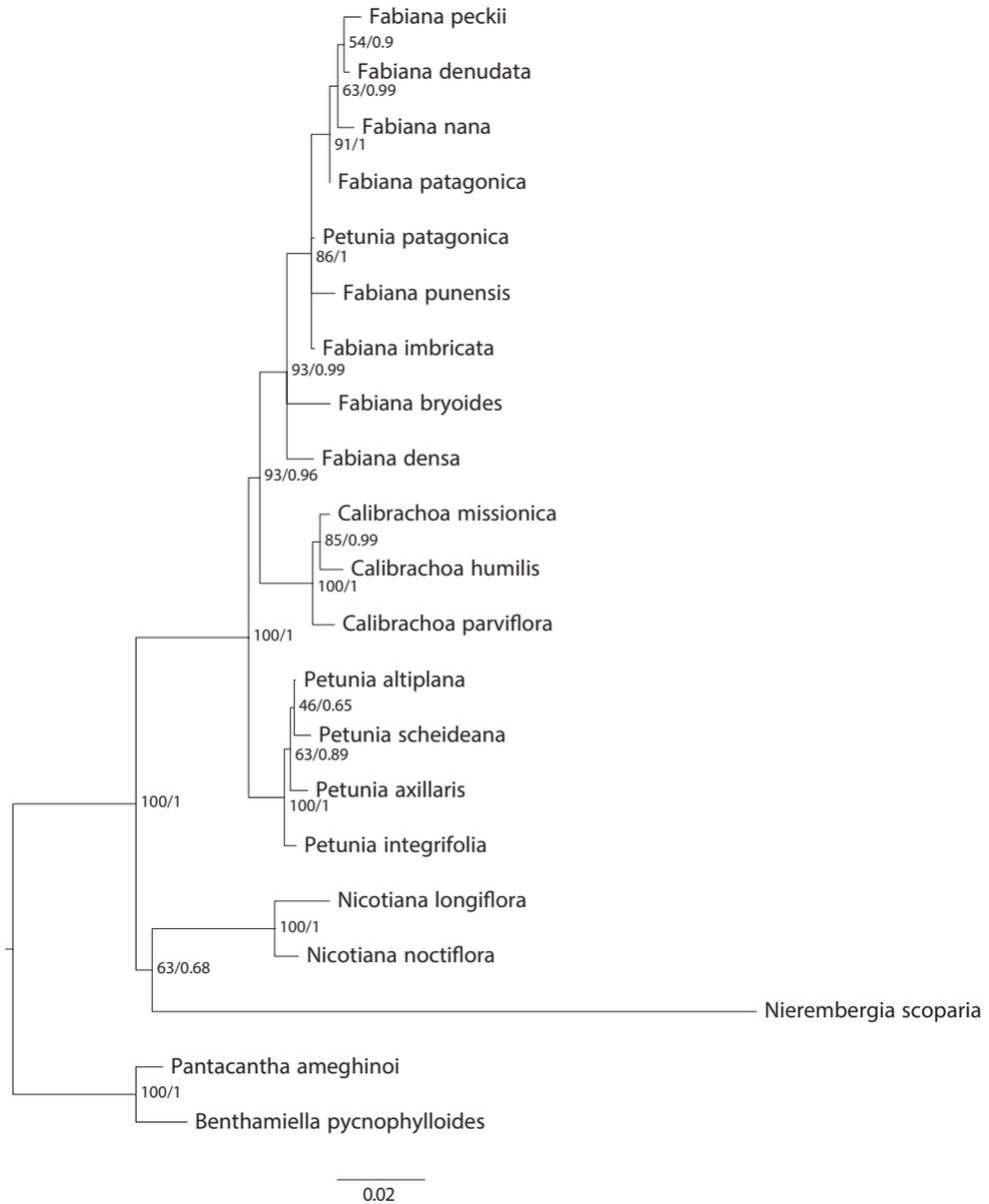


Figure 1. Chloroplast DNA tree of 17 Petunieae species and four outgroups. Phylogeny based on maximum likelihood analysis of concatenated *trnS-trnG*, *trnL-trnF*, and *psbA-trnH* chloroplast fragments. Maximum likelihood bootstrap values and Bayesian posterior probabilities shown at nodes.

be nested within *Fabiana*. This result is consistent with that of Reck-Kortmann et al. (2015), who found *P. patagonica* sister to *F. imbricata* and the combined clade sister to the rest of *Petunia*; but with only a single representative of *Fabiana*, that study was not able to reveal conclusively the placement of *P. patagonica* within *Fabiana*. Considering

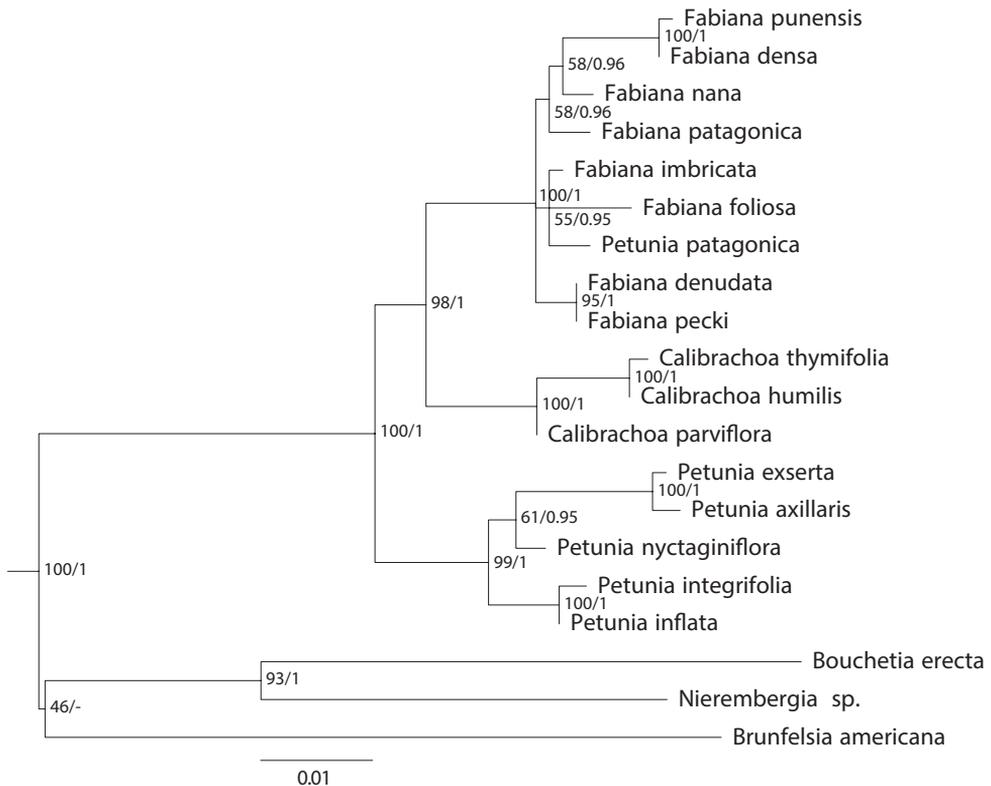


Figure 2. Nuclear waxy tree of 20 Petunieae species. Phylogeny based on maximum likelihood analysis of the nuclear waxy gene. Maximum likelihood bootstrap values and Bayesian posterior probabilities shown at nodes.

these phylogenetic results as well as shared morphological characteristics, geographical distribution, and chromosomal number between *Fabiana* and *Petunia patagonica* (Reck-Kortmann et al. 2015), the transfer of *Petunia patagonica* to the genus *Fabiana* is strongly supported.

Circumscription of *Fabiana* and transfer of *Petunia patagonica* to *Fabiana*

This study resolves the phylogenetic position of *Petunia patagonica*, an enigmatic species of controversial generic affinity. Spegazzini (1897) originally described this species in the genus *Nierembergia* based on a specimen collected in Gulf Saint George, Argentina. The corolla and androecium resemble other *Nierembergia* species, with a narrow base to the corolla tube that expands distally. Subsequently, in a review of *Nierembergia*, Millán (1941) transferred the species to *Petunia*, considering its floral characters as closer to this genus. The species has several characteristics that differentiate it from all other *Petunia* species and has been considered an outlier in the genus based on its geographic distribution and chromosome number (Stehmann and Greppi 2013;

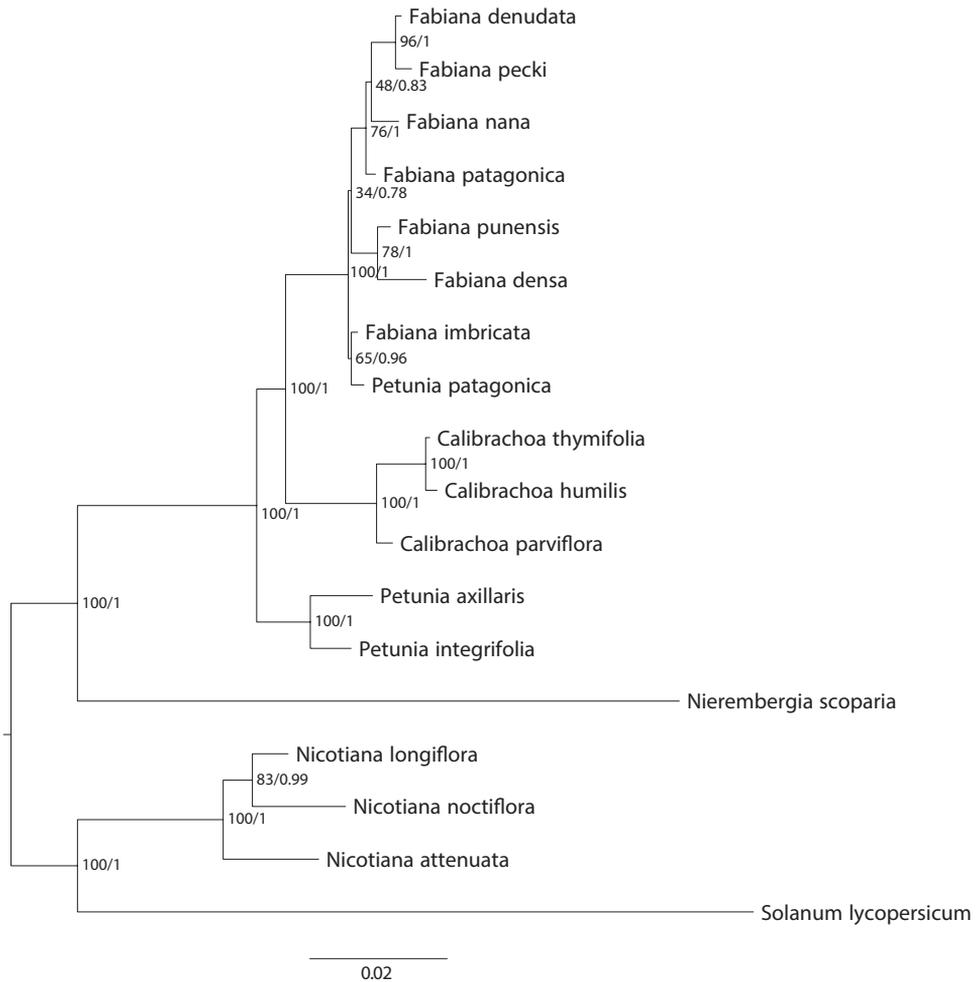


Figure 3. Combined chloroplast and nuclear DNA tree of 14 *Petunieae* species and four outgroups. Phylogeny based on maximum likelihood analysis of chloroplast fragments *trnS-trnG*, *trnL-trnF* and *psbA-trnH* and the nuclear waxy gene concatenated. Maximum likelihood bootstrap values and Bayesian posterior probabilities shown at nodes.

Reck-Kortmann et al. 2015). The species was included in a molecular phylogenetic study with several species of *Petunia*, two species of *Calibrachoa*, but only one species of *Fabiana* and was found in a small clade with *F. imbricata*, which was sister to a clade comprising the other species of *Petunia* (Reck-Kortmann et al. 2015).

Traditional classifications of *Petunia patagonica* have relied primarily on morphology, but with the insight gained from molecular phylogenetic studies, we can see that taxonomists weighed different floral traits in assigning the species first to *Nierembergia* and then to *Petunia*, while overlooking the similarities with *Fabiana*, including the resinous stems and dorsifixed anthers. Other characteristics, such as the chromosome number of

Petunia patagonica (n = 9), match those found in *Fabiana* species (Acosta et al. 2006). The particular distribution of this species in southern Patagonia (Fig. 4) and the environment where it grows, are similar to those of other *Fabiana* species (e.g., *F. imbricata*, *F. foliosa* (Speg.) S.C.Arroyo and *F. nana* (Speg.) S.C.Arroyo). The results of the molecular phylogenetic analyses obtained in this work also support its transfer to the genus *Fabiana*.

***Fabiana australis* Alaria, nom. nov.**

urn:lsid:ipni.org:names:77297063-1

Figures 4, 5

Petunia patagonica (Speg.) Millán. Darwiniana 5: 544 1941.

Basionym. *Nierembergia patagonica* Speg. Revista Fac. Agron. Univ. Nac. La Plata 3: 357. 1897, non *Fabiana patagonica* Speg. (1897). Type: Argentina. Prov. Santa Cruz, Golfo de San Jorge, C. Ameghino, Febr. 1896, “in campis aridis glariosis” (holotype: LP 006658!).

Description. Densely branched shrubs forming compact cushions of approximately 50 cm tall and up to 2.5 (-4) m in diameter; stems erect, leafy, glandular-pubescent, resinous. Leaves alternate but apparently verticillated by shortening of the internodes, sessile, fleshy, glandular; linear, elliptical or obovate, blade 3–4 (5) mm long by 1–2 mm wide. Flowers terminal, solitary, erect; flowering pedicels of 4–6 mm. Calyx tubular or slightly campanulate, (8–) 11–12 mm long, externally with dense glandulous indumentum, internally with scattered glandulous pubescence, short and broadly triangular lobes, 2–3 mm long by 2–3.5 mm wide, almost as long as wide. Corolla yellow with marked violet nerves, but also with color variation from light purple to deep violet, infundibuliform, 20–25 (–30) mm long, externally glandular, broad triangular lobes 2.5–5 mm long by 6–10 mm wide. Stamens heterodynamous, adhered to the middle third of the corolla, about 9 mm from the base of the corolla; filaments 2 short and 3 long, somewhat geniculate at the point of insertion with short glandular hairs scattered at the base; ellipsoid anthers of 1–1.5 mm. Ovary obovoid, 2.5–3 mm long, 1.2–1.5 mm wide, with nectariferous disc surrounding the base, 10–15 mm long style, truncated stigma, shallowly split. Capsule ovoid, 5–9 mm long by 3–5.5 mm wide. Seeds numerous, polyhedral in shape, 2–2.5 mm long.

Common name. “Mogote” meaning mound shape (Santa Cruz: Arroyo 1999)

Geographical distribution and habitat. Endemic to Patagonian Argentina, in the provinces of Chubut and Santa Cruz, from 90 to 700 m elevation, inhabiting dry and cold environments, on stony, sandy soils, sometimes rich in silt and clay, poor in organic matter. It forms large populations of cushion shrubs with numerous showy flowers (Fig. 6).

Taxonomic notes. The epithet *australis* was selected based on the restricted distribution of this species in southern Argentina. It is not possible to use *patagonica* as a specific epithet in *Fabiana*, because it is already in use in *Fabiana patagonica* Speg. *Fabiana australis* is one of the southernmost species of the genus. It shares with *F. foliosa* and *F. nana* a similar habit forming dense cushions in dry, cold, and poor soils of Patagonian Argentina.

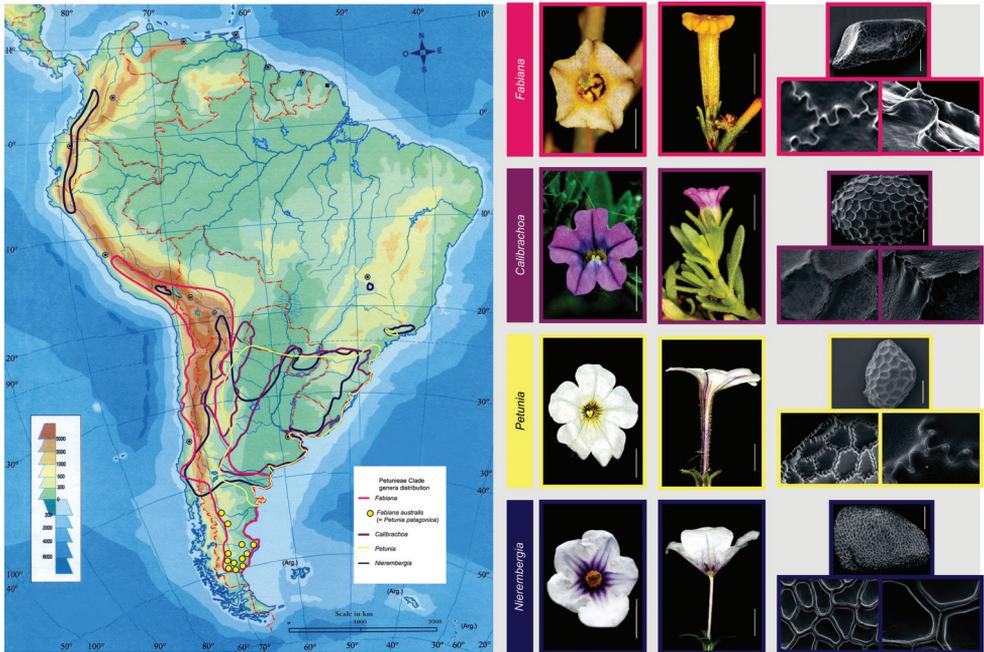


Figure 4. Geographic distribution of the genera *Fabiana*, *Calibrachoa*, *Petunia*, and *Nierembergia*, and the species *Fabiana australis* Alaria. Flowers and seeds of representative species: *Fabiana patagonica* Speng (first flower scale: 2.5 mm, second flower scale: 5 mm, seed scale: 0.5 mm, surface details magnifications 600× and 1,500×); *Calibrachoa parviflora* (Juss.) D'Arcy (first flower scale: 2.5 mm, second flower scale: 5 mm, seed scale: 0.5 mm, surface details magnifications 500× and 1,500×); *Petunia axillaris* (Lam.) Britton, Sterns & Poggenb. (first flower scale= 5 mm, second flower scale: 10 mm, seed scale: 0.5 mm, surface details magnifications 600× and 1,500×); *Nierembergia pulchella* Gillies ex Miers (first flower scale: 5 mm, second flower scale: 10 mm, seed scale: 0.5 mm, surface details magnifications 600× and 1,500×). Photograph IBODA, Flora Argentina database.

Representative specimens examined. ARGENTINA: **Santa Cruz:** Dpto. Corpen Aike, G.E. Barboza 3706 (CORD); O. Boelcke 16264 (BAB); A.A. Cocucci 3684 & 3723 (CORD); M.N. Correa 6527 (BAB); R.H. Fortunato 7492 (BAB); C.A. O'Donnell 3794 (CORD00015699). Dpto. Deseado, L.M. Bernardello & M.R. Figueroa Romero 335 (CORD00015696!); O. Boelcke, 12214 (BAB); A.A. Cocucci 4175 (CORD); M.N. Correa 2644 & 6697 (BAB); B.E. Leuenberger 4100 (B: D-14191 Berlin); M.C. Romanczuk 989 (UEC); F.B. Vervoort 5658 (CORD 00015700!). Dpto. Güer Aike, A. Soriano 5062 (BAB). Dpto. Lago Argentino, G.E. Barboza 3732 (CORD); A.A. Cocucci 471 (CORD 00015694!); R.H. Fortunato 4967 (BAB, ARIZ, NY, HRP); C. Guerrero 785 (SI). Dpto. Magallanes, G.E. Barboza 3704 (CORD); O. Boelcke 15394 (BAB); Iter Patagonicum 762 L. Hauman & C.M. Hicken (SI); B.E. Leuenberger & S. Arroyo 3710 (CORD 00015698!). Dpto. Río Chico, M.N. Correa & E.G. Nicora 3517 (BAB; CORD 00015697!); G.E. Barboza 3696 (CORD); G.E. Barboza 3746 (CORD; SI 063988!); G.E. Barboza 3748 (CORD); O. Boelcke 12810 (BAB); J.M. Bonifacino 2986 (SI); C.M. Hicken 10245 (SI); F.O. Zuloaga 13978 &

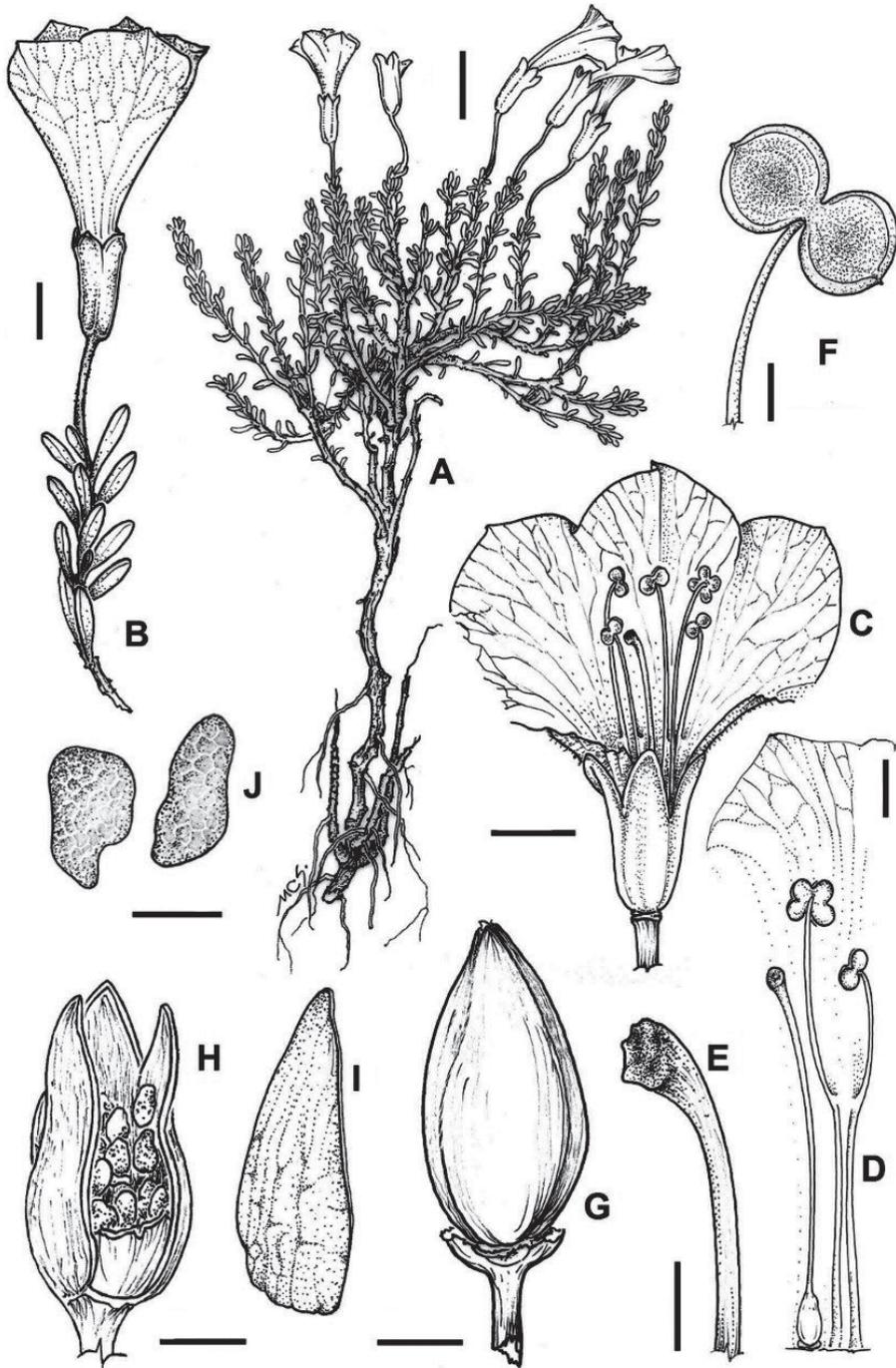


Figure 5. *Fabiana australis* Alaria. **A** plant **B** flowering branch **C** flower **D** corolla deployed showing gynoecium and stamens of different length **E** stigma **F** anthers **G** capsule **H** capsule showing seeds, **I** capsule valve **J** seeds. Scale bars: 10 mm (**A**); 4 mm (**B**); 5 mm (**C**); 2.5 mm (**D**); 1 mm (**E**); 0.5 mm (**F**); 2 mm (**G, H**). Illustration by Cecilia Scoones.



Figure 6. *Fabiana australis* Alaria. Plants habit and flower details (Zuloaga FO 13991, SI). Photograph IBODA, Flora Argentina database.

13991 (SI). Valle del Río Santa Cruz C. Burmeister s.n. & 95 (SI); M. Gentili 330 (BAB); J. Koslowsky 122 (CORD 00015695!). Without locality A. Donat 206 (SI); P.K.H. Dusén 5496 (SI); E. Molina Massey 31 (SI); Tessleff 5496 (SI). **Chubut:** Dpto. Futaleufú, A.A. Cocucci 3997 (CORD 00022097!); Dpto. Paso de Indios, S.C. Arroyo 208 (BAB, LIL, K); Dpto. Sarmiento, A. Alaria 321 (MERL). Dpto. Languíneo, A. Alaria 324 (MEN).

Taxonomic characters differentiating *Calibrachoa*, *Fabiana*, *Petunia*, and *Nierembregia* are described in the following key. Geographic distribution of the four genera and *Petunia patagonica*, as well as photographs of flowers and seeds of representative species of each genus, are illustrated in Figure 4.

Key to genera

- 1 Resinous shrubs to camephytes, stems densely leafy to partially foliated and even aphyllous; reduced membranaceous, slightly fleshy or leathery leaves. Dorsifixed anthers, usually elongated ***Fabiana***
- Non resinous, annual or perennial herbs, rarely subshrubs; leafy stems, developed membranaceous to fleshy leaves. Ventrifixed anthers with different shapes: reniform, globose, or ovate..... **2**

- 2 Hypocrateriform corolla with narrow and cylindrical tube. Androecium with 5 fertile stamens equal in length or heterodynamous, generally with 2 longer and 3 shorter stamens, adnate at the top edge of the corolla tube and generally connivent around the style; wide stigma usually tightly arranged between the anthers; staminal filaments and style apex usually straight. Nectary absent. Polyhedral seed, straight embryo.....*Nierembergia*
- Infundibuliform to campanulate, rarely hypocrateriform, corolla with wide tube. Androecium with 5 fertile heterodynamous stamens, generally with 2 longer, 2 medium length, and one shorter stamen, or 4 subequal and one shorter stamen, adnate at the top edge of the corolla tube but rarely connivent around the style; narrow stigma, staminal filaments and apex style usually curved. Nectary present. Ellipsoid, round, or reniform seed, straight or slightly curved embryo **3**
- 3 Corolla with reciprocative aestivation, the induplicated anterior lobe covering the other four conduplicated lobes, or rarely imbricate aestivation; calyx usually divided nearly to the middle, lobes narrowing towards the apex; seed episperm with straight anticlinal cell walls *Calibrachoa*
- Corolla with imbricate aestivation; deeply lobed calyx, lobes linear or spatulate, widening towards the apex; seed episperm with wavy anticlinal cell walls..... *Petunia*

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Appendix I

Voucher and locality information for specimens of species collected for this study and GenBank accession numbers for sequences used, with those generated for this study in bold.

Table A1.

Species	Voucher	Location	Coordinates	<i>trnL-trnF</i>	<i>trnS-trnG</i>	<i>psbA-trnH</i>	waxy
Pentstemonaceae							
<i>Bouchetia erecta</i> DC ex Dunal	D'Arcy 18213 MO	Mexico	–	–	–	–	OK120263
<i>Brunfelsia americana</i> L.	no voucher	Cultivated, USA, Marthaet Bot Gard	–	–	–	–	OK1166947
<i>Calibrachoa humilis</i> (R.E.Fr.) Stehmann & Semir	no voucher	Cultivated, Argentina, FCA, UNCuyo	33°00'28.2"S, 68°52'16.4"W	MZ855907	OK120228	MZ855925	OK120243
<i>Calibrachoa parviflora</i> (Juss) D'Arcy	Alaria 432 MERL	Cultivated, Argentina, FCA, UNCuyo	33°00'28.2"S, 68°52'16.4"W	MZ855908	OK120229	MZ855926	OK120244
<i>Calibrachoa missionica</i> Stehmann & Semir	no voucher	Cultivated, Argentina, FCA, UNCuyo	33°00'28.2"S, 68°52'16.4"W	MZ855909	OK120230	MZ855927	–
<i>Calibrachoa thymifolia</i> (A.St.-Hil.) Stehmann & Semir	no voucher	Cultivated, Argentina, FCA, UNCuyo	33°00'28.2"S, 68°52'16.4"W	MZ855910	–	MZ855928	OK120245
<i>Fabiana bryoides</i> Phil.	Alaria 444 MERL	Argentina, Jujuy	22°31'47.8"S, 66°18'45.4"W	MZ855911	OK120231	MZ855929	–
<i>Fabiana densa</i> J. Rémy	Alaria 365 MERL	Bolivia, Potosi	19°52'48.2"S, 65°40'44.4"W	MZ855912	OK120232	MZ855930	OK120246
<i>Fabiana demudata</i> Miers	Alaria 356 MERL	Argentina, Mendoza	32°29'16.3"S, 69°05'07.5"W	MZ855913	OK120233	MZ855931	OK120247
<i>Fabiana foliosa</i> (Speg.) S.C.Arroyo	Barboza 3760 CORD	Argentina, Santa Cruz	47°20'09"S, 70°59'05"W	–	–	MZ855932	OK120248
<i>Fabiana imbricata</i> Ruiz & Pav.	Alaria 397 MERL	Argentina, Mendoza	35°51'45.1"S, 69°48'27.5"W	MZ855914	OK120234	MZ855933	OK120249
<i>Fabiana nama</i> (Speg.) S.C.Arroyo	Alaria 316 MERL	Argentina, Chubut	45°47'44.5"S, 69°04'56.7"W	MZ855915	OK120235	MZ855934	OK120250
<i>Fabiana patagonica</i> Speg.	Alaria 359 MERL	Argentina, Jujuy	22°57'30"S, 65°25'39"W	MZ855916	OK120236	MZ855935	OK120251
<i>Fabiana peckii</i> Niederl.	Alaria 403 MERL	Argentina, Mendoza	34°31'55.2"S, 68°28'14.7"W	MZ855917	OK120237	MZ855936	OK120252
<i>Fabiana punensis</i> S.C.Arroyo	Alaria 048 MERL	Argentina, Tucumán	26°38'39.5"S, 65°49'12.5"W	MZ855918	OK120238	MZ855937	OK120253
<i>Nierembergia scoparia</i> Sendtn.	Alaria 431 MERL	Cultivated, Argentina, FCA, UNCuyo	33°00'28.2"S, 68°52'16.4"W	MZ855920	OK120240	MZ855939	OK120255
<i>Pentunia altilana</i> T. Ando & Hashim	–	–	–	AV772868	DQ792185	DQ791917	–
<i>Pentunia axillaris</i> (Lam.) Britton, Sterns & Poggenb.	Alaria 430 MERL	Argentina, Mendoza	32°58'45.7"S, 68°58'8"W	AY098702	JF918370	DQ225610	OK120258
<i>Pentunia exserta</i> Stehmann	Chau 312 WTU	Cultivated, USA, University of Washington	–	–	–	–	OK120259

Species	Voucher	Location	Coordinates	<i>tmlL-tmlF</i>	<i>tmS-tmG</i>	<i>psbA-tmH</i>	waxy
<i>Petunia inflata</i> R.E. Fr.	Olmstead S-62 WTU	Cultivated. USA, seed from Birmingham seed collection	—	—	—	—	OK120260
<i>Petunia integrifolia</i> (Hook.) Schinz & Tell.	Chau 311 WTU	Cultivated. USA, University of Washington	—	AY772873	JN565848	DQ208151	OK120261
<i>Petunia nyctaginiflora</i> Juss.	Olmstead S-63 WTU	Cultivated. USA, seed from Birmingham	—	—	—	—	OK120262
<i>Petunia patagonica</i> (Speg.) Millán	Alaria 321 MERL	seed collection Argentina, Chubut	45°56'16.8"S, 69°09'12.4"W	MZ855919	OK120239	MZ855938	OK120254
<i>Petunia scheideana</i> L.B. Sm. & Downs	—	—	—	AY772870	DQ792448	DQ792149	—
Outgroups							
<i>Benthameiella pycnophylloides</i> Speg.	Barboza 3688 CORD	Argentina, Santa Cruz	46°57'2"S, 67°22'24.6"W	MZ855921	OK120241	MZ855940	—
<i>Nicotiana attenuata</i> Torr. Ex S. Watson	—	—	—	AY098697	AJ584953	MG182422	KR083023
<i>Nicotiana longiflora</i> Cav.	Alaria 437 MERL	Argentina, Mendoza	32°59'47.7"S, 68°56'1.5"W	MZ855923	AJ584951	MZ855942	OK120256
<i>Nicotiana noctiflora</i> Hook.	Alaria 438 MERL	Argentina, Mendoza	32°59'49.4"S, 68°55'56.6"W	MZ855924	AJ584975	GQ248352	OK120257
<i>Pantacantha ameghinoi</i> Speg.	Barboza 3775 CORD	Argentina, Neuquén	38°52'0"S, 70°34'36.2"W	MZ855922	OK120242	MZ855941	—
<i>Solanum lycopersicum</i> L.	—	—	—	KY887587	HQ856092	KY887587	DQ169036

Hemilophia cardiocarpa (Brassicaceae), a new species from Yunnan, southwest China

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Abstract

Hemilophia cardiocarpa (Brassicaceae), the sixth species of the Chinese endemic genus *Hemilophia*, is described and illustrated. This plant is found in the Jiaozishan Mountains in Dongchuan District, northern Yunnan, southwest China. Morphologically, it shows close relationships with *H. rockii* and *H. pulchella*, but differs from it in the leaf shape and size, inflorescence, flower size, shape of fruit and length of its pedicel. An updated key to the taxa of *Hemilophia* is provided.

Keywords

Alpine flora, Cruciferae, endemism, *Hemilophia rockii*, Jiaozishan Mountains

Introduction

Hemilophia Franch. is a small genus established by the French botanist Adrien René Franchet based on Delavay's specimens from Lijiang, southwest China (Franchet 1889). Currently, five species of this genus are recognized, namely *H. franchetii* Al-Shehbaz, *H. pulchella* Franch., *H. rockii* O. E. Schulz, *H. serpens* (O. E. Schulz) Al-Shehbaz, *H. sessilifolia* Al-Shehbaz, Kats. Arai & H. Ohba (Zhou et al. 2001). All are restricted

to high-elevation habitats of the Hengduan Mountains in southwest China (Zhou et al. 2001; Al-Shehbaz 2015). The systematic position of *Hemilophia* in Brassicaceae is not well resolved up to now. Schulz (1936) placed it in the tribe Lepidieae DC., whereas this treatment was not supported by recent studies (Al-Shehbaz 2012; Nikolov et al. 2019; Walden et al. 2020; Francis et al. 2021; Liu et al. 2021).

During our botanical fieldwork to Jiaozishan Mountains in southwest China in August 2018, a showy plant of *Hemilophia* with cordiform fruits was collected. By thoroughly examining the specimens of this genus housed at herbaria KUN, PE and YUKU, and a survey of digital images available at the database Plant Photo Bank of China (<http://ppbc.iplant.cn/>), we found that this plant had been collected or photographed several times in Jiaozishan Mountains and was wrongly identified as *H. rockii* O. E. Schulz. Comparison with related species demonstrates that this plant actually represents a distinct species hitherto not described.

Materials and methods

The study followed the normal practice of plant taxonomic survey and herbarium taxonomy. Morphological studies of the new species were based on observation of living plants and specimens from the Jiaozishan Mountains in Dongchuan District, northern Yunnan, southwest China. Digital images of type specimens of genus *Hemilophia* available at the JSTOR Global Plants (<http://plants.jstor.org/>), as well collections housed at CDBI, KUN, PE, PYU and YUKU were examined and compared with the new species. Pertinent taxonomic literature (e.g., Al-Shehbaz 1999, 2002, 2015; Zhou et al. 2001) was extensively consulted. Measurements were carried out under a stereomicroscope (Olympus SZX2, Tokyo, Japan) using a ruler and a metric vernier caliper.

Taxonomy

***Hemilophia cardiocarpa* Huan C. Wang, Shao Y. Liu & Z. T. Ren, sp. nov.**

urn:lsid:ipni.org:names:77297069-1

Figs 1–3

Type. CHINA. Yunnan Province: Dongchuan District, Jiaozishan Mountains, on screes, 26°9'45"N, 102°56'7"E, alt. 3,970–4,000 m, 7 September 2018, *Huan-Chong Wang et al.* LQ4146 (Holotype: YUKU!; Isotypes: YUKU!)

Diagnosis. *Hemilophia cardiocarpa* is most similar to *H. rockii*, but clearly differs from the latter by its cauline leaves 5–8 mm long (vs. 3–6 mm long in *H. rockii*), 2–4 mm (vs. 1–3 mm) wide, pedicel of fruit shorter than or nearly equal to its leaflike bract (vs. significantly longer than leaflike bract) and fruit cordate (vs. spindle or narrowly oblong) in shape.

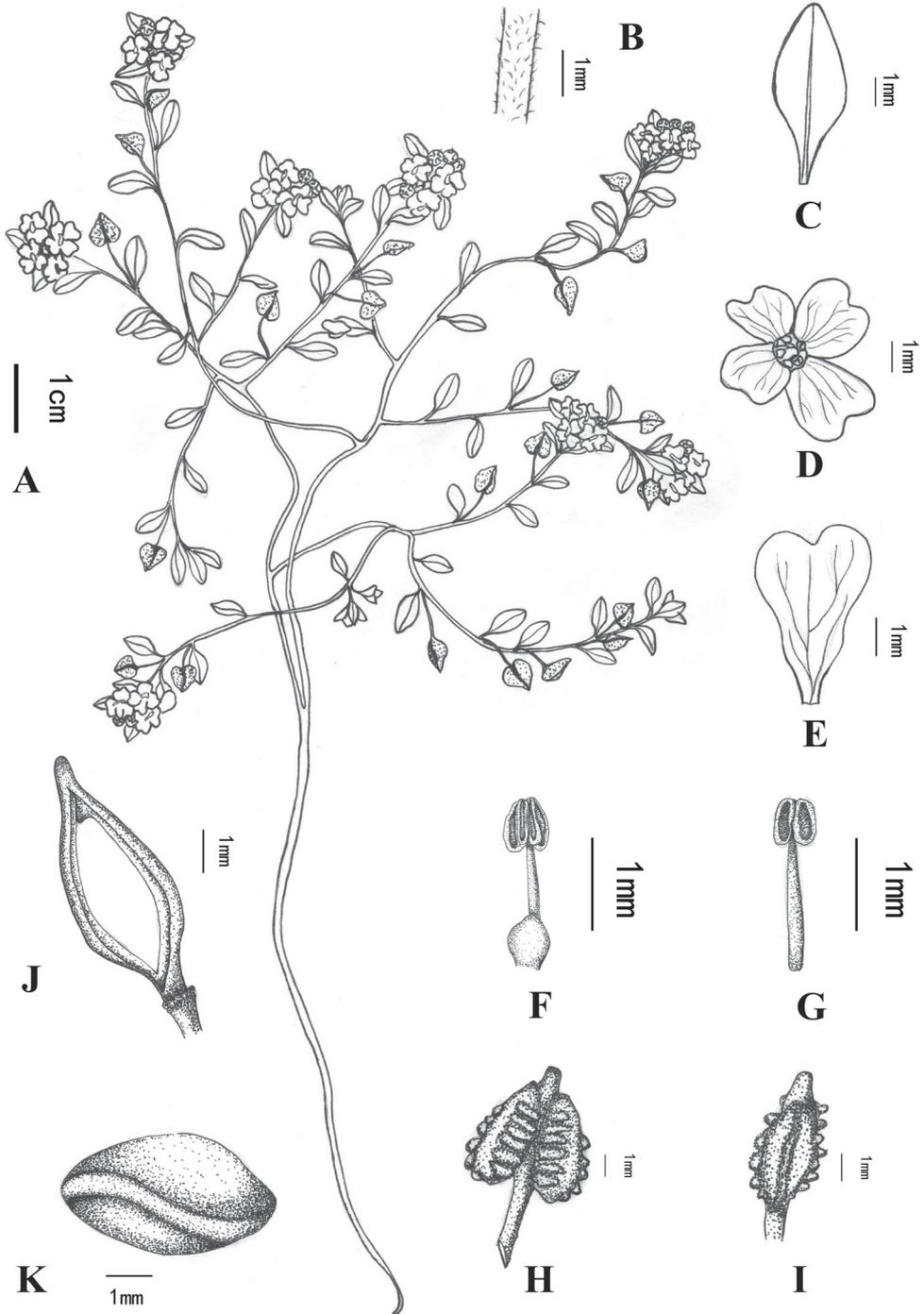


Figure 1. *Hemilophia cardiocarpa* **A** habit **B** a portion of stem showing trichomes **C** cauline leaf **D** flower (apical view) **E** petal **F** median filament **G** lateral filament **H** fruit (front view) **I** fruit (lateral view) **J** fruit after removal of valves and seeds **K** seed.

Description. Perennial herbs, cespitose, rhizomatous. Rhizomes slender, simple or branched, glabrous. Stems simple or few branched, 3–10 cm in length, with appressed, simple and minutely forked, 0.05–0.2 mm long trichomes. Basal leaves rosulate, obovate to oblanceolate, withering and deciduous at anthesis. Cauline leaves alternate; petioles blue-purple, glabrous, 1–2.5 mm long; blade ovate, obovate or oblong, 5–8 mm long, 2–4 mm wide, base cuneate, margin entire, apex usually acute to broadly acute, rarely obtuse; midveins sparsely pubescent adaxially, lateral nerves indistinct. Racemes terminal, usually 5–13-flowered. Pedicels 1–3 mm long, with dense and minute hairs. Sepals oblong or ovate, ascending, equal, caducous in fruit, 1–2 mm long, 0.8–1.25 mm wide, sparsely pubescent abaxially, margins membranous and ciliate, rounded at apex. Petals alternate with sepals, broadly obovate, bluish-white to white, with blue or a few purple veins on the lower half, 4–7 mm long, 2–3.5 mm wide, abruptly narrowed to claw at base, apex shallowly to deeply emarginate, apical notch up to 0.4–0.5 mm in depth. Stamens 6, in 2 whorls, slightly tetradynamous; filaments light yellow to purple, lateral pair slender, 1.2–1.5 mm long, median pairs 1.1–1.5 mm long, strongly dilated and appendaged at base; anthers nearly dark purple, 0.45–0.5 mm long, longitudinally dehiscent. Nectar glands surrounding base of lateral stamens, subtending base of median filaments. Pistil 2-carpelled, ovary sessile, ovate; style cylindrical, minutely papillate, nearly equal length to stamens. Pedicel of fruit elongated in fruit stage, 4–6 mm long, shorter than or nearly equal to leaflike bract in length. Fruit cordate in shape, glabrous, dehiscent, 5–5.5 mm long, 3–3.5 mm wide; valves navicular, thinly papery, with a crest of tubercles surrounding margin and extending along midvein; replum slender, 5.3–5.5 mm long; septum absent. Seeds 2 per fruit, usually ovoid, slightly flattened, 3.4–3.8 mm long.

Phenology. Flowering occurs from May to early September, and fruiting from August to October.

Etymology. The specific epithet *cardiocarpa* is derived from the Greek words “*kardio*” (heart) and “*karpos*” (fruit), referring to the fruit shape of this new species.

Vernacular name. Chinese Mandarin: xin guo ban ji ji (心果半脊芥).

Distribution and ecology. *Hemilophia cardiocarpa* appears to be a rare species endemic to the Jiaozishan Mountains, which are located in the northern Yunnan Province of southwest China with a highest elevation of 4344.1m and near to the Jinsha River. The new species grows on basaltic screes or open slopes at elevations ranging from 3900 to 4300 m, its association mainly include *Arenaria weissiana* Hand-Mazz. (Caryophyllaceae), *Draba amplexicaulis* Franch. (brassicaceae), *Scrophularia delavayi* Franch. (Scrophulariaceae), *Meconopsis integrifolia* (Maxim.) Franch. (Papaveraceae), *Ranunculus hirtellus* var. *orientalis* W. T. Wang (Ranunculaceae) and *Veronica szechuanica* subsp. *sikkimensis* (Hook.f.) Hong (Plantaginaceae).

Additional specimens examined (paratypes): CHINA. Yunnan: Dongchuan District, Jiaozishan Mountains, Lanniping, elev. 4300 m, 25 August. 1985, *Lan Shunbin* 547 (PE); Dongchuan District, Jiaozishan Mountains, Yaojingtan, elev. 4100 m, 25 June. 2020, *H. C. Wang et al.* DC8448 (YUKU); Dongchuan District, Jiaozishan Mountains, Jinfengkou, elev. 4200 m, 23 May 2021, *Ren Zhengtao et al.* DC12360 (YUKU).

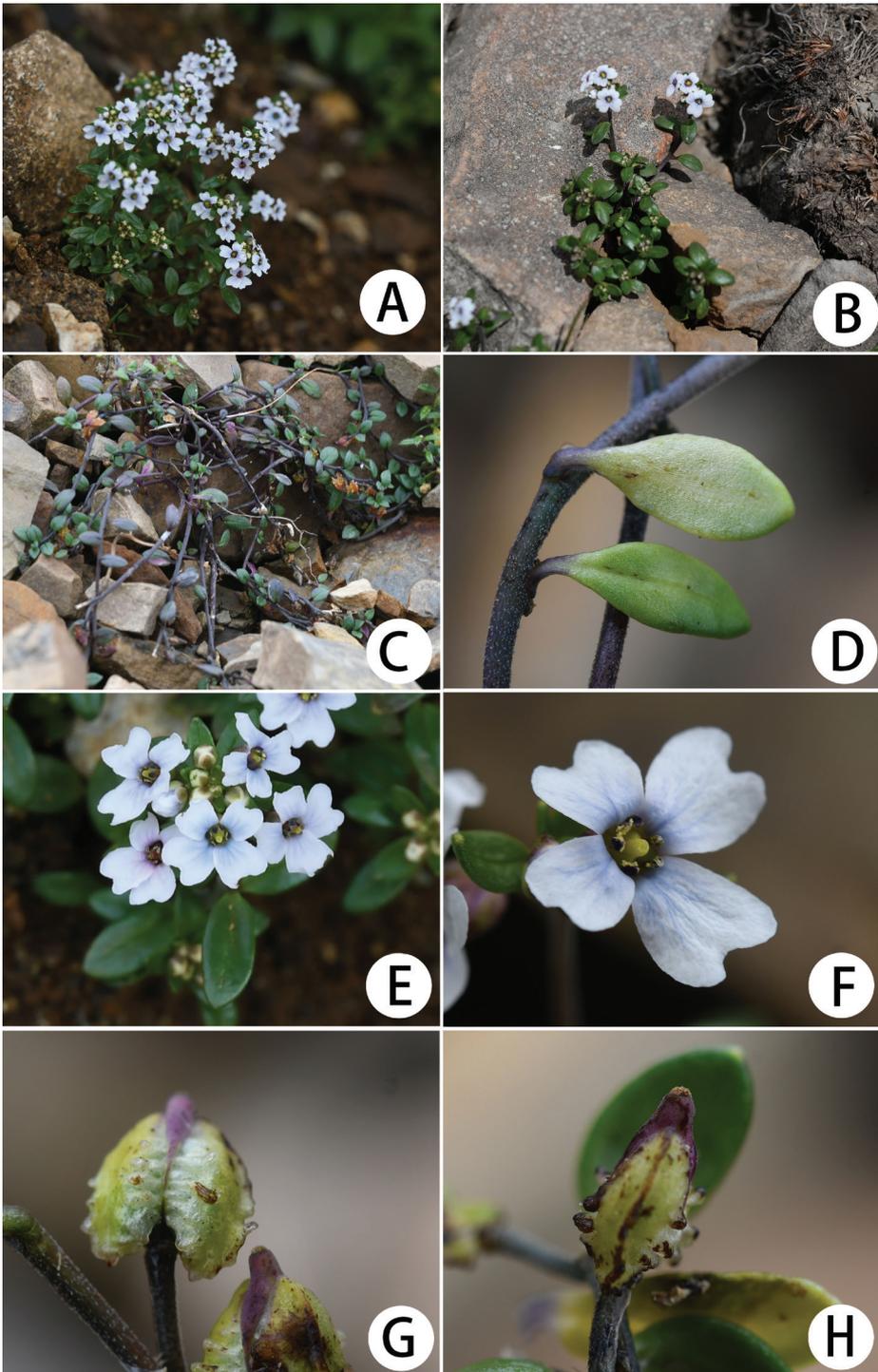


Figure 2. *Hemilophia cardiocarpa* **A–C** habit **D** a portion of stem with two cauline leaves **E** inflorescences in apical view **F** flower **G** fruit (front view) **H** fruit (lateral view).

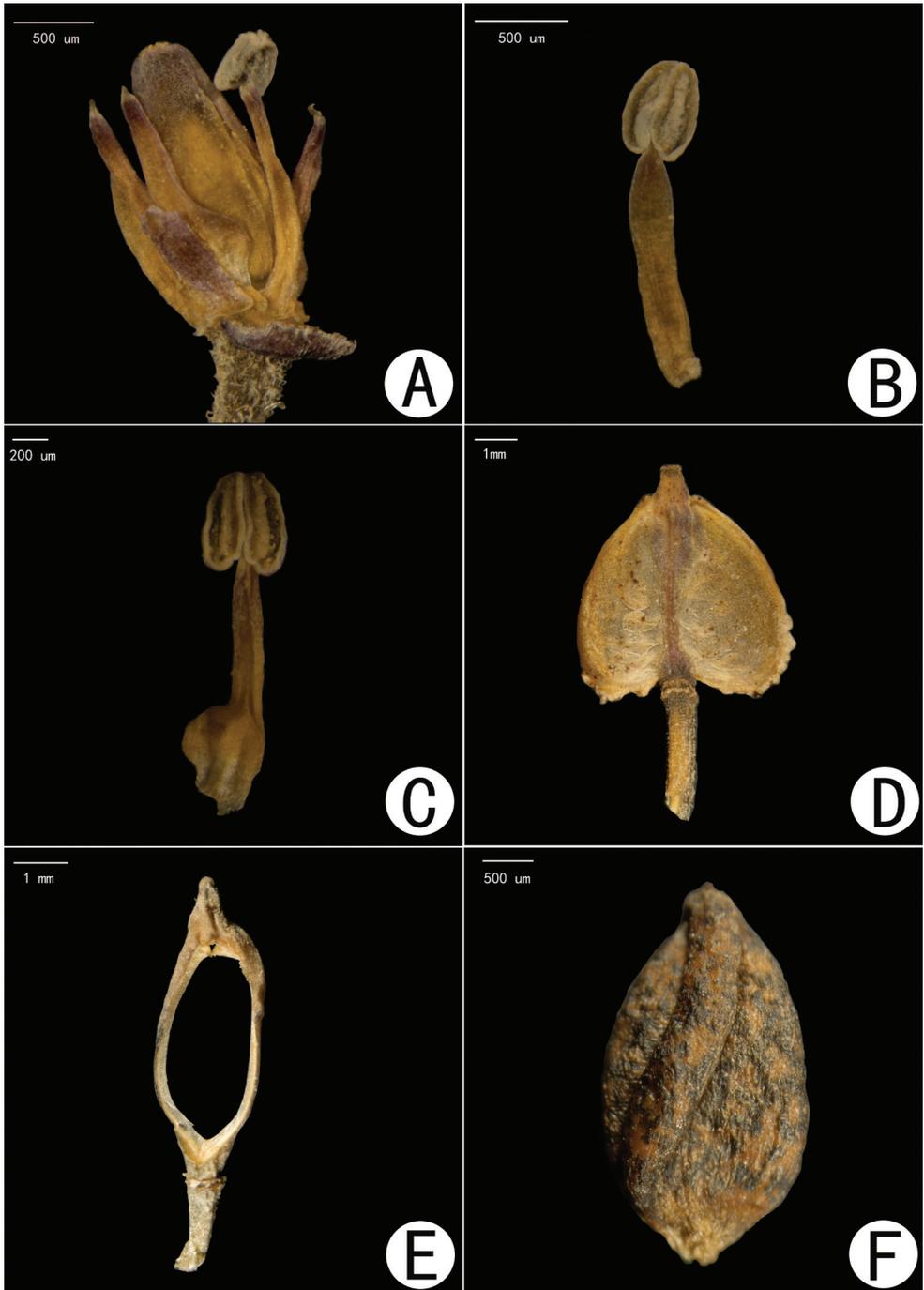


Figure 3. *Hemilophia cardiocarpa* **A** flower after removal of sepals and petals **B** lateral filament **C** median filament **D** fruit (front view) **E** fruit after removal of valves and seeds **F** seed.

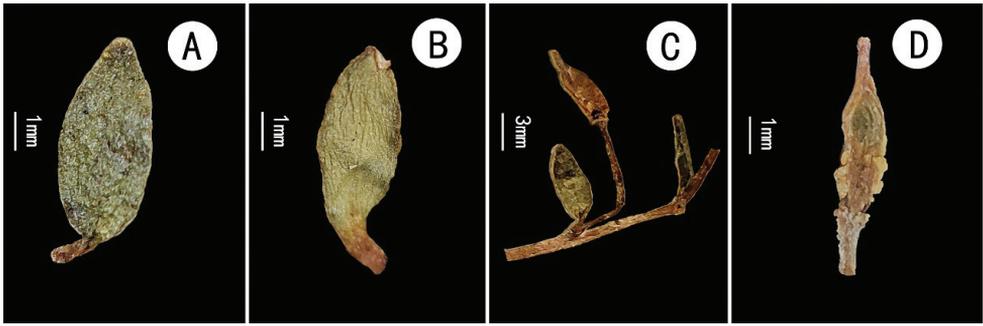


Figure 4. *Hemilophia rockii* **A** acauline leaf showing adaxial face **B** cauline leaf showing abaxial face **C** a portion of infructescence **D** fruit.

Taxonomic notes. The presence of cordiform fruits is the most remarkable character to distinguish *Hemilophia cardiocarpa* from other species of this genus. Morphologically, *H. cardiocarpa* is most similar to *H. rockii* (Fig. 4) in having a similar habit and sharing similar indumentum, leaf shape, as well as flower size and arrangement. Nevertheless, it clearly differs from the latter by its cauline leaves usually ovate, rarely obovate or oblong (vs. oblanceolate to narrowly elliptic, or rarely ovate in *H. rockii*), 5–8 mm (vs. 3–4 (6)) long, 2–4 mm (vs. 1–1.5 (3)) wide, racemes usually 5–13 (vs. 5–6) -flowered, pedicels of fruits shorter than or nearly equal to its leaflike bract (vs. significantly longer than leaflike bract) and fruit cordate (vs. spindle or narrowly oblong) in shape. *H. cardiocarpa* is also similar to the type species of the genus, *H. pulchella* Franch., from which it is readily distinguished by the racemes usually 5–13-flowered (vs. 2–3-flowered in *H. pulchella*), and petals bluish-white to white (vs. pink), 4–7 mm (vs. 2.5–3.5 mm) long, 2–3.5 mm (vs. 1.5–2 mm) wide. Taxa of *Hemilophia* can be distinguished through the morphological characters presented in the following identification key modified from Al-Shehbaz (2002, 2015).

Identification key to the species of *Hemilophia*

- 1a Cauline leaves sessile; sepals not ciliate at margin; style conical, glabrous.
- 2a Stems pubescent with simple trichomes only; petals creamy white with dark green veins; median filaments strongly inflated basally into an oblong appendage; ovules 2 per ovary..... *H. sessilifolia*
- 2b Stems pubescent exclusively with appressed, subsessile, forked and trifid trichomes; petals bright yellow with veins of same color as rest of limb; median filaments unappendaged at base; ovules 4 per ovary..... *H. serpens*
- 1b Cauline leaves petiolate; sepals ciliate at margin, if not then petals purple; style cylindrical, minutely papillate.
- 3a Petals purplish; leaves with setose, appressed trichomes; sepals not ciliate; stem trichomes malpighiaceae, not crisped..... *H. franchetii*
- 3b Petals pink, creamy white or yellowish; leaves glabrous or with crisped pilose trichomes; sepals ciliate; stem trichomes puberulent, crisped.

- 4a Petals 2.5–3.5 mm long, 1.5–2 mm wide, pink, narrowly obovate, shallowly emarginate; leaves glabrous or rarely sparsely pilose..... *H. pulchella*
- 4b Petals 4–7 mm long, 2–5 mm wide, creamy white to yellowish, obovate, deeply emarginate to nearly 2-lobed; leaves pilose or rarely glabrescent.
- 5a Petals creamy white; cauline leaves 5–8 mm long, 2–4 mm wide; pedicels of fruits shorter than or nearly equal length to leaflike bracts; fruits cordate in shape *H. cardiocarpa*
- 5b Petals creamy white to yellowish; cauline leaves 3–4 (6) mm long, 1–1.5 (3) mm wide; pedicels of fruits significantly longer than leaflike bracts; fruits spindle or narrowly oblong in shape..... *H. rockii*

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Terniopsis yongtaiensis (Podostemaceae), a new species from South East China based on morphological and genomic data

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Abstract

The new species *Terniopsis yongtaiensis* X.X. Su, Miao Zhang & Bing-Hua Chen, from Fujian Province, China, is described and illustrated. It is similar to *T. heterostaminata* from Thailand, but differs in its two fertile stamens, fewer but longer vegetative ramuli, fewer but shorter flowering ramuli, shorter pedicels, capsule-stalk and stamens. The complete chloroplast genome of the new species is 129,074 bp long and has a typical quadripartite structure, including two inverted repeat regions (IRs) of 18,504 bp in length, separated by a large single-copy (LSC) and a small single-copy (SSC) regions of 79,000 bp and 13,066 bp, respectively. The *ycf1* and *ycf2* genes were lost compared to most higher plants, leading to a substantial reduction in the IR. The phylogenetic analysis using both *matK* and nrITS revealed that *T. yongtaiensis* is sister to *T. heterostaminata* with moderate support, and formed a clade with other *Terniopsis* species, suggesting that the new species belongs to Tristichoideae.

Keywords

Biodiversity, chloroplast genome, morphology, phylogeny, taxonomy

Introduction

The Podostemaceae (river-weeds) are unique aquatic angiosperms that exist in various wetlands across the world's tropics and subtropics (Philbrick and Novelo 1995; Cook 1996; Koi et al. 2015). The plants grow immersed in rapid and turbulent currents and are tightly adhered to the surface of rocks during the rainy season, and then germinate, blossom, produce fruit and finally wither when the water level falls during the dry season. During the rainy season, the seeds are disseminated by wind, birds and running water; the seed coat becomes sticky and adheres to the rock surfaces, and then they germinate and develop seedlings (Täng and Kato 2020).

Three subfamilies, Podostemoideae, Weddellinoideae and Tristichoideae are recognized in the family Podostemaceae (Kita and Kato 2001; Koi et al. 2015). Morphologically, Tristichoideae has the least deviation in body plan, with a unique vegetative structure called “ramulus” that arises endogenously in the root tissue and is interpreted as leaf-stem intermediates because they combine typical leaf and typical stem characteristics (Fujinami and Imaichi 2009). There are five genera, viz. *Terniopsis* (= *Malaccotristicha*), *Tristicha*, *Indodalzellia*, *Indotristicha*, and *Dalzellia* in the subfamily Tristichoideae (Fujinami and Imaichi 2009; Koi et al. 2009) and only the genus *Terniopsis* is recorded in China (Chao 1948, 1980; Kato and Kita 2003).

Chao proposed *Terniopsis sessilis* H.C. Chao, a new genus and species. As name *Terniopsis* with the suffix *-opsis* means a plant similar to *Terniola* (= *Dalzellia*), Chao considered it as allied to *Terniola*. *Terniopsis* was described as a monotypic genus based on its floral traits (solitary or binary, sessile, axillary above the first basal leaves of flowering ramuli, two bracts, and cristate stigma), distinguishing it from Indian *Dalzellia* Wight (Chao 1948). Although the publication of Chao in 1948 was legitimate, it was unfortunately overlooked by authorities, so he redescribed it in 1980 (Chao 1980). Cusset and Cusset believed that the aforementioned characteristics were insufficient to support *Terniopsis* as a new genus, and reduced it under the genus *Dalzellia* Wight, which included *D. carinata* and *D. diversifolia* (Cusset and Cusset 1988). This view was accepted by the FOC (Flora of China) (Qiu and Philbrick 2003). Nevertheless, further molecular phylogenetic studies indicated that *T. sessilis* is sister to *Malaccotristicha* C. Cusset and G. Cusset (1988), and distant from *Dalzellia zeylanica* (type species of *Dalzellia*) (Kita and Kato 2001). Kato and Koi recognized the genus *Terniopsis* (Kato and Kita 2003), which was subsequently revised by Kato to include *Malaccotristicha* and *Dalzellia* sensu Cusset, pro parte, but excluded *D. zeylanica* (type species), as well as recognized *Terniopsis malayana* (= *Malaccotristicha malayana*). Furthermore, Kato included Australian *Tristicha australis* in *Terniopsis* as *T. australis* (Kato 2006). There are now 15 species in the *Terniopsis* genus around the world (Kato et al. 2003; Kato 2006; Kato and Koi 2009; Werukamkul et al. 2012; Koi and Kato 2015; Lin et al. 2016), including *T. australis* (C. Cusset & G. Cusset) Kato, *T. brevis* Kato, *T. chanthaburiensis* Kato & Koi, *T. filiformis* Werukamkul, Ampornpan, Koi & Kato, *T. heterostaminata* Werukamkul, Ampornpan, Koi & Kato, *T. malayana* (Dransfield & Whitmore, 1970) Kato, *T. microstigma* Koi & Kato, *T. minor* Kato & Koi, *T. ramosa*

Kato, *T. savannaketensis* Koi & Kato, *T. sesadensis* Koi & Kato, *T. sessilis*, *T. ubonensis* Kato, *T. vapyensis* Koi & Kato and *T. daoyinensis* Q.W.Lin, G. Lu & Z.Y.Li.

A *Terniopsis* species that resembles *T. heterostaminata* from Thailand was discovered during our field investigation in Yongtai County, Fujian Province. As a result of comprehensive research, we observed that the species has considerable variation in plant morphology, flower and fruit characteristics, and that its phylogenetic position is supported by molecular-level data. As a result, we conclude that it is a new species, *Terniopsis yongtaiensis*, based on morphological distinctions, geographical isolation, and molecular evidence.

Materials and methods

Morphological description

The morphological description of the new species was based on the specimens collected in a variety of localities in 2022. A stereoscopic zoom microscope (Carl Zeiss, Axio zoom. v.16, Germany), equipped with an attached digital camera (AxioCam), and a digital caliper were used to record the sizes of the morphological characters. Field observations provided habitats and phenology for the new species.

The leaf sample from Yongtai County, Fujian, China, was collected for DNA extraction.

DNA extraction, Genome sequencing, assembly, annotation and analysis

In this study, total DNA was extracted from freeze-dried material using DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Purified total DNA of the new species was fragmented, genome skimming was performed using next-generation sequencing technologies on the Illumina Novaseq 6000 platform with 150 bp paired-end reads and 350 bp insert size by Berry Genomics Co. Ltd. (Beijing, China), and 13.98 GB of reads was obtained.

The paired-end reads were filtered and assembled into complete plastome using GetOrganelle v.1.7.5 with appropriate parameters, with K-mer set “21,45,65,85,105” (Jin et al. 2020a). Following previous studies, our workflow includes five key steps as well (Camacho et al. 2009; Bankevich et al. 2012; Langmead and Salzberg 2012; Jin et al. 2020a). Graphs of the final assembly were visualized by Bandage to assess their completeness (Wick et al. 2015). Gene annotation was performed using CPGAVAS2 and PGA. Geneious v.2021.2.2 was used to manually calibrate the start and finish points for disputed positions (Jin et al. 2020a). The different annotations of protein coding sequences were confirmed using BLASTx. The tRNAs were checked with tRNAscan-SE v.2.0.3. Final chloroplast genome maps were created using OGDRAW.

The *matK* sequences were extracted using Geneious v.2021.2.2 from the chloroplast sequences deposited in the GenBank based on the annotated chloroplast genome. The nrDNA (18S-ITS1-5.8S-ITS2-26S) was assembled using GetOrganelle v1.7.5, with -R of 7 and k -merset of “35, 85, 115”, the embplant_nr library was selected as the reference genome database, then annotated and visualized using Geneious v2021.2.2.

Phylogenetic analysis

Phylogenetic analyses were conducted using Maximum likelihood (ML) and Bayesian Inference (BI) analyses, based on the *matK* and nrITS sequences. To construct the phylogenetic tree using *matK* sequence, 27 species (Suppl. material 1: Table S1) of *Terniopsis*, *Tristicha*, *Dalzellia*, *Weddellina*, *Polypleurum*, *Zeylanidium* and *Tristellateia* were included in our analysis. A species of *Tristellateia* was selected as outgroup. Each individual sequence was aligned using MAFFT 7.310 (Kato and Standley 2013) with default settings. A concatenated supermatrix of the two sequences was generated using PhyloSuite v.1.1.15 (Zhang et al. 2019) for the phylogenetic analysis. All missing data were treated as gaps. Gblocks 0.91b (Castresana 2000) was applied to eliminate poorly aligned regions of the concatenated supermatrix with gaps set as no different to other positions. The best nucleotide substitution model according to Bayesian Information Criterion (BIC) was TVM+F+G4, which was selected by Model Finder (Kalyanamoorthy et al. 2017) implemented in IQTREE v.1.6.8. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the model automatically selected by IQ-TREE ('Auto' option in IQ-TREE) for 1000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under GTR+F+G4 model (2 parallel runs, 2000000 generations), in which the initial 25% of sampled data were discarded as burn-in. Phylograms were visualized in iTOL v.5.

To construct the phylogenetic tree using nrITS, 13 species of *Terniopsis* and *Cladopus* (Suppl. material 1: Table S2) were included. A species of *Cladopus* was employed as outgroup. The study was carried out as described above, and according to the Bayesian Information Criterion (BIC), the optimal nucleotide substitution model was GTR+F+G4. The best nucleotide substitution model according to Bayesian Information Criterion (BIC) was HKY+F+G4, which was selected by Model Finder

Table 1. Comparison of two phylogenetically closely related and two other domestic species of *Terniopsis* from China.

Characteristics	<i>T. yongtaiensis</i>	<i>T. heterostaminata</i>	<i>T. sessilis</i>	<i>T. daoyinensis</i>
Root width (mm)	0.3–1.1	0.4–1.6	1–1.5	1–3
Root color	blackish-green	/	purplish-red	/
vegetative ramulus number	1	1–or2–(or3)	1	1
Flowering shoot associated ramulus number	1–2	1–4	1	2–or3
Ramulus length (mm)	1.8–22	1.4–14	7–9	3–30
Flower number per flowering shoot	1	1	1–2	1
Pedicle length (mm)	1.1–2.5	1.7–7	ca. 1.2	4–10
Capsule-stalk length (mm)	1.9–3.1	2.5–8	ca. 1	5–10
Stamen number	2	2 (rarely 3)	2,3	3
Stamen length (mm)	1.1–1.3	1.5–3	0.9–2.5	2–4
Ovary length (mm)	0.9–1.4	0.9–1.5	0.6–0.8	1.5–2
Stigma length (mm)	0.5	0.2–0.5	0.1–0.2	1
Stigma shape	Cristate	Cristate	cristate	multi-furcate
Capsule shape	Obovoid	Obovoid	elliptical	oblong-obovoid
Distribution	China	Thailand, Laos	China	China

(Kalyaanamoorthy et al. 2017) implemented in IQTREE v.1.6.8. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the model automatically selected by IQ-TREE ('Auto' option in IQ-TREE) for 1000 ultrafast (Minh et al. 2013) bootstraps.

Results

Taxonomic treatment

Terniopsis yongtaiensis X.X. Su, Miao Zhang & Bing-Hua Chen, sp. nov.

urn:lsid:ipni.org:names:77297070-1

Figs 1–4

Type. China. Fujian: Yongtai County, Fuquan Town, elevation 95 m, 25°51'N, 118°52'E, 2 January 2022, *Bing-Hua Chen* CBH 04587 (Holotype, FNU!, barcode FNU0041314; isotypes FNU!, Barcode FNU0041315).

Diagnosis. *Terniopsis yongtaiensis* is similar to *T. heterostaminata*, a remarkable species from Thailand, by having single flower per flowering ramulus, similar ovary length, same shape of stigma and capsule. However, *T. yongtaiensis* has 2 fertile stamens, less number (1 *vs.* 1–3) but longer (13.0–21.9 mm *vs.* 1.4–14 mm) vegetative ramuli, less (1–2 *vs.* 1–4) but shorter (1.8–5.5 mm *vs.* 1.2–15 mm) flowering ramuli, shorter (1.1–2.5 mm *vs.* 1.7–7 mm) pedicels, shorter (1.9–3.1 mm *vs.* 2.5–8 mm) capsule-stalk, and shorter (1.1–1.3 mm *vs.* 1.5–3 mm) stamens.

The variations in morphology between *T. yongtaiensis* and the other two *Terniopsis* species from China, *T. sessilis* and *T. daoyinensis*, are more obvious. *T. yongtaiensis* shows clear differentiation between vegetative and reproductive stems, the erectness of the ramuli, and the characteristics of flower and fruit are distinctive from those of *T. sessilis* from Changting County, Fujian Province (Table 1, Suppl. material 1: Figs S2–S4). However, *T. daoyinensis* from Hainan differs significantly from other species of the genus by its long (up to 1 mm) and distinctly multi-furcated stigmas (Table 1).

Description. Perennial herbs. Ribbon-like roots, flattened to subcylindrical, 0.59 (0.30–1.07) mm wide, 0.58 mm thick, monopodially branched, adhering to rock surface, dark green in water, turns purplish-red or brick-red at flowering or when water is shallow; vegetative ramuli on both flanks of roots, upright, 17.58 (3.00–21.90) mm long, ca. 0.28 mm wide; leaves 48 (39–55), elliptic or spatulate, flattened, sessile, entire, subdistichous; the top leaves are usually larger than the basal ones, 1.73 (0.96–1.66) mm long, 0.65 (0.56–0.76) mm wide, the basal leaves gradually fall off during growth; flowering shoots grow lateral to vegetative ramuli, with a single flower and 1–2 associated upright ramuli, 3.14 (1.76–5.53) mm long, 0.31 mm wide, each has 24 (17–32) leaves, 0.93 (0.61–1.24) mm long, 0.53 (0.35–0.75) wide, elliptic or broad-ovate, tristichous, subequal, smaller than leaves on vegetative ramuli (Fig. 1), all ramuli and leaves wither when fruiting. Flowers bisexual, small, solitary, petiolate, grows in axils

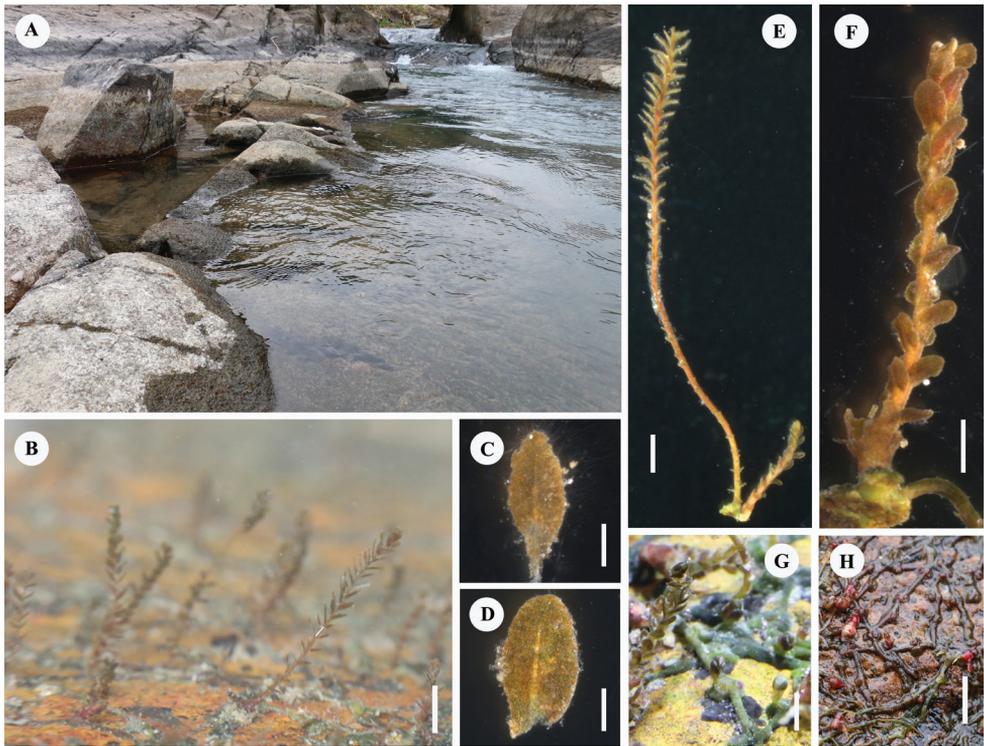


Figure 1. **A** habitat **B** vegetative ramulus, upright, subdistichous (photo in aquarium) **C** leaf on the vegetative ramulus **D** leaf on the fertile ramulus **E** vegetative ramulus (left, long) and fertile ramulus (right, short) **F** fertile ramulus with tristichous leaves **G** flattened ribbon-like roots, (dark green in water) **H** subcylindrical roots (purplish-red at flowering or when water is shallow). Scale bars: 4 mm (**B**, **H**); 0.4 mm (**C**, **D**); 2 mm (**E**, **G**); 0.2 mm (**F**).

of first leaves at base of flowering shoots; bracts 2, helmet-shaped, thinly membranous, pink or light red, 1.27 (1.08–1.61) mm long, 1.09 (0.80–1.45) mm wide; pedicel, 1.58 (1.13–2.52) mm long, ca. 0.41 mm in diameter; tepals, ca. 1.05 mm long, ca. 1.12 mm wide, shallowly lobed, lobes 3, red purple, semicircular, ca. 0.42 mm long, ca. 0.68 mm wide, lower part of tepals unite urceolated, turns to white when flowering; stamens 2, 1.21 (1.14–1.33) mm long, with introrse anthers, less than the perianth lobes, short filaments, segregate, base attached to ovary, 0.59 mm long; anthers 4, elliptic, 0.61 mm long, endocentric, rounded at the base. Ovaries elliptic, 3-locular, 1.13 (0.94–1.39) mm long, 1.03 (0.90–1.22) mm wide; ovules, 34 per locule; stigmas 3, padded, cristate, 0.16 mm tall, 0.49 mm long, 0.43 mm wide (Fig. 2). Capsule, 9-ribbed, obovoid, 1.15 (1.01–1.52) mm long, 0.98 (0.78–1.25) mm wide, fissured into 3 equal pieces at maturity; Capsules stalked, 2.48 (1.87–3.07) mm long; seeds ca. 25, green, teardrop-shaped, slightly concave at top, 0.21 (0.19–0.24) mm long (Fig. 3).

Florescence December to January, fruiting season January to February.

Distribution, habitat and conservation status. *Terniopsis yongtaiensis* is only known from Yongtai, Fujian, China (Suppl. material 1: Fig. S1), where it

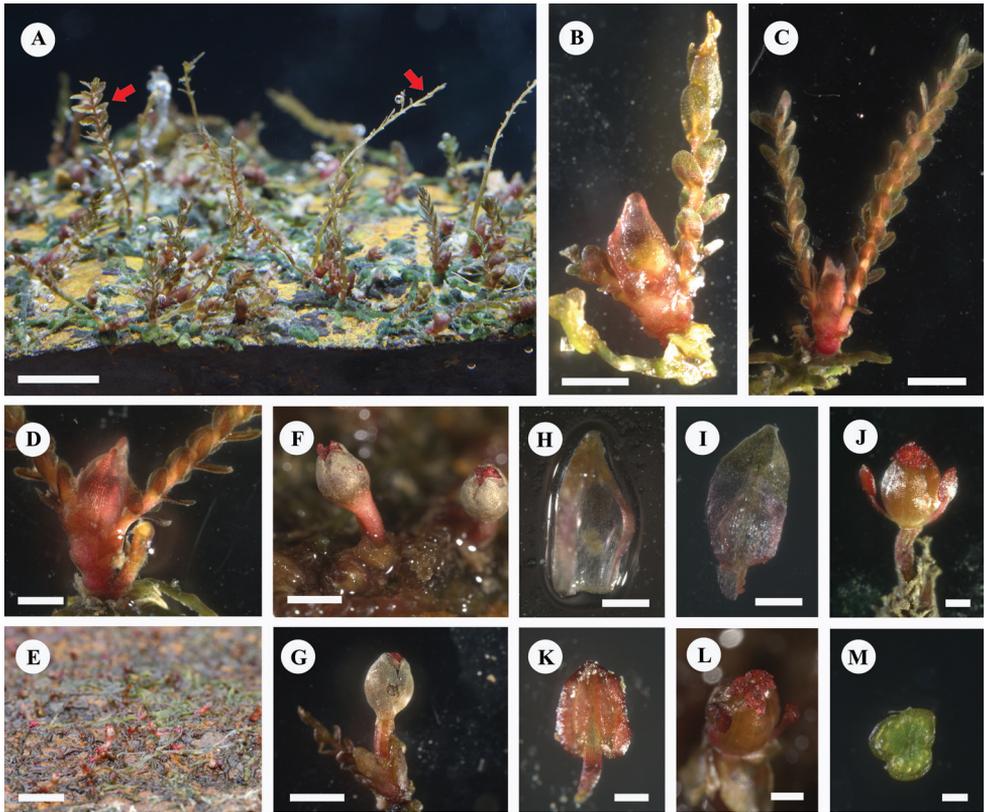


Figure 2. **A** branched flattened root with vegetative ramuli (red arrow) and young flower (shoot) on flank (photo in aquarium) **B, C** flower bud above bracts associated with short shoots (2-ramuli), showing leaves in 3 ranks **D** Young shoot associated with two ramuli and broken vegetative ramulus **E** flowers **F** two flowers at anthesis, showing withered ramuli **G** flower subtended with 2 bracts at base and associated with ramuli, showing pedicel and urceolate corolla **H** bract **I** tepal **J** flower with 2 stamens **K** stamen **L** top oblique view of flower, showing 3 cristate stigmas **M** cross section of the ovary, showing three locules. Scale bars: 5 mm (**A, E**); 1 mm (**B-D, F, G**); 250 μm (**H, I, J, L**); 100 μm (**K**); 200 μm (**M**).

grows on rocks in unpolluted streams, sometimes covering the entire surface of the rock. Many other plants grow in the surrounding habitat, whose tree layer includes *Ficus microcarpa* L. f. (Moraceae), *Prunus mume* Sieb. (Rosaceae), *Rhus chinensis* Mill. Anacardiaceae, *Schefflera heptaphylla* (Linnaeus) Frodin (Araliaceae) and others; the shrub layer includes *Ficus erecta* Thunb. (Moraceae), *Callicarpa kochiana* Makino (Lamiaceae), *Buddleja asiatica* Lour. (Scrophulariaceae), *Adina rubella* Hance (Rubiaceae) and others; the vegetation layer includes *Polygonum lapathifolium* L. (Polygonaceae), *P. chinense* L. (Polygonaceae), *Rubus hirsutus* Thunb. (Rosaceae), *Ludwigia epilobioides* Maxim. (Onagraceae), *Colocasia antiquorum* Schott (Araceae), *Panicum repens* L. (Poaceae), *Miscanthus floridulus* (Lab.) Warb. ex Schum et Laut. (Poaceae), *Neyraudia reynaudiana* (Kunth.) Keng (Poaceae), *Isachne globosa* (Thunb.) Kuntze (Poaceae), *Saccharum arundinaceum*

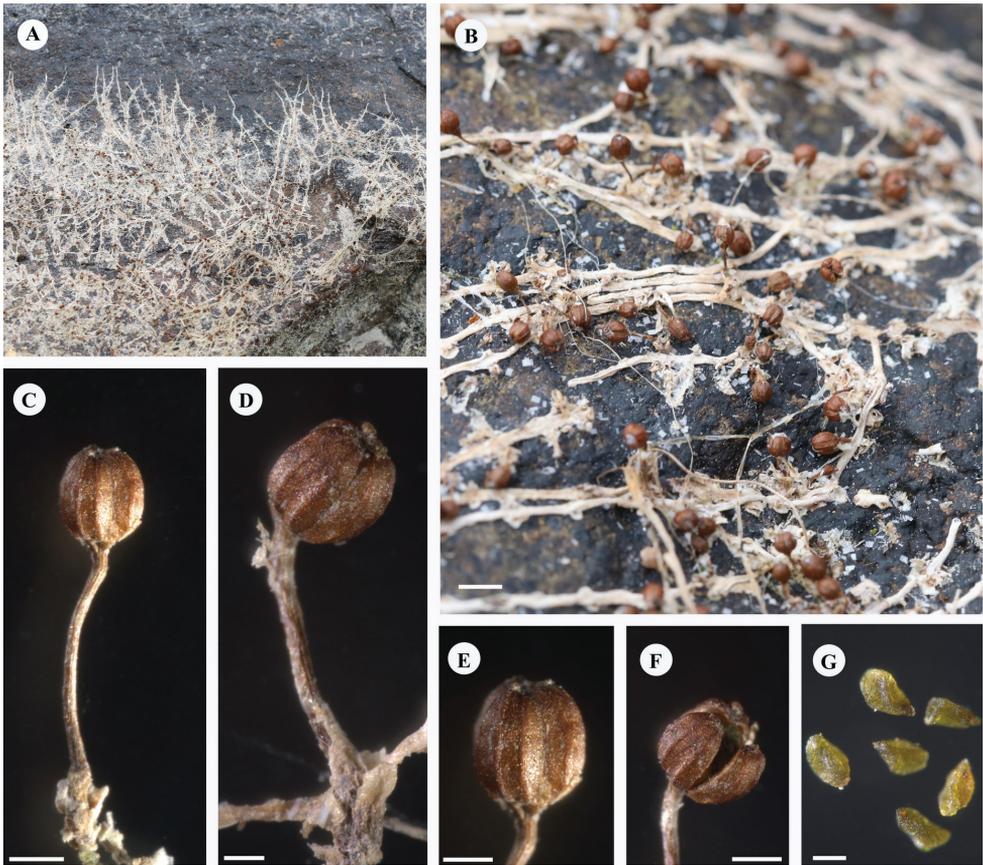


Figure 3. *Terniopsis yongtaiensis* **A** plants attached to stone surfaces in patches, withered after fruiting, banded-roots visible, in the dry season when the river level is reduced **B** habitat, showing ripe or nearly ripe fruits and withered roots **C, D** stalked fruit **E** fruit with 9 ribs **F** ripe fruits with dehiscent capsule, showing 3 lobes **G** seeds. Scale bars: 2 mm (**B**); 1 mm (**C**); 0.5 mm (**D, E**); 100 μ m (**F**).

Retz. (Poaceae), *Commelina communis* L. (Commelinaceae), *Musa nana* Lour. (Musaceae) and others; the interlayer plants includes *Cocculus orbiculatus* (L.) DC. (Menispermaceae), *Pueraria montana* (Loureiro) Merrill (Fabaceae) and others; and some exotic plants includes *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), *Bidens pilosa* L. (Asteraceae) and others.

Conservation status: According to our investigation, *Terniopsis yongtaiensis* was only found in a stream in Yongtai County, Fujian Province, China and hence, we suggest its placement in the Data Deficient category of IUCN (2022). In addition, according to the Updated List of National Key Protected Wild Plants (Decree No. 15) by the country's State Forestry and Grassland Administration and the Ministry of Agriculture and Rural Affairs, all of the known genera of Podostemaceae found in China are classified as under national secondary protection. This new species should

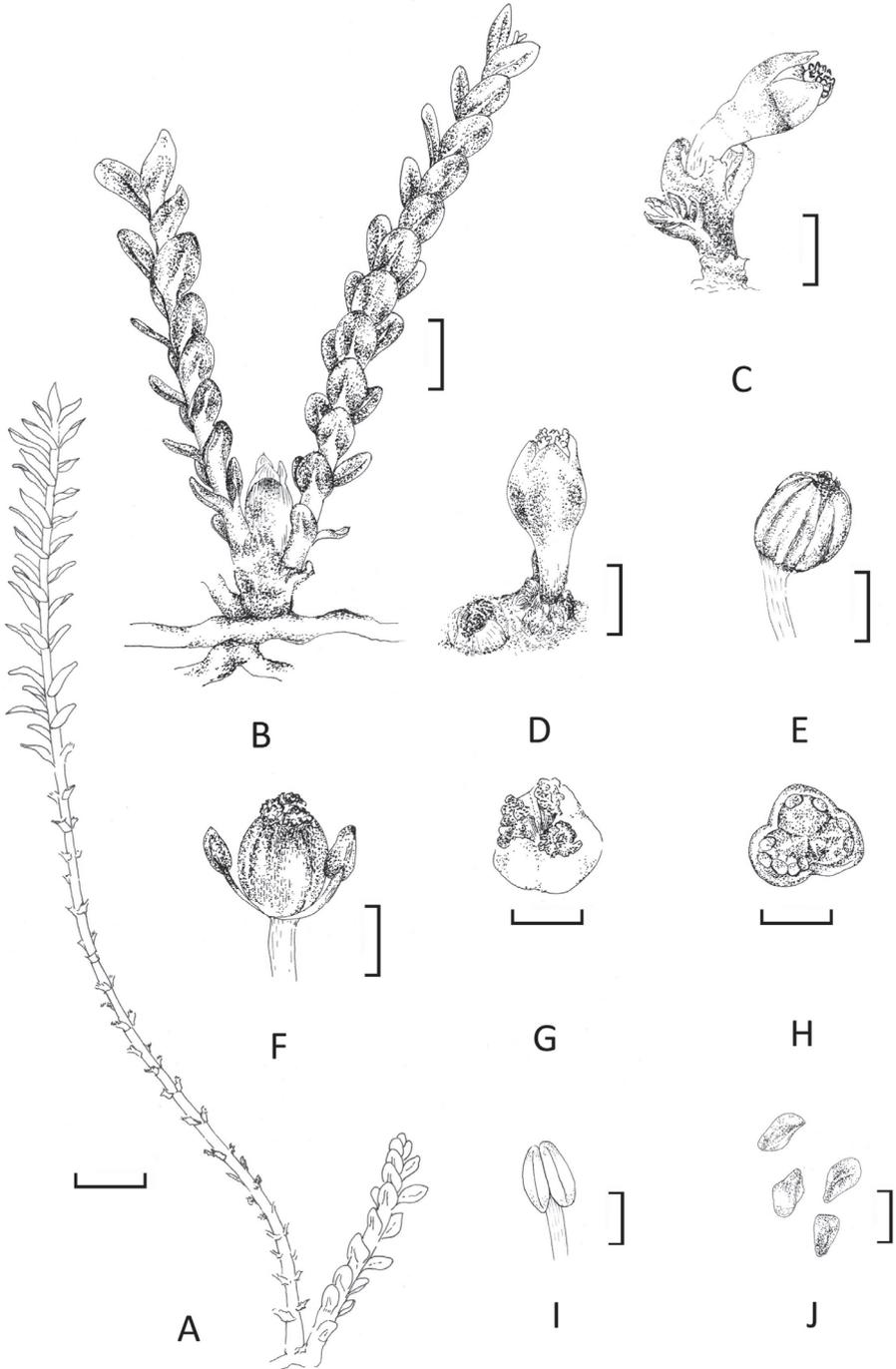


Figure 4. Illustration of *Terniopsis yongtaiensis* **A** vegetative ramulus (left, long) and fertile ramulus (right, short) **B** flower bud above bracts associated with short shoots (2-ramuli) **C** flower subtended with 2 bracts at base and associated with ramulus **D** flower at anthesis, showing withered ramuli **E** fruit with 9 ribs **F** flower with urceolate corolla removed, 2 stamens on side of ovary **G** cristate stigmas **H** cross section of the ovary **I** stamen **J** seeds. Scales bars: 1 mm (**A**); 500 µm (**B**); 250 µm (**C-H**); 100 µm (**I**); 50 µm (**J**).

Table 2. Gene contents in the plastid genome of *Terniopsis yongtaiensis*.

Category, Group of Genes	Gene Names
Photosynthesis:	
Subunits of ATP synthase	<i>atpA, atpB, atpE, atpF*, atpH, atpI</i>
Subunits of NADH dehydrogenase	<i>ndhA*, ndhB*(x2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
Cytochrome b/f complex	<i>petA, petB*, petD*, petG, petL, petN</i>
Subunits of photosystem I	<i>psaA, psaB, psaC, psal, psaj</i>
Subunits of photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbK, psbJ, psbL, psbM, psbN, psbT</i>
Large subunit of rubisco	<i>rbcL</i>
Other genes:	
Subunit of Acetyl-CoA-carboxylase	<i>accD</i>
c-type cytochrome synthesis gene	<i>ccsA</i>
Envelope membrane protein	<i>cemA</i>
Maturase	<i>matK</i>
Self-replication:	
Large subunit of ribosome	<i>rpl2*(x2), rpl14, rpl16*, rpl20, rpl23(x2), rpl33, rpl36</i>
DNA dependent RNA polymerase	<i>rpoA, rpoB, rpoC1*, rpoC2</i>
Small subunit of ribosome	<i>rps2, rps3, rps4, rps7(x2), rps8, rps11, rps12*(x2), rps14, rps15, rps18, rps19</i>
rRNA Genes	<i>rrn4.5S(x2), rrn5S(x2), rrn16S(x2), rrn23S*(x2)</i>
tRNA Genes	<i>trnA-UGC*(x2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnFM-CAU, trnG-GCC, trnH-GUG, trnI-GAU*(x2), trnI-CAU(x2), trnK-UUU*, trnL-CAA(x2), trnL-UAA*, trnL-UAG, trnM-CAU, trnN-GUU(x2), trnP-UGG, trnQ-UUG, trnR-ACG(x2), trnR-UCU, trnS-UGA*, trnS-GCU, trnS-GGA, trnT-CGU, trnT-GGU, trnT-UGU, trnV-GAC(x2), trnV-UAC*, trnW-CCA, trnY-GUA</i>
Unknown function:	
Conserved open reading frames	<i>ycf3*, ycfA, infA</i>

Note: * genes containing introns; (x2) genes present as two copies in the IR regions; * indicates trans-spliced gene.

also be included on the national secondary protection list during the upcoming revision process.

Etymology. The epithet *yongtaiensis* (永泰) refers to Yongtai County, Fujian Province where this new species was found.

Characteristics of the *Terniopsis yongtaiensis* plastome

The plastome of *Terniopsis yongtaiensis* (Fig. 5) is 129,074 bp in length, and exhibits a typical quadripartite structure, consisting of a large single copy (LCS) region of 79,000 bp and a small single copy (SSC) region of 13,066 bp, which were separated by a pair of 18,504 bp inverted repeat regions (IRs). The gene map of *T. yongtaiensis* is presented in Fig. 5. The gene composition in plastome of *T. yongtaiensis* would be divided into four categories: gene related to photosynthesis, genes related to self-replication, protein-coding genes with unknown functions, and other genes. A total of 106 unique genes were identified in the plastome; it contains 72 protein-coding genes, 30 tRNAs, and 4rRNAs. A total of 16 genes were duplicated in the IR regions, including *ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, *rrn4.5S*, *rrn5S*, *rrn16S*, *rrn23S*, *trnA-UGC*, *trnI-GAU*, *trnI-CAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, *trnV-GAC*. A total of six genes were lost, including *psbZ*, *clpP*, *rpl22*, *rpl32*, and uncommon losses of *ycf1* and *ycf2*. The annotated plastome was documented in GenBank (accession number OM717943).

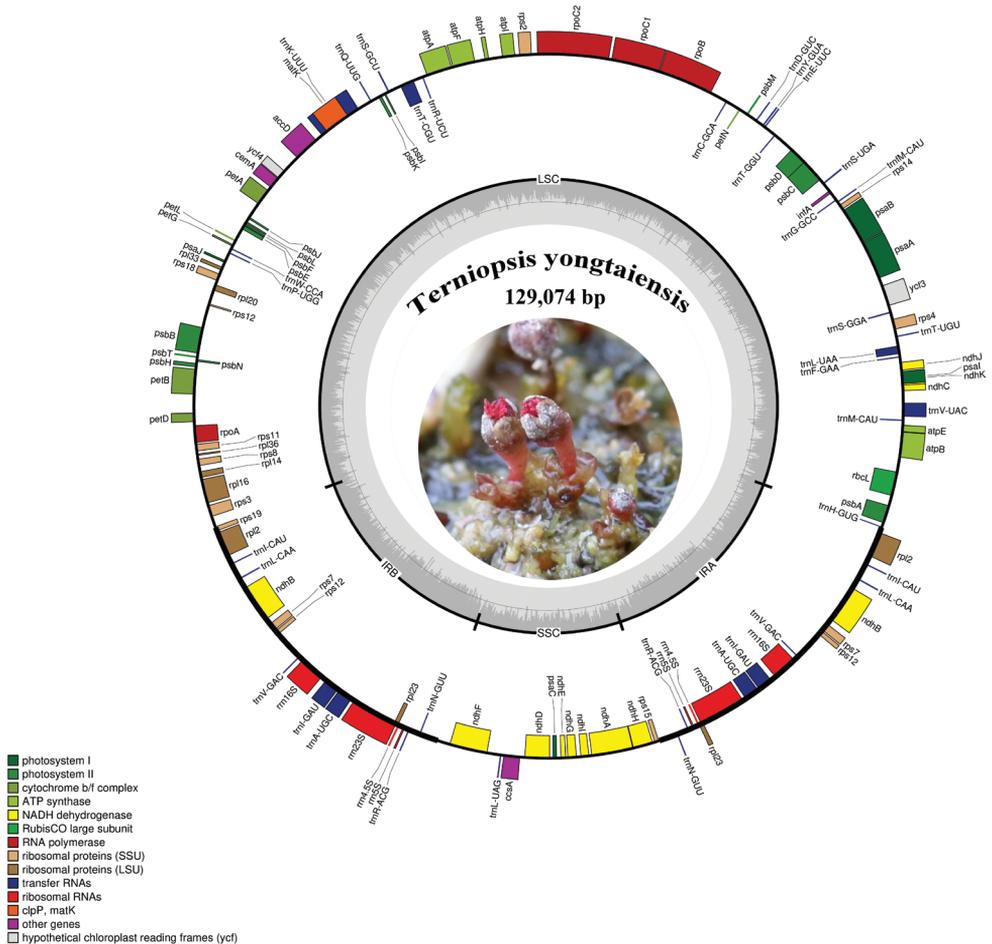


Figure 5. Circular gene map of the plastid genome of *Terniopsis yongtaiensis*. Genes inside the circle are transcribed clockwise, while those drawn outside are transcribed counterclockwise. Genes are color-coded according to their functional groups. The circle inside the GC content graph marks the 50% threshold.

Comparative analysis of the plastomes

A comparison of the plastome of *Terniopsis yongtaiensis* is made to five other species of Podostemaceae with available data (Table 3). The plastome lengths of the six species varied from 129,074 bp (*T. yongtaiensis*) to 134,912 bp (*Apinagia riedelii*), with *T. yongtaiensis* being the shortest. For the LCS and SSC regions, the extent of length variation between these species is not evident. The number of PCGs in these species is similar to that of most angiosperms, according to a comparative analysis of gene content (Jin et al. 2020b). The numbers of tRNA and rRNA genes, as well as the GC content, are substantially conserved in all of these plastomes, as shown by our findings. In all compared species, the *ycf1* and *ycf2* genes, which are two giant open reading frames found in most higher plants, are lost.

Table 3. Statistics on the basic features of the plastid genomes of *Terniopsis yongtaiensis* and related taxa.

Species	Voucher	Accession no.	Length (bp)	LSC (bp)	SSC (bp)	IR(bp)	GC content (%)	No. of PCGs	No. of tRNA	No. of rRNA
<i>Terniopsis yongtaiensis</i>	CBH 04587	OM717943	129,074	79,000 (-61.2%)	13,066 (-10.1%)	18,504 × 2 (-28.7%)	36.20	72	30	4
<i>Apinagia riedelii</i>	C.P. Bove 2513 (R)	MN165812	134,912	85,377 (-61.0%)	12,437 (-8.9%)	21,049 × 2 (-30.1%)	34.90	74	30	4
<i>Marathrum utile</i>	AMB 497 (ANDES)	MN165814	131,951	79,778 (-60.5%)	12,283 (-9.3%)	19,945 × 2 (-30.2%)	35.10	73	29	4
<i>Marathrum capillaceum</i>	C.P. Bove 2493 (R)	MN165813	134,374	79,990 (-59.5%)	12,302 (-9.2%)	21,041 × 2 (-31.3%)	35.00	75	30	4
<i>Marathrum foeniculaceum</i>	W. D. Stevens - 32072	MK995178	131,600	79,506 (-60.4%)	12,262 (-9.3%)	19,916 × 2 (-30.3%)	35.10	76	30	4
<i>Tristicha trifaria</i>	A. Mesterhazy MLI 128(Z)	MN165816	130,285	78,925 (-60.6%)	12,662 (-9.7%)	19,349 × 2 (-29.7%)	36.40	74	30	4

In *T. yongtaiensis* and *Tristicha trifaria*, the *rps15* gene is found at the SSC/IR border, but it is shifted to IRs in *Apinagia riedelii*, *Marathrum utile*, *M. capillaceum* and *M. foeniculaceum* due to the expansion at the IR/SSC boundary. In *T. yongtaiensis*, the *trnG-UCC* gene mutates to *trnT-CGU*, and in *M. capillaceum*, it is lost. Further, all the compared species have a gene inversion from *trnK-UUU* to *rbcL* in the LSC region, and the gene inversions are of similar size (ranging from 50.4 kb for *T. yongtaiensis* to 52 kb for *A. riedelii*). It represents an essential mechanism for plastome rearrangements (Mower and Vickrey 2018).

Phylogenetic analysis

Phylogenies were reconstructed by Maximum likelihood (ML) and Bayesian Inference (BI) analyses using the *matK* and nrITS sequences. The phylogenetic analysis based on *matK* sequences suggested that *Terniopsis yongtaiensis* is sister to *T. heterostaminata* with moderate support, and nested in a clade formed by *T. brevis*, *T. minor*, *T. malayana* with strong support (Fig. 6). Similar results showed by the phylogenetic analysis based on nrITS, suggested *T. yongtaiensis* is closely related to *T. heterostaminata* with moderate support, and sister to a clade comprising *T. chanthaburiensis*, *T. filiformis*, *T. vapyensis*, *T. microstigma*, *T. ubonensis*, *T. savannaketensis*, and *T. malayana* (Suppl. material 1: Fig. S5).

Discussion

The *Terniopsis sessilis* Chao was first discovered in 1948 in the Tingjiang River basin of Changting County in northwest Fujian Province (Chao 1948, 1980). The literatures indicated that this species has a wide distribution, but to date, 80 years after its report, it has not been found elsewhere after a long and continuous investigation, such as around the Min River, under the Wanshou Bridge (i.e. Jiefang Bridge) in Cangshan District, Fuzhou City, Fujian Province, where a distribution has been noted. This is possibly due

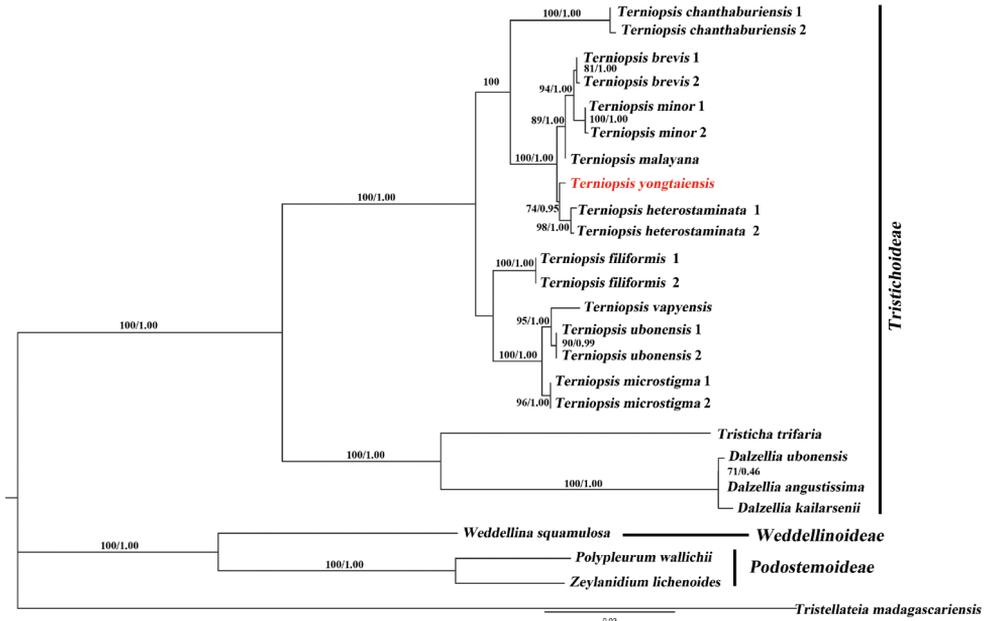


Figure 6. Phylogenetic tree of Asian Podostemaceae based on Bayesian Inference of *matK* sequences. Numbers above and below branches indicate RAxML (left) bootstrap probabilities (BP) and Bayesian (right) posterior probabilities (PP), respectively.

to environmental changes and urbanization. Fortunately, some botanical enthusiasts discovered plants that were morphologically similar in Guilin, Guangxi Zhuang Autonomous Region, which our team analyzed and determined were consistent with *T. sessilis* based on *matK* sequences (data not published).

While looking for other distribution sites of *T. sessilis* in Fujian Province, the new species *T. yongtaiensis* was discovered in Yongtai county; it differs greatly in appearance from *T. sessilis* (Suppl. material 1: Figs S2–S4), especially in the ramuli, flower and fruit. Roots of *T. yongtaiensis* are often dark green in water, and the vegetative and flowering ramuli can be clearly distinguished. There are more leaves on vegetative ramuli (up to 55), the leaves are spatulate, and they wither during flowering. The ramuli of *T. sessilis*, on the other hand, are often attached to rock surfaces, and are obviously shorter (7–9 mm long), and have fewer leaves (< 12). The number of flowering ramuli branches varies between *Terniopsis* species. The flowering ramuli of *T. yongtaiensis* are usually two-branched, with one flower. The flowering ramuli are shorter and single branched with one or two flowers, but the leaf shape is similar. And the flowering ramuli of *T. heterostaminata* are often single to four-branched, with one flower (Chao 1980; Fujinami and Imaichi 2009; Koi and Kato 2015)

The plastome of *T. yongtaiensis* was compared with the plastome of 5 other species within the Podostemaceae family. All of the studied species lack the *ycf1* and *ycf2* genes, which are giant open reading frames found in most higher plants, resulting in a

significant reduction of IR regions, thus reducing the size of their plastomes. Based on the available data, we believe that the absence of *ycf1* and *ycf2* genes is typical for Podostemaceae. The *ycf1* and *ycf2* genes were also lost in the plastome of Poaceae (Guisinger et al. 2010), Geraniaceae (Weng et al. 2014) and Ericaceae (Braukmann et al. 2017). There is still debate over the functions of the *ycf1* and *ycf2* genes, and they have yet to be classified as genes involved in genetic or photosynthetic systems (Drescher et al. 2000).

According to molecular data on *matK* comparison, the new species from Yongtai was closely related to *T. heterostaminata* from Thailand, and was in the sister group of the same cluster in the phylogenetic tree. Additionally, due to its geographical distance and the unique river habitat, this species was identified as a new species and named *T. yongtaiensis*. Investigations of other rivers in Yongtai and surrounding counties have revealed that the species was only found in the upper reaches of the first discovery site, indicating that the species has a very limited distribution area. Meanwhile, a whole-genome analysis will be carried out to ascertain its phylogenetic and evolutionary position among angiosperms.

Conclusion

Terniopsis yongtaiensis should be classified as a new species of Tristichoideae, based on the facts presented in the current study. The plastome of species of genus *Terniopsis* was studied for the first time, and the discovery of *T. yongtaiensis* provides new supporting materials for the phylogeny and evolution for the Podostemaceae family.

Key to the species of *Terniopsis* H. C. Chao

- 1 Stamens at least two times longer than ovary 2
- Stamens as long as ovary 5
- 2 Stamens 3; stigmas up to 1mm, distinctly multi-furcate..... 4. *T. daoyinensis*
- Stamens 2 or 3; stigmas less than 0.5mm, cristate 3
- 3 Ramulus 10–90mm long; stamen 5–6mm long 14. *T. ubonensis*
- Ramulus <5mm long; stamen <5mm long 4
- 4 Stamens 2, 2.5 times as long as ovary 11. *T. savannaketensis*
- Stamens 2 or 3, 2 times as long as ovary 15. *T. vapyensis*
- 5 Stigmas ≤ 0.2 mm long 6
- Stigmas more than 0.2 mm long 10
- 6 Stigmas simple to laciniate; pedicel 10–15 mm; capsule-stalk 15 mm 1. *T. australis*
- Stigmas cristate; pedicel < 1mm; capsule-stalk <10 mm 7
- 7 Pedicel ca. 0.5, ramulus 2–5..... 8. *T. microstigma*
- Pedicel >1mm, ramulus 1–4 8
- 8 Root 2 mm wide; shoot to 30mm long, many times branched; bracts several 10. *T. ramosa*
- Root <2 mm wide; shoot to 10mm long, bracts 2 9

- 9 Ramulus <10 mm long; ovary 0.6–0.8 mm; capsula elliptical .. **13. *T. sessilis***
 – Ramulus up to 30mm long; ovary 1.5–2.0 mm; capsula obovate
 **7. *T. matayana***
 10 Stamens 3, rarely 2; stigmas forked, filiform at maturity **5. *T. filiformis***
 – Stamens 2; stigmas cristate **11**
 11 Vegetative ramuli up to 14 mm long **12**
 – Vegetative ramuli less than 10 mm long **14**
 12 Pedicel 3–14 mm long **3. *T. chanthaburiensis***
 – Pedicel < 3 mm long **13**
 13 Ramuli associated with flowers 2–4, 2–6 mm long **6. *T. heterostaminata***
 – Ramulus associated with flowers 1, to 2 mm long **16. *T. yongtaiensis***
 14 Ramuli associated with flowers 4–7 mm long **9. *T. minor***
 – Ramuli associated with flowers 2–4 mm long **15**
 15 Pedicel 1.3–1.8 mm, ovary 1.3–1.5 × 0.8 mm **12. *T. sesadensis***
 – Pedicel 3 mm, ovary 0.8–1.3 × 0.5 mm **2. *T. brevis***

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Supplementary material I

Appendix

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Data type: doc file

Explanation note: **Fig. S1.** Distribution of *Terniopsis yongtaiensis*, *T. sessilis* and *T. daoyinensis* of genus *Terniopsis* from China. Legend ▲ *T. yongtaiensis*, ◆ *T. sessilis*, ● *T. daoyinensis*. **Fig. S2.** Habit and habitat of *Terniopsis sessilis*. **Fig. S3.** *Terniopsis sessilis*, showing stems (ramuli) arising laterally from root, distichous and leaves borne on ramuli in 3 ranks. **Fig. S4.** *Terniopsis sessilis*, showing two flower buds axillary to the basal leaf, sessile. **Fig. S5.** Phylogenetic tree of Asian Podostemaceae based on Bayesian Inference of nrITS sequences. Numbers above and below branches indicate RAxML (left) bootstrap probabilities (BP) and Bayesian (right) posterior probabilities (PP), respectively. **Table S1.** List of taxa from Podostemaceae and NCBI accession numbers (*matK*). **Table S2.** List of taxa from Podostemaceae and NCBI accession numbers (*nrITS*).

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Link: <https://doi.org/10.3897/phytokeys.194.83080.suppl1>

A phylogeny of the Triraphideae including *Habrochloa* and *Nematopoa* (Poaceae, Chloridoideae)

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Abstract

To investigate the evolutionary relationships among species of the tribe Triraphideae (including two monotypic genera, *Habrochloa* and *Nematopoa*), we generated a phylogeny based on DNA sequences from nuclear ribosomal (ITS) and four plastid markers (*rps16-trnK*, *rps16* intron, *rpl32-trnL*, and *ndhA* intron). *Habrochloa* and *Nematopoa* form a clade that is sister to *Neyraudia* and *Triraphis*. Members of the Triraphideae have paniculate inflorescences, 3-veined, marginally ciliate lemmas, usually with hairy lateral veins, that are apically bifid and awned from between a sinus. A description of the Triraphideae and key to the genera is provided, and the biogeography is discussed, likely originating in Africa.

Keywords

Classification, *Habrochloa*, molecular phylogenetics, *Nematopoa*, *Neyraudia*, Triraphideae, *Triraphis*

Introduction

Clayton and Renvoize (1986) pointed out that *Neyraudia* R. Br. was perhaps an ally of *Triraphis* R. Br. since both genera possess slender microhairs and the two have keeled lemmas that are villous on the lateral veins (Watson and Dallwitz 1992). Based on DNA sequence studies Bouchenak-Khelladi et al. (2008) were first to show strong support for *Neyraudia* and *Triraphis* as being sister in the subfamily Chloridoideae Kunth ex Beilschm. Hilu and Alice (2001) and Bouchenak-Khelladi et al.

(2008), using the same *matK* sequence marker placed these two genera in the subtribe Unioliinae Clayton, now a member of tribe Eragrostideae Stapf. Another DNA sequence study supported the placement of the *Neyraudia*–*Triraphis* clade as being sister to remaining species in the Chloridoideae and, subsequently, the tribe Triraphideae P.M. Peterson [based on subtribe Triraphidinae Stapf (1917)] was erected to include these two genera (Peterson et al. 2010). Using unpublished DNA sequence phylogenies (Peterson and Romaschenko, unpubl.), the monotypic *Habrochloa* C.E. Hubb., was added to the Triraphideae in the classification of all genera within the Poaceae (Soreng et al. 2015, 2017).

Hubbard (1935, 1957a, b) transferred *Triraphis longipes* Stapf & C.E. Hubb. to *Crinipes* Hochst. (Arundinoideae) since it possessed a bearded callus, then later moved it to a new monotypic genus, *Nematopoa* C.E. Hubb. *Nematopoa* was included in the Arundinoideae by Clayton and Renvoize (1986). In more recent classifications (Soreng et al. 2015, 2017), *Nematopoa longipes* (Stapf & C.E. Hubb.) C.E. Hubb. was placed as a synonym of *Triraphis* as originally described. Based on unpublished DNA sequence phylogenies (Peterson and Romaschenko, unpubl.), Soreng et al. (2022) and Gallaher et al. (2022) placed *Nematopoa* in the Triraphideae. Therefore, the current concept of the Triraphideae consists of four genera, *Habrochloa*, *Nematopoa*, *Neyraudia*, and *Triraphis*.

Habrochloa bullockii C.E. Hubb. is a small, delicate, African annual (culms 10–25 cm tall) with a fringe of hairs for a ligule and delicate panicles bearing 3–5-flowered spikelets, each including awned, apically bifid, marginally ciliate lemmas that bear trigonous caryopses, whereas *Nematopoa longipes* is a caespitose, southern African perennial (culms 30–80 cm tall) with ciliate, membranous ligules and capillary panicles bearing 4–7-flowered spikelets, each including awned, apically bifid, marginally ciliate lemmas (Clayton et al. 2016). *Neyraudia* consists of four reedlike perennials [culms (0.8–) 1–5 m tall], a cartilaginous ridge with a line of hairs apically for a ligule, and plumose panicles bearing 3–8-flowered spikelets, each including awned, apically bifid lemmas that are ciliate marginally and along lateral veins; three species in tropical and temperate Asia and one species in Africa (Watson et al. 1992; Filgueiras and Zuloaga 1999; Guala 2003; Clayton et al. 2016). *Triraphis* consists of eight annual or perennials (culms 4–140 cm tall) with membranous ligules or a fringe of hairs and open or contracted (rarely spiciform) panicles bearing 4–24-flowered spikelets, each including apically 3-lobed and 3-awned lemmas that are ciliate marginally and villous along the lateral veins, and trigonous caryopses; six species in Africa, one in Australasia and one in South America (Watson et al. 1992; Nightingale and Weiller 2005; Clayton et al. 2016).

In the present phylogenetic study, using DNA sequences from nuclear ribosomal (ITS) and four plastid markers (*rps16-trnK*, *rps16* intron, *rpl32-trnL*, and *ndhA* intron), we include for the first time *Habrochloa bullockii*, *Nematopoa longipes*, and *Neyraudia arundinacea* (L.) Henrard with two other species of *Neyraudia* and five species of *Triraphis*. In addition, we include a description of the Triraphideae, key to the genera in the tribe, and hypothesize its biogeographical history.

Materials and methods

Detailed methods for DNA extraction, amplification, and sequencing are given in Romaschenko et al. (2012) and Peterson et al. (2010, 2014a, b, 2015a, b, 2016). We used Geneious Prime 2020 (Kearse et al. 2012) for contig assembly of bidirectional sequences of *ndhA* intron, *rpl32-trnL*, *rps16* intron, *rps16-trnK* and ITS regions, and implemented in Geneious Muscle algorithm (Edgar 2004) to align the sequences and adjust the final alignment. The maximum likelihood parameters for each region were estimated with GARLI 2.0 (Zwickl 2006) and were used as priors in Bayesian calculations to infer overall phylogeny. The Bayesian tree was constructed using MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). All compatible branches were saved. The Bayesian analysis was initiated with random starting trees sampling once per 100 generations and continued until the value of the standard deviation of split sequences dropped below 0.01 indicating convergence of the chains. The effective sample size (ESS) value for all the parameters was greater than 200 and the first 25% of the sampled values were discarded. Maximum likelihood bootstrap analyses (Felsenstein 1985) were performed using GARLI with 1000 repetitions. The resulted file containing 1000 trees from the bootstrap analysis was then read into PAUP* v.5.0 (Swofford 2000) to compute the majority rule consensus tree.

Our study was designed to test relationships among species residing in four genera (*Habrochloa*, *Nematopoa*, *Neyraudia*, and *Triraphis*) attributed to the Triraphideae. Representative species from all remaining tribes (Centropodieae P.M. Peterson, N.P. Barker & H.P. Linder, Cynodonteae Dumort., Eragrostideae Stapf, and Zoysieae Benth.) in the Chloridoideae have been included to test the monophyly of the tribe (Peterson et al. 2010). In addition, the phylogeny includes two species from the Danthonioideae, *Danthonia compressa* Austin and *Merxmuellera drakensbergensis* (Schweick.) Conert, and one species from the Panicoideae, *Chasmanthium latifolium* (Michx.) H.O. Yates, which was used as an outgroup.

Results and discussion

Thirty-five new sequences (16%) from five species (nine individuals) are newly reported in GenBank, along with all other sequences for 48 individuals and 41 species included in this study (Table 1). Total aligned characters, numbers of sequences, proportion of invariable sites, and other parameters are noted in Table 2. The resulting plastid and ITS topologies were inspected for conflicting nodes with $\geq 95\%$ posterior probabilities. No supported conflict was found so plastid and ITS sequences were combined.

The Bayesian tree from the combined plastid and ITS regions is well resolved (Fig. 1). Most clades have posterior probabilities equal to 1.00 and additional bootstrap values of 90% or greater. There is strong support for *Habrochloa bullockii* + *Nematopoa longipes* sister to a monophyletic *Neyraudia* with three individuals of *N. reynaudiana* (Kunth) Keng ex Hitchc. sister to one individual of *N. arundinacea* (type of the genus)

Table 1. Taxon voucher (collector, number, and where the specimen is housed), country of origin, and GenBank accession for DNA sequences of *rps16-trnK*, *rps16* intron, *rpl32-trnL*, *ndhA* intron, and ITS regions; **bold** indicates new accession; a dash (–) indicates missing data, an asterisk (*) indicates sequences not generated in our lab.

Taxon	Voucher	Country	<i>rps16-trnK</i>	<i>rps16</i> intron	<i>rpl32-trnL</i>	<i>ndhA</i> intron	ITS
1	<i>Centropodia glauca</i> (Nees) Cope	South Africa	JF729075	–	JF729175	JF729164	JF729164
2	<i>Centropodia mossamedensis</i> (Rendle) Cope	South Africa	JF729076	JF729182	JF729176	–	–
3	<i>Chasmanthium latifolium</i> (Michx.) H.O. Yates	USA, Maryland	GU360517	GU360438	GU359891	GU359379	GU359319
4	<i>Chloris barbata</i> Sw.	Mexico, Sinaloa	GU360514	GU360435	GU359873	GU359377	GU359320
5	<i>Cottea pappophoroides</i> Kunth	Peru, Ancash	GU360600	GU360456	GU359842	GU359363	GU359237
	Fox (US)						
6	<i>Danthonia compressa</i> Austin	USA, North Carolina	GU360521	GU360483	GU359865	GU359370	GU359345
7	<i>Elaeusine indica</i> (L.) Gaertn.	Mexico, Mexico	GU360496	GU360472	GU359797	GU359473	GU359338
8	<i>Elaeusine poiflora</i> (Chiov.) Chiov.	Ethiopia	GU360601	GU360457	GU359843	–	GU359236
9	<i>Ellisochloa rangel</i> (Pilg.) P.M. Peterson & N.P. Barker	Namibia	JF729079	JF729184	–	JF729166	JQ345167
10	<i>Enneapogon scaber</i> Lehmann	South Africa, Western Cape	JQ345237	JQ345279	JQ345322	JQ345208	JQ345168
11	<i>Entoplocamia aristulata</i> (Hack. & Rendle) Stapf	South Africa	GU360492	GU360468	GU359793	GU359469	GU359342
12	<i>Engrostis kennehyae</i> F. Turner	Australia	JQ345238	JQ345281	JQ345323	JQ345209	JQ345169
13	<i>Engrostis wisiana</i> (C.A. Gardner & C.E. Hubb.) R.L. Barrett & P.M. Peterson	Australia, Western Australia	GU360703	GU360288	GU359986	GU359533	GU359137
14	<i>Gouinia virgata</i> var. <i>robusta</i> J.J. Ortiz	Mexico, Zacatecas	KF827775	KF827710	KF827639	KF827584	KF827521
15	<i>Gymnopogon grandiflorus</i> Roseng., B.R. Arill. & Izag.	Peru, Apurimac	GU360581	GU360383	GU359816	GU359436	GU359200
16	<i>Habrochloa bullockii</i> C.E. Hubb.	Tanzania, Ruvuma	ON012448	ON012442	ON012427	ON012435	OM980631
17	<i>Leptocarydium vulpiastrum</i> (De Not.) Stapf	Tanzania	KF827792	KF827725	KF827660	KF827595	KF827539
18	<i>Lepochloa digitata</i> (R.Br.) Domin	Australia, Northern Territory	JQ345246	JQ345289	JQ345331	JQ345213	JQ345178
19	<i>Lepathrium senegalense</i> (Kunth) Clayton	Kenya	KF827795	KF827728	KF827663	KF827597	KF827542
20	<i>Mersmanniella dhakensbergensis</i> (Schweikerdt) Conert	South Africa	JF729078	JF729183	–	JF729165	–
21	<i>Monechryum luederitzianum</i> Hack.	South Africa	GU360682	GU360421	GU359969	GU359459	GU359158
22	<i>Mosdenia leptostachys</i> (Ficalho & Hiern) Clayton	South Africa	GU360681	GU360420	GU359967	GU359458	GU359159
23	<i>Muhlenbergia japonica</i> Steud.	China, Yunnan	HM143667	HM143571	HM143183	HM143388	HM143081

Taxon	Voucher	Country	<i>rps16-trnK</i>	<i>rps16</i> intron	<i>rpl32-trnL</i>	<i>ndhA</i> intron	ITS
24 <i>Neesiochloa barbata</i> (Nees) Pilg.	Swallen 4491 (US)	Brazil	GU360724	GU360279	GU360005	—	GU359122
25 <i>Nematopoa longipes</i> (Stapf & C.E. Hubb.) C.E. Hubb.	Simon 2353	Africa	MF035992*	MF035992*	MF035992*	MF035992*	—
26 <i>Neyraudia arundinacea</i> (L.) Henraud	Peterson 23991, Soreng, Romaschenko & Abeid (US)	Tanzania, Njomba	ON012449	ON012443	ON012428	ON012436	OM980632
27 <i>Neyraudia reynaudiana</i> (Kunth) Keng ex Hitchc.	Columbus 5302 (RSA)		KF356392*	KF356392*	KF356392*	KF356392*	—
28 <i>Neyraudia reynaudiana</i> (Kunth) Keng ex Hitchc.	Soreng 5318, Peterson & Sun Hang (US)	China, Yunnan	—	GU360272	GU360003	GU359397	GU359124
29 <i>Neyraudia reynaudiana</i> (Kunth) Keng ex Hitchc.	Srisanga 97923, Norsaeangsi, Unwin, Rodda, Schuettpelz, Tin Tin Mu & Ling Shein Man (US)	China, Myanmar	—	—	ON012429	—	OM980633
30 <i>Pappophorum pappiferum</i> (Lam.) Kuntze	Peterson 21689, Soreng, La Torre & Rojas Fox (US)	Peru, Ancash	GU360700	GU360276	GU359996	GU359402	GU359128
31 <i>Pennis indica</i> (L.) Kuntze	Peterson 23880, Soreng & Romaschenko (US)	Tanzania	KF827801	KF827734	KF827669	KF827601	KF827546
32 <i>Ptilolemma jagleri</i> (Pilg.) S.M. Phillips	Peterson 24247, Soreng & Romaschenko (US)	Tanzania	KM011122	KM010919	KM010695	KM010535	KM010326
33 <i>Sporobolus virginicus</i> (L.) Kunth	Peterson 15683 & Soreng (US)	Chile, Region I	GU360610	GU360362	GU359892	GU359502	GU359215
34 <i>Tragus berteronianus</i> Schult.	FLSP 457 (US)	Peru	GU360616	GU360370	GU359898	GU359503	GU359224
35 <i>Tridens flavus</i> var. <i>chapmanii</i> (Small) Shimmers	McCauley 438 (MO)	USA, Missouri	KF827817	KF827751	KF827689	KF827615	KF827568
36 <i>Triplasis americana</i> P. Beauv.	Kral 12065 (MO)	USA, Georgia	KF827818	KF827752	KF827690	KF827616	KJ768887
37 <i>Triplasis andropogonoides</i> (Steud.) E. Phillips	Mennell s.n. (US)	South Africa, Cape Province	GU360654	GU360335	GU359949	ON012437	—
38 <i>Triraphis mollis</i> R. Br.	Lazarides 046 & Palmer (US)	Australia, Uluru National Park	—	—	ON012430	—	OM980634
39 <i>Triraphis mollis</i> R. Br.	Peterson 14344, Soreng & Rosenberg (US)	Australia, Western Australia	GU360669	GU360336	GU359933	GU359539	GU359187
40 <i>Triraphis mollis</i> R. Br.	Saarela 1608, Peterson, Soreng & Judziewicz (US)	Australia, Northern Territory	ON012450	ON012444	ON012431	ON012438	OM980635
41 <i>Triraphis mollis</i> R. Br.	Saarela 1615, Peterson, Soreng & Judziewicz (US)	Australia, Northern Territory	ON012451	ON012445	ON012432	ON012439	OM980636
42 <i>Triraphis mollis</i> R. Br.	Saarela 1648, Peterson, Soreng & Judziewicz (US)	Australia, Northern Territory	ON012452	ON012446	ON012433	ON012440	OM980637
43 <i>Triraphis mollis</i> R. Br.	Saarela 1656, Peterson, Soreng & Judziewicz (US)	Australia, Northern Territory	ON012453	ON012447	ON012434	ON012441	OM980638
44 <i>Triraphis purpurea</i> Hack.	Schweickert 2115 (US)	Namibia	GU360652	GU360337	GU359932	GU359549	—
45 <i>Triraphis ramosissima</i> Hack.	Seydel 4278 (US)	Namibia	GU360651	GU360338	GU359931	GU359541	GU359188
46 <i>Triraphis schinzii</i> Hack.	Smook 1933 (US)	South Africa	GU360650	GU360339	GU359930	—	—
47 <i>Umiola condensata</i> Hitchc.	Peterson 9342 & Judziewicz (US)	Ecuador, Chimborazo	GU360649	GU360340	GU359927	GU359534	GU359191
48 <i>Zypsia macrantha</i> subsp. <i>uakibii</i> M.E. Nightingale	Loch 435 (US)	Australia	GU360642	GU360345	GU359922	GU359548	GU359197

plus a monophyletic *Triraphis*. The *Triraphis* clade includes six individuals of *T. mollis* R. Br. (type of the genus as treated by Burbidge 1946 and Peterson et al. 2022) sister to *T. schinzii* Hack. and *T. ramosissima* Hack. sister to *T. andropogonoides* (Steud.) E. Phillips + *T. purpurea* Hack. Our molecular data clearly support independent recognition of *Nematopoa* since it is sister to *Habrochloa* and not a member of the *Triraphis* clade.

Habrochloa bullockii and *Nematopoa longipes* are clearly aligned within the Triraphideae, and together with *Neyraudia* and *Triraphis*, share the following salient morphological features: paniculate inflorescences, 3-veined, marginally ciliate lemmas, usually with hairy lateral veins, and lemmas that are apically bifid and awned from between the sinus (Watson and Dallwitz 1992; Watson et al. 1992; Peterson et al. 2010; Clayton et al. 2016). Another probable synapomorphy for these four genera is possession of panicoid-type bicellular microhairs (long, narrow basal and terminal cells; Amarasinghe and Watson 1988). Watson et al. (1992) verified the presence of panicoid bicellular microhairs for *Habrochloa*, *Nematopoa*, and *Triraphis* but indicate that they are absent in *Neyraudia arundinacea*. However, Clayton and Renvoize (1986) previously indicated that *Neyraudia* possesses slender microhairs similar to those in *Triraphis*.

Based on a sample containing *Nematopoa*, *Neyraudia*, and *Triraphis*, Gallaher et al. (2022) determined the crown age (10.62 Ma) and stem age (46.76 Ma) of the Triraphideae. Although at least three species of *Neyraudia* include tropical and temperate Asia in their distribution, Africa is the most likely area of origin for the Triraphideae since all four genera in the tribe include species distributed in Africa. In addition, the Triraphideae shares a common ancestor with Centropodieae, also from Africa and temperate Asia (Peterson et al. 2011). Because more than half of the genera of Chloridoideae reside in Africa and the larger tribes, i.e., the Eragrostideae

Table 2. Characteristics of the five DNA regions (*rps16-trnK*, *rps16* intron, *rpl32-trnL*, *ndhA* and ITS) and parameters used as priors in Bayesian analyses estimated with GARLI. 2.0.

Characteristic	<i>rps16-trnK</i>	<i>rps16</i> intron	<i>rpl32-trnL</i>	<i>ndhA</i> intron	Combined plastid data	ITS	Overall
Total aligned characters	887	1046	844	1146	3923	769	4692
Number of sequences	45	45	46	42	178	41	219
Number of new sequences	6 (13%)	6 (13%)	8 (17%)	7 (17%)	27 (15%)	8 (20%)	35 (16%)
Likelihood score (-lnL)	3909.0	3405.6	3778.7	4281.4		7973.0	
Number of substitution types	6	6	6			6	
Model for among-sites rate variation	gamma	Gamma	gamma			gamma	
Substitution rates	1.2071	1.2951	1.0625	0.9848	–	1.1422	
	2.7093	1.2876	1.7914	2.5216		2.6273	
	0.4083	0.3028	0.3251	0.2912		1.7222	
	1.5405	1.1547	1.4401	1.9389		0.6568	
	2.9778	2.0746	1.5146	2.3679		4.5253	
	1.0000	1.0000	1.0000	1.0000		1.0000	
Character state frequencies	0.3088	0.3779	0.3693	0.3669	–	0.2404	
	0.1363	0.1226	0.1380	0.1348		0.2374	
	0.1462	0.1743	0.1222	0.1484		0.2582	
	0.4084	0.3251	0.3703	0.3497		0.2641	
Proportion of invariable sites	0.1666	0.3154	0.0413	0.2537	–	0.2547	
Gamma shape parameter (α)	2.1848	1.0833	0.9498	1.0636	–	0.9409	

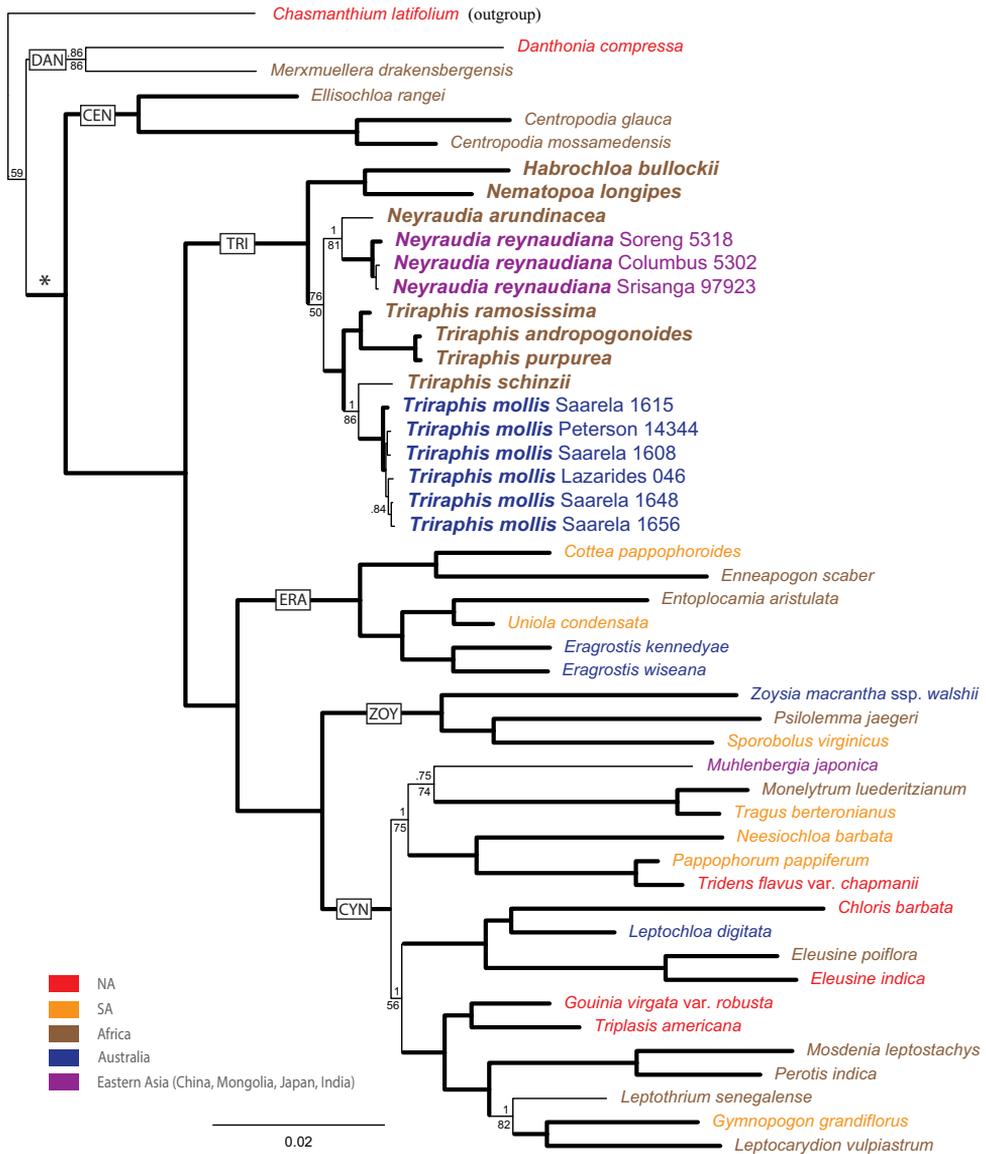


Figure 1. Maximum-likelihood tree inferred from combined plastid (*rps16-trnK*, *rps16* intron, *rpl32-trnL*, and *ndhA* intron) and ITS sequences. Numbers above branches are posterior probabilities; numbers below branches are bootstrap values; thick branches indicate bootstrap $\geq 90\%$ and posterior probabilities of 1.00; DAN = Danthonioideae; tribes within the Chloridoideae = *, include: CEN = Centropodieae, TRI = Triraphideae, ERA = Eragrostideae, ZOY = Zoysieae, and CYN = Cynodonteae. Scale bar: 2%.

and Zoysieae have centers of diversity there, Hartley and Slater (1960) earlier concluded that the subfamily probably originated on the African continent and spread to other parts of the world (Bouchenak-Khelladi et al. 2008; Peterson et al. 2007, 2010, 2011, 2014c).

Taxonomy

Triraphideae P.M. Peterson, *Molec. Phylogen. Evol.* **55**(2): 591. 2010 ≡ **Triraphidinae** Stapf, *Fl. Trop. Afr.* **9**: 22. 1917 – **Type:** *Triraphis* R. Br., *Prodr.* **185**. 1810.

Description (emendation). Annuals or perennials, sometimes rhizomatous or reed-like (*Neyraudia*) culms 4–500 cm tall, erect or decumbent; ligules membranous and ciliate or a fringe of hairs; inflorescence a panicle, open to contracted, rarely spiciform; spikelets 2–15 mm long, 3–24-flowered, laterally compressed; glumes usually shorter than the spikelets or upper glume 2 × as long as adjacent lemma (*Habrochloa*), 0-, 1- or 3-veined, membranous, sometimes hyaline, apex entire to mucronate, rarely awned; lemmas membranous, rarely cartilaginous, 3-veined with ciliate or pilose margins, lateral veins, if present, usually hairy and sometimes extending as awns (*Triraphis*), apex bifid and awned from between the sinus; paleas 0.5 to as long as lemma, 2-veined; stamens 3; Caryopses with adherent pericarp, often trigonous to ellipsoid, sometimes linear.

Included genera. *Habrochloa*, *Nematopoa*, *Neyraudia*, *Triraphis*.

Key to the genera

- 1 Lemmas 3-awned, the lateral veins extending into awns..... *Triraphis*
- Lemmas 1-awned, the lateral veins never extending into awns **2**
- 2 Culms (80–) 100–500 cm tall, generally 1–1.5 cm wide at base, often woody; plants perennial, reedlike; ligules cartilaginous at base, apically with a line of hairs; panicles 30–80 cm long, plumose..... *Neyraudia*
- Culms 10–80 cm tall, ≤ 3 mm wide at base, herbaceous; plants annual not reedlike; ligules membranous with a fringe of hairs, not cartilaginous at base; panicles 2–30 cm long, not plumose..... **3**
- 3 Spikelets 2–2.5 mm long; lemmas 1–1.3 mm long, 3-veined, awned, the awns 4–6 mm long; upper glumes 2 × as long as adjacent lemma *Habrochloa*
- Spikelets 6–10 mm long; lemmas 3.5–4.3 mm long, 1-veined, awned, the awns 8–13 mm long; upper glumes 0.5–0.6 × as long as adjacent lemma *Nematopoa*

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