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Research Article

## Two new varieties of Agapetes (Ericaceae) from Xizang, China

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#### Abstract

Two new varieties from Xizang, China, i.e. *Agapetes interdicta* var. *flaviflora* and *A. forrestii* var. *parvifolia*, are described and illustrated. *Agapetes interdicta* var. *flaviflora* differs from the nominate variety in having yellow and smaller corollas with shorter lobes and anthers with shorter appendages at the base. *Agapetes forrestii* var. *parvifolia* is distinguished from the nominate variety in the smaller leaves with an acute apex, nearly transverse secondary veins and puberulent peduncle. Taxonomic notes on these taxa are also provided.

Key words: Medog County, morphology, taxonomy

#### Introduction

The genus Agapetes D. Don ex G. Don (Ericaceae-Vaccinioideae-Vaccinieae) comprises ca. 115 species worldwide, with 63 distributed in China (Fang and Stevens 2005; Tong 2014; POWO 2024; Yang et al. 2024). General overviews of the historic taxonomic studies of this genus in China were presented by Tong (2016) and Tong et al. (2019). Xizang Autonomous Region, with 42 species and one variety including the recently published A. huangiana Bin Yang, Y. H. Tan & Y. H. Tong and A. rhuichengiana Bin Yang & Y. H. Tan, harbours the most species of Agapetes in China (Huang 1986; Fang and Stevens 2005; Tong 2014; Yang et al. 2022, 2024). Most Agapetes species from Xizang are concentrated in Medog County, which possesses diverse topography as well as high biodiversity. Nowadays, as road access to Medog is improving, scientists have more opportunity to conduct explorations in this area. New species of plants, fungi and animals have been continuously discovered in Medog County in recent years (e.g. Tong et al. (2021); Song et al. (2022); Yang et al. (2022, 2024); Pei et al. (2024)). During two recent field trips to Medog, the authors encountered undescribed variation within two species of Agapetes, which is described as two new varieties and illustrated below.



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#### Materials and methods

Specimens were collected from Medog County, Xizang Autonomous Region, China during two field expeditions in January 2020 and April 2024, respectively. Descriptions were based on both field observations and dried specimens. The studied specimens were mainly deposited at the Herbaria of Institute of Botany, Chinese Academy of Sciences (PE), Kunming Institute of Botany, Chinese Academy of Sciences (KUN) and South China Botanical Garden, Chinese Academy of Sciences (IBSC). Measurements were performed with a ruler and small plant parts were observed and measured under a stereomicroscope (Mshot-MZ101). The concepts of infraspecific ranks follow Christensen (1987).

#### **Taxonomic treatment**

#### 1. Agapetes interdicta (Hand.-Mazz.) Sleumer in Bot. Jahrb. Syst. 70: 106. 1939.

- = Pentapterygium interdictum Hand.-Mazz. in Anz. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 60: 186. 1923. Type: China, Yunnan, above Schutsche on the Taron [Dulongjiang River, upper course of the Irrawaddy], 2400–2800 m elev., 9 Jul 1906, H. F. v. Hand.-Mazz. 9465 (holotype: W, barcode no. WU0044070, image!; isotypes: W, image!, E, barcode no. E0078234, image!).
- Agapetes interdicta var. stenoloba (W. E. Evans) Sleumer in Bot. Jahrb. Syst. 70: 106. 1939. = Pentapterygium interdictum var. stenolobum W. E. Evans in Notes Roy. Bot. Gard. Edinburgh 15: 207. f. 1A. 1927. Type: Myanmar, Salween-Kiu Chiang [Dulongjiang River] divide, 3050-3350 m elev., Jul 1924, *G. Forrest 25678* (lectotype: E, barcode no. E00078236, image!, designated here; isoloectotypes: K, barcode no. K000357906, image!, P, barcode no. P06672383, image!); remaining syntype: ibid., Oct. 1924, *G. Forrest 25802* (K, barcode no. K000357907, image!).

Description. Evergreen shrub, 0.3-0.8 m tall, epiphytic on trees, with inflated root tubers. Twigs angled, 1-3 mm in diam., pubescent and glandular-setose, glabrescent when old. Leaves alternately scattered, often pseudo-whorled; petiole 1-2 mm long, glabrous, glaucous; blades leathery, oblanceolate to elliptic, 2-4.5 × 0.8-2.3 cm, glabrous, mid-veins conspicuously raised on both sides, secondary veins 7-9 pairs, at an angle of 40-50° with the mid-vein, conspicuous and raised adaxially, slightly raised abaxially, veinlets conspicuous and raised adaxially, inconspicuous abaxially, base cuneate, with 1 basal gland each side at a distance of 1.5-3 mm away from the junction of blade and petiole, margin slightly revolute when dry, nearly entire, except the apex with 2-3 serrulas on each side, each serrula terminated with a gland, apex acute or apiculate. Inflorescences shortly racemose, 1-3(-7)-flowered, often cauline, sometimes also axillary; peduncle 1-7 mm long, puberulent, with 2 or 3 sterile bracts; floral bracts ovate-triangular, ca. 0.7 × 0.6 mm, margin entire, apex acute; pedicels 3-11(-13) mm long, densely puberulent, sometimes intermixed scattered shortly glandular setose, apex slightly expanded; bracteoles 2, basal, similar to floral bracts, but smaller, deciduous. Hypanthium glabrous or sparsely pubescent at base; tube 2-4 mm long, 4-5.5 mm wide (including

wings), sparsely pubescent, conspicuously winged, wings to 1 mm wide; limb 4.5–6 mm long, glabrous, nearly divided to 2/3, lobes narrowly ovate-triangulate to ovate, 4–10 mm long, apex apiculate. Corolla red, rarely yellow-green or yellow, with 4–5 inconspicuous V-shaped veins, tubular, 5-angled, 1.3-3(-3.4) cm long, glabrous; lobes slightly reflexed, greenish or yellow, triangular-subulate to triangular, 2–9 mm long. Stamens 10; filaments flat, 2–5 mm long, glabrous; anthers 1.2-2.6(-3.2) cm long, thecae echinate, each locule with a very small appendage at the bottom, appendages 0.2-1.1 mm long, tubules  $2-4\times$  as long as thecae, without spurs on the back. Style slender, as long as the corolla or slightly exerted; stigma truncate; ovary 10-pseudoloculed, each locule with several ovules; disc glabrous. Fruit stalk densely puberulent; berry ca. 1 cm in diam., subglabrous, with persistent calyx lobes and wings.

**Distribution and habitat.** Southeast Xizang and northwest Yunnan of China and north Myanmar. It grows on the tree trunks under evergreen forests at elevations of 2300–2700(–2900) m.

Phenology. Flowering in March to April; fruiting in August.

#### 1a. Agapetes interdicta var. interdicta

**Description.** Corolla usually red, rarely yellow-green, 2.2–3 cm long, lobes 6–9 mm; anther appendages ca. 1.1 mm long.

**Distribution and habitat.** Southeast Xizang and northwest Yunnan of China and north Myanmar. It grows on the tree trunks under evergreen forests at elevations of 2300–2700(–2900) m.

Phenology. Flowering in March to April; fruiting in August.

Examined specimens. CHINA. Yunan: Gongshan County, foot of Gaoligong Mountains, 1960 (without date), Su-Kung Wu s.n. (KUN, barcode no. 0230889) • [Gongshan County], Taron-Taru Divide [Gaoligong Mountains], Tehgai to Ahtehmai; 2300 m elev.; 30 October 1938; Te-Tsun Yu 20890 (KUN, barcode no. 0034482, PE, barcode no. 01907994) • Gongshan County, Gaoligong Mountains; 8 March 2023 (fl.), Yi-Hua Tong, Jing-Bo Ni, Bing-Mou Wang, Wei-Hao Pan TYH-2615 (IBSC) · Gongshan County, Dulongjiang, Erduibei; 2300 m elev.; 16 May 1991; Dulongjiang Expedition 6839 (KUN, barcode no. 0231130) · Gongshan County, Dulongjiang, Maku; 2000 m elev.; 16 December 1990 (fl. bud); Dulongjiang Expedition 1117 (KUN, barcode nos. 0231128 & 0231131), 1118 (KUN, barcode nos. 0231124 & 0231125) • ibid.; 8 March 1991 (fl.), Dulongjiang Expedition 4269 (KUN, barcode nos. 0231122 & 0231123), 4280 (KUN, barcode no. 0231133) • Gongshan County, Dulongjiang, Qiawudang; 2650 m elev.; 25 March 1991; Dulongjiang Expedition 4926 (KUN, barcode no. 0230893), 4986 (KUN, barcode nos. 0230890 & 0230891), 4987 (KUN, barcode no. 0231126) · Gongshan County, Dulongjiang, Sandui; 2550 m elev.; 27°34'N, 98°21'E; 11 September 1979 (fr.); Hong Wang 64917 (HITBC) • Gongshan County, Dulongjiang, Xishaofang; 3200 m elev.; 30 March 1991; Dulongjiang Expedition 5387 (KUN, barcode nos. 0231134 & 0231135). Xizang: Medog County, near Hanmi; 1900 m elev.; August 1974 (fr.); Qianghai-Xizang Expedition s.n. (KUN, barcode no. 0230892) • Zayu County, Delei Valley; 28°10'N, 96°30'E; 7000-8000 ft elev.; 12 April 1924 (fl.); F. Kingdon-Ward 8082 (K, barcode no. K000639646, image). MYANMAR. Kachin State: Kangfang; 7000–9000 ft elev.; 9 January 1939 (fl.); *F. Kingdon-Ward 206* (NY, barcode no. 02651412, image) • Top of Hpare Vally; 8000 ft elev.; 5 April 1938 (fl.); *C. W. D. Kermod 17166* (K, barcode no. K000639647, image) • Tzi-tzo-ti; 26°58'N, 98°28'E; 7000–8000 ft elev.; May 1925; *G. Forrest 26585* (K, barcode no. K000639648, image).

**1b.** *Agapetes interdicta* var. *flaviflora* Y.H.Tong, B.M.Wang & X.L.Guo, var. nov. urn:lsid:ipni.org:names:77351464-1 Fig. 1

**Type.** CHINA • Xizang Autonomous Region: Medog County, Beibeng Xiang, De'ergong Village; 2300–2700 m elev.; 3 April 2024 (fl.); *Xiang-Long Guo TYH-2898* (holotype IBSC, isotype PE).

**Diagnosis.** This new variety differs from the nominate variety in having a yellow and smaller (1.3–1.8 cm) corolla with shorter (2–2.5 mm) lobes and anthers with shorter (ca. 0.2 mm) appendages at the base.

**Etymology.** The variety epithet is derived from its striking yellow flowers. The Chinese name is given as 黄花中型树萝卜(Chinese pinyin: huáng huā zhōng xíng shù luó bo).

**Distribution and habitat.** This species is currently known only from the type locality, i.e. Medog County, Xizang, China. It grows on the tree trunks under broadleaved forests at elevations of 2300–2700 m.

Phenology. The new variety flowers in March to April.

Taxonomic notes. Compared to this new variety, the nominate variety, Agapetes interdicta var. interdicta, has a much wider distribution including southeast Xizang and northwest Yunnan of China, and north Myanmar (Fang and Stevens 2005) (Fig. 2). Although the two varieties differ markedly in the colour, size and lobes of corollas, their vegetative parts are nearly the same so that it is difficult to identify them if the material is sterile. It is worth noting that Agapetes interdicta var. interdicta also rarely bears yellow-green flowers, but the differences in corolla size and lobes remain consistent (Fig. 3). The same kind of corolla colour variation also occurs in other Agapetes species, such as A. hosseana, which usually bears red or orange flowers, but some populations have green flowers (Watthana 2015). Except for the corolla colour variation, the differences between this taxon and A. interdicta are mainly presented in some quantitative characters, viz. the size of corollas and the length of appendages at the base of anthers, which seem not significant enough to differentiate them as distinct species. Thus, we recognise this taxon (A. interdicta var. flaviflora) as a new variety rather than a new species. Another variety with narrower calyx lobes, A. interdicta var. stenoloba (W. E. Evans) Sleumer, was merged with the nominate variety by Fang and Stevens (2005). We agree with this treatment because the shapes of calyx lobes of this species can vary from narrowly laceolate to ovate even in a population, such as the Dulongjiang population. A lectotype for this name was designated here incidentally, since two collections were cited in the protologue without clear type designation (Evans 1927). Due to the striking yellow corollas, A. interdicta var. flaviflora has high ornamental value.



Figure 1. Agapetes interdicta var. flaviflora A flowering plant B root tuber C leafy branch D flower E longitudinal section of flower with corolla and stamens removed to show style and ovary F androecium, abaxial view G androecium, adaxial view H calyx and disc, top view I calyx, bottom view J transection of ovary. Scale bars: 1 cm (D–G); 5 mm (H–I); 3 mm (J). A, B, E, G and I by Zi Wang, others by Xiang-Long Guo.



Figure 2. Distribution map of Agapetes interdicta var. interdicta (black circle), A. interdicta var. flaviflora (white circle), A. forrestii var. forrestii (black triangle) and A. forrestii var. parvifolia (white triangle).

# 2. *Agapetes forrestii* W. E. Evans in Notes Roy. Bot. Gard. Edinburgh 15: 202. t. 220. 1927.

**Type.** CHINA. Yunnan: Lung-fan [Long Fang]; 25°34'N, 98°33'E; May 1925, *G. Forrest 26583* (holotype E, barcode no. E00078232, image!; isotypes: K, barcode no. K000357892, image!, NY, barcode no. 00008156, image!, PE, barcode no. 00195226!).

**Description.** Evergreen shrub, 0.25–1 m tall, epiphytic on trees, with inflated root tubers. Tubers globose or spindle-like. Twigs slightly angled, 1-2 mm in diam., densely pubescent and sparsely setose. Leaves spirally scattered, glabrous; petiole 0.5-5 mm long; leaf blades leathery, ovate to ovate-lanceolate,  $1.1-4 \times 0.5-1.2$  cm, glabrous, mid-veins conspicuously raised on both sides, secondary veins 2-3 pairs, nearly transverse, strongly impressed adaxially, slightly raised abaxially, veinlets strongly impressed adaxially, inconspicuous abaxially, base rounded, without basal glands, margin slightly revolute when dry, each side with 5-7 serrulae, each serrula terminated with a gland, apex acute. Inflorescences corymbose, (2-)3-10-flowered, axillary; peduncle slender, 0.8-2 cm long, glabrous or puberulent, with several sterile bracts; floral bracts ovate-triangular, ca. 0.5 × 0.5 mm, margin serrulate, apex acute; pedicels 7-15 mm long, nearly glabrous, slightly expanded upwards; bracteoles 2, basal, similar to floral bracts, but smaller, deciduous. Hypanthium nearly cupular, 0.9-4.5 × 1-3.5 mm, glabrous; limb 1.5-2 mm long, glabrous, nearly divided to base, lobes triangular to narrowly triangular,  $1.5-2 \times 0.8-2$  mm, glabrous, apex acute.



Figure 3. Agapetes interdicta var. interdicta A flowering plant B leafy branch C inflorescence D variation of yellow-green corolla. All by Yi-Hua Tong. A–C from Gongshan County of Yunnan Province D from Tengchong City of Yunnan Province.

Corolla carmine, with 5–6 zig-zag transverse veins, green at the apex, tubular, 5-angled, 9–20 × 2.5–5 mm, glabrous; lobes green, slightly reflexed, ovate-triangular, 0.8–2 mm long. Stamens 10, 9–15 mm long; filaments flat, 1–1.8 mm long, pubescent; anthers 8–13 mm long, thecae 1.5–5.5 mm long, echinate, each locule with a very small appendage at the bottom, tubules 6–8 mm long, each with an oblique pore ca. 3.5 mm long, without spurs on the back. Style slender, 1.2–1.9 cm long, glabrous; stigma truncate; ovary 10-pseudoloculed, each locule with several ovules; disc glabrous. Mature fruit white, more or less with crimson dots, subglobose.

#### 2a. Agapetes forrestii var. forrestii

**Description.** Leaf blades  $3-4 \times 0.8-1.2$  cm, apex acuminate, secondary veins arcuate. Peduncle glabrous. Corolla ca. 2 cm long.

**Distribution and habitat.** Southeast Xizang and west Yunnan of China and north Myanmar. It grows on the tree trunks under evergreen forests at elevations of 1800–2700 m.

Phenology. Flowering in December to May of next year.

**Examined specimens.** CHINA. Yunnan: Tengchong City, Langya Mountain, 2200–2400 m elev.; 14 April 1984; *Wen-Zheng Li & Yin-Qing Guo 8411* (SWFC). Xizang Autonomous Region • Medog County, Beibeng Xiang, Xirang, De'endong; 2300–2400 m elev.; 29 April 1983 (fl.); *Bo-Sheng Li & Shu-Zhi Cheng 4396* (PE,

barcode nos. 01907990 & 01907988) • ibid., Xirang, Sangxing; 2300 m elev.; 26 April 1983 (fl.); *Bo-Sheng Li & Shu-Zhi Cheng 4705* (PE, barcode no. 01907985) • ibid., Xirang, Xidengshan; 2300 m elev.; 1 May 1983 (fl.); *Bo-Sheng Li & Shu-Zhi Cheng 4770* (PE, barcode no. 01907989). МYANMAR. Kachin State: Near Panwa Pass; 7000–9000 ft elev.; 11 March 1939 (fl.); *F. Kingdon-Ward 388* (NY, barcode nos. 02651408 & 02651409, image).

#### 2b. Agapetes forrestii var. parvifolia Y.H.Tong & B.M.Wang, var. nov.

urn:lsid:ipni.org:names:77351465-1 Fig. 4

**Type.** CHINA • Xizang Autonomous Region: Medog County, Damu Xiang, Km 80 on Zhamo Road, epiphytic on trees in evergreen broad-leaved forest; 29°40'59.9"N, 95°30'6.3"E; 2191 m elev.; 3 January 2020 (fl.); *Yi-Hua Tong & Bing-Mou Wang TYH-2363* (holotype IBSC, isotypes IBSC, PE).

**Diagnosis.** This new variety differs from the nominate variety in having smaller leaves  $(1.1-1.8 \times 0.5-0.7 \text{ cm})$  with an acute apex, nearly transverse secondary veins, puberulent peduncle and shorter corollas (9–12 mm).

**Etymology.** The variety epithet is derived from its small leaves compared with the nominate variety. The Chinese name is given as 小叶伞花树萝卜(Chinese pinyin: xiǎo yè sǎn huā shù luó bo).

**Distribution and habitat.** This species is currently known only from the type locality, Medog County, Xizang, China. It grows on the tree trunks under broad-leaved forests at elevations of 2190–2700 m.

**Phenology.** The new variety flowers in November to January the next year and fruits in May to July.

Additional specimens examined. (paratypes): CHINA. Xizang Autonomous Region: Medog County: Damu Xiang, Km 54 on Zhamo Road, epipetric; 29°41'31.77"N, 95°31'00.6"E; 2337 m elev.; 17 November 2016 (fl.); *Yong-Jie Guo, Qiao-Rong Zhang, Shao-Fa Qin, Feng-Qiong Zhang, Sangjie Pingcuo 16CS14481* (KUN, barcode no. 1448873) • ibid., Km 74 on Zhamo Road; 29°41'34.88"N, 95°31'1.96"E; 2380 m elev.; 30 May 2013 (fr.); *Jie Cai, En-De Liu, Yong-Jie Guo 13CS7653* (KUN, barcode no. 1375387) • ibid., Km 80 on Zhamo Road; 4 July 2013 (fr.); *Yi-Hua Tong XZ060* (IBSC) • Dexing Xiang, Lage to Hanmi; 18 October 2012 (fl. bud); *Yarlung Zangbo Expedition Team 934* (BJM, barcode no. 0230310, IBSC) • Jialasa Xiang, Gudeng Gongshe; 2300 m elev.; 11 December 1982 (fl.); *Bo-Sheng Li & Shu-Zhi Cheng 2100* (PE, barcode nos. 01907986 & 01907987) • Pangxin District, west bank of Yarlung Zangbo; 2700 m elev.; 15 December 1982 (fl.); *Bo-Sheng Li & Shu-Zhi Cheng 3389* (PE, barcode nos. 0191009 & 0191010).

**Taxonomic notes.** This new variety looks very much like a smaller version of the nominate variety. Although the nominate variety is also distributed in Medog County (Huang 1986), its distribution area is a little more south compared to that of *A. forrestii* var. *parvifolia*, and no overlapping area was found (Fig. 2). This species also has potential ornamental values, due to its showy carmine corollas with short green lobes at apex.



Figure 4. Agapetes forrestii var. parvifolia A habit B root tubers C flowering branch D leafy branch E opened corolla, abaxial view F flowers with corolla and some stamens removed to show style G stamens, lateral (left), adaxial (middle) and abaxial (right) view H infructescence. Scale bars: 5 mm (E–G). All by Yi-Hua Tong, except H by Jie Cai.

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#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

YHT: writing original manuscript, finance support, data collection, field investigation. XLG: field investigation, data collection. BMW: field investigation, writing original manuscript. ZW & YJG: field investigation.

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#### **Data availability**

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

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#### **Research Article**

## Mallotus bullatus (Euphorbiaceae), a new species from Southwest China based on morphological characters and phylogenetic evidence

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Abstract

Mallotus bullatus M.T.An & J.H.Yu, sp. nov. (Euphorbiaceae), a species new to science discovered in Guizhou, China, is described and illustrated here, and its phylogenetic position among other Mallotus species is presented. Morphological, micro-morphological, and molecular evidence is presented as attestation of its novelty. The new species morphologically resembles M. philippensis var. reticulatus and M. philippensis var. philippensis, but it clearly differs by having bullate leaf surfaces (vs. not bullate), leaf margins entire or nearly so (vs. entire or nearly so in M. philippensis var. philippensis and coarsely serrate in M. philippensis var. reticulatus), leaf margins sometimes bearing red glands (vs. red glands absent), 5 sepals in staminate flowers (vs. 3-4 in M. philippensis var. philippensis and 4 in M. philippensis var. reticulatus), fruits with spines (vs. spines absent in M. philippensis var. philippensis and present in M. philippensis var. reticulatus), and abaxial leaf epidermal scattered and clustered vein hairs 0.1-0.8 mm long (vs. 0.04-0.28 mm long in M. philippensis var. philippensis and 0.05–0.1 mm long in M. philippensis var. reticulatus). Molecular phylogenetic analysis (BS = 100% / BS = 96%, PP = 1 / PP = 1) provides strong evidence supporting M. bullatus as a new species within the genus Mallotus and supports its placement in M. sect. Philippinenses as sister to M. philippensis.

Key words: Euphorbiaceae, Guizhou province, karst, molecular identification

# Introduction

Mallotus Lour. (Euphorbiaceae) is a large genus comprising approximately 150 species (Sierra et al. 2005), predominantly consisting of shrubs or trees, seldom climbers. It is mainly distributed in tropical and subtropical regions of Asia, Australia, and the Pacific, with a few species found in tropical Africa and Madagascar (Kulju et al. 2007a; Sierra et al. 2007). In China, there are approximately 30 species of Mallotus, mainly distributed in southern provinces and regions. The bark of some species is used for making ropes, and the seed oil



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Copyright: © Jiang-Hong Yu et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0). is used for soap and industrial oils (Liao and Liu 1958). Additionally, *Mallotus* species are important medicinal plants and sources of dye (Kumar et al. 2006; Sharma and Varma 2011; Dhaker and Sharma 2014). The genus *Mallotus* is an important component of forest vegetation (Slik et al. 2003; Eichhorn 2006), exhibiting a variety of life history strategies. Some species act as early successional pioneers, while others are climax species. The genus occurs in a wide range of habitats at low elevations (Sierra et al. 2007).

The genus Mallotus was established by De Loureiro in 1790 based on Mallotus cochinchinensis Lour. (Loureiro 1790). In the latest taxonomic studies of Euphorbiaceae (Webster 1994; Radcliffe-Smith 2001), the genus Mallotus is classified in the subtribe Rottlerinae Meisn. In addition, Mallotus has morphological, distribution and ecological similarities with Macaranga Thouars, another large genus in the Euphorbiaceae. Two phylogenetic studies (Slik and van Welzen 2001; Kulju et al. 2007b) specifically investigated the relationships of Mallotus with related genera. Kulju et al. (2007b) identified three clades, with the majority of Mallotus (Mallotus sensu stricto [s.str.]) forming a sister group to several small genera within the Macaranga clade. Sierra et al. (2010) compiled various datasets including plastid (matK) and nuclear (ITS) DNA sequences, macro-morphological characteristics, and leaf anatomical data, providing a detailed analysis of the phylogeny of Mallotus. The study revealed that Mallotus sect. Mallotus, sect. Polyadenii Pax & K.Hoffm., and sect. Stylanthus Pax & K.Hoffm. are monophyletic, while sect. Axenfeldia (Baill.) Pax & K.Hoffm. and sect. Rottleropsis Müll.Arg. are polyphyletic, and sect. Philippinenses Pax & K.Hoffm. is paraphyletic.

In 2023, during a botanical survey in the Maolan National Nature Reserve in Guizhou, China, we discovered a possibly new species of Euphorbiaceae. After more than a year of field investigations and specimen collection (GZAC-MU-0001), we conducted a field investigation on new species in Maolan National Nature Reserve, Guizhou Province, including photographing its characteristics and collecting seven live specimens. We found that its morphological characteristics resemble those of the genus *Mallotus*. To effectively distinguish the new species from other *Mallotus* species, this study utilized morphology, including pollen and leaf epidermal micromorphology, and molecular phylogenetics using ITS and matK sequences. The results led to the conclusion that the putative new species represents a new taxon.

#### Materials and methods

#### Morphology

Morphological features of leaves, inflorescences, flowers, and capsules were carefully observed and measured in the field, followed by detailed examination in the laboratory. Additionally, we compared specimens based on field observations and photographs taken, as well as studied related species using FAA-fixed materials and dried specimens (GZAC).

#### Leaf epidermis and pollen grains

The mature, complete pollen grains and leaves collected from the field were used to measure characters through a dissecting microscope. Subsequently,

they were mounted on stubs with double-sided tape, coated with a layer of gold, and then photographed using a Hitachi S-4800 scanning electron microscope. The micro-morphological characteristics of the pollen grains are described according to Wang and Wang (1983) and Nowicke and Takahashi (2002). The average size of the pollen grains is calculated based on 20 samples. The micromorphological features of the leaf epidermis of the genus *Mallotus* are stable genetic characteristics that show certain interspecific differences, reflect certain phylogenetic relationships, and can provide a basis for classification and species identification within the genus (Raju and Rao 1977; Alyas et al. 2020). Therefore, this study also investigates the leaf epidermal micromorphology of *Mallotus philippensis* H.Karst., including both var. *philippensis* and var. *reticulatus* (Dunn) F.P.Metcalf. The description of leaf micromorphological features follows Fiala et al. (1994) and Živa et al. (2012), and based on seven specimens collected in the field, including the holotype and the two paratypes.

#### Taxon sampling and DNA sequencing

We used a total of 36 species of *Mallotus* (Euphorbiaceae) in this study, including two individuals of the new species, and one outgroup species: *Macaranga trichocarpa* (Zoll.) Müll.Arg. We chose to use two molecular markers: ITS (ITS-1, 5.8S, and ITS-2) and matK. The ITS sequence, a highly reiterated tandem sequence in the nuclear genome, exhibits rapid changes, providing abundant variation and informative sites (Nürk et al. 2015) and a high level of species resolution accuracy (Chinese Plant Bol Group et al. 2011). The matK gene is one of the fastest-evolving genes in the chloroplast genome. It is easy to align and widely used in the study of families, genera and species (Khidir and Hongping 1997).

We extracted DNA sequences from fresh leaves of the new species and *M. philippensis* var. *reticulatus* using a modified CTAB protocol from Doyle and Doyle (1987), followed by PCR amplification and sequencing following the protocols in referred the methods of White et al. (1990) and Taberlet et al. (1991). We downloaded DNA sequences from GenBank for the two molecular markers for the remaining species used. Taxa and GenBank accession numbers are listed in Suppl. material 1: table S1.

#### **Phylogenetic analysis**

Sequences were aligned using default parameters in Clustal X v.1.83 (Thompson et al. 1997), followed by manual adjustments in BioEdit v.7.0 (Hall 1999). The phylogenies were constructed using Maximum Likelihood (ML) as implemented in PhyloSuite (Zhang et al. 2020) and Bayesian Inference (BI) as implemented in MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003), with the ITS and matK sequences analyzed separately. For the the nucleotide substitution model was chosen using the Akaike Information Criterion (AIC) in Modeltest v.3.06 (Posada and Crandall 1998), with the GTR+I+G model chosen for ITS and the GTR+I model for matK. For the ML analyses, the nucleotide substitution model was chosen using AIC in ModelFinder (in PhyloSuite), with the GTR+G model chosen for both regions.

#### Results

#### **Taxonomic treatment**

*Mallotus bullatus* M.T.An & J.H.Yu, sp. nov. urn:lsid:ipni.org:names:77351570-1 Fig. 1

**Type.** CHINA. • Guizhou Province, Libo County, Dawn township aquatic animals, 25°19'N, 107°56'E, alt. 700 m, 29 April 2024, *Ming-tai An, Jiang-hong Yu, Jian Xu, Feng Liu GZAC-MU-001* (holotype GZAC!).

**Diagnosis.** *M. bullatus* can be distinguished from *M. philippensis* var. *reticulatus* and *M. philippensis* var. *philippensis* by having leaves with bullate surfaces and entire or nearly so margins, sometimes bearing red glands, 5 sepals in the staminate flowers, fruits with spines, pollen grains tricolporate with obvious furrows containing protrusions in the apertures, and abaxial leaf scattered and clustered vein hairs 0.1–0.8 mm long (Table 1).

Description. Shrubs, 1.5-2.5 m tall; twigs, young leaves, and inflorescences densely covered with yellowish-brown disc-shaped glandular hairs. Leaves simple, alternate, ovate or lanceolate, 5-18 (-22) × 3-6 cm, thickly papery, apex acuminate, base rounded or cuneate, margins entire or nearly so, sometimes bearing red glands, surface bullate, upper surface glabrous, lower surface densely grayish-yellow clustered-tomentose, with long soft solitary or clustered hairs on the veins, and scattered red disc-like glands; basal veins 3, lateral veins 3-4 pairs, looped and joined near the margin; extrafloral nectaries, 2-4, brown, near the base; petiole round 2-5 (-9) cm long, slightly pulvinate at both ends, covered with clustered hairs. Inflorescences racemose, terminal, solitary or clustered, solely staminate or pistillate, or mixed with pistillate flowers in lower part and staminate ones in upper part; sometimes apparently bisexual flowers also present. Staminate inflorescences 5-10 cm long, bracts ovate, ca. 1 mm long, pedicel 1-2 mm long, calyx lobes 5, oblong, ca. 2 mm long, densely covered with stellate hairs, with red disc-like glands; stamens 28-30. Pistillate inflorescences s 3-8 cm long, bracts ovate, about 1 mm long; pedicels ca. 1-2 mm long; calyx lobes 4, ovate, densely covered with stellate hairs outside, ca. 3 mm long; ovary hairy, stigmas 3 split, 3-4 mm long, stigmas densely set with feather-like papillae on upper surface; some pistillate flowers sometimes bisexual, then with 1 or 2 stamens, the filaments almost as long as the anthers. Bisexual inflorescences 5-10 cm long, with 3-6 staminate flowers at the apex, lower part entirely pistillate; bracts ovate. Capsule subglobose, with spines, ca. 6-8 mm in diameter, fruit wall thickness ca.1-2 mm, 3 carpellate, densely covered with red disc-like glands; seeds black, ovate or globose, naked with late mature stage.

**Distribution and habitat.** This species is known only from the karst landscape of Libo County, Guizhou Province, China (Fig. 2), alt. 700–900 m.

**Phenology.** Flowering from April to May, and fruiting from May to August. **Etymology.** "Bullatus" specifically refers to the convex leaf areoles.

Local name. Simplified Chinese: 荔波野桐; Chinese Pinyin: lì bō yě tóng.

**Leaf epidermis and palynology.** Pollen grains of *M. bullatus* are spheroidal, with a size of  $22 \times 20 \ \mu\text{m}$ , L(long)/W(width) = 1.1, and tricolporate. They feature tricolporate furrows containing protrusions (Fig. 3A–C). Pollen grains of *M. philippensis* var. *reticulatus* are also spheroidal, 19 × 20  $\mu\text{m}$ , L/W ratio



Figure 1. *Mallotus bullatus* A habitat B, C plant D leaf from above E leaf from below F, G leaf lower surface H–J inflorescences K pistillate flower L, M staminate flower sepals N infructescence with mature capsules O capsule with part removed to show seed P capsule in transverse section.



Figure 2. Geographical distribution of Mallotus bullatus.

0.95, without distinct furrows (Fig. 3D–F). The lower epidermis of *M. bullatus* leaves bears evenly distributed elliptical glands measuring  $100 \times 70 \ \mu m$  (E1 × E2: length of long equatorial axis × length of short equatorial axis), is densely covered with short clustered hairs, and has long (0.1–0.8 mm long) solitary or clustered hairs on the veins (Fig. 3G, H). Similarly, the lower epidermis of *M. philippensis* var. *reticulatus* exhibits elliptical glands measuring  $80 \times 70 \ \mu m$  (E1 × E2), is densely covered with short clustered hairs, and has solitary or clustered hairs 0.05–0.15 mm long on the veins (Fig. 3J, K). The upper epidermis of both *M. bullatus* and *M. philippensis* var. *reticulatus* leaves is smooth (Fig. 3I, L).

**Conservation status.** During the period of 2023–2024, we sampled the population of *M. bullatus* and discovered two additional distribution points near the species initial discovery location (Fig. 2). Each site contained approximately 30 plants. The habitat of *M. bullatus* mainly occurs in karst scrublands, distributed from the foothills to the middle of the mountains. The plant habitat features poor soil fertility, low water retention capacity, and frequent drought conditions. Due to our current insufficient comprehensive assessment of the survival status and threats to *M. bullatus*, we cannot provide specific distribution information about this population. Therefore, we recommend categorizing *M. bullatus* as Data Deficient "DD" (IUCN 2022).

#### Morphological comparisons

Morphologically, the new species is similar to *M. philippensis* in having alternate leaves, basal veins 3, and racemose inflorescences. However, the new species can be distinguished from *M. philippensis* by its bullate leaf surface (vs. not bullate), leaf margins entire or nearly so (vs. entire or nearly so in *M. philippensis* var. *philippensis*, and coarsely serrate in *M. philippensis* var. *reticulatus*), leaf margins sometimes bearing red glands (vs. not red glands), fruits with spines (vs. absent in *M. philippensis* var. *philippensis* var. *reticulatus*), 5 sepals in staminate flower (vs. 3–4 in *M. philippensis* var. *philippensis* and 4 in *M. philippensis* var. *reticulatus*) (Table 1, Suppl. material 1: fig. S1).



**Figure 3.** Scanning electron microscope images of *Mallotus* leaf epidermis and pollen grains **A**–**C** pollen grains of *M. bullatus* **D**–**F** pollen grains of *M. philippensis* var. *reticulatus* **G**–**I** lower leaf epidermis of *M. bullatus* **J**–**L** lower leaf epidermis of *M. philippensis* var. *reticulatus*.

#### **Phylogenetic position**

Nuclear data phylogenetic analyses

The length of the aligned ITS sequences of *M. bullatus* is 760 bp. Based on a dataset of 28 ITS sequences with 182 informative loci, phylogenetic relationships were analyzed using both Bayesian Inference (BI) and Maximum Likelihood (ML) methods (Fig. 4). The two sequences from the new species both originate from the same population, forming a strongly supported monophyletic clade (Fig. 4: BS = 100%, PP = 1). *Mallotus philippensis* var. *philippensis* and *M. philippensis* var. *reticulatus* are sister taxa with strong support (Fig. 4: BS = 100%, PP = 1), and they form a strongly supported sister group relationship with *M. bullatus* (Fig. 4: BS = 98%, PP = 1).

**Table 1.** Comparison of characteristic of three species of *M. bullatus*, *M. philippensis* var. *philippensis*, and *M. philippensis* var. *reticulatus*.

Character	M. philippensis var. philippensis	M. philippensis var. reticulatus	M. bullatus	
Habit	Small tree or shrub	Shrub	Shrub	
Upper leaf midrib hairys	No	Yes	No	
Leaf margins	Entire or nearly so	Coarsely serrate	Entire or nearly so	
Leaf margins bearing red glands	No	No	Sometimes	
Leaf surface bullate	No	No	Yes	
Length of solitary or clustered hairs on leaf abaxial veins	0.04-0.28 mm (Zhang, 2018)	0.05-0.1 mm	0.1-0.8 mm	
Number of staminate sepals	3-4	4	5	
Pollen size	15.5(17.5)-(11.6)15.5 μm (Nowicki and Takahashi 2002)	19 × 20 μm	22 × 20 μm	
Fruits with spines	No	Yes	Yes	

#### Plastid data phylogenetic analysis

The aligned matK sequences of *M. bullatus* are 2000 bp in length. Based on a dataset of 27 matK sequences with 557 informative loci, both Bayesian Inference (BI) and Maximum Likelihood (ML) analyses indicate that the two sequences from the new species form a strongly supported monophyletic clade (Fig. 5: BS = 96%, PP = 1). *Mallotus philippensis* var. *philippensis* and *M. philippensis* var. *reticulatus* forms a weakly supported sister clade (Fig. 5: BS = 80%, PP = 0.82), and they form a strongly supported sister group relationship with *M. bullatus* (Fig. 5: BS = 100%, PP = 1).

#### Discussion

According to the classification in Sierra et al. (2010), *M. bullatus* belongs to sect. *Philippinenses*, characterized by shrubby habit, alternate leaves, 3 basal leaf veins, and capsules densely covered with orange-red glands. Both phylogenetic trees (ITS and matK; Figs 4, 5) indicate that *M. bullatus* is a distinct member of the genus, and furthermore, support its sister group relationship with *M. philippensis* var. *philippensis* plus, *M. philippensis* var. *reticulatus*; these three species form a weakly supported clade with *M. repandus* (Figs 4, 5), also in sect. *Philippinensis*, thus corroborating the evidence provided by the morphological and micro-morphological observations. The two known populations of *M. bullatus* show no consistent morphological differences. Although *M. bullatus* forms a clade with *M. philippensis* var. *philippensis* var. *reticulatus*, it differs from both taxa in its bullate leaf surfaces, length of hairs on the leaf abaxial veins, and number of sepals in the staminate flowers (Table 1).

#### Additional specimen examined

*Mallotus philippensis* var. *reticulatus* (Dunn) F. P. Metcalf. –CHINA. Fujian: Collected on Mr. Dunn's expedition to Central, China. Apr. to Jun., 1905, 3429 (HH); West lake, Chenxiang town, Changtai district, Zhangzhou city. Jun. 11, 1976, *Wang QJ*, 012320 (AU). Jangxi: Yangling, Chongyi County, Ganzhou City, 24°29'N, 103°54'E alt. 1092 m, May 15, 2024, *Yu JH*, *Tang YB*, *Wang YR*, 20240501 (GZAC). *M. philippensis* var. *philippensis* (Lam.) Müll. Arg. – Yunan: roadside at the edge of forests, alt. 800 m. 2000, *Shui YM*, *Chen WH* 13773 (PE).





Sichuan: Jinjia Village, Sutie National Nature Reserve, Panzhihua, Sichuan Province, 26°37'29.7"N, 101°33'03.1"E, alt. 1653 m. Sept. 16, 2010. *Yang Y*, *Huang JH*, *Yang YQ*, *Liu B*, *Ye JF* 551 (PE). **Paratypes.** *Mallotus bullatus* — Guizhou: Rao gu village, Dawn township aquatic animals, Libo County, 25°19'N, 107°56'E, alt. 800 m, Apr. 29, 2024, *An MT*, *Yu JH*, *Xu J*, *Liu F* 202306-1 (GZAC); La nei village, Libo County, 28°21'N, 107°56'E, alt. 750 m, Apr. 30, 2024, *An MT*, *Yu JH*, *Xu J*, *Liu F* 202306-2 (GZAC). **Other specimens.** *Mallotus bullatus* — Guizhou: Yiba Mountain, Lane Village, Dawn township aquatic animals, Libo County, 25°16'N, 107°55'E, alt. 950 m, Jul. 26, 2024, *Yu JH*, *Tang YB*, *Liu F* 001 (GZAC); Yiba Mountain, Lane Village, Dawn township aquatic animals, Libo County, 25°16'N, 107°55'E, alt. 780 m, Jul. 28, 2024, *Yu JH*, *Tang YB*, *Liu F* 002 (GZAC); Dawn township aquatic animals, Libo County, 25°16'N, 107°55'E, alt. 780 m, Jul. 28, 2024, *Yu JH*, *Tang YB*, *Liu F* 002 (GZAC); Dawn township aquatic animals, Libo County, 25°16'N, 107°57'E, alt. 780 m, Jul. 28, 2024, *Yu JH*, *Tang YB*, *Liu F* 002 (GZAC); Dawn township aquatic animals, Libo County, 25°16'N, 107°57'E, alt. 840 m, Jul. 22, 2024, *Yu JH*, *Tang YB*, *Liu F* 004 (GZAC).





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#### Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Jiang Hong Yu and Zheng Ren Chen completed all the work of this paper together, they contributed equally to this work and are the first co- authors of this paper. MingTai An planned and guided the writing of the whole paper, participated in the field investigation and identification work, acted as the corresponding author of the paper. Deng Li Yu participated in the field investigation and completed part of the data processing and content writing and guide the writing of the paper. Feng Liu Collect plant specimens in the field and take photographs for identification. Jian Xu participated in field investigation and compared plant specimens to identify species. Yu Bin Tang participated in field investigation and data collation. Hua Kai Zou participated in field investigation and content writing.

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#### Data availability

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

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#### Supplementary material 1

#### Additional information

Authors: Jiang-Hong Yu, Zheng-Ren Chen, Ming-Tai An, Deng-Li Yu, Feng Liu, Jian Xu, Yu-Bin Tang, Yi-Ran Wang, Hua-Kai Zou

Data type: docx

- Explanation note: table S1. Taxa and GenBank accession numbers for thesequences used in this study. fig. S1. Mallotus philippensis var. philippensis (Lam.) Müll.Arg. and M. philippensis var. reticulatus (Dunn) F.P.Metcalf. A, B leaf of Mallotus philippensis var. reticulatus C holotype of Mallotus philippensis var. philippensis var. philippensis var. reticulatus E merotype of Mallotus philippensis var. reticulatus.
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Research Article

# Discovery of *Whittieria hengduanensis* sp. nov. (Ophioglossaceae) from Southwest China demonstrates a unique intercontinental disjunct pattern in plants between the Himalaya and the Americas

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#### Abstract

A new fern species, *Whittieria hengduanensis* (Ophioglossaceae), from Sichuan, Xizang, and Yunnan, Southwest China (eastern Himalaya), is described and illustrated. This species is similar to *W. engelmannii* in the Americas in having a cylindrical rhizome and complex-reticulate venation. In addition, both species grow in open habitat on basic soil. However, the two species are distinguishable in root number per rhizome and the number of the larger areolae per trophophore. Our molecular study also supports that they are sister to each other but divergent at the molecular level. The discovery of *W. hengduanensis* shows that the genus is intercontinentally disjunct between the Himalaya and the Americas, a unique pattern not having been documented in the literature.

Key words: Adder's tongues, fern biogeography, intercontinental disjunctions

#### Introduction

Ophioglossaceae or Adder's tongue ferns are known for their extremely simplified morphological characteristics (Clausen 1938; Wagner 1990; Zhang et al. 2020; Zhang and Zhang 2022) and the highest known chromosome numbers among known organisms (Ghatak 1977). Following the most recent phylogenetic analysis based on Sanger DNA sequence data, plastome sequences, and analyses of 34 morphological/ecological traits, this family was divided into four subfamilies and 15 genera (Zhang and Zhang 2022). Among the four subfamilies, Ophioglossoideae are the most difficult taxonomically because of their extremely simple morphology. Zhang and Zhang (2022) recognized seven genera in Ophioglossoideae: *Cheiroglossa* C. Presl, *Goswamia* Li Bing Zhang & Liang Zhang, *Haukia* Li Bing Zhang & Liang Zhang, *Ophioderma* (Blume) Endl., *Ophioglossum* L., *Rhizoglossum* C. Presl, and *Whittieria* Li Bing Zhang & Liang Zhang. Based on plastome and mitogenome data, Kuo et al. (2024) resolved the morphologically well-defined South African *Rhizoglossum* as sister to *Whittieria* and found the overall relationships to be consistent to those found by



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**Copyright:** © Zhen-Long Liang & Li-Bing Zhang. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Zhang and Zhang (2022), albeit with lower support values, especially for the monophyly of *Ophioglossum* s.l. (31% maximum likelihood bootstrap value). Kuo et al.'s (2024) results are suggestive that the recognition of the seven genera in the subfamily is well founded.

Among the seven genera in Ophioglossoideae, *Whittieria* was believed to be monospecific and endemic to North to Central Americas. This genus is well defined morphologically, ecologically, and physiologically in Ophioglossoideae by having complex-reticulate venation (large areoles of the trophophore subdivided into smaller areoles; Wagner and Wagner 1994), growing in basic soil, and having relatively shorter spore germination time (70 days vs. 90–100 days of species in *Ophioglossum* s.s.; Whittier 1981).

During a field trip in western Sichuan and southeastern Xizang in 2021, we collected some materials of a species of Ophioglossoideae from several localities. This species has quite clear complex-reticulate venation, similar to the American *Whittieria engelmannii*, but about 8000 km away from the latter species's distribution in air distance. We then conducted detailed morphological and phylogenetic analyses and found that this represents a new species of *Whittieria*.

#### Materials and methods

#### Morphology study

Plant materials were collected from the field trips in Sichuan and Xizang in 2021. The collected specimens were compared with other herbarium specimens deposited at CDBI, and PE, as well as digital images from online sources such as CVH (https://www.cvh.ac.cn/), PPBC (https://ppbc.iplant.cn/), and POWO (https://powo.science.kew.org/).

A preliminary morphological study showed that the newly collected material had complex-reticulate venation with large areolae including several small areolae, which resembled the American genus, *Whittieria*.

#### DNA sequencing and phylogenetic study

To resolve the relationships of the newly collected materials from western Sichuan, we included 32 samples of Ophioglossoideae representing six out of the seven genera recognized by Zhang and Zhang (2022). The South African *Rhizoglossum* C. Presl was not sampled. One species from each of *Cheiroglossa* and *Ophioderma* was used as outgroups following the most recent phylogenies of the family published by Zhang et al. (2020) and Zhang and Zhang (2022).

Silica gel-dried materials were collected in the field. Total genomic DNA was extracted from silica gel-dried samples using the FOREGENE Plant Genomic DNA Isolation Kit. Three plastid markers were sequenced for the phylogenetic analysis, the *rbcL* gene and two intergenic spacers, *rps4-trnS* and *trnL-F*, were separately amplified using the standard PCR protocol. The lab work, sequence alignments, and phylogenetic analysis followed Zhang et al. (2020) and Zhang and Zhang (2022).

The resulting DNA sequences were deposited in GenBank. The information on the plant materials used in the sequencing along with GenBank accession numbers are listed in the Appendix 1.

#### Results

Nine sequences of three samples were newly sequenced. The combined dataset of *rbcL*, *trnL-F*, and *rps4-trnS* contained 3,029 bp of which 462 sites were parsimoniously informative. In spite of only three markers and limited taxon sampling used to construct the phylogeny (Fig. 1), all four segregated genera of *Ophioglossum* s.l. recognized by Zhang and Zhang (2022) were recovered as monophyletic except the monospecific *Haukia*, of which only one accession was included. *Goswamia* was resolved as sister to the rest, and *Whittieria* was sister to a clade containing *Ophioglossum* s.s. and *Haukia*. The newly sampled three accessions of the southwestern Chinese (eastern Himalayan) species were resolved as monophyletic and were sister to *W. engelmannii*.



**Figure 1.** The maximum likelihood tree based on the combined plastid dataset of *rbcL*, *trnL-F*, and *rps4-trnS*. Maximum likelihood bootstrap support (MLBS) and Bayesian inference posterior probability (BIPP) are given above and below the branches, respectively. Voucher information is indicated behind the species name.

#### Discussion

Consistent with previous findings (Zhang et al. 2020; Zhang and Zhang 2022; Kuo et al. 2024), *Haukia* is resolved as sister to *Ophioglossum* s.s. (Fig. 1). However, *Goswamia* is resolved as sister to the rest of the four genera, which differs from a resolution in which *Goswamia* was found to be sister to *Whittieria* (Zhang et al. 2020; Zhang and Zhang 2022). We consider this conflict as minor because our current resolution of *Goswamia* received low statistical support. This conflict might have been the result of different sampling sizes and characters included.

It is quite striking that the eastern Himalayan species (hereafter referred as Whittieria hengduanensis) is resolved as sister to the American endemic W. engelmannii. The genus Whittieria was believed to contain a single species in America (Zhang and Zhang 2022). With the second species discovered here, this genus now has an intercontinental disjunction between the Himalaya and the Americas. Wen (1999) listed 65 genera of seed plants displaying the eastern Asian-eastern North American disjunctions. Xiang et al. (2015) hypothesized that 31 groups of ferns and lycophytes showed eastern Asian-North American disjunctions. However, none of these taxa are endemic to the Himalaya and have their sister in the Americas (southern North America and Central America for Whittieria). Therefore, the intercontinental disjunction pattern in Whittieria has not yet been documented in any group of plants at any taxonomic ranks. The divergence time between Whittieria and the rest of Ophioglossoideae and that between two species in Whittieria are of great biogeographical significance. Where is the ancestral area of Whittieria then? How did the two species form today's disjunct pattern? The discovery of *W. hengduanensis* is surely interesting.

*Whittieria hengduanensis* is indeed similar to *W. engelmannii* in having complex-reticulate venation, which is unique in Ophioglossaceae and in fact defines the genus *Whittieria* (Zhang and Zhang 2022). The morphological differences between the two species are minute (see below) because of the generally simple morphology in the subfamily. Both species grow in basic soil, which is unique in the family, too (Zhang and Zhang 2022). However, the two species are so widely displaced geographically (ca. 8000 km in air distance), with *W. hengduanensis* being in the eastern part of the Himalaya and *W. engelmannii* being endemic to North and Central America (Wagner and Wagner 1994; Wan et al. 2022). In addition, the two species occur in very different elevations with *W. hengduanensis* in elevations between 2500–4000 m with temperate and alpine climates and *W. engelmannii* in those between 200–2200 m with tropical, subtropical, and temperate climates.

For a long time, this species has been confused with *Ophioglossum nudicaule* L.f. They overlap in geographical distribution. However, *O. nudicaule* has no persistent old leaf stalks at the base of the rhizomes and fewer roots per rhizome, and the sporophore base is not slightly attached to the trophophore. Importantly, they have different venation patterns with *W. hengduanensis* with complex-reticulate venation and *O. nudicaule* with common reticulate venation, although Wagner and Wagner (1994) reported complex-reticulate venation for the North American *O. nudicaule*. It is unclear whether the materials from Africa (type locality), Asia, and the Americas of "*O. nudicaule*" represent the same species.

#### **Taxonomic treatment**

#### Whittieria hengduanensis Z.L.Liang & Li Bing Zhang, sp. nov.

urn:lsid:ipni.org:names:77351700-1 Figs 2, 3

**Type.** CHINA • Sichuan: Yajiang County, Jiaonibao Village, elev. 2750 m, 30°6'10.93"N, 101°1'49.79"E, in the shrubs in dry and hot river valleys, 15 July 2021, *Z.-L. Liang, L.-S. Jiang & Q. Yu LZL1959* (holotype CDBI!).

**Diagnosis.** Whittieria hengduanensis is similar to W. engelmannii but the former has up to 25 (-50) roots per rhizome and 10–20 large areolae per trophophore, whereas the latter has fewer than 15 roots per rhizome and 0–8 areolae per trophophore.

**Description.** Plants 10–13 cm tall. Rhizomes erect, cylindrical, diam. 6–7 mm, with numerous black residual trophophore stalks. Leaves 1–2 per rhizome. Roots fleshy, up to 25 (–50) per rhizome, ca. 0.6 mm diam. Leaf stalks 4.5–7.5 cm long, 1.5–2 mm diam.; the lower part is buried in the soil, grey. Trophophores nearly circular to ovate, papery, 1.6–6 cm long, 1–3 cm broad at the middle, apex acute, narrowed toward the base. Venation complex-reticulate (also called "double venation" with small areolae inside a large areola), with included veinlets. Sporophores arising at ground level, the base is slightly attached to sporophores; stalks 3.5–7 cm long; spike 1.2–1.8 cm long, ca. 2.5 mm diam., 16–23 pairs of sporangia per spike.

**Geographical distribution.** This species is found in western Sichuan (Batang, Daocheng, Daofu, Derong, Maerkang, Xiangcheng, Yajiang, Yuexi), northwestern Yunnan (Lijiang, etc.), and eastern Xizang (Mangkang, Zayu).

**Habitat.** This species grows in basic soil under shrubs in dry and hot river valleys at elevations of 2500–4000 m.

**IUCN Red List category.** Based on the field investigations, this species has a relatively wide distribution and large population sizes in southwest China and should be classified as Least Concern (LC), based on current information and following the International Union for Conservation of Nature and Natural Resources guidelines (IUCN 2024).

**Etymology.** The species epithet *henduanensis* is based on the distribution of this species in the Hengduan Mountain.

Additional specimens examined. CHINA • Sichuan: Daocheng county, Aug. 23, 1937, *T.T.Yu* 12889 (PE01622937); 23 Aug. 1981, *Qingzang Exp.* 4234 (PE01384314) • Aug. 17, 1973, s.c. 2519 (KUN0803070 2519) • Daofu, 18 Sept. 1934, *Harry Smith* 12236 (PE00405282) • 16 Aug. 1960, *Sichuan Drug Exp.* 15532 (NAS00143448) • Derong, 03 Aug. 1981, *Qingzang Exp.* 3174 (PE01384316, PE01384315, HITBC053830) • Maerkang, 18 Jul. 1960, *Sichuan Drug Exp.* 22092 (NAS00143451) • Yuexi, 22 Sept. 1960, *Sichuan Drug Exp.* 26220 (NAS00143450) • Xiangcheng, 31 Jul. 1981, *Qingzang Exp.* 3115 (PE01384313, HITBC053829) • 12 Sept. 1937, *T.T.Yu* 13319 (PE00405281, PE00405283, PE00405286) • Xizang: Zayu, Sept. 1935, *C.W.Wang* 66172 (PE00405289); Sept. 1935, *C.W.Wang* 66236 (KUN0803067) • 13 Aug. 1961, *Xizang Complex Exp.* 2703 (PE00405290) • 16 Aug. 1965, *Yongtian Zhang & Kaiyong Lang* 1541 (KUN0803072) • Yunnan: Lijiang, 04 Jul. 1965, *Jinsha River Exp.* 4539 (PE01593067); *T.T.Yu* 13319 (KUN0803063).



Figure 2. Whittieria hengduanensis A, B habitat C lower portion of plant showing stem and roots D individuals in different sizes E trophophores and sporophore stalks F sporophore G veins showing complex-reticulate venation.



Figure 3. Whittieria hengduanensis **A**, **B** habit **C** trophophore and base of sporophore **D** sporophore spike **E** veins showing complex-reticulate venation.

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#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

No funding was reported.

#### Author contributions

L-BZ defined the project, supervised the execution, revised the first draft; Z-LL conducted the fieldwork, lab work, and analysis, prepared the figures and wrote the first draft.

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#### Data availability

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

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#### Appendix 1

Taxon sampling, voucher information, and GenBank accession numbers used in this phylogenetic analysis.

Species name	Voucher	Provenances	references	chloroplast complete genome	rbcL	trnL intron & trnL-F spacer	rps4-trnS spacer
Cheiroglossa palmata	Eric Schuettpelz 1771	Unknow			MW620387		
Goswamia aletum	M. Patel MP765	India	Patel et al. 2018		MK120497		
Goswamia costatum	S. Petchsri 2018-29 (BCU)	Unknow			MN524768		MN524788
Goswamia costatum		India				MW666160	
Goswamia eliminatum	H.K. Goswami HK004	India	Patel et al. 2018		MK120496		
Goswamia gomezianum	Burrows 5767 (K)	Zambia	Hauk et al. 2003		AY138419		
Goswamia gujaratense	K.S. Rajput et al. MIPDG023-18	India	Patil et al. 2018		MH229473		
Goswamia hitkishorei	M. Patel s.n. (CAL)	India	Patel and Reddy 2019		MK360156	MK358465	
Goswamia indicum		India			MW666155	MW666168	
Goswamia malviae	M. Patel BLAT112082 (BLAT)	India	Patel et al. 2018		MF184998		
Goswamia reticulatum	NL7	India			MW666154	MW666164	
Haukia crotalophoroides	A. Grusz 112 (DUKE)	San Jose, Costa_Rica	Zhang et al. 2020		MN524769		
Ophioderma pendula		Khanh Hoa, Vietnam	Zhang et al. 2020		MN524766		MN524718
Ophioglossum californicum		USA		KC117178			
Ophioglossum chaloneri	MPHKG001	Jharkhand, India	Goswami et al. 2020		MH605181	MH605183	
Ophioglossum coriaceum	NSW:Papadopulos AP708	Lord Howe Island, Australia	Papadopulos et al. 2011		JF950813	JF950935	
Ophioglossum gramineum	RGB Kew 1981-6838 (K)	London, UK	Hauk et al. 2003		AY138412	MW666162	
Ophioglossum kawamurae		Japan	Shinohara et al. 2013		AB626648		
Ophioglossum namegatae	TNS:764351	Ibaraki, Japan	Ebihara et al. 2010		AB574675		
Ophioglossum nudicaule	W. M. Chu et al. 15999	Yunnan, China	Zhang et al. 2020		MN524772		MN524792
Ophioglossum parvifolium	BAS10	India			MW666157	MW666167	

Table A1. A new fern species, Whittieria hengduanensis (Ophioglossaceae) from Sichuan, Xizang and Yunnan, China.

Species name	Voucher	Provenances	references	chloroplast complete genome	rbcL	trnL intron & trnL-F spacer	rps4-trnS spacer
Ophioglossum parvum	R. Knapp 4647 (P, MO)	Taiwan Island	Zhang et al. 2020		MN524787		MN524805
Ophioglossum petiolatum	L.B. Zhang et al. 9022	D´a ăk L´a ăk, Vietnam	Zhang et al. 2020		MN524777		MN524797
Ophioglossum petiolatum	Z.R.He et al. MT-168	Xizang, China	Zhang et al. 2020		MN524774		MN524794
Ophioglossum pusillum	Nekola 8069 (COE)	lowa, USA	Hauk et al. 2003		AY138413		
Ophioglossum pycnostichum	SCBI-SIGEO-13_0145	Unknow	Erickson et al. direct submission		KP644048		
Ophioglossum richardsiae	Burrows 5756 (K)	Zambia	Hauk et al. 2003		AY138415	AY138451	
Ophioglossum thermale var. nipponicum	TNS:1108349	Japan			AB574680		
Ophioglossum thermale var. thermale	TNS:764007	Okinawa, Japan	Ebihara et al. 2010		AB574679		
Ophioglossum vulgatum	Z.L.Liang 1283	Sichuan, China	Zhang et al. 2022		OL539473	OL519781	OL519796
Whittieria engelmannii	BRIT:Gostel485	Texas, USA			OL537713		
Whittieria engelmannii	MEXU:1163958	Mexico			MH028789		
Whittieria engelmannii	MO:G. Yatskievych 15-025	Unknow		OR777260			
Whittieria hengduanensis	Z.L. Liang et al. 1956	Mangkang, Xizang	this study		PQ407856	PQ407862	PQ407859
Whittieria hengduanensis	Z.L. Liang et al. 1958	Batang, Sichuan	this study		PQ407857	PQ407863	PQ407860
Whittieria hengduanensis	Z.L. Liang et al. 1959	Yajiang, Sichuan	this study		PQ407858	PQ407864	PQ407861


#### **Research Article**

## Wedelia figueiredoana (Asteraceae, Heliantheae), a winged cypselae new species of Wedelia for Brazil

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### Abstract

We describe one new species from Ceará state, Brazil: *Wedelia figueiredoana*. It is morphologically associated with *W. bonplandiana*, but distinguished by 0.25–1.05 cm leaf blade width (vs. 1.5–2.6 cm), linear to narrow oblong leaf blade (vs. elliptic to spatulate), 3-seriate involucre (vs. 2-seriate), and cypselae 3.9–4 mm long (vs. 6–7 mm). An anatomical analysis of cypselae is provided, and we propose a more accurate terminology to describe similar wings to *Wedelia* species. We also provide a scientific illustration of the new species, photos of habitat, a map of its geographic occurrence, and its taxonomic affinities are discussed with a taxonomic key to the *Wedelia* species with the apically pronounced wings in the cypselae.

Key words: Auricular projections, Cerrado, Compositae, fruit anatomy

## Introduction

*Wedelia* Jacq. is inserted in Heliantheae Cassini. This genus occurs mainly in the Neotropics (Strother 1991; Panero 2007), and occasionally in the south USA and Tropical Africa (Strother 1991). We adopted the *Wedelia* circumscription of Turner (1992), which includes *Aspilia* Thouars as its synonym. Brazil is the most rich-species country of the world for the genus with the occurrence of 89 species, 56 of them endemic (Flora e Funga do Brasil 2024).

Wedelia can be characterized by herbaceous or shrubby habit, opposite leaves, blades lanceolate to ovate, oval or elliptic; capitula solitaires or in cymes, radiate, rarely discoid; 2–4-seriate involucre, paleaceous receptacle; pistillate or sterile ray florets, yellow or yellow to orange corolla; bisexual disc florets, yellow to orange corolla; black anthers, yellow styles arms; black cypselae, winged or no-winged, coroniform pappus, 0–3 awns (Panero 2007).

During field expeditions in the municipality of Graça, Ceará state, Brazil in February 2023, specimens with yellow florets of Asteraceae were found. After



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**Copyright:** © Vinicius R. Bueno et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). extensive literature review, morphological analysis of specimens from herbaria, and anatomical studies led to the conclusion that this is a new species with unusual cypselae structures, when compared with the Brazilian *Wedelia* species. Thus, we propose here a new species of *Wedelia* for the Brazilian flora. We provide morphological description, an illustration plate, photos and information on the habitat, distribution map, preliminary data on its conservation status, taxonomic key to the Brazilian *Wedelia* species with winged cypselae, and the morphological relationship with the most similar species is discussed. In addition, an anatomical plate is provided and the taxonomic implications for *Wedelia* of its results are discussed.

## Material and methods

Herbarium specimens from BHCB, BHZB, CEN, HUFU, ICN, R, RB, SPF (acronyms according to Thiers 2024) were analyzed. Several online herbarium databases were consulted, including the following: C. V. Starr Herbarium Virtual (https://sweetgum.nybg.org/science/vh/), Reflora (http://reflora.jbrj.gov.br/ reflora/), Smithsonian Virtual Herbarium (https://collections.si.edu/search/), SpeciesLink (https://specieslink.net/), and Tropicos (https://tropicos.org). The types of numerous *Wedelia* species were consulted for taxonomic comparisons. The literature of *Wedelia* was reviewed (Baker 1884; Turner 1988, 1992, 2004; Strother 1991; Pruski and Robinson 2018; Bueno et al. 2019; Bueno and Nakajima 2020; Remor et al. 2022; Flora e Funga do Brasil 2024).

The morphological description was based on vegetative and reproductive material from herbaria specimens, for which a stereomicroscope was used to perform the measurements with a caliper rule. The vegetative structures were described from dried material, whereas reproductive structures were characterized after rehydration, immersing the structure in warm water for 1 minute at 100 °C. The outliers of measures were based on calculation of medians, quartiles, and interquartile deviations. "Rarely" is applied for characters that occur in up to 10% of the specimens studied; "sometimes" is adopted for features that occur in between 10.01% and 25% of the specimens analyzed; "often" is used for the characters that occur between 25.01% and 40% of the specimens studied; and "Or" is applied for traits that occur between 40% and 60% of the specimens (Bueno and Heiden 2022).

The general morphological terminology follows Hickey (1973), Ellis et al. (2009), and Beentje (2016). The specialized Compositae terminology follows Funk et al. (2009), and specific literature about the terminology of receptacle (Bueno et al. 2022) and pappus (Bueno and Heiden 2022). Although Bueno and Heiden (2022) proposed the terminology of pappus based on pappus scales, they refer to the size of pappus scales and the frequency of different lengths in the same heads. We believe that this terminology contributes to the description of the aristate pappus of *Wedelia*. The GeoCAT analysis (Bachman et al. 2011) and IUCN guidelines (2022) were used for preliminary conservation status assessment. The distribution maps were prepared in QGIS v. 3.0 (QGIS Development Team 2015).

We used a technique for scanning electron microscopy (SEM) to observe the details of the surface of the cypsela. Mature ray and disk cypsela were placed on aluminum stubs and then covered by gold old using a sputter coater (Leica EM SCD050). Pictures of the cypsela were taken from a SEM (Tescan VEGA 3 LMU).

Anatomical studies were made from exsiccatae, therefore, the cypselae were rehydrated in a solution containing 5% NaOH for four hours (Anderson 1963 modified). After that time, the material was washed with distilled water for 24 hours, and dehydrated in an increasing ethylic series. Dehydrated cypsela was included in historesin (Leica®) following the manufacturer protocol. The material was 8–10  $\mu$ m thick on a rotary microtome and the sections obtained were stained in toluidine blue at pH 4.7 with acetate buffer (O'Brien et al. 1964 modified). Finally, the sections were mounted in synthetic resin and images were taken using a light microscope (Olympus BX51). The anatomical terminology pericarp follows Roth (1977).

## Results

## Taxonomy

Wedelia figueiredoana V.R.Bueno, sp. nov. urn:lsid:ipni.org:names:77351701-1 Figs 1-5

**Type.** BRAZIL • **Ceará:** Graça, área de Cerrado rupestre; 4°05'18.1"S, 40°43'25.4"W; 26 February 2023; *L.S. Rodrigues 201* (holotype: HUFU00082144!, isotypes: HUVA!, HCDAL!, RB!).

**Diagnosis.** Wedelia figueiredoana morphologically resembles W. bonplandiana by 0.25–1.05 cm leaf blade width (vs. 1.5–2.6 cm), linear to narrow oblong leaf blade (vs. elliptic to spatulate), 3-seriate involucre (vs. 2-seriate), and cypselae 3.9–4 mm long (vs. 6–7 mm).

Description. Herbaceous habit, prostrate, 0.15–0.3 m tall. Stems cylindrical, strigose to sparsely hirsute or densely hirsute, castaneous, internodes 0.84-6.7 cm long. Leaves decussate, rarely reduced in the base, sessile; blades 2.25-10.3 × 0.25-1.05 cm, linear to narrow oblong, base attenuate, apex attenuate, often acute, venation hiphodromous, often acrodromous basal, margins entire, flat; abaxial surface hirsute to densely strigose, veins hirsute to densely strigose, eglandular, adaxial surface hirsute to densely strigose, eglandular; olivaceous, concolorous to slightly discolorous, chartaceous. Capitula solitaries, terminal, peduncle (1.85-) 5.3-13.2 cm long, sparsely hirsute to hirsute, often strigose to sparsely hirsute, eglandular. Capitula heterogamous, radiate; involucre campanulate, (5.3-) 6.7-9.5 mm × 6.7-12.7 mm. Phyllaries 3-seriate; blades lanceolate, margins entire, flat, eglandular surfaces; outermost series apex acute or attenuate, densely hirsute to hirsute or densely hirsute to densely strigose, first series blades 9.4-12 × 1.9-2.3 mm, margin ciliate or not ciliate, olivaceous, foliaceous; second series blades 7.6-10.7 × 2.2-3.1 mm, margin ciliate or not ciliate, olivaceous or pale yellow to olivaceous, foliaceous or scarious with apex foliaceous; innermost series blades 5.5-7.6 × 1.6-2.6 mm, apex attenuate, sparsely hirsute to strigose, pale yellow to olivaceous, scarious with apex foliaceous or scarious. Receptacle flat, holopaleaceous, paleae 6.7-8.9 × 2-2.6 mm, narrow elliptic or narrow oblong, apex acute, concave or conduplicate, pale yellow to yellow. Ray florets 6-8, neutral, corolla ligulate, 10.3-14.3 mm long, tube 1.2-2.5 mm long, limb 9.1-12.3 × 6-6.3 mm, obovate or wide elliptic, apex 3-lobulate, 3-6 veins, tube pilose or glabrous, surface abaxial glabrous, surface adaxial glabrous, nerves sparsely pilose or glabrous, yellow.



**Figure 1.** Wedelia figueiredoana sp. nov. **A** flowering branch **B** abaxial surface leaf **C** first series of phyllaries **D** second series of phyllaries **E** third series of phyllaries **F** capitulum with ray florets removed to show involucre, paleae, and disc floret arrangements **G** ray floret **H** disc floret **J** cypselae with apically pronounced wings **K** apically pronounced wing **A**–**K** drawn from L. S. Rodrigues 201 (HUFU) **A**–**K** millimeter scale. Illustration by Débora Dalzotto.



Figure 2. Wedelia figueiredoana sp. nov. A linear blade leaf B head (top view) C flowering branch D head and phyllaries (side view) E habit.



**Figure 3**. Geographic occurrence of *Wedelia figueiredoana* sp. nov. in Brazil. CE: Ceará, PB: Paraíba, PE: Pernambuco, PI: Piauí, RN: Rio Grande do Norte.



Figure 4. Wedelia figueiredoana sp. nov. habitat A enclaves of Cerrado B Wedelia figueiredoana in the ferrugineous soil C floral visitor in the W. figueiredoana D prostrate habit E solitary herbaceous individual of W. figueiredoana.

Disc florets 25–40, monoclinous, corolla tubular, 4.8–6.2 mm long, tube 1.3– 1.9 mm long, lobes 0.9–1.1 mm long, glabrous, yellow; anthers 2.2–3 mm long, apical anther appendages ovate, black; style arms 1–1.2 mm long, linear, yellow. Cypselae 3.9–5.6 mm long, obovoid, flattened, densely sericeous, blackish, wings present throughout the pericarp, but apically pronounced, 0.04–0.4 mm long, yellow; pappus constrict at base, coroniform 0.5–0.6 mm long, 2-aristate, bitypic, monolength or bilength, 1.2–2.9 mm long, yellow.

**Distribution and habitat.** *Wedelia figueiredoana* is a microendemic species, which is known to occur exclusively in one municipality of Ceará state: Graça (Figs 3, 4). The new species grows in ferruginous open areas of Cerrado enclaves in elevations around 370–600 m a.s.l.. These enclaves also occur in the Ibiapaba plateau (Moura-Fé 2017), in the municipality of Graça, Ipu, Ipueiras, Pacujá, Pires Ferreira, and Reriutuba (municipalities from Ceará state). This new species occurs in testimonial hills and small inselbergs associated with the plateau (Moura-Fé 2017; Claudino-Sales et al. 2020), forming a Cerradão interspersed with open areas, rocky outcrops and ferruginous soils. This area is a transition zone between the Caatinga vegetation in low-elevation areas to the humid and subhumid forests in the high-elevation areas.

**Conservation status.** The GeoCAT analysis (Bachman et al. 2011) considers that each collection point has 4.0 km<sup>2</sup> of Area of Occupancy, but the species occurs in patches of open areas in the midst of closed vegetation, which greatly restricts its habitat. The Extent of Occurrence (Bachman et al. 2011) was not calculated because there were only two collection points available. Through satellite images and QGIS v.3.0 mensuration, we believe that the areas so far known for the species are not larger than 0.5 km<sup>2</sup>. According to the IUCN (2022) criteria, *W. figueiredoana* meets the following requirements for the Critically Endangered status: the criteria B1 (less than 100 km<sup>2</sup> of extent of occurrence), B2 (less than 10 km<sup>2</sup> of area of occupancy) and the condition A of criterion B (because it has one known location); criterion C (less than 250 mature individuals), conditions i and ii of C2 (about 50 individuals were seen, all mature individuals); criterion D (about 50 individuals were seen). Therefore, *Wedelia figueiredoana* is proposed as Critically Endangered (B1; B2a; C, C2i, ii; D).

Phenology. The specimens were collected with florets and fruits in February. Etymology. The epithet "figueiredoana" is in honor of Dr. Marlene Feliciano Figueiredo, born in 1963, a dedicated educator at Universidade Estadual Vale do Acaraú (UVA). Her dynamic engagement in teaching, research, and outreach, notably the Pensando Verde project, has left an indelible mark. Noteworthily, she was pivotal in founding and curating the Herbário Francisco José de Abreu Matos-HUVA (1998-2004), a vital botanical resource in Northwest Ceará. In botanical research, Figueiredo specializes in phanerogam taxonomy, floristics, ethnobotany, and seed germination ecophysiology, fostering a deep understanding of Northwestern Ceará's intricate flora through her mentorship.

### Anatomical and morphological cypsela studies

The cypsela of *Wedelia figueiredoana* is flattened-obovoid (Fig. 5A) with lateral wings (Fig. 5A, B). The pappus is coroniform (Fig. 5A–C) with two aristae and several smaller bristles setose (Fig. 5C). The carpopodium is conspicuous and bilobed with elaiosomes on both sides (Fig. 5A, D). Biseriate trichomes are distributed throughout the cypsela (Fig. 5E). In cross section, the cypsela is rhombic (Fig. 5F, I). The exocarp is composed of juxtaposed and periclinally flattened cells. The mesocarp is divided into three regions: the outer one is formed by approximately two parenchymatic layers, the median one has approximately five



Figure 5. Cypsela of *Wedelia figueiredoana* sp. nov. in scanning electron microscope (SEM) (A–E) and light microscopy (F–K). A General view B apex detail C coroniform pappus detail, note the outer awn and inner bristles fused at the base D basal region detail, observe the presence of elaiosomes E indumentum detail highlighting the biserial tector trichomes F–H middle region F overview G pericarp detail, note the sclereids of the middle mesocarp and the presence of phytomelanin H lateral region detail, observe the vascular bundle immersed in the mesocarp I–K cypsela apex I general view J pericarp detail K lateral region detail. in: external mesocarp; ex. exocarp; im: inner mesocarp; mm: medium mesocarp; se: seed; arrow: vascular bundle; asterisk: crashed layer (part of the inner mesocarp and endocarp); black arrowhead: sclereid; white arrowhead: phytomelanin. Cypsela analyzed from L. S. Rodrigues 201 (HUFU).

layers of sclereids and the inner one has composed of parenchymatic cells consumed in the median region of the cypsela and maintained in the apical region. (Fig. 5G, J). Four collateral bundles are immersed in the sclereids (Fig. 5H). The outermost layer of the median mesocarp has anticlinal projections that are responsible for the schizogenous space filled with phytomelanin (Fig. 5G, H, J, K). The endocarp appears consumed by seed growth. The wing is composed of the entire pericarp with a projection of the median mesocarp at the apex (Fig. 5H, K).

## Discussion

The herbaceous habit, decussate leaves, monocephalous heads, overlapped phyllaries in the involucre, and the neutral ray florets resembles the *Wedelia foliacea* group: *Wedelia foliacea* (Sprengel) B.L. Turner, *W. montevidensis* (Sprengel) B.L.Turner, and *W. riedelii* (Baker) B.L.Turner. However, *W. figueiredoana* can be differentiated from this group by the 3-seriate involucre (vs. 2-seriate, rarely 3-seriate), second series of phyllaries hirsute (glabrous, rarely pilose), and pronounced apically winged cypselae (vs. wings absent).

Wedelia figueiredoana, W. bonplandiana (Gardner) B.L.Turner, Wedelia brachylepis Griseb., and Wedelia rudis (Baker) H.Rob. are the unique Brazilian Wedelia species with winged cypselae. Wedelia figueiredoana and W. bonplandiana have neutral ray florets, while W. brachylepis and W. rudis have pistillate ray florets. Wedelia figueiredoana and W. bonplandiana are the only two species from Brazil with a pronounced apically winged cypselae, they also share more characters as the herbaceous and prostrate habit, sessile leaves, both surfaces with strigose indumentum, solitaries capitula, and paleae about 7 mm long (Santos 2001). Nevertheless, W. figueiredoana can be individualized from W. bonplandiana by 0.25–1.05 cm leaf blade width (vs. 1.5–2.6 cm), linear to narrow oblong leaf blade (vs. elliptic to spatulate), 3-seriate involucre (vs. 2-seriate), and cypselae 3.9–4 mm long (vs. 6–7 mm).

Blake (1931) was the first author who described "auriculate" projections to the Wedelia cypselae for W. penninervia S.F.Blake. Strother (1991) described three taxa with the same projections: W. acapulcencis var. hispida (Kunth) Strother, W. greenmanii B.L.Turner, and W. strigosa Hook. & Arn. The same terminology was applied by Pruski and Robinson (2018) to describe the cypselae of Wedelia filipes Hemsl. Until then, these were the only known Wedelia species with such structures described. The theoretically same structure can be seen in the cypselae of W. bonplandiana (Gardner) B.L.Turner. In the first moment of the morphological descriptions, it was believed to be the same projections in the cypselae of W. figueiredoana.

Strother (1991) and Pruski and Robinson (2018) made the distinction between species with winged cypselae and cypselae with auriculate projections. Our anatomical results found internal tissues in the supposed projections so we will treat these projections as wings. Based on this, we hypothesize that the species aforementioned with such projections also have winged cypselae, but further studies will elucidate this. We propose that this specific type of wing, described here for *W. figueiredoana*, be treated as "apically pronounced wings" to differentiate them from the wings that are more developed and that can be easily seen along the cypselae commonly found in *Wedelia* species, such as the wings described by Strother (1991). Elaiosomes are more common in seeds but can be found in fruits of Asteraceae, such as *Centaurea* (Roth 1977). For *Wedelia*, elaiosomes proved to be very important for new classification of this genus, proposed by Alves (2019). In this classification, *Wedelia* now includes the genera *Aspilia*, *Coronocarpus* Schumach. & Thonn., *Oyedaea* DC., *Steiractinia* S.F. Blake, *Zexmenia* La Llave, *Zyzyxia* Strother, and two species of *Elaphandra* Strother (Alves 2019). According to Alves (2019), clades within *Wedelia* that do not present elaiosomes are considered as a derived character state. The anticlinal projections present in the outermost layer of the median mesocarp of *W. figueiredoana* were also described for *Clibadium*, *Desmanthodium*, *Ichthyothere* (Stuessy and Liu 1983) and *Calea* (Marques et al. 2022).

## Key to the species of Brazilian winged cypselae Wedelia species

1	Herbaceous habit, prostrate or decumbent, shorter than 50 cm tall; ray
	florets neutral
-	Shrubby habit, erect, longer than 50 cm tall, ray florets pistillate3
2	Leaf blade width 0.25-1.05 cm, linear to narrow oblong; 3-seriate involu-
	creW. figueiredoana
-	Leaf blade width 1.5–2.6 cm, elliptic to spatulate; 2-seriate involucre
3	Stems hirsute or pubescent; leaf base attenuate Wedelia rudis
-	Stems strigose; leaf base rounded Wedelia brachylepis

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## **Additional information**

## **Conflict of interest**

The authors have declared that no competing interests exist.

## **Ethical statement**

No ethical statement was reported.

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## Author contributions

Conceptualization: VRB, DM. Data curation: VRB, DM, JM. Formal analysis: VRB. Investigation: VRB, DM, JM, LSR. Resources: JM. Software: FDS, ICS. Supervision: VRB, DM, JM. Writing – original draft: VRB, DM, JM. Writing – review and editing: VRB, DM.

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### Data availability

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

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## PhytoKeys

**Research Article** 

# *Elymus multiramosus* (Poaceae), a new species from the north-western Qinghai-Tibetan Plateau, China

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#### Abstract

A new species from China, Elymus multiramosus Y.C. Zhang, sp. nov. is described and illustrated herein, based on morphological characters and molecular phylogenetic analysis. The taxonomic descriptions of E. multiramosus and the comparison with related species are presented. The taxonomic distinctiveness of this new species was inferred by Maximum Likelihood (ML) analysis and Bayesian phylogenetic analysis, based on the complete chloroplast genome sequence. It is assigned to the Elymus section and bears similarity to Elymus nutans Griseb. However, it can be easily distinguished from other species by its compound spike, in contrast to the simple spike inflorescence typical of those species. The compound spike is characterised by rhachillas that are extended at the base of the main axis, giving rise to 3-6 mini-spike-like branches. Notably, these branches significantly increase in length from the top towards the bottom of the compound spike. In the molecular phylogeny, Elymus multiramosus from Qinghai, north-western China, is phylogenetically positioned as a distinct lineage. The lineage comprising Elymus sinosubmuticus from Sichuan, east of the Tibetan Plateau and Elymus nutans from the Himalayas forms a sister group to Elymus multiramosus, suggesting that these three species share a common ancestor that is distinct from the lineage leading to Elymus atratus from Gansu, north of the Tibetan Plateau.

Key words: Molecules, morphology, new species, phylogeny, Poaceae

## Introduction

Poaceae (grasses) is one of the largest flowering plant families in angiosperms, including many economically important crops, such as rice (*Oryza sativa* L.; He et al. (2011)), barley (*Hordeum vulgare* L.; Pan et al. (2023)) and maize (*Zea mays* L.; Chen et al. (2022a) (Hodkinson 2018). Evolutionary changes in the organisation and structure of grass inflorescence have resulted in their different morphologies from those of core eudicots and non-grass monocots (Zanis 2007). The basic inflorescence unit of grasses is the spikelet, which is a short branch with leaf-like organs called glumes enclosing one or more florets. Grass plants develop distinct inflorescences and spikelets that determine grain yields



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**Copyright:** © Yong-Chao Zhang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). (Gao et al. 2013). The combined number and size of seeds contribute to higher fitness in the wild and higher yield in cultivation. Accordingly, inflorescence structure and flower/seed production have been the target of both natural and human selection (Kellogg 2022).

Elymus L., as defined in the seminal works of Löve and Dewey (Dewey 1984; Löve 1984), stands as the most expansive genus within the Triticeae tribe, marked by its polyploid, mostly short-lived perennial grasses (Jensen and Chen 1992). Spanning about 172 species, Elymus extends from the Arctic to subtropical regions, adapting to diverse environments like grasslands, semi-deserts and mountainous areas. The genus' taxonomy is complex, shaped by its morphological diversity, affected by environmental and developmental factors, polyploid origins and frequent interspecies hybridisation. Some lineages comprise many species with a wide range of ecological variation, while others encompass small groups which vary little from one another (Darwin 1951). Morphological innovations are critical for the diversification of plants to adapt to new environments (Linder and Rudall 2005). Through the genome-wide DArTseqLD data from 57 taxa for analysis of the genetic structure and relationships within Elymus and some associated taxa, two major clades were identified, separating American and Eurasian species, suggesting at least two independent origins. Subclades were found within both regions and the species likely migrated multiple times between North and South America (Leo et al. 2024).

Inflorescence structure has been studied intensively in rice, maize and especially in wheat. The 'Miracle wheat' produces lateral meristems that sprout mini-spike-like branches predominantly from the basal part of the main axis of the spike. The mini-spike-like branches can produce their own spikelets in a distichous arrangement resulting in an indeterminate number of spikelets per spike (Poursarebani et al. 2015). In rice, mutants were created to study the inflorescence morphology of primary and secondary branches (Gao et al. 2013). In addition to the plant's genetic basis, environmental factors play an important role in modification of the inflorescence. During inflorescence development, most stress response genes are highly expressed, resulting in spikelet differentiation (Li et al. 2018; Kellogg 2022).

Central Asia is an important centre of diversity for the family Poaceae (Tzvelev 1983; Nobis et al. 2020), including the genus *Elymus* L. which is the largest in the tribe Triticeae (Hodkinson 2018). The principal argument on the circumscription of *Elymus* is whether there are single or multiple spikelets per rachis node (Sun and Salomon 2009). In the description of *Elymus* L. in the web of Plants of the World Online, the inflorescence of *Elymus* L. is either composed of racemes, fertile sessile spikelets and with a barren rhachilla extension or with diminished florets at the apex.

*Elymus* provides important perennial forage in temperate regions of the world and especially in the Qinghai-Tibetan Plateau. In October 2020, in the city of Delingha, west of the Qinghai-Tibetan Plateau, a plant with a compound spike was discovered in a dry, rocky area of alpine meadow. This plant appeared to represent a new species of the genus *Elymus* L. This species occurs in the central region of the north-western margin of the Tibetan Plateau, defined by the Kunlun Mountains and is characterised by high altitudes, low temperatures and minimal precipitation. The eastern segment of the Kunlun

Mountains receives more precipitation, ranging from 300 to 600 mm annually, while the central and western segments are much drier. Most of the region experiences average annual temperatures below 0 °C, with particularly harsh winters. These extreme climatic conditions have intensified ecological pressures, resulting in unique vegetation and ecosystems. Multiple glaciation events during the Quaternary Period have further shaped the region's geomorphology and water resources. The Kunlun Mountains' ecosystems, formed since the Pliocene, feature distinctive vegetation types, including high-altitude grasslands, temperate deciduous forests and glacier-adapted vegetation, with marked differences between the southern and northern slopes due to climatic variations (Du 2021).

The aim of this paper is to describe and classify this newly-discovered species, distinguished by its unique compound spike. The study involves comparing this species with existing species within the genus, particularly *Elymus nutans* and providing molecular evidence to confirm its status as a distinct species. Additionally, the research seeks to introduce the compound spike as a significant characteristic for the classification within the genus *Elymus*.

## Materials and methods

## **Plant materials**

The new species *Elymus multiramosus* described below, is known only from a small area of Delingha City, west of Qinghai Province (37°29'14"N, 97°23'27"E). Presently, only five populations with approximately 26 individuals had been found. Every individual of this new species possessed over 60 tillers and each tiller featured a compound spike. Morphological observations and dissections of this new species were made under a stereoscopic microscope (Nikon SMZ18, Tokyo, Japan). Karyotype analysis was carried out and compared with two germplasm of *Elymus sibiricus* L. with the sample numbers 15–262 and 16–118 provided by Qinghai Academy of Animal Science and Veterinary Medicine, Qinghai University, China.

## Chloroplast sequencing and genome annotation

DNA extraction was performed by utilising the TianGen CTAB method kit. Following the DNA extraction, we prepared the sequencing library, ensuring its integrity through PCR amplification and subsequent quality assessment. Once the library met the quality standards, it underwent sequencing on the Illumina NovaSeq platform and employed 150 bp pair-end (PE) read length for sequencing, ensuring adequate coverage depth and accurate sequence information. The sequence data were processed using GetOrganelle1.7.5 software. Finally, genome annotation was performed using the Geneious and Geseq software. The data reported in this paper have been deposited in the GenBase at the National Genomics Data Center (Members and Partners 2023), Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession number C\_AA070531 that is publicly accessible at https://ngdc.cncb.ac.cn/genbase.

### **Phylogenetic analysis**

To conduct this study, the chloroplast genome was selected for phylogenetic analysis, with the removal of inverted repeat regions to enhance the accuracy of the analysis. Sequence alignment was then performed using the MAFFT software on these selected regions. To improve alignment quality, Gblocks software was employed to extract highly-conserved regions, which are more suitable for phylogenetic inference. Following alignment, the ModelFinder tool was utilised to identify the best-fit substitution model for the dataset. The GTR+F+R3 model was selected as the optimal choice for the analysis (Nguyen et al. 2015). Based on the selected substitution model, the phylogenetic tree was constructed using IQ-TREE software. To ensure robustness and reliability, Bayesian Inference (MrBayes) was also used to construct a phylogenetic tree. The trees generated by the Maximum Likelihood method (IQ-TREE) and Bayesian Inference (MrBayes) were compared, providing comprehensive validation of the consistency and robustness of the phylogenetic inferences.

To determine the phylogenetic position of *Elymus multiramosus*, the complete cp genome sequences of 39 species were analysed. These included 36 species from Poaceae family and three species from Solanaceae Juss., Brassicaceae Burnett and Fabaceae Lindl. families, which served as the outgroup. Amongst the 36 Poaceae species, 24 belonged to *Elymus* genus, three to the *Campeiostachys* Drobow, two to Hordeum L., one species each from *Pseudoroegneria* (Nevski) Á. Löve, *Triticum* L., *Thinopyrum* Á. Löve, *Aegilops* L., *Secale* L., *Agropyron* Gaertn., *Thinopyrum* Á.Löve and *Brachypodium* P. Beauv. Table 1 provides detailed information on the species and their corresponding GenBank accession numbers.

## Results

Specimens of the potential new species were collected in Baishu Mountain, Delhi City, Qinghai, China. Morphological comparisons between the new species and its morphologically similar species of the genus *Elymus* were found in the Flora Reipublicae Popularis Sinicae (https://www.iplant.cn/frps) and the Flora Qinghaiica. Diagnostic characters involved in inflorescence morphology, spikelet number and glume, palea apex and lemma, leaf sheath and leaf characteristics, culm characteristics are shown in Tables 2–6. Morphological descriptions mainly referred to the Flora Reipublicae Popularis Sinicae.

*Elymus multiramosus* is distinguished by its compound spike, which is slightly lax and pendulous, measuring 17–19 cm in total length, with 3–6 branches, each 2–4 cm long. This contrasts sharply with the other species, which predominantly exhibit a simple spike with no branches. For instance, *Elymus breviaristatus* has a spike that is lax, but unbranched and *Elymus sibiricus* shows a similarly unbranched lax spike. In addition, *Elymus excelsus* has a spike of comparable length (15–22 cm), but it also lacks the branched structure that characterises *Elymus multiramosus*, further emphasising the unique morphological traits of the latter (Table 2). In terms of spikelet number, *Elymus multiramosus* typically bears 1–2 spikelets per node, which is similar to species such as *Elymus sinosubmuticus* and *Elymus sibiricus*. However, the glume morphology sets *Elymus multiramosus* apart, as its glumes are lanceolate, 4–7 mm in length,

Family	Genus	Species	Accession number
Poaceae	Elymus	Elymus ciliaris (Trin.) Tzvelev	MK775252.1
Poaceae	Elymus	Elymus submuticus (Keng) Á.Löve	MT644143.1
Poaceae	Elymus	Elymus repens (L.) Gould	NC_058753.1
Poaceae	Elymus	Elymus grandis (Keng) S. L. Chen	MN703669.1
Poaceae	Elymus	<i>Elymus nodosus</i> subsp. <i>caespitosus</i> (K.Koch) Melderis	MK775251.1
Poaceae	Elymus	<i>Elymus kamoji</i> (Ohwi) S. L. Chen	NC_051511.1
Poaceae	Elymus	Elymus tauri (Boiss. & Balansa) Melderis	MT385864.1
Poaceae	Elymus	Elymus sibiricus L.	MK775250.1
Poaceae	Elymus	Elymus gmelinii (Ledeb.) Tzvelev	NC_066043.1
Poaceae	Elymus	Elymus breviaristatus (Keng) Keng f.	MT644142.1
Poaceae	Elymus	Elymus trachycaulus (Link) Gould ex Shinners	MW752517.1
Poaceae	Elymus	Elymus sinosubmuticus S. L. Chen	MT644146.1
Poaceae	Elymus	Elymus pendulinus (Nevski) Tzvelev	NC_066045.1
Poaceae	Elymus	Elymus strictus (Keng) S. L. Chen	MZ736600.1
Poaceae	Elymus	Elymus alashanicus (Keng) S. L. Chen	OL444890.1
Poaceae	Elymus	Elymus hystrix L.	NC_058749.1
Poaceae	Elymus	Elymus cognatus (Hack.) T. A. Cope	MT385860.1
Poaceae	Elymus	Elymus libanoticus (Hack.) Melderis	MT385861.1
Poaceae	Elymus	Elymus stipifolius (Trautv.) Melderis	MT385862.1
Poaceae	Elymus	Elymus nutans Griseb.	NC_058918.1
Poaceae	Elymus	Elymus atratus (Nevski) HandMazz.	MT610373.1
Poaceae	Elymus	Elymus longearistatus (Boiss.) Tzvelev	MN703670.1
Poaceae	Elymus	Elymus virginicus L.	NC_058750.1
Poaceae	Elymus	Elymus magellanicus (É.Desv.) Á.Löve	MZ337548.1
Poaceae	Campeiostachys	<i>Campeiostachys kamoji</i> (Ohwi) B.R.Baum, J.L.Yang & C.Yen	MW043483.1
Poaceae	Campeiostachys	<i>Campeiostachys dahurica</i> (Turcz. ex Griseb.) B.R.Baum, J.L.Yang & C.Yen	NC_049159.1
Poaceae	Campeiostachys	Campeiostachys dahurica var. tangutorum	MN420499.1
Poaceae	Thinopyrum	Thinopyrum elongatum (Host) D.R.Dewey	MW888707.1
Poaceae	Pseudoroegneria	Pseudoroegneria spicata (Pursh) Á.Löve	MH285855.1
Poaceae	Triticum	Triticum aestivum L.	KJ614396.1
Poaceae	Aegilops	Aegilops speltoides var. speltoides	KJ614406.1
Poaceae	Secale	Secale cereale L.	KC912691.1
Poaceae	Agropyron	Agropyron cristatum L.	MN703653.1
Poaceae	Hordeum	Hordeum vulgare subsp. vulgare	NC_008590.1
Poaceae	Hordeum	Hordeum bogdanii Wilensky	NC_043839.1
Poaceae	Brachypodium	Brachypodium distachyon (L.) P.Beauv.	NC_011032.1
Solanaceae	Solanum	Solanum tuberosum L.	NC_008096.2
Brassicaceae	Arabidopsis	Arabidopsis thaliana (L.) Heynh.	AP000423.1
Fabaceae	Melilotus	Melilotus albus Medik	NC 0414191

Table 1. Basic information on species involved in Phylogenetic analysis.

with three veins and bear awns of 1.5–2.2 mm. In contrast, *Elymus canadensis* features linear glumes with significantly longer awns (7–18 mm). Additionally, *Elymus rosthornii* and *Elymus villifer* exhibit distinct glume shapes and awn lengths, highlighting further differences amongst these species (Table 3).

	Inflorescence							
Species	Туре	Tightness	Upright or Bent	Total length	Branch number	Branch length		
Elymus multiramosus Y.C. Zhang	compound spike	slightly lax	pendulous	17-19	3-6	2-4		
Elymus breviaristatus (Keng) Keng f.	spike	lax	tender and pendulous	10-15	no	no		
Elymus sinosubmuticus S. L. Chen	spike	laxer	curved	3.5-7.5	no	no		
Elymus sibiricus L.	spike	laxer	pendulous	15-20	no	no		
Elymus atratus (Nevski) HandMazz.	spike	denser	flexuous, pendulous	5-8	no	no		
Elymus nutans Griseb.	spike	denser	flexuous, apex pendulous	5-12	no	no		
Elymus canadensis L.	spike	denser	pendulous	12-20	no	no		
Elymus villifer C. P. Wang & H. L. Yang	spike	denser	slightly curved	9-12	no	no		
Elymus purpuraristatus C. P. Wang & H. L. Yang	spike	denser	erect or slightly curved	8-15	no	no		
<i>Elymus dahuricus</i> Turcz. var. violeus C. P. Wang & H. L. Yang	spike	denser	curved	18.5-25.5	no	no		
Elymus dahuricus Turcz. var. dahuricus	spike	denser	erect	14-18	no	no		
Elymus excelsus Turcz.	spike	laxer	erect	15-22	no	no		
Elymus dahuricus var. cylindricus Franch.	spike	denser	erect	7-14	no	no		
Elymus tangutorum (Nevski) HandMazz.	spike	denser	erect	8-15	no	no		
Elymus dahuricus Turcz.	spike	denser	erect	14-18	no	no		
Elymus barystachyus L. B. Cai	spike	denser	erect	8-18	no	no		

Table 2. Morphological inflorescence comparison of *Elymus multiramosus* with related *Elymus* species.

**Table 3.** Morphological comparisons of spikelet number and glume comparison of *Elymus multiramosus* with related*Elymus* species.

Species	Spikelet	Glume						
	number	Туре	Length (mm)	Veins number	Apex type	Apex awn length (mm)		
Elymus multiramosus Y.C. Zhang	1-2	Lanceolate	4-7	3	awned	1.5-2.2		
Elymus breviaristatus (Keng) Keng f.	2	oblong or ovate lanceolate	3-4	1-3	acuminate or mucro	1		
Elymus sinosubmuticus S. L. Chen	1-2	oblong	2-3	3	acute or acuminate	no cuspidate		
Elymus sibiricus L.	1-2	Narrowly lanceolate	4-5	3-5	acuminate or a short awn	4		
Elymus atratus (Nevski) HandMazz.	2	Narrowly oblong or lanceolate	2-4	1-3	acuminate	< 1		
Elymus nutans Griseb.	1-2	oblong	3-4	3-4	acuminate or a short awn	1-4		
Elymus canadensis L.	2-3	Linear	3-4	3-4	awned	7-18		
Elymus villifer C. P. Wang & H. L. Yang	1-2	Narrowly lanceolate	4.5-7.5	3-4	acuminate to an awned tip	1.5-2.5		
Elymus purpuraristatus C. P. Wang & H. L. Yang	2	Lanceolate to linear-lanceolate	7–10	3	mucro	1		
<i>Elymus dahuricus</i> Turcz. var. violeus C. P. Wang & H. L. Yang	1-2	Lanceolate	7–11	3-5	awned	3-6		
Elymus dahuricus Turcz. var. dahuricus	1-2	Lanceolate or linear-lanceolate	8-10	3-5	awned	5		
Elymus excelsus Turcz.	2-4	Narrowly lanceolate	10-13	5-7	awned	7		
Elymus dahuricus var. cylindricus Franch.	1-2	Lanceolate to linear-lanceolate	7-8	3-5	acuminate	4		
Elymus tangutorum (Nevski) HandMazz.	1-2	Lanceolate to linear-lanceolate	7–10	5	acuminate	1-3		
Elymus dahuricus Turcz.	1-2	Lanceolate to linear-lanceolate	7–10	3-5	acuminate or awned	5		
Elymus barystachyus L. B. Cai	2	linear-lanceolate	7-10	4-7	acuminate or pointed	1.5		

		Lemmas				
Species	Palea apex	Туре	Vein number	First lemmas length (mm)	Awn length (mm)	
Elymus multiramosus Y.C. Zhang	rounded or flattened	lanceolate	3	7-10	9-12	
Elymus breviaristatus (Keng) Keng f.	obtuse-rounded or slightly concave	lanceolate	5	8-9	1-5	
Elymus sinosubmuticus S. L. Chen	obtuse-rounded	lanceolate	5	7-8	2	
Elymus sibiricus L.	2-lobed	lanceolate	5	8-11	10-15	
Elymus atratus (Nevski) HandMazz.	obtuse-rounded	lanceolate	5	7-8	10-17	
Elymus nutans Griseb.	obtuse-rounded or truncate	oblong-lanceolate	5	10	12-20	
Elymus canadensis L.	pointed or obtusely rounded and retuse	lanceolate	5	10-17	20-30	
Elymus villifer C. P. Wang & H. L. Yang	-	oblong-lanceolate	5	7–11		
Elymus purpuraristatus C. P. Wang & H. L. Yang	_	oblong-lanceolate		6-9	7-15	
<i>Elymus dahuricus</i> Turcz. var. violeus C. P. Wang & H. L. Yang	_	lanceolate		9-21	9-21	
Elymus dahuricus Turcz. var. dahuricus	truncate	lanceolate	5	9	10-20	
Elymus excelsus Turcz.	_		5	8-12	15-40	
Elymus dahuricus var. cylindricus Franch.	obtuse-rounded	lanceolate	5	7-8	6-13	
Elymus tangutorum (Nevski) HandMazz.	obtuse-headed	lanceolate	5	8-12	3-11	
Elymus dahuricus Turcz.	narrowly truncate	lanceolate		7-9	2-20	
Elymus barystachyus L. B. Cai	_	oblong-lanceolate		7-8	1-2	

Table 4. Morphological comparisons of palea apex and lemmas of Elymus multiramosus with related Elymus species.

The palea apex of Elymus multiramosus is rounded or flattened, while its lemmas are lanceolate with three veins, the first lemmas measuring 7-10 mm in length and bearing awns 9-12 mm long. Other species, such as Elymus sibiricus and Elymus canadensis, have longer lemma awns, reaching up to 20-30 mm, much longer than those of Elymus multiramosus. Additionally, Elymus nutans has truncate palea apices, with awns measuring 12-20 mm, creating a notable morphological distinction from Elymus multiramosus. These differences play a critical role in distinguishing species within this genus (Table 4). Elymus multiramosus has glabrous leaf sheaths and flattened blades, with leaves measuring 18-22 cm in length and 5-7 mm in width. This contrasts with species like Elymus breviaristatus and Elymus sinosubmuticus, which have shorter and narrower leaves. For instance, Elymus villifer has leaves 9-15 cm long and 3-6 mm wide and their margins may be involute, unlike the consistently flattened leaves of Elymus multiramosus. Additionally, Elymus purpuraristatus has even narrower leaves, measuring only 2.5-4 mm in width. These morphological differences help in identifying and differentiating species within the group (Table 5). The culms of Elymus multiramosus are tufted, with an erect base that is slightly decumbent and they range in height from 82 to 95 cm. This is contrasted with the culms of Elymus canadensis, which are taller, reaching up to 100 cm and have a more erect and less decumbent base. Elymus purpuraristatus, with culms up to 160 cm, far exceeds the height of Elymus multiramosus, showcasing the range of variation in culm height across species. Elymus sibiricus, on the other hand, has shorter culms (60-90 cm) with a more inclined base, creating a stark difference in growth habit when compared to Elymus multiramosus (Table 6).

Species	Leaf-sheath type	Leaf type	Leaf length (cm)	Leaf width (mm)
Elymus multiramosus Y.C. Zhang	glabrous	blade flattened	18-22	5-7
Elymus breviaristatus (Keng) Keng f.	glabrous	blade flattened	4-12	3-5
Elymus sinosubmuticus S. L. Chen	glabrous	blade flattened or involute	3-6	1.5-3
Elymus sibiricus L.	smooth and glabrous	blade flattened	10-20	5-10
Elymus atratus (Nevski) HandMazz.	smooth and glabrous	blade or involute	3-10	2
Elymus nutans Griseb.	glabrous	blade flattened,sparsely pilose above, scabrous or smooth below	6-8	3-5
Elymus canadensis L.	glabrous	blade flattened	20-30	7-15
Elymus villifer C. P. Wang & H. L. Yang	densely villous	flattened or margins involute	9-15	3-6
<i>Elymus purpuraristatus</i> C. P. Wang & H. L. Yang	glabrous	blades often involute	15-25	2.5-4
<i>Elymus dahuricus</i> Turcz. var. violeus C. P. Wang & H. L. Yang	base densely white villous	blade flattened or drying involute	20-35	8.7-13.6
Elymus dahuricus Turcz. var. dahuricus	smooth and glabrous	blade flattened, sparsely involute	15-25	5-12
Elymus excelsus Turcz.	glabrous	flattened	20-30	10-16
Elymus dahuricus var. cylindricus Franch.	glabrous	blade flattened	5-12	5
Elymus tangutorum (Nevski) HandMazz.	smooth	blade flattened	10-20	6-14
Elymus dahuricus Turcz.	glabrous, or densely pilose at base	blade glaucous, flat, rarely rolled	5-25	5-12
Elymus barystachyus L. B. Cai	glabrous	blade glabrous on both surfaces	7-22	4-8

Table 5. Morphological comparisons of leaf sheath and leaf of *Elymus multiramosus* with related *Elymus* species.

Table 6. Morphological comparisons of culm of *Elymus multiramosus* with related *Elymus* species.

Species	Culms bushy type	Culms type	Culms height (cm)
Elymus multiramosus Y.C. Zhang	tufted	erect, base slightly decumbent	82-95
Elymus breviaristatus (Keng) Keng f.	sparsely tufted	erect or basally geniculate, short, decurrent rhizomes	70
Elymus sinosubmuticus S. L. Chen	tufted	erect or base slightly geniculate, weak	25-45
Elymus sibiricus L.	solitary or sparsely tufted	erect or base slightly inclined	60-90
Elymus atratus (Nevski) HandMazz.	sparsely tufted	erect, weak	40-60
Elymus nutans Griseb.	tufted	erect, base slightly geniculate	50-70
Elymus canadensis L.	few tufted	erect or base slightly geniculate	100
Elymus villifer C. P. Wang & H. L. Yang	sparsely tufted	erect	60-75
<i>Elymus purpuraristatus</i> C. P. Wang & H. L. Yang	tufted	erect, stout	160
<i>Elymus dahuricus</i> Turcz. var. violeus C. P. Wang & H. L. Yang	sparsely tufted	erect	145-225
Elymus dahuricus Turcz. var. dahuricus	sparsely tufted	erect	70-140
Elymus excelsus Turcz.	tufted	erect, robust	140
Elymus dahuricus var. cylindricus Franch.	tufted	erect, weak	40-80
Elymus tangutorum (Nevski) HandMazz.	tufted	erect, tall and stout, base geniculate	120
Elymus dahuricus Turcz.	tufted	erect, base geniculate	40-140
Elymus barystachyus L. B. Cai	laxly tufted or solitary	erect, base geniculate	50-80

#### **Taxonomic treatment**

#### Elymus multiramosus Y.C.Zhang, sp. nov.

urn:lsid:ipni.org:names:77351702-1 Figs 1–3

**Type.** CHINA • Qinghai, Delhi City, Baishu Mountain. 37°29.23N, 97°23.45'E, 3722 m a.s.l., 10 October 2020, *Yong-Chao Zhang* (holotype at Qinghai-Tibetan Plateau Museum of Biology, HNWP 371720).

Diagnosis. Excluding the compound spike characteristic, Elymus multiramosus shares notable similarities with Elymus sibiricus in several traits. Both species have 1-2 spikelets per node, comparable glume lengths and vein numbers and similar lemma awn lengths. Their leaf morphology is also guite alike, with glabrous sheaths and comparable leaf blade dimensions. Additionally, their culm heights overlap, as both species exhibit tufted, erect culms, further highlighting their morphological resemblance. Similarly, Elymus multiramosus closely resembles Elymus nutans, especially in terms of spikelet number and glume features. Both species have 1-2 spikelets per node and nearly identical lemma awn lengths, though *Elymus nutans* tends to have slightly longer awns and an oblong-shaped glume (Hua 2007). Their leaf blades are also alike, being smooth or nearly smooth, with similar dimensions, further emphasising the parallels between these two species. Lastly, Elymus dahuricus var. dahuricus and Elymus multiramosus display significant similarity in leaf morphology. Both species have glabrous sheaths and flattened leaf blades with matching lengths and widths. Their culm height ranges overlap considerably and both have tufted, erect culms. Despite these similarities, the subtle differences in their overall structure allow for their differentiation.

*Elymus* species are characterised by their perennial, tufted growth, typically lacking rhizomes and erect culms, sometimes decumbent at the base, with leaf sheaths split to the base. The leaves are linear or lanceolate, either flat or rolled, with membranous, non-ciliate ligules. The inflorescence is a spike, either erect or nodding, with 1–2 laterally compressed spikelets per node, each containing 2–10 florets that disarticulate below the fertile floret at maturity. The glumes are linear-lanceolate with 1–9 veins, often awned, and the lemmas are 5-veined, rounded on the back and typically awned at the apex. The caryopsis fruit adheres to both the lemma and palea (Hua 2007).

The new species is similar to *Elymus* sp. but it can be easily distinguished from that species by its compound spike, which has 3–4 spikelets with extended rhachillas at the base nodes. These rhachillas become much longer from the top to the base of the compound spike (Figs 4–6). The glumes of *Elymus multiramosus* are noticeably shorter than the first floret. The awns at the lemma apex range in length from 9 to 12 mm, surpassing the length of the lemma body. *Elymus multiramosus* is distinguished by stouter plants and longer inflorescences, which measure 17 to 19 cm, with spikelets primarily arranged on one side of the rachis. The glume apices are awn-tipped and the glumes themselves are lanceolate, exceeding the length of those in *Elymus nutans* by 4 to 7 mm.

**Description.** Culms are usually erect, sometimes slightly decumbent at base, 82-95 cm tall. Leaf-sheath glabrous; leaf blade flat,  $18-22 \times 0.5-0.7$  cm, glabrous or adaxial surface slightly pubescent. Compound spike pendulous,





slightly lax, 17-19 cm; rachis margin scabrous, no ciliolate, rachis knot dilated. Compound spike includes a clear main shoot axis and a series of lateral branches produced by the main shoot. Flower formed from the top of the main axis and primary branches from the base to the middle of the main axis. A total of 3–6 primary branches are formed in the main axis, 2–4 cm length. Each primary branch has 3–7 nodes. Spikelet usually 2 per node, with 2 or 3 florets. Glumes lanceolate, 4–7 mm, 3-veined, glabrous, scabrous along veins, apex with awn 1.5–2.2 mm. Lemma lanceolate, 3-veined, obscurely at the base, scabrous or puberulent at the apex and edge; first lemma 7–10 mm; awn 9–12 mm. Palea equalling lemma, ciliolate along keels, puberulent between keels.



**Figure 2**. One holotype specimen of *Elymus multiramosus* (371720), collected and preserved at Qinghai-Tibetan Plateau Museum of Biology (HNWP).

**Phenology.** *Elymus multiramosus* flowers in early September and bears fruit in early October.

**Etymology.** The specific epithet multiramosus is a compound of the Latin words multi meaning many and ramosus meaning branches to indicate a specific type of inflorescence.



**Figure 3**. *Elymus multiramosus* in the wild, the top left is the locality found; the top right and bottom left are the compound spikes; the bottom right is the stem and leaf. Photographs by Yongchao Zhang.

Vernacular name. Duō Zhī Pī Jiǎn Cǎo (Chinese pronunciation); 多支披碱草 (Chinese name).

**Distribution and habitat.** The species is presently known only from a small area of Delingha City, west of Qinghai Province (37°29'14"N, 97°23'27"E). It grows on a dry rocky area of alpine, at an elevation of 3722 m a.s.l. Other plants in the vicinity of the plant include *Juniperus przewalskii* Kom., *Agropyron cristatum* J.Gaert.,*Elymus dahuricus* Turcz. var. *cylindricus* Franchet, *Neotrinia splendens* (Trin.) M. Nobis, P. D. Gudkova & A. Nowak etc.

## Karyotype analysis

A total of 42 chromosomes were obtained by DAPI fluorescence staining, with a length of  $5.0-8.5 \,\mu$ m, mainly proximal middle and proximal centromeres, the end of the chromosome being rich in heterochromatin and the genome is large (Fig. 7A).

In situ fluorescence hybridisation using Oligo-pSc119.2 and Olig-pTa535 probes shown that 14 chromosomes have a strong Olig-pTa535 signal at the end and possibly belong to H chromosome group (Fig. 7B).

5SrDNA and 18SrDNA repeat probes were used for fluorescence in situ hybridisation and it was found that six chromosomes had 5SrDNA hybridisation signals (red) and four chromosomes had 18SrDNA hybridisation signals (green) (Fig. 7C), confirming that the sample was hexaploid material (2n = 6x =42) with large-scale repetitive amplification (Table 7, Fig. 7D).



**Figure 4**. The compound spike of *Elymus multiramosus* **A**–**C** are three compound spikes from *Elymus multiramosus* and **D**–**F** are compound spikes after seed threshing of the **A**–**C** separately, the mini-spike-like branches can be seen.

 Table 7. Genome size and ploidy of Elymus multiramosus compared with two germplasm of Elymus sibiricus with sample numbers 15-262 and 16-118.

Germplasm	Reference	Fluorescence intensity of reference	Fluorescence intensity of germplasm	Ratio	Genome (Gb)	Ploidy
Elymus multiramosus	Corn	64.26	263.87	4.11	9.44	6X
Elymus sibiricus 15-262	Corn	63.26	163.04	2.58	5.93	4X
Elymus sibiricus 16-118	Corn	63.66	170.26	2.67	6.15	4X



Figure 5. Detail spikelet of *Elymus multiramosus* during the mature stage **A** part of compound spike with a branch **B** branch **C** spikelet **D** lemma **E** glumelle **F** seed back **G** seed ventral **H** glume ventral **I** glume back **J** glume awn and vein.



Figure 6. Details of compound spike of *Elymus multiramosus* during the flowering stage **A** spikelet **B** flower **C**, **D** stamens, pistil, anthers **E** anther **F** pollen, stigmas.

## Compound spike development

*Elymus multiramosus* seeds (five inflorescences included about 80 seeds) were sown in May 2021 and, after the rejuvenation in April 2022, the development of compound spike was detected from more than 30 inflorescences.

In the development of the compound spike of *Elymus multiramosus*, its stages have been identified - the stages of the initial floret, the stage of the beginning of the spikelet protuberance on the branches, branching from the base of the compound spike and finally branches elongation and spikelet formation (Fig. 8A–G).



**Figure 7.** Chromosome fluorescence staining with DAPI *Elymus multiramosus* (**A**). Chromosome repeat fluorescence in situ hybridisation using Oligo-pSc119.2 (green) and Olig-pTa535 (red) probe of *Elymus multiramosus* (**B**). rDNA fluorescence in situ hybridisation of *Elymus multiramosus* chromosomes, 5SrDNA is red and 18SrDNA is green (**C**). Root tip chromosomal tableting, from left to right is *Elymus multiramosus* (2n = 6x = 42) (**D**). *Elymus sibiricus* 16-118 (2n = 4x = 28) (**E**). *Elymus sibiricus* 15-262 (2n = 4x = 28) (**F**).

#### **Phylogenetic analysis**

The chloroplast genome of *Elymus multiramosus* is 135,059 bp in length with an average sequencing depth of 112×h. It exhibits a typical four-level structure consisting of a large single-copy (LSC) region of 80,667 bp in length, a small single-copy (SSC) region of 12,766 bp in length and two inverted repeat regions (IRa/IRb) of 20,813 bp in length (Fig. 9A). The whole chloroplast genome has a CG content of 38.3% and encodes a total of 134 genes, including 88 protein-coding genes, 38 tRNA genes and eight rRNA genes. Amongst them, seven protein-coding genes (rps16, atpF, rpl2, ndhB, ndhA, ndhB, petB and rpl2) contained one intron and one gene ycf3 had two introns (Fig. 9B). In addition, the single 5' end of the trans-spliced gene rps12 is located in the large single-copy region, whereas the duplicated 3' end exons are located in the two trans-repeat regions (Fig. 9C).

In the molecular phylogeny, *Elymus multiramosus* from Qinghai, north-western China, is phylogenetically positioned as a distinct lineage. The lineage comprising *Elymus sinosubmuticus* from Sichuan, east of the Tibetan Plateau and *Elymus nutans* from the Himalayas forms a sister group to *Elymus multiramosus*, suggesting that these three species share a common ancestor that is distinct from the lineage leading to *Elymus atratus* from Gansu, north of the Tibetan Plateau (Fig. 10). The final phylogenetic analysis revealed consistent evolutionary relationships, with results from both methods corroborating each other, thereby ensuring the credibility of the conclusions.

The new species is similar to *Elymus nutans*, a perennial herbaceous plant is primarily distributed in the temperate regions of Asia, especially common in the alpine meadows of the Tibetan Plateau and its surrounding areas in China. It can be easily distinguished from that species by its compound spike. With



Figure 8. The compound spike development **A**, **B** are the stages of the initial floret **C** is the stage of the start of the spikelet protuberance on the branches **D**–**F** show the stage of branching starting from the base of the compound spike **G** is the stage of branches elongation and spikelet formation.

the molecular evidence, thus, we confirmed that it represented a new species discovered within the alpine regions. It is necessary to introduce the compound spike into *Elymus* L. for classification.

## Discussion

Inflorescences amongst the Triticeae tribe, exemplified by barley, likely evolved from an ancestral compound spike into the more typical unbranched spike observed today. Grass inflorescences, according to proposed evolutionary models, originated from a primitive ancestral form featuring a relatively small panicle-like branching system, comprising primary and secondary branches standing alone at nodes (Vegetti and Anton 1995). This ancestral structure is a compound spike, still evident in tribes like Oryzeae and Andropogoneae, which maintain complex



**Figure 9.** Schematic circular map of overall features of *E. multiramosus* chloroplast genome. Graphic showing features of its plastome was generated using CPGview. The map contains three tracks. From the inner circle, the first track depicts the sizes of the inverted repeats (IRa and IRb), small single-copy (SSC) and large single-copy (LSC). The second track plots the distribution of GC contents along the plastome. The third track displays the genes belonging to different functional groups with different coloured boxes. The outer and inner genes are transcribed in the clockwise and counter-clockwise directions, respectively (**A**). Cis-splicing gene distribution map (**B**). Trans-splicing gene distribution map (**C**).

compound shapes with true-lateral long primary and secondary branches. Conversely, other grasses, such as *Brachypodium distachyon*, exhibit reduced inflorescence complexity, characterised by smaller lateral pedicels that culminating in single multi-floweret spikelets (Kellogg et al. 2013; Remizowa et al. 2013).

It was found that the inflorescence differentiation of *Elymus sibiricus* encompasses the initiation, elongation, single-ridged or double-ridged spikelet and flower differentiation stages (Mao et al. 2004). However, the development of the compound spike in *Elymus multiramosus* passes through distinct stages. In the inflorescence differentiation of *E. multiramosus*, the base of the spikelet, located in the middle and lower part of the inflorescence axis, functions as an independent component of the entire inflorescence. It undergoes a specialised process of branch initiation and elongation, unlike the upper part of the inflorescence and related species, which do not exhibit this process. This process initiates with branching at the base of the compound spike, followed by branch elongation and subsequent spikelet formation. These stages are critical for understanding the reproductive and developmental intricacies of *E. multiramosus* within its genus. This process of compound spike formation is crucial for crop yield formation and the synchronous maturation of seeds (Wang et al. 2021).

The inflorescences of *Elymus* are described as racemes. In the Flora of China (Hua 2007), *Elymus* has been described as: the spike erect to nodding, spikelets 1 or 2(-4) per node, rarely very short pedicellate, appressed to rachis, clearly laterally compressed, usually all similar, with 2–10 or more florets. The spike is one type of the racemes. The compound spike has branches in the rhachis, each branch being like a mini spike, which is the key to the new species.



**Figure 10.** Phylogenomic reconstruction result of *E. multiramosus* with 1000 bootstraps in RAxML. Solanum tuberosum, *Arabidopsis thaliana* and *Melilotus albus* are the outgroup.

*Elymus multiramosus* is characterised by distinctive morphological features, especially the glumes, which are shorter than the first floret and the awns, which are 9 to 12 mm long and exceed the lemma. The robust stature and longer inflorescences, along with the predominantly unilateral arrangement of spikelets, further distinguish *Elymus multiramosus* from other species. These features are crucial for identifying and distinguishing *E. multiramosus* from closely-related species like *Elymus nutans*.

Moreover, *Elymus multiramosus* is distinguished by its compound spike inflorescence, which is a key feature that distinguishes it from other *Elymus* species with simpler inflorescences. This compound spike is defined by a distinct main shoot axis from which lateral branches emerge, in contrast to simpler *Elymus* inflorescences where flowers form directly from the main axis (Benlloch et al. 2007). In *Elymus multiramosus*, these lateral branches, or rhachillas, originate at the base of the main axis, extending into 3–6 mini-spike-like branches arranged distichously. Notably, the length of these branches increases progressively from the top towards the bottom of the spike, demonstrating a complex structural adaptation. These morphological differences underline the divergence between the species and are vital for identifying and differentiating *E. multiramosus* from its relatives.

As the world's highest and youngest plateau, the Qinghai-Tibetan Plateau has had a profound impact on the phylogeny of *Elymus* species due to its unique geographical and climatic conditions. Notably, larger genome size variations occurred in mid-altitude populations (3900–4300 m) compared with populations at other

altitudes, suggesting a distinct altitudinal pattern in genome size variation. This variation plays a crucial role in shaping genome evolution according to altitude and supports that mid-altitude regions serve as centres of genetic richness, facilitating species adaptation to highland environmental conditions and providing valuable germplasm for utilisation and conservation (Chen 2022b). *Elymus* species originated through a typical allopolyploidy process, involving the combination of different genomes. Cytological studies suggest that five basic genomes – St, Y, H, P and W – exist in various combinations across *Elymus* species. The evolutionary history of *Elymus* species on the Qinghai-Tibetan Plateau involve multiple origins due to the introduction of different H genome donors (Liu et al. 2006).

*Elymus multiramosus* has only been found in a small area at the north-western Qinghai-Tibetan Plateau, growing in a dry, rocky area at an elevation of 3722 metres. Its restricted geographical distribution positions it as a distinct lineage in phylogenetic analyses. *Elymus sinosubmuticus* and *Elymus nutans*, found in Sichuan and the Himalayas, respectively, form a sister group with *Elymus multiramosus*, sharing a common ancestor, distinct from *Elymus atratus* from northern Gansu. This lineage divergence reflects the natural selection and adaptive evolution on *Elymus multiramosus*. Furthermore, phylogenetic analyses have shown that *Elymus* species on the Qinghai-Tibetan Plateau have experienced multiple origins and gene flow events during their evolution (Yan et al. 2024). The uniqueness of this species reflects its independent evolutionary history in the specific ecological context of the north-western Qinghai-Tibetan Plateau.

Through detailed examination of morphological, phylogenetic and developmental characteristics, it is supported that *Elymus multiramosus* is classified within the section *Elymus*. This comprehensive analysis supports its classification and provides insights into its evolutionary development and agronomic potential (Vegetti and Anton 1995). Further research, particularly in the domain of genetic diversity and environmental adaptation, would enrich our understanding of its role within its ecosystem and its agricultural value.

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## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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## Author contributions

Writing-original draft: Yong-Chao Zhang. Formal analysis: Xiao-Xing Wei. Data curation: Yan Qin. Investigation: Yong Liu. Investigation: Shu-Zhen Zhang. Validation: Zhi-Feng Jia, Wen-Hui Liu. All authors have read and approved the final manuscript.

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## Data availability

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

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# **Supplementary material 1**

#### Supplementary image

Authors: Yong-Chao Zhang, Xiao-Xing Wei, Yan Qin, Yong Liu, Shu-Zhen Zhang, Zhi-Feng Jia, Wen-Hui Liu

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#### **Research Article**

# Reinstatement of *Struthanthus venetus* (Loranthaceae): an endemic species of central Mexico

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#### Abstract

Struthanthus Mart. is a challenging genus because the abundance of reproductively incomplete specimens (lacking mature pistillate and staminate flowers and fruit) has inspired the description of many species, resulting in a long list of names to be verified. In addition, the genus currently lacks a monographic treatment. *Struthanthus venetus* (Kunth) Blume was previously considered a heterotypic synonym of *S. interruptus* (Kunth) G. Don. However, examination of herbarium specimens and observation of living plants demonstrate that *S. venetus* is a distinct species distinguishable from *S. interruptus*. Here, we propose the reinstatement of *S. venetus* along with a complete description of the species and the designation of an epitype to complement the reproductively incomplete (lacking flowers) and severely fragmented type.

**Key words:** Dioecious, epitype, hemiparasitic, mistletoe, nomenclature, Psittacanthinae, taxonomy

#### Introduction

*Struthanthus* Mart. (Martius 1830) is a neotropical genus of mistletoe belonging to the tribe Psittacantheae subtribe Psittacanthinae in Loranthaceae (Nickrent et al. 2010; Kuijt 2012; Nickrent et al. 2019). Being closely related to *Cladocolea* Tiegh. and *Peristethium* Tiegh., members of *Struthanthus* are mainly dioecious and consist of approximately 60 to 70 species (Kuijt 1981; Vidal-Russell and Nickrent 2008; Kuijt 2012; Robles et al. 2016; Galván-González 2016; Grímsson et al. 2018; Caires and Dettke 2020). A key requisite for the identification of *Struthanthus* is the presence of flowers, especially staminate ones. Taxonomic studies in *Struthanthus* have been challenging because the genus currently lacks a monographic treatment (Dettke and Waechter 2012; Kuijt 2016; Robles et al. 2016, Maldonado-Borja et al. 2023). In addition, the genus contains a long list of synonyms and many taxa that were described from fruiting individ-



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Maria Guadalupe Maldonado-Borja et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). uals or from specimens containing only pistillate or staminate inflorescences, these sometimes at the bud stage (Kuijt 2016; Caires and Dettke 2020). Moreover, herbarium specimens of putative species of *Struthanthus* are often sterile, which causes uncertainties for classification at the generic and species levels.

In Mexico, 15 species of *Struthanthus* are currently accepted (Maldonado-Borja et al. 2023), and dozens more have been proposed. One of these is *S. venetus* (Kunth) Blume, a species that has been treated as one of the eight to 11 synonyms of *S. interruptus* (Kunth) G.Don (Kuijt 1980, Kuijt 2009; Kuijt 2016; Tropicos.org 2024; POWO 2024). Both *S. venetus* and *S. interruptus* were described by Kunth (Humboldt et al. 1818[1820]) based on an incomplete specimen that has obscured the proper identification of the species. Therefore, this paper seeks to reestablish *S. venetus*, providing evidence from morphological characters and germination of pollen grains to distinguish it from similar species from Mexico, particularly *S. interruptus*.

# Methods

# Morphological analysis

The study is based on specimens from the herbaria CIIDIR, ENCB, HUAP, HUMO, IEB, MEXU, UAMIZ, and XAL (acronyms following Thiers 2024). Digitalized images from COLO, DES, F, GH, K, MA, MO, NY, P, RSA, SMU, U, UC, US, and VT were also studied. Morphological characters were analyzed from field-collected plants. We encountered several flowering individuals in March 2022, and then proceeded to carry out fieldwork from May 2022 to October 2023 to collect specimens with flowers and fruits. Comparisons between fresh and dried morphological characters were performed with a stereomicroscope using specimens collected from the states of Ciudad de México, Estado de México, Guanajuato, Guerrero, Morelos, and Puebla.

Relevant literature was reviewed, as well as the protologues of all taxa involved in this study. Nomenclatural decisions were based on the rules and recommendations provided in the International Code of Nomenclature for algae, fungi, and plants (ICBN; Turland et al. 2018). A distribution map was generated with QGIS (QGIS Development Team 2024; version 3.26.2) with coordinates based on data from fieldwork, herbarium specimens, and observations obtained from iNaturalist (2024).

# Fluorescence microscopy

Flowers of *S. venetus* were collected in central Mexico from March 2022 to October 2023 and consisted of 43 accessions at different stages of anthesis. In addition, fresh inflorescences and flowers at different stages were collected in the field and fixed in FPA (1:1:18 37% formaldehyde: propionic acid: 70% ethanol). Flowers from each specimen were examined to verify the presence of fully developed reproductive structures. Pollen viability was assessed through a germination test by fluorescence microscopy to determine if *S. venetus* accessions had functionally bisexual flowers. This analysis was carried out at the Laboratorio de Desarrollo en Plantas in the Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), in August 2023. The pistils of flow-

ers were removed and washed in distilled water followed by a wash in sodium hydroxide (NaOH 1N) for four days to soften the pistil tissue. The styles were rinsed in distilled water and softly macerated for five minutes on a slide with 1 or 2 drops of cotton blue pigment from aniline at 0.1% in 0.1 N potassium phosphate (O'Brien and McCully 1981, Torres and Murashige 1985). The stigma and style were observed under a fluorescence microscope (Olympus Provis AX750) searching for pollen tubes. Cotton blue has a great affinity to callose, allowing identification of the pollen tubes (Martin 1959), and pollen grains that emitted a bright green tube were considered to have germinated.

# Results

A total of 102 specimens of *S. venetus* were examined, 59 from herbaria and 43 collected during fieldwork. Forty percent of these specimens have only flowers, whilst 34% have only fruit, 10% are with both flowers and fruit, 10% possess flower buds, and 6% are sterile. *Struthanthus venetus* is a monoclinal species whose flowers reach 6–8 mm in length at full anthesis. Flowers are formed by six unfused petals, valvate, and green to yellowish in color; six well-developed stamens in two alternating series (three long and three short, all with viable pollen); and a pistil composed of an inferior ovary, a convolute style that is the same length of the first stamen series ending in a differentiated stigma, and a thick hexagonal ring-shaped nectary (Fig. 1A–F).

Our observations indicate that flowers of *S. venetus* at early and late anthesis display male and female organs that are functional and well-developed (Fig. 2A–C). Stamens in early anthesis have white anthers with yellowish pollen, whereas in late anthesis anthers become brownish with few pollen grains. Interestingly, inflorescences contain flowers at different stages of maturity (Fig. 2C). On the other hand, *S. interruptus* has clearly unisexual flowers (Fig. 2D–E) with vestigial organs of the opposite sex. Pistillate flowers have a convolute style with staminodes poorly distinguishable (Fig. 2D), while male flowers show a wavy style and well-developed anthers with pollen.

Regarding non-reproductive morphological characters, leaves of *S. venetus* are green and crasso-coriaceous (Fig. 3A) and fruit are ovoid to subglobose, granulated, and rusty colored when ripe,  $4.0-6.0 \times 6.5-10.0$  mm (Fig. 3B). This contrasts with *S. interruptus*, a species with bluish-green tinge and papery or chartaceous leaves (Fig. 3C) and ovoid to ellipsoid smooth fruit that ripen orange-reddish,  $6.6-7.2 \times 10.8-12.8$  mm (Fig. 3D).

Flowers at full anthesis of *S. venetus* examined using fluorescence microscopy confirmed that pollen grains were viable, capable of germinating on a receptive stigma and developing a specialized tube up to two-thirds the length of the style tissue as a conduit to the embryo sac (Fig. 4A, B).

# **Epitypification**

In 1820, Karl S. Kunth (Humboldt et al. 1818[1820]) described *Loranthus venetus* Kunth based on the specimen *Humboldt & Bonpland 3985* (P-00215992!; Fig. 5A), which was collected during the expedition to New Spain by Alexander von Humboldt & Aimé Bonpland between 1799 to 1804 (Humboldt 1802–1804, 1827; Humboldt et al. 1818 [1820]; Stoddard 1859; Minguet 1969; Labastida



Figure 1. Flower morphology of *Struthanthus venetus* (Loranthaceae) **A** lateral view of flower at early anthesis **B** upper view of flower at full anthesis showing ascendant petals, the pistil with stigma in the center, and functional stamens with tufts of trichomes (t) behind the anthers **C** lateral view of flower at full anthesis with a petal removed to show the contour of the convolute style (black dashed line) **D** lateral view of the convolute style with brownish stigma **E** long (S1) and short (S2) series of stamens next to the whole pistil (right side) **F** hexagonal nectary (ne) at the base of the style (central circular scar).

2016). According to Minguet (1969), Humboldt and Bonpland visited Cuernavaca between April 9<sup>th</sup> and 11<sup>th</sup>, 1803; hence, the collection of *L. venetus* was made during those dates, at an elevation of 1,555 m ("altitudo 850 hexapedalis", from the protologue).

The holotype of *L. venetus* only contain leaves and infructescences (Fig. 5A), and the species diagnosis prepared by Kunth (Humboldt et al. 1818 [1820]) reads as follow: "foliis elliptico oblongis aut abovatis, apice rotundatis, crasso-coriaceis, glaucescentibus...". Because of its elliptic-oblong or ovate leaves, Kunth suggested that *L. venetus* was closely related to *L. ovalifolius* Ruiz & Pav. (= *Oryc-tanthus ovalifolius* (Ruiz & Pav.) J.F.Macbr.), which is illustrated in plate CCLXX-VIIb by Ruiz and Pavón (1802). This plate depicts leaf blades whose shape is consistent in both young and mature leaves. However, *S. venetus* shows heterophylly, with leaf blades varying in size and shape on the same individual along a branch. In addition, although the diagnosis for *S. venetus* mentions that the species blooms in April ("Floret Aprili"), Kunth mentioned that flowers were not seen ("Flores haud vidi"; Humboldt et al. 1818 [1820]). Given that most species in *Struthanthus* are dioecious and the flowers of *L. venetus* were not examined when preparing its protologue, it was assumed that the species was dioecious.



**Figure 2.** Comparative reproductive morphology between *Struthanthus venetus* and *S. interruptus* (Loranthaceae). *S. venetus*: **A** dissected flower at early anthesis. Note that all stamens are well-developed and arranged in two alternating series (S1 and S2). Pistil showing the convoluted style at right **B** dissected flower during late anthesis **C** inflorescence with flowers in early and late anthesis. *S. interruptus*: **D** dissected pistillate flower showing vestigial, non-functional staminodes and pistil with convoluted style **E** dissected petals of a staminate flower showing two series of alternating stamens with pollen grains visible and an undifferentiated, wavy pistillode **F** pistillate inflorescence showing some flowers without petals **G** staminate inflorescence with flowers at full anthesis exhibiting bright white anthers. Black arrows in pistils are pointing to the first convolution in styles.

In 1830, Blume transferred *L. venetus* Kunth along with 35 more taxa to the genus *Struthanthus*. In this publication there is no association between the generic name and the epithet, where only the basionyms were listed. However, this is a valid publication following article 41.3 of the ICBN (Turland et al. 2018): "Before 1 January 1953 an indirect reference to a basionym or replaced synonym is sufficient for valid publication of a new combination, name at new rank, or



Figure 3. Leaves and fruits of *Struthanthus venetus* and *S. interruptus* (Loranthaceae). *S. venetus*: A leaves B infructescence with mature and unmatured fruits. *S. interruptus*: C leaves D infructescence, fruit reddish, glaucous.

replacement name." Therefore, S. venetus (Kunth) Blume is a valid name, along with the other species of Struthanthus that Blume transferred in the same work.

Struthanthus venetus was cited for the first time in the book Trees and Shrubs of Mexico by Standley (1922), where a diagnosis for the genus was prepared to include the Mexican species. Standley mentioned that the type of *S. venetus* was from Cuernavaca, Morelos and that *L. volubilis* Sessé & Moc., described later in 1888, could represent the same taxon. *Loranthus volubilis* was described from specimens collected in Cuernavaca (as "Cuahunahuacae") during the Royal Botanical Expedition to New Spain (Sessé and Mociño 1888). The description of the taxon indicates voluble stems with opposite, rarely alternate, ovate, fleshy, glabrous, shortly petiolate leaves; inflorescences in axillary clusters with trifloral pedicels; white to greenish flowers; and oval fruits. In addition, the Nahuatl name *Teapizmictiquahuitl* is recorded for this species.

But which is the type for *L. volubilis*? A recurrent problem in the Sessé & Mociño specimens is that plants are not accompanied with data about location, date, collection number, and collector name. In 1997, Nelson designated the specimen *M. Sessé et al. s.n.* (MA-600707!; Cuatrec. No. 924; Negative/Types: F-46623) as the lectotype of *L. volubilis* (Fig. 5C). At the herbarium of the Real Jardín Botánico de Madrid (MA), there are two additional collections identified as *L. volubilis*: *M. Sessé et al. s.n.* (MA-606685!; 168-PB) and *M. Sessé & J.M. Mociño s.n.* (MA-606815!; Cuatrec. No. 4952), both determined by Standley (McVaugh 2000) as *S. haenkeanus* Standl. ( $\equiv$  *S. haenkei* (Presl.) Engl.), which is a synonym of *S. interruptus*. An examination of the specimen *M. Sessé &* 



**Figure 4.** Fluorescence microscopy showing germinated pollen grains and pollen tubes of *Struthanthus venetus* (Loranthaceae) flowers at full anthesis **A** pollen grains germinating and growing pollen tubes (pt) into the stigma **B** pollen tubes (pt) growing through the style to reach the embryo sac (es). Reference specimen: *M. I. Miguel V. 1300* (HUMO, accession number 40028).

J.M. Mociño 4952 reveals that its characters does not match the description of L. volubilis of having shortly petiolate leaves, with petioles on the specimen measuring 0.8–1.5 cm. On the other hand, the specimens M. Sessé et al. 168-PB and M. Sessé et al. 924 have leaves with petioles of 4.0–6.0 mm and leaves with acute apex, which coincides with the description of S. venetus by Kunth (Humboldt et al. 1818[1820]): "...Folia opposita, breviter petiolata, elliptico-oblonga aut obovata, apice rotundata... Petioli 2 lineas longi." (translated as "Leaves opposite, shortly petiolate, elliptical-oblong or obovate, round apex... Petiole 4.2 mm long."). This description mostly agrees with the one provided for L. volubilis by Sessé and Mociño (1888): "Folia opposita, raro alterna, ouata, carnosa, utrinque glabra, uenosa, breuiter peciolata." (translated as "Leaves opposite, rarely alternate, ovate, fleshy, glabrous on both sides, veined, shortly petiolate").

The specimen M. Sessé et al. s.n. (MA-606685!; 168-PB) has flower buds while M. Sessé et al. 924 has infructescences. The most likely explanation is that both specimens were collected in the second expedition conducted in Cuernavaca, Morelos, and other localities visited in Guerrero. Botanists began this expedition in March of 1789 and established their headquarters in Cuernavaca until December of the same year (García 2011). The fact that the two specimens collected during this expedition were either flowering or fruiting shows that explorers were present in the area for several months. All these botanical expeditions were carried out by Sessé et al. from 1787 to 1790 to New Spain and the localities of specimens collected in Cuernavaca match with the known distribution of S. venetus (Fig. 6) (Álvarez 1952, 1953; McVaugh 1969; Blanco Fernández de Caleya 1995; Maldonado 2000; Blanco Fernández de Caleya et al. 2010). According to the morphological comparison of the herbarium material, L. volubilis and S. venetus present similar morphological characters such as petiole length, leave and apex shape, and have the same distribution. For the above mentioned reasons, we are considering here L. volubilis to be conspecific with S. venetus.

Struthanthus venetus is not a synonym of S. interruptus.



**Figure 5.** Types of Struthanthus venetus, S. volubilis, and S. interruptus (Loranthaceae) **A** holotype of Loranthus venetus ( $\equiv$  Struthanthus venetus), Humboldt & Bonpland 3985 (P-00215992!) **B** holotype of S. interruptus, Humboldt & Bonpland s.n. (P-00215992!) **C** lectotype of L. volubilis ( $\equiv$  S. volubilis), Sessé 924 (MA-600707!).

In 1975, Kuijt proposed Struthanthus haenkei (Presl.) Standl. as a synonym of S. venetus. In his study he examined 16 specimens, of which and to our understanding, only four correspond to S. venetus: Bonpland 3985 (P00215992!), Nagel 8026 (MEXU-103129!, MEXU-103130!, MEXU-103131!; GH 13259!), Pringle 6185 (MEXU-11956!; UC-116705!; UVMVT-242343!; COLO-02212413!; US-930060!), and Rose 8584a (GH!). As for the other 12 cited specimens, Brandegee s.n. ( $\bigcirc$  fr, UC-116703!) agrees with S. condensatus Kuijt because the inflorescences have two triads and a cymbiform bract that embraces the bracteoles. Also, it matches the distribution range reported for this taxon (Pacific coast of México). The two duplicate specimens of *Hinton 1281* (♀ fl, GH!; US-1636506!; DES-00025368!) match with S. crassipes (Oliv.) Eichler since the leaves have an acute apex and inflorescences are racemes with eight pairs of pedicellate triads and subsessile flowers. The Lumholtz s.n. (GH!) specimen is sterile, which makes uncertain its identification. The two specimens by Rowell 2935 (SMU) and Turner 2106 (SMU) were not located. The seven remaining material examined correspond to S. interruptus: Gregory & Eiten 318 (♂ fl, NY!, SMU), Haenke 146 (♀ fl, fr, MO!, P!, F!, GH!, PR), King 1194 (2 fl, UC!, NY!), Leavenworth & Hoogstraal 1656 (MO!), *Moseed M-1398* (♂ fl, GH!), *Palmer 337* (♀ fr, UC!), and *Palmer 982* (♀ fr, UC!).

Five years later, Kuijt (1980) changed his opinion and treated *S. venetus* as synonym of *S. interruptus* (Kunth) Blume. The basonym of *S. venetus* is described before *S. interruptus* (Humboldt et al. 1818[1820]), and according to the principle of priority (Art. 11.3; Turland et al. 2018), *S. venetus* would have priority over *S. interruptus*. *Struthanthus interruptus* and *S. venetus* were described based on incomplete material (types lacking flowers and severely fragmented), which has added confusion and misunderstanding for both species. It should be noted that Blume (1830) did not transfer *L. interruptus* to *Struthanthus*, but to *Spirostylis* Presl; in fact, it was G. Don in 1834 who transferred *L. interruptus* to *Struthanthus*.

Therefore, as a measure to allow interpretation of the primary type of *L. venetus*, we propose an epitype, in which the inflorescences and reproductive characters (gynoecium and androecium) can be studied (Fig. 7). This action follows art. 9.9 of the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018), as well as the recommendations of Lendemer (2020) and Sennikov (2022), which are: (1) the taxonomic link between the epitype and the protologue and the type it supports; (2) same location as the type; (3) explanation of why the type is ambiguous and justification of epitype; and (4) a statement that the designated epitype represents the same taxon as that to which the name currently applies.

An attempt was made to collect specimens of *S. venetus* growing on the same species or genus as the host recorded in the type collection (*Diospyros nigra* (J.F. Gmel.) Perr., Ebenaceae). However, *Diospyros* was not found at the desired elevation, and the individuals found in the region were not being parasitized by *S. venetus*. Therefore, the material selected as epitype was collected at the type locality of Cuernavaca, Morelos, at an elevation comparable to the one recorded for the holotype.

#### **Taxonomic treatment**

Struthanthus venetus (Kunth) Blume, Syst. Veg. (ed. 15 bis) 7(2): 1731. 1830. Fig. 1A-F

Loranthus venetus Kunth, Nov. Gen. Sp. (quarto ed.) 3: 434. 1818[1820].
Loranthus volubilis Sessé & Moc., Pl. Nov. Hisp.: 51. 1888. Type: Quahunahuacae arboribus [Cuernavaca, Morelos], M. Sessé et al. s.n. (lectotype, designated by Nelson 1997, pg. 401: MA-600707!; Cuatrec. No. 924; Neg./Types: F-46623).

**Type material.** MEXICO • Morelos: crescit in Regno Mexicano, prope Cuernavaca, altitudo 850 hexapedalis [1554.5 m], *Humboldt & Bonpland 3985* (holotype, photography in JSTOR: P-00215992!; Fig. 5A); (**epitype**, designated here; Fig. 7). MEXICO • Morelos: Cuernavaca, Jardín etnobotánico Acapatzingo, calle Mariano Matamoros esquina con Degollado, col. Acapatzingo, 18°54'27.91"N, 99°13'29.95"W [18.9079155, -99.2243666], 1462 m, on *Gliricidia sepium* (Jacq.) Kunth (Fabaceae), 8 May 2023 (fl), *M.I. Miguel V. 1300* (HUMO-40028).

**Description.** *Shrub* hemiparasitic, perennial, woody, scandent, monoecious-monocline. *Haustorium* with terete epicortical roots, emerging from the base of the plant, secondary haustoria. *Stems* terete, glabrous, glaucous when young, brown to brown-grayish with lenticels; green, voluble with nodes flat when young; with epicortical roots. *Leaves* opposite, subopposite or alternate, decussate, sessile to subpetiolate, petioles 1.0-6.0 mm long, resupinate; leaves shape variable on the same individual, ovate to widely lanceolate, obovate, oblong, elliptic or rarely rotund,  $2.0-5.5 \times 4.0-12.0$  cm, apex obtuse to rounded, truncate to subemarginate with a small mucron, base attenuate, margin hyaline; glabrate, glaucous, crasso-coriaceous, pinnate venation, middle vein fading; leaves prehensile when young. *Inflorescences* racemes, 1(2) per axil, indeterminate, 2.0-6.2 cm long, peduncle 0.5-1.0 cm long, rachis subterete, glabrous, green; 6-8(10) triads, opposite to subopposite, decussate, peduncle



Figure 6. Distribution range of *Struthanthus interruptus* (Loranthaceae; white circles) and *S. venetus* (black circles) in México. AGS= Aguascalientes, CDMX= Ciudad de México, EDO. DE MÉXICO= Estado de México, MOR= Morelos, OAX= Oaxaca.

triad 0.6-2.0 mm long; bracts and bracteoles cymbiform, deciduous in anthesis. Flower buds cylindric to widely clavate with a rounded apex, 5.0-6.2 mm long. Mature flowers bisexual, sessile, hexamerous, 2.2-3.5 × 6.0-8.0 mm; petals linear, reflexed in the middle part to 45° up to 90°, 0.8-1.0 × 4.2-7.0 mm, white greenish to yellowish, petal apex rarely brown to reddish. Androecium with dimorphic stamens in two series, alternate, adnate to petals, filament free on middle part, papillate; first series with a filament 0.5-1.0 mm long, second series 0.3-0.6 mm long; symmetric anthers, dorsifixed, connective apiculate, anthers of the second series with a prominent connective; trichomes (villous) on the abaxial side of the anther up to 1.0 mm. Gynoecium with a convolute style, 3.2-5.0 mm long, 2(3) longitudinal folds, stigma capitate, slightly obligue; ovary inferior, cylindric 1.4-1.8 × 1.5-2.0 mm; nectary or nectariferous ring with 6 protuberances, globose, around the style; calyculus green-yellowish, irregular to dentate margin. Fruit a single-seeded berry, ovoid to subglobose, 4.6-7.5 × 7.8-12.0 mm, granulated, rusty; pedicel accrescent on the fruit, granulated. Seeds ovoid, 4.0-6.0 × 6.5-10.0 mm (Figs 2, 3).

**Distribution, habitat, and hosts.** *Struthanthus venetus* is known from oak and pine-oak forests, tropical deciduous and subdeciduous forests, urban areas, and crop fields. It occurs in central Mexico, mainly in the states of Morelos, Ciudad de México, Estado de México, Guanajuato, Guerrero, and Puebla (Fig. 5), at elevations from 850 to 2,445 m. It parasitizes species of at least 26 families, including gymnosperms and angiosperms: Adoxaceae E. Mey., Asteraceae



Figure 7. Epitype of *Loranthus venetus* ( $\equiv$  *Struthanthus venetus*), *M.I. Miguel V. 1300* (HUMO-40028!); collected in Cuernavaca, Morelos at the type locality.

Bercht. & J. Presl, Bignoniaceae Juss., Burseraceae Kunth, Casuarinaceae R. Br., Convolvulaceae Juss., Cupressaceae Gray, Fabaceae Lindl., Fagaceae Dumort., Meliaceae Juss., Moraceae Gaudich., Myrtaceae Juss., Oleaceae Hoffmanns. & Link, Opiliaceae Valeton, Petiveriaceae C. Agardh, Pinaceae Spreng. ex Rudolphi, Proteaceae Juss., Punicaceae Bercht. & J. Presl, Rosaceae Juss., Rubiaceae Juss, Rutaceae Juss, Salicaceae Mirb., Tiliaceae Juss., and Verbenaceae J. St.-Hil. Host records include other genera of parasitic plants such as *Phoradendron* (Santalaceae) and *Schoepfia* (Schoepfiaceae).

**Phenology.** Flowering from April to November and with fruits from June to April. **Notes.** Older individuals have larger leaves near the base of the stem, leaf shape and size of variable size thorough the whole plant, and thick and corky petioles. Also, petals of some flower buds become reddish near the apex.

Additional specimens examined. MEXICO. Ciudad de México · Coyoacán, Universidad Nacional Autónoma de México (UNAM, between Institute of Biomedical Research and Sports Complex Buildings, 19.32437, -99190957, 2229 m a.s.l., 22 Feb 2018 (fr), M.A. Caraballo 3383 (US) · Cuauhtémoc, alameda central a 10 m de la avenida Hidalgo. Colonia Centro, 19°26'08.2"N, 99°08'42.65"W [19.435561, -99.14518], s.d., L. Agonizante N. s.n. (UAMIZ) • ibid., San Antonio Abad y avenida del Taller, col. Tránsito, 19°24'50.61"N, 99°08'4.66"W, 2,250 m a.s.l., 23 Feb 1974 (fr), J. Gimate L. s.n. (ENCB) • Iztapalapa, dentro del campus de la UAM-Iztapalapa, calle San Rafael Atlixco, 19°21'36.8"N, 99°04'17.8"W, 2,240 m a.s.l., 4 Aug 2022 (fl), R. Cerros T. s.n. (HUMO) · Gustavo A. Madero, Sierravista 353, jardinera frente a tienda. En Lindavista, 19°29'55.25"N, 99°08'42.65"W [19.49868, -99.12861], 2,240 m a.s.l., 15 Oct 2023 (fl, fr), M.G. Maldonado B. 183 (HUMO). Estado de México · Ixtapan de la Sal, 18°50'31.49"N, 99°40'48.11"W, 1,876 m a.s.l., 15 May 2023 (fl), M.I. Miguel V. 1305 (HUMO) • ibid., 18°50'53.45"N, 99°40'52.27"W, 1,913 m a.s.l., 15 May 2023 (fl), M.I. Miguel V. 1306 (HUMO) • Temascaltepec, Chorrera [Carnicería], 19°02'40"N, 100°01'19.5"W, 1,230 m a.s.l., 8 Aug 1932 (fl), G.B. Hinton 1281 (DES, GH, US) • Tejupilco, en el cerro de la Muñeca, 18°54'40"N, 100°08'20"W, 1,500 m a.s.l., 27-28 Feb 1954 (fr), E. Matuda 30519 (MEXU) • Tepetlixpa, carretera Atlatlahucan-Tepetlixpa, 1 km antes de la desviación a Nepantla, 18°58'47.87"N, 98°51'10.81"W [18.979963, -98.853003], 1,964 m a.s.l., 2 Aug 2022 (fl), M.I. Miquel V. 1027 (HUMO) · Tonatico, salida de Tonatico (N-S), 18°48'22.68"N, 99°39'52.27"W, 1,668 m a.s.l., 15 May 2023 (fl), M.I. Miguel V. 1307 (HUMO). Guanajuato · León de los Aldama, blvd. Adolfo López Mateos, en barranca frente a Mobil 1, cerca del parque los Cárcamos, 21°09'49.32"N, 101°41'3.44"W, 1,829 m a.s.l., 5 Jul 2023 (fl), M.G. Maldonado B. 142 (HUMO) • ibid., en parque Cárcamos, Av. Adolfo López Mateos, esquina con blvd. José María Morelos, en árboles frente al lago, 21°10'3.14"N, 101°41'3.88"W, 1,822 m a.s.l., 5 Jul 2023 (fl), M.G. Maldonado B. 143 (HUMO) · ibid., en parque Cárcamos, av. Adolfo López Mateos esquina con blvd. José María Morelos, en árboles frente al lago, 21°10'2.89"N, 101°40'59.99"W, 1,823 m a.s.l., 5 Jul 2023 (fl), M.G. Maldonado B. 144 (HUMO). Guerrero · Chilpancingo de los Bravo, col. Juquila, en terreno baldío en la calle Cuauhtémoc, esquina con Miguel Hidalgo, 17°31'52.20"N, 99°30'40.82"W, 1,337 m a.s.l., 15 Jan 2023 (fr), M.G. Maldonado B. 83 (HUMO) • ibid., 15 Jan 2023 (fl), M.G. Maldonado B. 84 (HUMO). ibid., 15 Jan 2023 (fl), M.G. Maldonado B. 85 (HUMO) • ibid., 27 Feb 2023 (fl), M.G. Maldonado B. 86 (HUMO) · Leonardo Bravo, Corral de Piedra, a lado del tecorral que delimita el patio trasero del sr. Hipólito Maldonado, 17°39'45.01"N, 99°56'36.35"W, 1,563 m a.s.l., 23 Apr 2023 (fl, fr), M.G. Maldonado B. 95 (HUMO) · Pilcaya, el Mogote, 18°40'55.31"N, 99°33'57.27"W, 1,504 m a.s.l., 15 May 2023 (fl), M.I. Miguel V. 1303 (HUMO) • ibid., loc. Piedras Negras, costado de la carretera, 18°43'23.59"N, 99°36'30.28"W, 1,557 m a.s.l., 15 May 2023 (fl), M.I. Miguel V. 1304 (HUMO) • Taxco de Alarcón, Axixintla; km 194 carretera Taxco-Cuernavaca, 18°37'43.9"N, 99°30'39.9"W, 1,940 m a.s.l., 13 May 2021 (fl, fr), M.G. Maldonado B. 47 (HUMO) · ibid., Palmillas; km 29 carretera Taxco-Cuernavaca, 18°30'52.6"N, 99°25'35.3"W, 1,940 m a.s.l.,13 May 2021 (fl, fr), M.G. Maldonado B. 48 (HUMO). Morelos · Amacuzac, km 18 de la carretera de cuota a Acapulco, a 2 km de Casahuatlán, 100 m a orilla de carretera, 18°33'57"N, 99°24'27"W, 987 m a.s.l., 3 Jul 2007 (fl, fr), L.G. Galván-González 123 (MEXU, UAMIZ, IEB) • Atlatlahucan, carretera Cuautla-Amecameca, 10 m adelante de la desviación a Yecapixtla, a un costado de la carretera, 18°53'52.61"N, 98°54'29.13"W, 1,518 m a.s.l., 2 Aug 2022 (fl), M.I. Miguel V. 1026 (HUMO) • ibid., carretera Atlatlahucan-Totolapan, en la esquina con la calle Galeana, a un costado de la carretera, 18°56'44.9"N, 98°53'52.3"W, 1,680 m a.sl., 2 Aug 2022 (fl), M.I. Miguel V. 1028 (HUMO) · Coatlán del Río, rumbo al parque ecoturístico el Hoyanco, a orillas del canal de agua, 18°43'21"N, 99°25'51"W, 1,219 m a.s.l., 24 Feb 2013 (fr), L.G. Galván-González 212 (HUMO) • Cuautla, 420 m antes de la empresa Campi, en dirección de sur a norte, a un costado de la carretera Izúcar de Matamoros-Cuautla, 18°50'32.78"N, 98°55'37.56"W, 1,367 m a.s.l., 30 May 2023 (fl), M.I. Miguel V. 1309 (HUMO) • Cuernavaca, on shrubs, hillsides above Cuernavaca, 9 Nov 1895 (fl), C.G. Pringle 6185 (MEXU; UC; HUH; US; P; VT; COLO) • ibid., near Cuernavaca, 2 Jun 1939 (fl), O. Nagel 8026 (GH, MEXU) • ibid., about 4.5 mi E of Ocotepec on road from Cuernavaca to Tepoztlán, [18°58'39.85"N, 99°09'39.85"W, 1,643 m a.s.l.], 4 Aug 1971 (fl), W.D. Stevens 1385 (ENCB) • ibid., Colonia del Bosque, 18°54'49.4"N, 99°15'17.1"W, 1,510 m a.s.l., 31 May 2014 (fr), N.R. Rueda 2 (HUMO) • ibid., en barranca de la col. la Unión, 18°54'49.4"N, 99°15'17.1"W, 1,490 m a.s.l., 31 May 2014 (fl), L.G. Galván-González 221 (HUMO) • ibid., Facultad de Ciencias del Deporte, campus Chamilpa de la UAEM, 18°59'7.03"N, 99°14'18.46"W, 1,932 m a.s.l., 21 Nov 2016 (fl, fr), L.G. Galván-González 367 (HUMO) · ibid., la Carpa brecha a la barranca del Túnel, 18°57'2.99"N, 99°14'29.92"W, 1,908 m a.s.l., 5 Jul 2021 (fl), M.I. Miguel V. 964 (HUMO) · ibid., jardín botánico de la UAEM, 18°59'11.4"N, 99°17'15.3"W, 1,906 m a.s.l., 18 May 2022 (fl, fr), M.G. Maldonado B. 60 (HUMO) · ibid., jardín etnobotánico Acapatzingo, calle Mariano Matamoros esquina con Degollado, 18°54'27.91"N, 99°13'29.951"W, 1,462 m a.s.l., 8 May 2023 (fl), M.I. Miguel V. 1300 (HUMO) • Emiliano Zapata, Tetecalita, entrando por vv. Las Granjas, 18°46'26.7"N, 99°10'0.2"W, 1,194 m a.s.l., 18 Dec 2014 (fr), Y. Montoya M. 369 (HUMO) · Jantetelco, faldas del cerro del Chumil, 18°42'42"N, 98°45'16"W, 1,428 m a.s.l., 1 Sep 2006 (fl, fr), L.G. Galván-González 15 (HUMO) • ibid., Chalcatzingo zona arqueológica, 18°40'40.38"N, 98°46'14.18"W, 1,365 m a.s.l., 9 May 2023 (fl), M.I. Miguel V. 1301 (HUMO) • ibid., desviación junto al puente Jantetelco, en la carretera Puebla-Jantetelco, frente al parque, 18°42'58.54"N, 98°46'1.1"W, 1,435 m a.s.l., 19 Jun 2023 (fl), M.G. Maldonado B. 123 (HUMO) · Jiutepec, parque estatal el Texcal, 250 m al noreste de la entrada principal, cerca del borde de la reserva, a un costado de una brecha, 18°53'46.79"N, 99°08'26.12"W, 1,408 m a.s.l., 31 May 2023 (fl), M.I. Miguel V. 1324 (HUMO) · Jonacatepec, 20 m antes de la planta tratadora de agua, a un

costado de la carretera Tepalcingo-Jonacatepec, 18°39'41.09"N, 98°48'37.88"W, 1,295 m a.s.l., 30 May 2023 (fl), M.I. Miguel V. 1308 (HUMO) • Miacatlán, Xochicalco, 1.85 km al S de la zona arqueológica, 18°47'35.4"N, 99°17'48.53"W, 1,198 m a.s.l., 10 May 2023 (fl), M.I. Miguel V. 1302 (HUMO) • Puente de Ixtla, Tilzapotla. El Espinazo del Diablo, 1.5 km al este de Tilzapotla, brecha de terracería mina la Parreña, entronque con la Piaña-carretera el Salto, [18°29'31.66"N, 99°14'25.92"W, 1,410 m a.s.l.], 30 Jun 1998 (fl), J.C. Juárez 993 (HUMO) · ibid., carretera Tilzapotla-Caxintla, 300 m pasando la cantera, 1.5 km antes de la desviación hacia el Salto, 18°28'51.7"N, 99°15'50"W, 1,037 m a.s.l., 9 Feb 2023 (fr), M.I. Miguel V. 1251 (HUMO) • Temixco, barranca los Sabinos, 18°48'35"N, 99°15'58"W, 1,215 m a.s.l., 10 Nov 2006 (fl buds), R. Cerros T. 2725 (UAMIZ) • Tepoztlán, carretera Tepoztlán-Oacalco, 18°58'10"N, 99°04'25"W, 1,560 m a.s.l., 28 Sep 2006 (fr), R. Cerros 2676 (CIIDIR, MEXU, UAMIZ) · ibid., Cerro de la Cruz, cerca de San Andrés de la Cal. [18°57'35.73"N, 99°6'59.76"W, 1,200 m a.s.l.], 4 Apr 2009 (fl), M. Cházaro B. 9401 (XAL) · ibid., alrededor del poblado de San Andrés de la Cal, 18°57'09"N, 99°06'36.1"W, 1,783 m a.s.l., 7 Jun 2020 (fl), R. Hernández C. 2439 (HUMO) • Tlaltizapán, balneario Sta. Isabel, área de acampado, 18°43'58.44"N, 99°06'51.22"W, 954 m a.s.l., 15 Jun 2023 (fl), M.I. Miguel V. 1346 (HUMO) • Yautepec, huerta de una casa particular (la familia Chávez) a ca. 1 km al NE de Yautepec [Tabachines], 18°53'55.6"N, 99°02'16.9"W, 1,231 m a.s.l., 4 Apr 1986 (fr), D.H. Lorence 5033 (RSA, US) · ibid., carretera Tlayacapan-Oaxtepec, 400 m adelante de la desviación a Atlatlahucan, 18°55'39.15"N, 98°57'59.59"W, 1,481 m a.s.l., 2 Aug 2022 (fl remains), M.I. Miguel V. 1029 (HUMO) · ibid., 200 m adelante de la excaseta de Oacalco, autopista Oaxtepec-Tepoztlán, 18°55'59.23"N, 99°01'39.22"W, 1,367 m a.s.l., 30 May 2023 (fl), M.I. Miguel V. 1310 (HUMO) · Yecapixtla, Yecapixtla, [18°52'9.13"N, 98°49'47.98"W], 1,700 m a.s.l., 10 Oct 2014 (fl), L. Ortega C. 148 (HUMO) • Zacualpan de Amilpas, Campos de cultivo de Tlacotepec, 18°48'46"N, 98°44'48"W, 1,770 m a.s.l., 22 Sep 2006 (fr), L.G. Galván-González 44 (UAMIZ). Puebla · Atlixco, camino Puebla-Atlixco, 200 m después de la caseta de cobro Siglo XXI, 18°56'09.01"N, 98°26'58.31"W, 1,927 m a.s.l., 19 Jun 2021 (fl, fr), M.G. Maldonado B. 122 (HUMO) · Puebla, jardín botánico de la BUAP, 18°59'57.51"N, 98°11'49.65"W, 2,150 m a.s.l., 12 Dec 2017 (fr), A.J. Coombes 1808 (HUAP) • Puebla, camellón frente al jardín botánico de la BUAP, 18°59'57.6"N, 98°11'53.15"W, 2,132 m a.s.l., 30 Sep 2022 (fl, fr), M.I. Miguel V. 1032 (HUMO) • ibid., camellones de la BUAP, 18°59'56"N, 98°11'50.06"W, 2,134 m a.s.l., 9 Jun 2023 (fl), M.I. Miguel V. 1345b (HUMO) · Tepexco, al N de loc. de Ixtlala, 18°35'37.58"N, 98°42'8.3"W, 1,151 m a.s.l., 24 Aug 2016 (fr), L. Caamaño O. 9052 (HUAP, HUMO) • 1.5 km al N del balneario Ixtatlala, sobre la brecha al poblado de Tepexco, 18°36'16.2"N, 98°42'20.2"W, 1,154 m a.s.l., 28 Aug 2022 (fl), M.I. Miguel V. 1030 (HUMO) • ibid., 28 Aug 2022 (fl, fr), M.I. Miguel V. 1031 (HUMO).

# Discussion

The findings obtained in this study support the idea that *S. venetus* should be reinstated and no longer be considered a synonym of *S. interruptus*. The unique combination of bisexual flowers, granular fruits, and crasso-coriaceous leaves help to recognize this taxon from other Mexican species of *Struthanthus*. Other

characters that can help separate *S. venetus* from *S. interruptus* include young branches with glaucous stems that become brown to brown-grayish when mature (vs. young branches green with brown to reddish-brown stems, and glossy with lenticels when mature in *S. interruptus*); leaf petioles that are subsessile to decurrent and 0.1-0.6 cm long (vs. leaf petioles decurrent and 0.6-1.0 (1.5) cm long in *S. interruptus*); and bisexual flowers with accrescent pedicels bearing granulated fruit ripening rusty red (vs. unisexual flowers with non-accrescent pedicels bearing smooth fruits ripening reddish in *S. interruptus*; Fig. 3C, D). In addition, the distributional range recorded for *S. venetus* does not overlap with the one known for *S. interruptus* (Fig. 6).

Struthanthus venetus was described based on a fruiting individual. Similarly, S. interruptus was collected with pistillate flowers (no longer extant in the specimen) and unripe fruits, and its diagnosis remarked that male flowers and fruit were insufficiently known (Fig. 5A, C). Incomplete specimens have led to numerous synonyms in Struthanthus through the description of new taxa that are subsequently merged (Caires and Dettke 2020). It is noteworthy to mention that proper identification of specimens in Struthanthus is very difficult in the absence of material containing both staminate and pistillate flowers (for dioecious species) and fruit. Our results from the pollen germination test provide evidence that pollen of S. venetus can germinate and develop pollinic tubes of considerable length. Additional studies on pollen germination in Struthanthus can help to understand better the reproductive mechanisms of fertilization, especially for species with convolute styles. We did not conduct pollen viability tests in S. interruptus because we considered that differences in flower morphology with S. venetus (dioecious vs. bisexual flowers) were enough evidence that both taxa represent separate biological entities. It is worthy to note that none of the species of Struthanthus from México have been the subject of studies on pollen germination. Only S. flexicaulis Mart. and S. vulgaris (Vell.) Mart. (both from Sao Paulo, Brazil) have been the subject of embryological studies (Venturelli 1981, 1984).

The study by Kuijt in 1975 showed that *S. venetus* has been misidentified as *S. interruptus*, *S. condensatus*, and *S. crassipes*. However, *S. venetus* can be separated from all these taxa by its distinctive bisexual flowers. Also, *S. venetus* has a racemose inflorescence with free bracts and deciduous bracteoles that contrast with the capitate inflorescence with fused bracts and bracteoles that persist after anthesis seen in *S. condensatus*. Additionally, *S. venetus* bear one or rarely two racemes per axil, leaves with obtuse to rounded, truncate or subemarginate apex and attenuated base, and granulated fruiting pedicels. These features diverge from the ones found in *S. crassipes*, which has one to three (rarely five) racemes per axil, leaves with attenuate to acuminate apex and obtuse to round base, and smooth fruiting pedicels (Table 1).

Struthanthus venetus has a spotty distribution with the core found in central México at the states of Ciudad de México, Estado de México, Guerrero, Morelos, and Puebla. There are also a handful of disjunct specimens from León de los Aldama in Guanajuato, about 280 km away from the westernmost edge of the main distribution in the Estado de México (Fig. 6). These collections were made on ornamental plants in public parks, and it is possible that these ornamental trees originated from Morelos or the Estado de México, given that these states are the main producers of ornamental plants for the country (SIAP 2020).

Characters	S. condensatus	S. interruptus	S. crassipes	S. venetus
Plant sexuality	dioecious	dioecious	dioecious	monoecious-monocline
Leaf shape	lanceolate, ovate to rounded, rarely elliptic	oblong to ovate- elliptical, rarely obovate or rounded	lanceolate to elliptic- lanceolate	ovate to widely lanceolate, obovate, oblong, elliptic, rarely rounded
Leaf apex	obtuse to rounded	obtuse to rounded, rarely truncate or acute	attenuate to acuminate	obtuse to rounded, truncate to subemarginate with a small mucro
Leaf base	obtuse to rounded	decurrent	obtuse to rounded	attenuate
Leaf size (cm)	2.0-5.0 × 4.0-7.2	1.5-5.0 × 5.5-10.2	1.8-5.5 × 5.5-12.5 (13.5)	2.0-5.5 × 4.0-12.0
Leaf texture	coriaceous to crasso-coriaceous	papery or chartaceous	coriaceous	crasso-coriaceous
Petiole length (cm)	0.2-0.6	0.6-1.0 (1.5)	0.3-1.2	0.1-0.6
Inflorescences type	capitate	spiciform	racemose	racemose
Inflorescences per axil	1	1-2	1–3 (5)	1 (2)
Triads per inflorescence	2-6	8-12 (14)	6-16	6-8 (10)
Peduncle triad length (mm)	0	0	4.0-10.0	0.6-2.0
Bracts and bracteoles	fused into a cup, persistent	not fused, deciduous	not fused, bracteoles persistent in anthesis	not fused, deciduous
Flower length from base of ovary (mm)	3.5-5.5	4.0-7.5	4.0-7.0	6.0-8.0
Fruit pedicel	not applicable (fruit sessile)	accrescent, smooth	evidently accrescent, pendent and smooth	accrescent, granulated
Fruit shape	ovoid to ellipsoid	ellipsoid	ovoid to obovoid	ovoid to subglobose
Fruit size (mm)	4.0-6.0 × 5.0-7.5	6.5-7.0 × 10.8-12.0	3.8-6.0 × 6.5-8.6	4.0-6.0 × 6.5-10.0
Fruit surface	smooth	smooth	smooth	granulated
Color of ripe fruit	maroon	reddish	orange-reddish to rusty	rusty

Table 1. Morphological differences among *Struthanthus condensatus*, *S. crassipes*, *S. interruptus*, and *S. venetus* (Loran-thaceae).

The taxonomy of *Struthanthus* and related genera needs to be investigated from an interdisciplinary perspective, and future work with expanded sampling covering *Struthanthus* and related genera should help clarify their taxonomy and evolutionary history. These future studies should incorporate a strong component of fieldwork, given that examining living plants is very important to detect morphological variation and recognize overlooked taxa, especially when type specimens are incomplete and/or severely fragmented. Finally, we would like to mention that in this study we did not address directly the taxonomy of *S. interruptus* because our surveys indicate that, as currently defined, it seems to involve at least three more overlooked taxa. Therefore, we decided to discuss the taxonomy of *S. venetus* by itself and address the rest of the *S. interruptus* complex in a separate publication.

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# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### **Author contributions**

All authors contributed to the preparation of the manuscript by providing data and reviewing and editing the text. MGMB, MIMV, and RCT conducted the fieldwork, RCT provided photos of living plants, and MGMB prepared stereoscopic photographs. AES aided with nomenclature and interpreting historical collections.

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#### Data availability

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

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**Research Article** 

# Morphological and molecular characterization of Halamphora vantushpaensis (Bacillariophyceae, Amphipleuraceae), a new diatom species widely dispersed on the shores of the soda Lake Van (Türkiye)

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#### Abstract

In this study, we describe *Halamphora vantushpaensis* **sp. nov.**, a newly identified diatom species found in the highly alkaline Lake Van in Eastern Turkey (Türkiye). This new species is characterized morphologically by light and scanning electron microscopy, performed on both wild and cultivated samples. Two monoclonal cultures were submitted to a genome-skimming approach, giving access to the complete sequence of their nuclear rRNA cluster of genes, mitochondrial and plastid genomes. Both strains were highly similar from the genomic point of view, with few mutations noted, although in organellar genomes some of them concerned protein coding genes and were non-silent. Also, the group II intron in the mitochondrial *cox1* gene was found to display a relatively high number of polymorphisms. The plastome also distinguishes itself from other *Halamphora* spp. by the extension of its inverted repeat at the expense of the two single copy regions of the genome. Maximum likelihood molecular phylogeny inferred from a concatenated three genes dataset (*18S*, *psbC* and *rbcL*) positions this species within the K clade, which is known to contain hypersaline to freshwater species.

**Key words:** Alkaline lake, group II introns, inverted repeat, mitochondrial genome, multigene phylogeny, nuclear rRNA genes, plastid genome

# Introduction

Soda lakes are among the rarest and most geochemically distinctive wetlands on Earth. They are characterized by their alkaline waters containing high levels of carbonate and bicarbonate ions, typically resulting in elevated pH levels. Lake Van is the largest soda lake in the world (Glombitza et al. 2013), with water that is both saline (21.4‰) and alkaline (155 m mEq<sup>-1</sup>, pH 9.81) (Aydar et al. 2003; Kempe et al. 1991). The lake has existed for 600,000 years, spanning multiple glacial-interglacial cycles (Stockhecke et al. 2014; North et al. 2018) and hosts endemic species of fishes (e.g. Akku et al. 2021). However, studies on the phytoplanktonic flora of the lake, and especially diatoms, have been rather scarce.

For a long time, investigations into the contemporary diatom flora from Lake Van were restricted to a single study by Legler and Krasske (1940). These authors worked on samples brought to them in Germany from Lake Van and, based on light microscopy, they found 24 diatom taxa. Among them, two belonged to the genus *Amphora*. The first one was described as '*Amphora coffeiformis* Ag.' (more correctly referred to as *Amphora coffeiformis* (C.Agardh) Kützing, 1844). This is now a non-accepted synonym of *Halamphora coffeiformis* (C.Agardh) Levkov following the recent revisions of the amphoroid diatoms in which Cleve's sect. *Halamphora* has been recognized at the genus level (Cleve 1895; Levkov 2009; Stepanek and Kociolek 2013, 2014, 2019). The second was '*Amphora commutata* Grun.', published in Van Heurck (1880), considered a valid species as of today. Both taxa are known for being brackish species and both have wide distributions (Guiry and Guiry 2024).

In the last few years, new investigations have been conducted on Lake Van diatoms using an integrative approach that combines light/scanning electron microscopy (LM/SEM) and molecular phylogenies derived from next generation sequencing results. With these data, three new species already have been discovered and described, namely *Nitzschia anatoliensis* Górecka, Gastineau and Solak (Solak et al. 2021), *Navicula vanseea* Yılmaz, Gastineau, Solak and Witkowski (Yilmaz et al. 2024) and *Halamphora witkowskii* (Yilmaz et al. in press). Lake Van can be divided into four different basins of various depths: the shallow Erçis basin (northeast), the Van basin (southeast), the Ahlat basin (northwest) and, at the centre of the lake, a deeper fourth basin (Kaden et al. 2010). *Navicula vanseea* and *Ni. anatoliensis* were both described from material from the Erciş basin but have not been observed yet in other parts of the lake. *Halamphora witkowskii* is known so far only from the Ahlat basin (Yilmaz et al. in press).

In the current article, we describe another *Halamphora* species, *Halamphora* vantushpaensis sp. nov., using the same integrative approach and tools previously used for *Na. vanseea* and *Ni. anatoliensis*.

# Material and methods

#### Sampling, isolation and cultivation

Live samples were collected by scraping stones at four different stations around Lake Van: Ahlat, Bitlis ( $38^{\circ}75'45.748"N$ ,  $42^{\circ}50'71.257"E$ ); Erciş, Van ( $39^{\circ}00'07.9"N$ ,  $43^{\circ}25'40.4"E$ ); Adilcevaz, Bitlis ( $38^{\circ}79'83"N$ ,  $42^{\circ}72'16"E$ ); and Edremit, Van ( $38^{\circ}42'07.09"N$ ,  $43^{\circ}23'74.39"E$ ) (Fig. 1). Individual diatom cells were isolated from the Ahlat samples using a micropipette under a Nikon TS100 inverted microscope (NIKON, Tokyo, Japan). The strains were subsequently moved into 250 mL Erlenmeyer flasks containing F/2 medium (Guillard, 1975), which had been adjusted to 18% salinity. The cultures were maintained in conditions promoting active growth, with a light intensity of 60 µmol photons m<sup>-2</sup> s<sup>-1</sup>,



**Figure 1.** Map of the sampling location **A** location of Lake Van in Turkey. The red frame indicates the position of Lake Van **B** general view of the lake. The areas indicated by arrows are the sampling stations. (Esri. (2023). ArcGIS Pro 3.1.0. Environmental Systems Research Institute)

and a photoperiod of 14 hours of light and 10 hours of darkness at a temperature of 18 °C. The two monoclonal cultures were obtained and registered in the Szczecin Diatom Culture Collection as SZCZEY 2166 and SZCZEY 2167.

#### Light and scanning electron microscopy

Light microscopy (LM) documentation was obtained at the University of Szczecin with a Zeiss Axio Scope A1 (Carl Zeiss, Jena, Germany) using a Canon EOS 500D camera and Canon EOS Utility software (Canon, Tokyo, Japan). Images were obtained using a 100× Plan Apochromat oil immersion objective (numerical aperture = 1.4).

For the preparation of diatom frustules for both LM and scanning electron microscope (SEM) observations, samples (pellets of cells from monoclonal cultures or wild samples) were moved into 20 mL beakers and 10 mL of 10% HCl added. Over a 24-hour period, the samples were washed with distilled water four times, allowing the material to sediment naturally between washes. Next, the samples were re-suspended in 30% H<sub>2</sub>O<sub>2</sub> and boiled on a hotplate at 170 °C for approximately four hours. The final step involved washing the samples four times with distilled water in 24 h, as before. For LM, the material was airdried on cover glasses and then affixed to a glass slide using Naphrax (Brunel Microscopes, Chippenham, UK). LM measurements were done on a total of 91 valves. For SEM, a drop of cleaned sample was placed on a Nuclepore Track-Etch membrane (Whatman, Maidstone, U.K.). Following air-drying overnight, the membranes were mounted on aluminium stubs using carbon tape and coated with gold using a Q150T coater (Quorum Technologies, Laughton, UK). SEM imaging was conducted at the Faculty of Chemical Technology and Engineering, Western Pomeranian University of Technology in Szczecin (Poland) on a Hitachi SU8020 field emission microscope (Tokyo, Japan). The imaging was conducted with an accelerating voltage of 5kV and a working distance of 8500-8600 µm.

#### Next generation sequencing and bioinformatic analyses

Clones SZCZEY2166 and SZCZEY2167 were harvested by centrifugation and DNA was extracted following Doyle and Doyle (1990). Total DNA was sent to the Beijing genomics Institute (BGI) in Shenzhen (China) where they were sequenced on a DNBSEQ platform for a total for each clone of ca. 40M 150 bp paired-end clean reads. Assemblies were conducted using SPAdes 3.15.0 (Bankevich et al. 2012) with a k-mer parameter of 125. The contigs corresponding to the plastid and mitochondrial genomes or nuclear rRNA clusters were data-mined by standalone blastn queries. The subunits of the plastid genome were merged with each other with the help of Consed (Gordon and Green 2013). Annotation of protein coding genes was done as described in Gastineau et al. (2023).

# Molecular phylogeny

Maximum likelihood phylogenies were inferred from two different datasets. One contained a concatenated alignment of 18S, rbcL and psbC genes representing 214 taxa downloaded from GenBank appended with those obtained in the course of this study. Two strains of Triparma pacifica (Guillou and Chrétiennot-Dinet) Ichinomiya and Lopes Santos were used as an outgroup. Among the diatom taxa, two were lacking rbcL data, 22 strains lacked psbC and 21 strains lacked 18S. A rbcL-only tree was built as well, in order to compare the tree topologies. Sequences were aligned using MAFFT 7 (Katoh and Standley 2013) and trimmed automatically with trimAl (Capella-Gutiérrez et al. 2009). The best model of evolution was selected separately for each gene with ModelTest-NG (Darriba et al. 2020). In case of three-gene dataset, trimmed alignments of 18S, rbcL and psbC were concatenated with Phyutility 2.7.1 (Smith and Dunn 2008). Maximum Likelihood phylogenes were computed using IQ-TREE 2.2.0 (Minh et al. 2020) with 1000 ultrafast bootstrap replicates; the dataset was partitioned based on the best models of evolution found for each gene. The trees were visualised with MEGA11 (Tamura et al. 2021). Lists of the sequences with their corresponding accession numbers can be accessed as explained in the data availability statement.

## Results

#### Taxonomy

Phylum Bacillariophyta Haeckel Class Bacillariophyceae Haeckel Family Amphipleuraceae Grunow Genus Halamphora (Cleve) Levkov

Halamphora vantushpaensis Yilmaz, Solak & Gastineau, sp. nov. Figs 2-4

**LM** (Figs 2A–M). Valves semi-lanceolate, dorsiventral with arched dorsal margin and slightly tumid ventral margin. Valve ends protracted and capitate in larger specimens (Figs 2A–F); but less protracted and not clearly separated



**Figure 2. A–M** *Halamphora vantushpaensis* sp. nov. LM micrographs **A–F** cleaned valves of the larger specimen (SZCZEY2167) **G–M** cleaned valves of the smaller specimen (SZCZEY2166). Scale bar: 10 µm

from the rest of the valve in smaller specimens (Figs 2G–M), ventrally bent. Valve length 24.0–42.0  $\mu$ m, valve width 4.0–5.0  $\mu$ m (n = 35). Axial area very narrow, wider on the ventral side. Central area visible in larger specimens: indistinct on the dorsal side, semi-lanceolate on the ventral side. Raphe almost straight, slightly arched, appearing to be located near the median line of the valve or slightly dorsal in valve view (Fig. 2). Sometimes the proximal raphe



Figure 3. A–F Halamphora vantushpaensis sp. nov. SEM micrographs of strain SZCZEY2167 A External view of the entire valve B details of central area showing simple proximal raphe endings and regular shortened striae C details of apex showing the terminal fissure D internal view of the entire valve Edetails of central area showing fused central helictoglossae in proximal raphe endings F details of apex showing well-developed helictoglossae. Scale bars: 10  $\mu$ m (A, D); 5  $\mu$ m (E, F); 3  $\mu$ m (B); 2  $\mu$ m (C).

endings can be seen to be slightly dorsally bent (Fig. 2B). Striae hard to resolve in LM, dorsally slightly radiate over the entire valve (see SEM images for clearer demonstration of this), 27-32 in  $10 \,\mu$ m.

**SEM (Figs 3A–F, 4A–F, 5A–J).** Externally, the valve face is arched, merging gently into the mantles (Figs 3B, 4B, C, 5H). Raphe ledge narrow and linear, present on the dorsal side of the raphe, with a prominent groove separating it from the valve face. The proximal raphe endings are slightly expanded into central depressions



Figure 4. A–F Halamphora vantushpaensis sp. nov. SEM micrographs of strain SZCZEY2166 A external view of the entire valve B details of central area showing simple proximal raphe endings and regular shortened striae C details of apex showing the terminal fissure D internal view of the entire valve E details of central area showing fused central helictoglossae in proximal raphe endings F details of apex showing well-developed helictoglossae. Scale bars: 10 μm (A, D); 3 μm (B, C, E, F).

and are dorsally deflected (Figs 3B, 4B, 5H). The distal raphe endings are dorsally deflected and hook around to link with the groove bordering the raphe ledge (Figs 3C, 4C, 5J). The striae are simple and uniseriate, containing small round or slightly elongate poroids (Figs 3B, 4B, C, 5H), which are somewhat irregularly spaced (Fig. 4B, 5H and see also the internal views in Figs 3E, 4E, 5B, E).

The internal view of the valve shows the overall structure (Figs 3D, 5A). The central area is easier to detect than the external area and appears symmetrical



Figure 5. A–J SEM images of a cleaned valve from wild material A external view of the entire valve of the larger specimens B details of central area showing simple proximal raphe endings and regular shortened striae C details of apex showing the terminal fissure D internal view of the entire valve of the smaller specimens E details of central area showing fused central helictoglossae in proximal raphe endings F details of apex showing well-developed helictoglossae G external view of the entire valve of the smaller specimens H details of central area showing simple proximal raphe endings and regular showing simple proximal raphe endings and regular showing simple proximal raphe endings and regular showing simple proximal raphe endings A details of central area showing simple proximal raphe endings and regular shortened striae J details of apex showing the terminal fissure. Scale bars: 10 μm (A, D, G); 3 μm (B, C, J); 2 μm (E, H, F).

and large on the dorsal side in larger specimens (Fig. 3E, 5B); but very small on both sides in smaller specimens (Fig. 4E, 5E). Proximally, the raphe terminates within a fused central helictoglossa (Figs 3E, 4E, 5E). The distal raphe endings are slightly deflected ventrally and terminate in well-developed helictoglossae (Figs 3F, 4F, 5C, F). Internally, the poroids have round to elliptical internal openings (Figs 3E, 4E, 5B, E). These characteristics are summarized and compared with those of similar species in Table 1.

Phycobank. http://phycobank.org/104935.

**Holotype.** Slides number SZCZEY2167 in the collection of Andrzej Witkowski at the University of Szczecin, Poland. Valves representing the holotype population here illustrated in Fig. 2D.

**Isotype.** Slide number TR\_Erciş\_Van\_2021 deposited in Kütahya Dumlupınar University (Türkiye).

**Type locality.** Erciş Van, Turkey (39°00'07.9"N, 43°25'40.4"E) leg. Elif Yılmaz, 31 July 2021.

**Etymology.** The species is named with regard to both Lake Van and the city of Tushpa, capital of the Iron Age kingdom of Urartu, which was located in the vicinity of the lake.

**Distribution.** The presence of this taxon has been assessed and confirmed at four different stations around Lake Van: Ahlat (North West of the lake), Adilcevaz (North), Erciş (North East), and Edremit (South East).

Table 1. Morphological characteristics of Halamphora vantushpaensis sp. nov. and similar Halamphora ( represents n	0
information) (for <i>H. vantushpaensis</i> measurements, n = 35).	

	Halamphora vantushpaensis sp. nov.	H. atacamana	H. borealis	H. gasseae	H. salinicola	H. sardiniensis	H. thermalis
Valve length (µm)	24.0-42.0	29.0-45.0	19.0-40.0	19.0-35.0	20.0-34.0	13.0-27.5	18.0-40.0
Valve width (µm)	4.0-5.0	4.5-8.0	3.0-4.0	3.5-4.5	2.5-3.7	3.0-4.5	4.0-6.5
Stria density (in 10 µm)	27-32	25-28	20-24	20-24	21-26	36-42	26-36
Valve outline	semi-lanceolate with arched dorsal margin, slightly tumid ventral margin	semi-lanceolate, arched dorsal margin, concave or straight to weakly tumid ventral margin	semi-lanceolate, arched dorsal margin, straight to weakly tumid ventral margin	semi-lanceolate, smoothly arched dorsal margin, straight to weakly concave ventral margin	semi-lanceolate, smoothly arched dorsal margin, straight to weakly concave ventral margin	semi-lanceolate, strongly arched dorsal margin and straight to slightly concave ventral margin	semi-lanceolate to lanceolate, smoothly arched dorsal margin, straight to slight- ly tumid ventral margin
Valve ending	subprotract- ed in smaller specimens; protracted, capitate in larger specimens and ventrally bent	slightly subprotract- ed and ventrally bent	protracted, capi- tate and slightly ventrally bent	shortly protract- ed and capitate	shortly protract- ed and capitate	shortly protract- ed and capitate	attenuate
Raphe ledge	narrow, arched with equal width throughout	narrow, equal width throughout and dor- sally elevated from the valve face	narrow, linear		narrow, expand- ed on both valve sides	narrow, expand- ed on both valve sides	narrow, equal width throughout
Axial area	narrow, widening ventrally	narrow, widening ventrally	narrow, widening ventrally	narrow, widening ventrally	narrow, widening ventrally	narrow, widening ventrally	narrow, slightly dorsally bent
References	in this study	Levkov 2009	Levkov 2009	Levkov 2009	Levkov 2009	Levkov 2009	Levkov 2009

#### Genomics - the cluster of nuclear ribosomal RNA genes

Complete clusters of the rRNA genes, containing *18S*, internal transcribed spacer 1 (ITS1), *5.8S*, internal transcribed spacer 2 (ITS2) and *28S*, were obtained for both strains and registered with GenBank accession numbers PP726705 and PP726703 for SZCZEY2166 and SZCZEY2167 respectively. Their sizes and sequences were identical except for a single T/G SNP in the ITS1. The sizes of the different parts of the cluster are indicated in Table 2 and compared with results obtained on the same set of species as in Hamsher et al. (2019), which concern *Halamphora americana* Kociolek, 2014, *Halamphora calidilacuna* Stepanek & Kociolek, 2018 and *H. coffeiformis*. Lengths of *18S*, *5.8S* and *28S* were very conserved among species, except for *H. americana*, which has a group II intron in its *18S* that also contains on ORF coding for a putative reverse transcriptase protein. *Halamphora vantushpaensis* sp. nov. displays a longer ITS1 when compared to other species.

#### **Genomics - mitochondrial genome**

Complete mitogenomes were obtained on both strains and registered with Gen-Bank accession numbers PP962256 (SZCZEY2166) (Fig. 6) and PP962257 (SZC-ZEY2167) (Fig. 7). The genomes are 42,659 bp and 43,152 bp long (SZCZEY2166 and SZCZEY2167 respectively). The genomes both contain 35 conserved protein coding genes (PCGs), two rRNA and 26 tRNA genes. The mitogenomes Table 2. Length (in bp) of the different portions of the nuclear rRNA cluster for four species of *Halamphora* spp. The length of the *18S* gene of *H. americana* is indicated with and without the intron.

Species	Halamphora vantushpaensis	Halamphora calidilacuna	Halamphora americana	Halamphora coffeiformis
Accession number	PP726705 and PP726703	MH810165	MH810166	MH810167
Total length	5932	5764	9254	5938
18S	1767	1769	1783 (5241 with intron)	1767
ITS1	431	223	229	347
5.8S	156	155	155	154
ITS2	368	405	419	454
28S	3210	3212	3210	3217



Figure 6. Map of the mitochondrial genome of *Halamphora vantushpaensis* sp. nov. SZCZEY2166

harbour the conserved open reading frame (ORF) orf151, although its position differs from most know species among which it is included in a conserved cluster of genes together with *rps11* and *mttb/tatC* (Pogoda et al. 2019) whereas here it is located between *rps11* and *rps8*. There is also a non-conserved ORF (orf115) between *cob* and *nad5*. The *cox1* gene contains a group II intron with an ORF coding for a putative reverse transcriptase. There are discrepancies in the length of this putative protein which is 632 amino-acid long in SZCZEY2166



Figure 7. Map of the mitochondrial genome of *Halamphora vantushpaensis* sp. nov. SZCZEY2167

while it is 604 amino-acid long in SZCZEY2167, the extra-length being at the C-terminal part of the putative protein entirely. The polymorphisms between both strains mostly occurred in intergenic parts, hence the slight differences in lengths of the mitogenomes. The conserved protein coding genes were strongly conserved with some of them completely identical, although a certain number of polymorphisms could still be spotted in PCGs in the following genes, with the number of SNPs/lengths indicated between brackets: *cob* (6/1287), *nad2* (1/1536), *nad4* (1/1473), *rpl2* (1/810), *rpl6* (1/573), *rps3* (2/1038), *rps10* (1/540). These mutations were silent in *nad4* and *rpl2*, but led for each of the other encoded protein to one amino-acid substitution. Several variations were otherwise found in the *cox1* intron, whose size varied because of indels (four in total). It otherwise displays 17 SNPs for a total length of 3433/3435 bp, with nine of them being found in the 1815 bp shared between the two putative reverse transcriptase encoding ORF, leading to seven amino-acid substitutions.

#### **Genomics - plastid genome**

Both plastome were also obtained. Their lengths are 133,866 bp long for strain SZCZEY2166 (GenBank: PP962255) and 133,852 bp long for strain SZC-ZEY2167 (GenBank: PP727123). The two plastomes came out as different isomers after assembly, hence the difference of strand of the large single copy region (LSC) that can be observed between SZCZEY2166 (Fig. 8) and SZC-ZEY2167 (Fig. 9). There were slight differences of lengths for LSC and SSC.



Figure 8. Map of the plastid genome of Halamphora vantushpaensis sp. nov. SZCZEY2166

The LSC is 61,711/61,691 bp long and display 26 indels and six SNPs. Out of these six SNPs, five were found in PCGs (*psbC*, *ycf90*, *petB*, *rps20* and *rpoC2*) and were silent only in *petB* and *rpoC2*. The short single copy (SSC) is 39,615 bp long for both strains, with no indels and 17 SNPs, all located in intergenic area. The inverted repeat had identical lengths and displayed six consecutive polymorphisms in the intergene between *rpl32* and *ycf35*. The LSC contains 70 PCGs, a single non-conserved open reading frame (ORF), and 17 tRNAs. The SSC encodes for 46 PCGs, also a single non-conserved ORF and six tRNAs. The inverted repeat IR contains 10 PCGs, three rRNA and four tRNA.

Three plastid genomes are available in GenBank for the genus *Halamphora*, all originating from the same study (Hamsher et al. 2019). In Table 3, the total lengths of these genomes and the lengths of their different compartments are compared.

Halamphora vantushpaensis has shorter LSC and SSC but its IR is consequently longer when compared to other species. The gene content of the IR is compared for all these species in Table 4. The restricted set of conserved genes found among *H. calidilacuna* or *H. americana* and which consists of a single PCG (ycf89), three tRNA and three rRNA seems to be shared by many unrelated species and genera such as Navicula veneta Kützing 1844 or Tryblionella apiculata Gregory 1857 (Gastineau et al. 2021a). As with *H. americana*, an extension of the IR may result from the presence of non-conserved ORF or putative genes of plasmid origin, as exemplified by its ORF9 and the putative



Figure 9. Map of the plastid genome of Halamphora vantushpaensis sp. nov. SZCZEY2167

**Table 3.** Lengths (in bp) of the different compartments of the plastid genomes of four species of *Halamphora* spp.

Species	Length of the LSC	Length of the SSC	Length of the IR	Total length
Halamphora calidilacuna	82,227	49,698	9,407	150,739
Halamphora americana	77,289	44,724	10,269	142,551
Halamphora coffeiformis	64,938	41,485	7,752	121,927
Halamphora vantushpaensis	61,705/61,691	39,615/39,615	16,273	133,866/133,852

**Table 4.** Gene composition of the inverted repeats of the plastid genomes of four species of *Halamphora* spp. Genes highlighted in bold for *Halamphora* vantushpaensis sp. nov. are genes found in the LSC in other species. Genes in bold italic concern genes usually found in the SSC. Genes marked by an asterisk are non-conserved genes of probable plasmidic origin.

Species	Gene composition of the IR
Halamphora calidilacuna	tRNA-Pro, ycf89, rrs, tRNA-Ile, tRNA-Ala, rrl, rrf
Halamphora americana	tRNA-Pro, ycf89, ORF9*, rrs, tRNA-Ile, tRNA-Ala, rrl, rrf, tyrC*
Halamphora coffeiformis	tRNA-Pro, ycf89, rrs, tRNA-Ile, tRNA-Ala, rrl, rrf, ycf35
Halamphora vantushpaensis	psaA, psaB, tRNA-Pro, ycf89, rrs, tRNA-Ile, tRNA-Ala, rrl, rrf, psb28, syfb, thiS, tRNA-His, rps16, ycf35, rpl32

integrase/recombinase encoded by the gene labelled as *tyrC* by Hamsher et al. (2019). The case of *H. vantushpaensis* is entirely different in the sense that the extension of the IR is a consequence of the incorporation of several conserved PCGs plus one tRNA. When compared with the gene content of the other species, it appears that this extension has been done at the expense of both the LSC and the SSC, which distinguishes it from species like *Climaconeis* spp. (Gastineau et al. 2021b) among which the IR seemed to have only taken over the SSC. Indeed, among the other *Halamphora* spp., *psaA* and *psaB* are located in the LSC while the other genes belong to the SSC in which they form a well-conserved cluster.

# Molecular maximum likelihood phylogeny

Fig. 10 presents the Halamphora clade as a sub-tree derived from the threegene inferred phylogeny. The complete three-gene tree and the rbcL-only tree can be found as indicated in the data availability statement. In the three-gene tree, H. vantushpaensis strains appear as a highly supported (99%) longbranched sister group to a larger cluster composed of 18 Halamphora species, namely H. subacutiuscula, H. angustiformis, H. foramina, H. sydowii, H. tumida, H. witkowskii, H. bonnewillensis, H. americana, H. calidilacuna, H. intramaritima, H. incelebrata, H. banzuensis, H. bistriata, H. pertusa, H. subtropica plus three unidentified Halamphora species. The topology of the rbcL-inferred tree slightly differs regarding the species sister to H. vantushpaensis, which are, in this case, H. angustiformis (bv = 93) and H. subacutiuscula (bv = 96). These strains are further nested in a clade with H. maritima, H. pecensa, "Amphora" caribeana, H. exilis, H. subtropica, H. pertusa, H. banzuensis and H. bistriata with low support (bv < 50) and, together with these, sister to H. tumida, H. witkowskii, H. bonnewillensis, H. americana, H. calidilacuna, H. intramaritima, H. incelebrata, H. foramina, H. sydowii and Halamphora sp. SZCZCH45

# Discussion

# Morphological comparison with similar taxa

Halamphora vantushpaensis sp. nov. is a new species, characterized through the extensive study of two distinct cultivated clones as well as examination of wild samples. The findings indicate that the morphological characteristics of *H. vantushpaensis* can strongly vary and that LM observations might not be sufficient. Halamphora atacamana (Patrick) Levkov, *H. borealis* (Kützing) Levkov, *H. gasseae* Levkov, and *H. salinicola* Levkov and Diaz have been identified as the most similar species. In terms of outline, *H. borealis* exhibits a semi-lanceolate shape similar to *H. vantushpaensis* (Table 1). However, distinguishing features of *H. vantushpaensis* such as the larger ventral side and the indistinct striae with a higher density (more than 27 striae in 10 µm) set it apart. Additionally, SEM images reveal differences in striae composition between *H. vantushpaensis* and *H. borealis*, further supporting their taxonomic differentiation. Halamphora atacamana exhibits a similar outline, especially to smaller specimens of *H. vantushpaensis*, with slightly protracted valve endings; however, larger specimens of *H. vantushpaensis* have elongated valve ends. Halamphora atacamana also


**Figure 10.** Maximum Likelihood phylogenetic tree inferred from concatenated alignments of *psbC*, *rbcL* and *18S*. The figure represents the sub-tree that contains the *Halamphora* clade.

tends to have lower stria densities (< 28 in 10  $\mu$ m). *Halamphora gassae* and *H. salinicola* are further similar taxa, but both have smaller valves (< 35  $\mu$ m in length, < 4.5  $\mu$ m in width), lower stria densities (< 27 in 10  $\mu$ m), and smoothly arched dorsal margins. Moreover, we observed that *H. salinicola* has larger

areolae on the dorsal side, one elongate areolae on the ventral side, and a raphe ledge that extends continuously over the entire length of the valve. Among other species that might be compared with *H. vantushpaensis*, *H. sardiniensis* has smaller valves and a higher stria density (> 36 striae in 10  $\mu$ m) and strongly arched dorsal margin. Additionally, *H. sardiniensis* has elongate areolae on the dorsal side, one elongate areola on the ventral side, and a large central area on the ventral side (visible in SEM: Levkov 2009, pl.245, fig. 4). *Halamphora thermalis* has a smoothly arched dorsal margin and a more visibly tumid ventral margin. In SEM, *H. thermalis* has larger irregularly rounded elongate areolae on dorsal side and rounded areolae on ventral side and areolae become smaller toward the central area ventrally. Also, the proximal raphe endings open into larger depressions (Levkov 2009, pl. 230, figs 1–6).

# Genomic results and phylogenies

Initially, when comparing SZCZEY2166 and SZCZEY2167 by the means of LM, it was unclear whether or not they belonged to identical or different species, particularly because of the differences of shape of their apices. Of course, SEM brought supplementary clues of their identity, but in the end, molecular methods provided the decisive argument. With regards to this, it should be noted that within a single round of short-reads sequencing, complete nuclear rRNA clusters, mitochondrial genomes and plastid genomes were obtained, which allowed to derive accurate phylogenies. The *rbcL*-inferred phylogeny strictly positions H. vantushpaensis within a clade of species previously described as 'K clade' (Stepanek and Kociolek 2019). This is a noteworthy result, because this clade is known to contain species with extremely different preferences for what regards salinity, ranging from freshwater to hypersaline. As such, this clade is regarded as an interesting model to study transition between habitats, although it is noteworthy that such a transition seemed to have occurred repeatedly and independently among the genus Halamphora. The genomic approach we employed, sometimes described as 'genome-skimming', has reliably provided results on diatoms (for recent examples, see Gastineau et al. 2021c; Solak et al. 2021 ; Dabek et al. 2022 ; Yilmaz et al. 2024). Aside from their interest in molecular phylogeny, the availability of full-length RNA operon reference sequences from duly identified organisms could become increasingly valuable with the development of long-read metabarcoding (e.g. Jamy et al. 2020). In the current case, it was interesting to see that the only polymorphism between both strains of H. vantushpaensis was located in one of the internal transcribed spacers, a portion that does not participate to the final 3D structure of the ribosome and as such, is more likely to display variations.

When comparing the plastomes, the low number of polymorphisms slightly misled us at first into thinking that these SNPs might only have concerned non-coding parts. Surprisingly, it was not the case, and although the number of SNPs is rather low, interestingly, several among them were not silent. However, this variability between the two strains is consequently lower than what was observed with the previously published Lake Van-species *N. vanseea* (Yılmaz et al. 2024). A gene such as *psbC* displayed three times more polymorphisms between the two strains studied at that time when compared to what was unveiled between both strains of *H. vantushpaensis*. At the time *N. vanseea* was being investigated, it was possible to sequence the mitogenome for only one of the strains, for reasons that remain unknown but might be related to the amount of bacterial contamination in the DNA pool. This time, with *H. vantushpaensis*, sequencing of both strains was successful. We note that the *cox1* gene, which seems to be a sensitive marker for the study of diatoms at the subspecific level (Gastineau et al. 2013, 2021c; Dąbek et al. 2022) was entirely conserved in its exonic parts. The position of the *cox1* intron was perfectly conserved, unlike previous reports on other species (Gastineau et al. 2021c), but there were noticeable differences in its sequence. This is exemplified by the changes in length and sequence of the putative reverse-transcriptase it contains. Among land plants, introns in organellar genomes have been documented to be relevant as population markers (e.g. Spaniolas et al. 2010; Grosser et al. 2023), but no such work seems to exist for diatoms.

# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

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#### **Author contributions**

Conceptualisation: EY. Data curation: EY. Funding acquisition: AW. Investigation: EY, RG, CNS, EG, CL, MT, CO. Methodology: AW, RT, DGM. Project administration: CNS, AW. Supervision: AW, DGM, RT, CNS, RG. Visualisation: EY, CNS, EG. Writing - original draft: EY. Writing - review and editing: DGM, RT, CL, MT, CO, CNS, RG.

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#### **Data availability**

All sequences have been deposited and are already available on GenBank with accession numbers PP726705, PP726703, PP962256, PP962257, PP962255, PP727123. The complete 3-gene tree, the rbcL-inferred trees and the lists of sequences used for phylogeny can be found on Zenodo following this link: https://doi.org/10.5281/zenodo.12963401.

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**Research Article** 

# Insights to the taxonomy and phylogeny of the genus *Ptilagrostis* worldwide (Poaceae, Stipeae) with a key to species identification, checklist and outlines for further studies

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#### Abstract

Ptilagrostis (false needlegrass) is a genus of high-mountain grasses distributed in Central and North-East Asia, as well as in North America. The phylogenetic position of the genus Ptilagrostis within the Stipeae is well defined based on micromorphological patterns of lemma epidermis and moleculs. However, there is a lack of a comprehensive taxonomic revision of the genus in its entire distribution range. In this study, we performed comprehensive analyses using integrative taxonomic methods, aiming at both micromorphological and macromorphological analyses, and molecular analyses based on SNPs obtained from DArT genome-wide sequencing, in which we considered all taxa representing the genus in order to establish phylogenetic relationships between its members. We analysed all species possessing the characteristic 'Ptilagrostis pattern' of the lemma epidermis, with a particular reference to species possessing the terminal segment of the awn (seta) covered by short (up to 1 mm long) hairs that, until now, were treated as representatives of the genus Achnatherum. Following with the result of our molecular, morphological and anatomical analyses, the genus Ptilagrostis is represented by 15 species, one subspecies and five varieties organised in three well supported phylogenetic clades corresponding to the three sections: Ptilagrostis, Barkworthia and Chenella. In this paper, we provide an original key to identifying false needlegrass species, together with a checklist containing the intrageneric speciesorganisation. In addition, for each species, we present the data regarding nomenclatural types, morphological description, and information on the geographical distribution, habitat preferences and altitudinal ranges. We proposed two new varieties within the genus Ptilagrostis, P. glabrifolia var. himalayensis and P. concinna var. xizangensis, and the new section Chenella comprising three species with awns scabrous or covered by very short hairs up to 0.3 mm long. Additionally, we transfer Stipa chingii to Ptilagrostis chingii, Achnatherum chingii var. laxum to P. chingii var. laxum, and Ptilagrostis concinna subsp. schischkinii to P. junatovii var. schischkinii. Lectotypification was made for three taxa, Stipa mongholica var. minutiflora, P. czekanowskii, and P. tibetica.

**Key words:** Distribution, false needlegrass, micromorphology, morphology, phylogeny, *Ptilagrostis*, taxonomy



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#### Introduction

Grasses (Poaceae) are one of the most prevalent flowering plants, thriving on every continent worldwide (Gibson 2009; Hodkinson 2018). With an impressive presence, they rank as the fifth most abundant family among angiosperms, boasting a rich diversity of approximately 11,000 species spread across over 700 genera (Gibson 2009; Bhatt and Thaker 2021). Within the family, the Stipeae emerges as a noteworthy and widely distributed group. Species representing the tribe are found in grassland ecosystems across nearly all continents, excluding Antarctica (Tzvelev 1977; Romaschenko et al. 2012; Nobis et al. 2020; Barkworth 2007; Cialdella et al. 2010, 2013; Everett et al. 2009). Recognised as a monophyletic and well-defined taxon, Stipeae contributes significantly to the global diversity of grasses (Soreng et al. 2022). There are approximately 680 species within Stipeae (Barkworth 2007), organised into 21 to 28 genera (Romaschenko et al. 2008; Peterson et al. 2019; Soreng et al. 2022).

One of the genera representing Stipeae and comprised of high mountain species occurring on fresh and wet meadows, swards, alpine steppes, rocky grasslands, and screes is Ptilagrostis Griseb. (false needlegrass) (Tzvelev 1976; Wu and Phillips 2006; Johnston 2006; Zhang et al. 2016a, 2016b, 2017; Tzvelev and Probatova 2019; Nobis et al. 2019a). In the middle of the 19th century, Grisebach (1852) described the genus Ptilagrostis, into which he transferred Stipa mongholica Turcz. ex Trin. The representatives of the genus Ptilagrostis are characterised by having erect stems, spikelets with solitary florets, short, plumose and geniculately-bent awns, and lemmas discontinuously covered by long hairs, usually scabrous and hairless in the middle part (Tzvelev 1976; Freitag 1985; Barkworth 1983; Romaschenko et al. 2012; Tzvelev and Probatova 2019; Nobis et al. 2019a). The current species-organisation within the genus Ptilagrostis is based on a combination of macromorphology, micromorphological patterns of the lemma epidermis, and molecular analyses. To date, species representing the genus Ptilagrostis are divided into two sections, namely sect. Ptilagrostis and sect. Barkworthia M. Nobis, A. Nobis & A. Nowak (Nobis et al. 2015).

Micromorphological patterns of the lemma epidermis (LEP) are regarded as conservative and important for understanding evolutionary relationships within Stipeae (Tzvelev 1977; Barkworth and Everett 1987; Romaschenko et al. 2012; Nobis and Nobis 2013; Nobis et al. 2019a, 2020). Within this tribe, two types of LEP are found. The first one, called the maize-like epidermal pattern, is characterised by numerous, square to rounded silica bodies and short fundamental cells, and occurs in representatives of achnatheroid grasses (Romaschenko et al. 2012, 2014; Nobis et al. 2019a, 2020). The second, called saw-like epidermal pattern, is characterised by elongated fundamental cells and reniform, ovate, oblong to elongated silica bodies sometimes associated with cork cells. This type of pattern occurs in stipoid grasses, such as Stipa L., Orthoraphium Nees, Neotrinia (Tzvelev) M. Nobis, P.D. Gudkova & A. Nowak, Trikeraia Bor, Piptatherum P. Beauv. and Ptilagrostis, although there are slight differences among particular genera (Romaschenko et al. 2012, 2014; Nobis et al. 2019a, 2019b, 2020). Based on the LEP analysis, several species have recently been transferred from the genera Stipa, Piptatherum and Ptilagrostis to Achnatherum P. Beauv. and from the genus Achnatherum to Neotrinia or Ptilagrostis (Nobis and Nobis 2013; Banfi et al. 2018; Nobis et al. 2019a, 2019b, 2020). However,

patterns of the lemma micromorphology are still understudied in many species, and further research is required to identify their generic affiliation. To date, within the genus *Ptilagrostis*, lemma micromorphology has been analysed in eight species: *P. alpina* (F. Schmidt) Sipliv., *P. concinna* (Hook. f.) Roshev., *P. contracta* Z.S. Zhang & W.L. Chen, *P. duthiei* (Hook.f.) M.Nobis & P.D.Gudkova, *P. malyschevii* Tzvelev, *P. mongholica* (Turcz. ex Trin.) Griseb., *P. porteri* (Rydb.) W.A. Weber and *P. yadongensis* Keng & Tang (Barkworth 1983; Romaschenko et al. 2012, 2014; Nobis and Nobis 2013; Nobis et al. 2019a, 2019b, 2020; Zhang et al. 2017). Species belonging to the genus *Ptilagrostis* are characterised by having elongated fundamental cells with sinuate to lobate sidewalls, frequent silica bodies with constricted side walls and cork cells (Romaschenko et al. 2012, 2014; Nobis and Nobis 2013; Nobis et al. 2019a). The pattern of lemma micromorphology confirmed both the affiliation of *Ptilagrostis pelliotii* (Danguy) Grubov to *Achnatherum*, as well as *Achnatherum duthiei* (Hook. f.) P.C. Kuo & S.L. Lu and *Stipa bhutanica* Noltie to *Ptilagrostis* (Nobis et al. 2019a, 2020).

Characters of leaf anatomy play an important role in taxonomy within the Poaceae. Examples include: the outline of the leaf cross-section, number of ribs, presence of bulliform cells, arrangement of sclerenchyma in relation to the vascular bundles and number of vascular bundles (Renvoize 1985; López and Devesa 1991; Conert 1998; Namaganda et al. 2009; Martínez-Sagarra et al. 2017). Their application aims to improve the taxonomy of morphologically very similar species. Leaf anatomy was utilised mainly to examine the genus *Festuca* L. and other taxonomically challenging genera within the Poaceae, such as *Stipa, Anthoxanthum* L. or *Sesleria* Scop. (e.g., Pimentel and Sahuquillo 2003; Kuzmanović et al. 2009; Namaganda et al. 2009; Martínez-Sagarra et al. 2017; Gudkova et al. 2023). Analysis of the morphological structures of leaves has also been applied in some species of the genus *Ptilagrostis*, including *P. concinna*, *P. junatovii* Grubov, *P. malyschevii*, *P. mongholica* and *P. porteri* (Malyschev 1965; Tzvelev 1974; Barkworth 1983).

Recently, molecular studies have shed light on the phylogenetic relationships of the Stipeae and have also led to further changes in the taxonomy of the genus *Ptilagrostis*. The latest studies, based on the ITS and cpDNA sequences, were conducted by Romaschenko et al. (2008, 2012, 2014), Hamasha et al. (2012), and Zhang et al. (2017). They analysed 13 species representing the genus *Ptilagrostis*, however, in different combinations and with different sets of species in particular studies. Based on the results of molecular analyses, Hamasha et al. (2012) transferred *Ptilagrostis pelliotii* (Danguy) Grubov to *Achnatherum*, whereas Peterson et al. (2019) transferred *P. kingii* to *Ptilagrostiella*. However, in most cases, analyses failed to resolve intrageneric relationships between taxa since some of the species were organised in polytomies (Hamasha et al. 2012; Zhang et al. 2017) or the findings obtained from plastid and nuclear sequences were not consistent (Romaschenko et al. 2014). Thus, further research that takes into account traditional macro- and micromorphological studies combined with modern wide-genome analyses is needed.

Previous studies on the genus *Ptilagrostis* were often limited to specific geographic regions, selected species or subsets of species. In this study, we aim to fill this gap by summarising all of the *Ptilagrostis* species worldwide. Because there is a lack of current comprehensive treatment of taxa representing this genus, the main goals of this study are to provide: i) morphological and molecular analysis (latest based on SNPs derived from genome-wide DArT sequencing) of the species representing *Ptilagrostis* with the particular reference to the species having upper segments of the awn scabrous or covered with up to 1 mm long hairs; ii) micromorphological analysis of the lemma epidermal structures of the members of this genus; iii) analysis of vegetative leaves' cross-sections; iv) an identification key for all members of the genus; v) taxonomic and no-menclatural summary that also includes morphological description, notes on habitat preferences and distribution of particular false needlegrasses; (vi) intrageneric species organisation.

# Methods

# Plant material

The research was conducted utilising plant material preserved in the following herbaria: Academy of Science, Uzbekistan Central Herbarium (TASH), Botanische Staatssammlung München Herbarium (M), Herbarium of the Institute of Botany, Jagiellonian University (KRA), Institute of Botany, Chinese Academy of Sciences Chinese National Herbarium in Beijing (PE), Kunming Institute of Botany, Chinese Academy of Sciences Herbarium (KUN), Ludwig-Maximilians-Universität München Herbarium (MSB), Missouri Botanical Garden Herbarium (MO), Herbarium of the Institute of Applied Ecology, Academia Sinica in Shenyang, China (IFP), P.N. Krylov Herbarium of Tomsk State University (TK), Herbarium of the Institute of Botany, Kyrgyz Academy of Sciences in Bishkek (FRU), Herbarium of the Institute of Botany, Kazakh Academy of Sciences in Almaty (AA), Herbarium of the Moscow State University (MW), Central National Herbarium known also as CNH or Calcutta herbarium in India (CAL), Museum of Evolution in Uppsala (UPS), New York Botanical Garden Herbarium (NY), Royal Botanic Garden Edinburgh Herbarium (E), Royal Botanic Gardens Herbarium in Kew (K), The Herbarium at the Natural History Museum, London (BM), University of Colorado Museum Herbarium (COLO), Utah State University Intermountain Herbarium (**UTC**), V.L. Komarov Botanical Institute Herbarium in St. Petersburg (LE) (acronyms of the herbaria are used according to Index Herbariorum, Thiers 2024). We reviewed over 400 herbarium specimens representing all species of Ptilagrostis during these studies.

#### Morphological measurements and multivariate analyses

The herbarium material was examined using biometric analysis. Measurements were conducted using a stereomicroscope (Nikon SMZ800) with a graduated scale eyepiece and ruler. In total, we used 342 selected, well-developed and undamaged specimens for morphological measurements. In detail, we examined 281 specimens of *Ptilagrostis* sect. *Ptilagrostis*, species and the number of specimens for each are as follows: *P. alpina* (8), *P. arcuata* Z.S. Zhang & W.L. Chen (4), *P. concinna* (25), *P. concinna* var. *xizangensis* M. Nobis & Krzempek (2), *P. dichotoma* Keng ex Tzvelev var. *dichotoma* (38), *P. dichotoma* var. *roshevitsiana* Tzvelev (5), *P. glabrifolia* X.Y. Zhang & W.L. Chen var. *glabrifolia* (5), *P. glabrifolia* var. *himalayensis* M. Nobis & Krzempek (2), *P. junatovii* var. *schischkinii* (Tzvelev) M. Nobis &

Krzempek (1), P. luquensis P.M. Peterson, Soreng & Z.L. Wu (4), P. malyschevii (84), P. mongholica subsp. mongholica (63), P. mongholica subsp. porteri (Rydb.) Barkworth (6) and P. tibetica (Mez) Tzvelev (8). Moreover, we included 62 specimens of false needlegrasses having awn with hairs on seta up to 1 mm long, including 20 specimens representing sect. Barkworthia with P. bhutanica (Noltie) M. Nobis (11) and P. yadongensis (9), and 42 specimens representing sect. Chenella, described below, with specimens of P. chingii (Hitchc.) M. Nobis & Krzempek (27), P. contracta Z.S. Zhang & W.L. Chen (6) and P. duthiei (9). Moreover, we included six specimens of Ptilagrostiella kingii (Bol.) Romasch. as an outgroup. List of examined specimens is presented in Suppl. material 1. Each specimen was analysed on the basis of 28 quantitative characters (length of the lemma and palea, length of lemma lobes, callus length, callus base length and width, length of hairs on the dorsal part of the callus, length of hairs on the ventral part of the callus, awn length, length of lower segment of the awn (column), length of terminal segment of the awn (seta), width of the awn base, length of hairs on the lower segment of the awn (in the middle part of the segment), length of hairs on the terminal segment of the awn (near geniculation), length of culms, length of vegetative leaves, width of the leaves, number of vascular bundles on the leaf cross-section, length of ligule on the lower culm sheath, length of ligule on the middle culm sheath, length of ligule on the upper culm sheath, length of the longest ligules on the external leafsheaths on the vegetative shoot, length of the longest ligules on the internal leaf-sheaths on the vegetative shoot, length of bracts below the panicle, length of panicle, length of the lower pedicles within the panicle, length of the lower glume, length of the upper glume, number of spikelets in lower pedicle within the panicle) and nine qualitative traits (character of the lower, middle and upper culm sheaths (glabrous/pubescent), character of leaf-sheaths on the vegetative shoots (glabrous/pubescent), width of panicle (narrow/wide), character of pedicles (glabrous/pubescent), character of the abaxial and adaxial surface of leaves (glabrous, scabrous, pilose), presence of hairs on the top of the anthers). In addition, three ratios were measured: the length of the lemma to the length of the palea, the length of hairs on the lower segment of the awn to the length of hairs on the terminal segment of the awn and the length of the lower glume to the length of upper glume. In accordance with the principles of numerical taxonomy, every individual specimen was treated as an operational taxonomic unit (OTU) (Sokal and Sneath 1963). Of 40 characters studied, the 10 most informative traits (i.e., those having the strongest factor loadings (>0.60) that allowed the best distinction of the Ptilagrostis species) were selected in the Principal Component Analysis (PCA) of the entire dataset and 18 key morphological characters, that led to distinguish examined taxa, were used in the UPGMA analysis (Table 1). Moreover, to visualise the differences in the species group that have a terminal segment of the awn (seta) covered with up to 1 mm long hairs and belonging to sections Chenella and Barkworthia, 11 characters allowed for the best distinction for analysed species, and at the same time having the strongest factor loadings (>0.60), were chosen for the final PCA. Due to generally high morphological similarity among particular Ptilagrostis representatives, we decided to also use qualitative characters in addition to the quantitative characters. Thus, for the Principal Coordinates Analysis (PCoA) we selected 12 of the most important

characters (11 quantitative and one qualitative), enabling better differentiation of the studied species. The characteristics selected for analysis are presented in Table 1. The findings derived from all biometric studies are summarised in the key to species identification and in morphological descriptions of the taxa. In order to reveal significant differences among the means of characters across investigated species having up to 1 mm long hairs on the seta, a Kruskal-Wallis test was performed for all characters included in PCA. Subsequently, post-hoc evaluations were conducted using a multiple comparison test. The analyses were performed in Statistica 13 (TIBCO Software, USA) and PAST v. 4.03 (Hammer et al. 2001).

# **Micromorphology analysis**

The upper surface of the lemma epidermis was subjected to micromorphological observations. Samples were obtained from the middle part of the panicle and were examined from the base to the top. The presence, location and shape of prickles and hooks, the distribution and length of macro-hairs, the length and shape of long cells and the shape of silica bodies were examined. Using a JCF-1100E ion sprayer (JEOL, Japan), the dried material was coated with gold and then photographed under various magnifications on a Hitachi S-4700 scanning electron microscope. Measurements were taken using ImageJ software (LOCI, University of Wisconsin, USA). Terminology was adopted from Nobis et al. (2019a, 2020). Studied samples are indicated in Suppl. material 1.

#### Vegetative leaf cross-section analysis

Cross-sections through the middle part of the leaf blade of the studied species were made using a razor blade, followed by microscopic observations under a Nikon Eclipse 80i compound microscope. Leaf blade length and width, number of vascular bundles and ribs, location and appearance of sclerenchyma, and presence of hairs were analysed. Specimens of the following species are represented in the analysis: *P. alpina* (8), *P. arcuata* (3), *P. bhutanica* (9), *P. chingii* (27), *P. concinna* (15), *P. contracta* (2), *P. dichotoma* (35), *P. duthiei* (5), *P. malyschevii* (81), *P. mongholica* (57) and *P. yadongensis* (8) were reviewed. Studied samples are indicated in Suppl. material 1.

# Genomic library preparation and DArT sequencing

Molecular analyses were based on 31 samples belonging to *P. alpina*, *P. arcuata*, *P. bhutanica*, *P. chingii*, *P. concinna*, *P. contracta*, *P. dichotoma* var. *dichotoma*, *P. dichotoma* var. *roshevitsiana*, *P. duthiei*, *P. glabrifolia*, *P. junatovii*, *P. luquensis*, *P. malyschevii*, *P. mongholica* and *P. yadongensis* (see Suppl. material 1). Two additional samples of *Ptilagrostiella kingii* were selected for outgroup comparison. Whole genomic DNA was isolated using a Genomic Mini AX Plant Kit (A&A Biotechnology, Poland). NanoDrop One (Thermo Scientific, USA) was used to perform the quantification check. Following the DArTseq methodology, each sample was diluted up to a concentration of 50–100 ng/µL. Purified DNA (1–2 µg for each sample) was shipped to Diversity Arrays Technology Pty Itd (Canberra, Australia) for sequencing and marker identification.

Abbreviation	Character	PCA	UPGMA	PCA*	PCoA*
PL	Length of palea (mm)	+	+	+	+
L/P	Ratio: length of lemma to the length of palea	+	+	+	+
LL	Length of the lobes (mm)		+	+	+
AL	Length of the awn (mm)	+	+	+	+
UL	Length of hairs on the terminal segment of the awn (seta) (mm)	+	+	+	+
HR	Length of hairs on the lower segment of the awn (column) to length of hairs on the terminal segment of the awn (seta) ratio (mm)	+	+		
CL	Length of culm (cm)	+	+	+	+
LW	Width of the leaf (mm)		+	+	+
CN	Number of vascular bundles		+	+	+
IL	Length of the longest ligules on the internal leaf-sheaths on the vegetative shoot	+	+		
PeL	Length of the lower pedicle within the panicle (cm)	+	+	+	+
LP	Length of panicle	+			
GL	Length of the lower glume (mm)		+	+	+
GU	Length of the upper glume (mm)	+			
GL/U	Ratio: length of lower glume to length of upper glume		+	+	+
PW	Width of the panicle		+		+
HA	Presence of hairs on the top of the anthers		+		
HL	Presence of hairs on the lower segment of the awn		+		
НМ	Presence of hair on the middle part of the lemma		+		
HP	Presence of hairs on the pedicles		+		
PB	Presence of bracts below the panicle		+		
No. of characters examined		10	19	11	12

**Table 1.** Morphological characters used in the numerical analyses provided for all the taxa representing *Ptilagrostis*. Analyses marked with the asterix (\*) were done for the *Ptilagrostis* species having seta covered with hairs up to 1 mm long.

DArTseq is a hybrid of DArT complexity reduction techniques with next-generation sequencing technologies, tuned for each organism and application to pick the most appropriate complexity reduction strategy (Sansaloni et al. 2011; Kilian et al. 2012; Cruz et al. 2013). Based on the results of testing several enzyme combinations for complexity reduction for *Ptilagrostis*, Diversity Arrays Technology Pty Ltd. chose the Pstl-Msel enzyme combination. This section was performed according to the procedures previously published (Baiakhmetov et al. 2020; Nobis et al. 2023; Sinaga et al. 2024). All DNA samples were processed in digestion/ligation reactions as described by Kilian et al. (2012), but replacing a single Pstl-compatible adaptor with two different adaptors corresponding to two different restriction enzyme overhangs. The Pstl-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence, and "staggered", varying length barcode region, similar to the sequence reported previously (Elshire et al. 2011). The reverse adapter contained a flowcell attachment region and Msel-compatible overhang sequence. Only "mixed fragments" (Pstl-Msel) were effectively amplified by PCR using an initial denaturation step of 94 °C for 1 min, followed by 30 cycles with the following temperature profile: denaturation at 94 °C for 20 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s, with an additional final extension at 72 °C for 7 min. After PCR, equimolar amounts of amplification products

from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina, USA) bridge PCR, followed by sequencing on Hiseq2500 (Illumina, USA). The sequencing (single read) was run for 77 cycles. Sequences generated from each lane were analysed utilising proprietary DArT analytical pipeline methods. The poor-quality sequences were filtered away from fastq files, with more stringent selection criteria for the barcode region than for the rest of the sequence. Thanks to this, the assignments between the sequences and specific samples during the "barcode split" were reliable. During the marker calling step, ca. 2.5 mln sequences per barcode/sample were identified. As a result, short read sequences were obtained, which, after removing the restriction site-associated adapter, were 20–69 bp long, depending on the quality.

#### **SNP data analysis**

For the downstream analyses, we applied co-dominant single nucleotide polymorphisms (SNP) markers, which were analysed using the RStudio package "dartR" (Gruber et al. 2018) and "devtools" (Wickham et al. 2022). Data filtering included the following steps: 1) removing all monomorphic loci, 2) removing loci identified (=called) in less than 95% of all individuals, 3) removing loci with reproducibility below a predetermined threshold (<1), 4) filtering sequence tags contained more than one SNP, to keep randomly selected one of them, and 5) filtrating loci based on the criteria of a minor allele frequency (MAF) (threshold 1%). Maximum Likelihood (ML) tree was generated based on 12,502 SNP loci (processed using R) for 33 samples of seventeen taxa (P. alpina, P. arcuata, P. bhutanica, P. chingii, P. concinna, P. contracta, P. dichotoma var dichotoma, P. dichotoma var. roshevitsiana, P. duthiei, P. glabrifolia, P. junatovii, P. luquensis, P. malyschevii, P. mongholica, P. yadongensis and Ptilagrostiella kingii as outgroup). The genlight object was converted to the FASTA file (package dartR), and heterozygous locations were replaced with standard ambiguity codes. The FASTA file was then analysed using MEGA version 11.0.13 (Tamura et al. 2021), with the GTR (General Time Reversible) model chosen as the best-fitting substitution model based on AIC values and the bootstrap method as the phylogenetic test with 1,000 replications.

# Results

# **Numerical analysis**

Principal Component Analysis (PCA) based on 10 most informative quantitative characters (PL, L/P, AL, UL, HR, CL, IL, PeL, LP, GU) for all species representing *Ptilagrostis*, revealed that the first three principal components accounted for 77.14% of all character variation (Table 2) (PC1–39.11%, PC2–25.32%, PC3–12.71%). A high correlation ( $\ge 0.7$ ) with the first axis is displayed by four characters: PL, CL, LP and GU, while the one, AL, is highly correlated with the second axis (Table 2). The most significant values of H statistics were observed for AL and UL. The scattered diagram of principal components defined by the first two axes PC1 vs. PC2 constitutes the best two-dimensional representation of the data and shows within the cloud of points the three groups (Fig. 1a). The first group located in the right part of the plot, comprises the taxa representing

Character	PC1	PC2	PC3	H value	p value
PL	-0.86	0.04	0.01	224.80	<0.05
L/P	-0.21	0.45	-0.72	229.92	<0.05
AL	0.07	-0.74	-0.45	270.88	<0.05
UL	0.62	-0.59	0.02	250.31	<0.05
HR	-0.61	0.47	-0.26	131.44	<0.05
CL	-0.79	-0.36	0.32	197.82	<0.05
IL	-0.21	-0.66	-0.58	228.23	<0.05
LP	-0.80	-0.41	0.24	216.45	<0.05
PeL	-0.67	-0.56	0.02	191.36	<0.05
GU	-0.75	0.40	-0.07	218.86	<0.05
PL	-0.86	0.04	0.01	224.80	<0.05

**Table 2.** Results of the Principal Component Analysis (PCA) of all studied species of *Ptilagrostis* for ten morphological characters (the highest factor loadings are bolded); Kruskal-Wallis test with H and p values. For character abbreviations, see Table 1.

sect. Ptilagrostis, whereas in the left part of the diagram there are two discreet groups comprising OTUs of the five species P. bhutanica P. chingii, P. contracta, P. duthiei and P. yadongensis that represent sections Barkworthia and Chenella. These species are characterised by the upper segment of the awn (seta) covered with short (up to 1 mm long) hairs. A similar organisation of Ptilagrostis representatives divided into three main clades (Fig. 2) is revealed by the cluster analysis (UPGMA) based on 19 quantitative and qualitative morphological characters (Table 1). For better readability we presented in the diagram up to 20 specimens per examined taxon (Fig. 2; complete result is presented in Suppl. material 2: fig. S1). Ptilagrostiella kingii differed from all analysed false needlegrasses by having the first segment of the awn covered by short, ca. 0.2 mm long, and hard prickles as opposed to hairs 0.4–3.0 mm long and soft prickles. Ptilagrostis species are divided into three clades. The first clade includes OTUs of P. bhutanica and P. yadongensis that represent sect. Barkworthia, while the second comprises the OTUs of P. chingii, P. contracta and P. duthiei that are members of the sect. Chenella. These two clades are sister to the third clade that is most numerous in species and includes all the remaining Ptilagrostis species and comprises sect. Ptilagrostis. These OTUs are organised into two subsequent subclades. The first includes P. alpina, P. arcuata, P. concinna var. concinna, P. concinna var. xizangensis, P. dichotoma var. dichotoma, P. dichotoma var. roshevitsiana, P. junatovii var. junatovii, P. junatovii var. schischkinii and P. malyschevii. The second subclade includes P. glabrifolia var. glabrifolia, P. glabrifolia var. himalayensis, P. luquensis, P. mongholica subsp. mongholica, P. mongholica subsp. porteri and P. tibetica.

Separate PCA and PCoA analyses were carried out to clarify the variation among *Ptilagrostis* species with terminal awn segment (seta) covered with hairs up to 1 mm long (Fig. 3). The PCA analysis, based on 11 quantitative characters (PL, L/P, AL, LL, UL, CL, LW, CN, PeL, GL, GL/U), with the highest scores of the first three principal components provides the greatest contribution to explaining differences within and between groups. Variation within examined species is best explained by four of 11 traits ( $\geq$  0.7, Table 3). The first three components explain 76.83% of all trait variation (PC1–34.77%, PC2–27.60%, PC3–14.46%).



**Figure 1.** Scatter plot of (**a**) the Principal Component Analysis (PCA) performed on 10 quantitative characters (**b**) with projection of the variables on the factor plane PC1 × PC2 for all *Ptilagrostis* species and all specimens examined. List of specimens examined is presented in Suppl. material 1.

**Table 3.** Results of the Principal Component Analysis (PCA) of the studied *Ptilagrostis* species representing sect. *Barkworthia* and sect. *Chenella*, for 12 morphological characters (the highest factor loadings are bolded); Kruskal-Wallis test with H and p values. For character abbreviations, see Table 1.

Character	PC1	PC2	PC3	H value	p value
PL	0.89	0.05	0.03	36.42	<0.05
L/P	-0.65	-0.50	-0.36	41.11	<0.05
LL	0.30	0.61	0.62	50.76	<0.05
AL	0.20	-0.72	0.30	25.20	<0.05
UL	0.47	-0.50	0.37	26.25	<0.05
CL	0.87	0.34	0.00	43.01	<0.05
LW	0.52	-0.65	-0.35	44.56	<0.05
CN	0.68	-0.50	-0.40	49.72	<0.05
PeL	0.68	0.36	-0.08	27.52	<0.05
GL	0.31	-0.74	0.37	34.66	<0.05
GL/U	-0.44	-0.43	0.65	26.56	<0.05

A high correlation with the first axis is displayed by two characters: PL and CL, while the two characters, AL and GL, are highly correlated with the second axis (Table 3). The PCA scatter plot of the first two axes showed distinctly separate clusters comprising OTUs in four groups (Fig. 3a). The PCoA analysis performed using one qualitative and 11 quantitative characters shows a separation of five groups of OTUs corresponding to each of the examined species (Fig. 3b). Significant differences were detected across all quantitative characters studied also according to the Kruskal-Wallis test (Table 3). The most significant values



Figure 2. Cluster analysis (UPGMA) of selected specimens of all *Ptilagrostis* species. List of specimens examined is presented in Suppl. material 1.

of H statistics were observed for L/P, LL, CL, LW and CN. The results of multiple comparison post-hoc tests are presented in Table 4. Various characters were identified as significant depending on the taxon. All the examined characters proved effective in distinguishing at least one pair of taxa. The most distinguishable species combinations were *P. chingii – P. yadongensis* and *P. duthiei – P. yadongensis* differed in nine and eight characters, respectively. Five characters were able to distinguish among the species combinations of *P. bhutanica – P. chingii, P. bhutanica – P. contracta* and *P. contracta – P. chingii,* four characters to distinguish among *P. bhutanica – P. duthiei, P. bhutanica – P. duthiei, None of the characters examined effectively distinguished the <i>P. contracta – P. duthiei* pair of species (Table 4).



**Figure 3.** Scatter plot of (a) the Principal Component Analysis (PCA) performed on eleven quantitative characters with projection of the variables on the factor plane PC1 × PC2 for *Ptilagrostis* species with seta covered with short hairs up to 1 mm long (b) the Principal Coordinates Analysis (PCoA) performed on one qualitative and eleven quantitative characters with projection of the variables on the factor plane PC1 × PC2.

	post-hoc test									
Character	bhu-chin	bhu -con	bhu -dut	bhu -yad	chin-con	chin-dut	chin-yad	con-dut	con-yad	dut-yad
PL	+	+	+	ns	ns	ns	+	ns	+	+
L/P	+	+	+	ns	ns	ns	+	ns	ns	+
LL	+	+	ns	ns	ns	+	+	ns	ns	ns
AL	ns	ns	ns	ns	+	ns	+	ns	ns	ns
UL	ns	ns	ns	+	ns	ns	+	ns	ns	+
CL	+	+	+	ns	ns	ns	+	ns	+	+
LW	+	ns	ns	ns	+	+	ns	ns	+	+
CN	ns	ns	ns	+	+	+	ns	ns	+	+
PeL	ns	ns	+	ns	ns	ns	+	ns	ns	+
GL	ns	+	ns	+	+	+	+	ns	ns	ns
GL/U	ns	ns	ns	+	ns	ns	+	ns	+	+
No. of significant differences	5	5	4	4	4	4	9	0	5	8

**Table 4.** The results of multiple comparison post-hoc tests: + – significant, p < 0.05, ns – not significant (abbreviations: *Ptilagrostis bhutanica* – bhu, *P. chingii* – chin, *P. contracta* – con, *P. duthiei* – dut, *P. yadongensis* – yad). For character abbreviations, see Table 1.

#### Micromorphology of the lemma epidermis

All of the studied species representing sections *Barkworthia*, *Chenella* and *Ptilagrostis* possessed a saw-like lemma epidermal pattern. The majority of cells that build the upper (abaxial) lemma epidermis are long cells with a rectangular, oblong shape (Figs 4b, e, h, k, n, r, 5b, e, h, k, n, r). The side walls of the long cells are raised with  $\Omega$ -shaped indentations. Silica bodies are rectangular to elongated in shape with sinuate edges constricted one, two or (less commonly) three times. Silica bodies are usually adjacent to cork cells, these latter being more or less frequently distributed than the silica bodies. Hooks are heterogeneously distributed, frequently near the base and the apex of the lemma, and usually absent in the middle section. Moving closer to the lemma apex, the hooks turn into prickles. Macro-hairs are straight and cylindrical (Figs 4a, d, j, m, p, 5a, d, j, m, p). In all the species studied, the lemma, beyond its central part, is covered with macro-hairs. The only exceptions are *P. glabrifolia* and *P. tibetica*, which have hairs distributed throughout the lemma. The hairs reach the top of the lemma, forming there an irregular corolla of hairs (Figs 4c, f, i, I, o, s, 5c, f, i, I, o, s).

#### Leaf cross-section analysis

The examined Ptilagrostis species differ in the number of vascular bundles present in the vegetative leaf and the number of ribs on its adaxial (upper) surface. Species in sect. Barkworthia had between three and seven vascular bundles with P. bhutanica usually possessed seven (rarely five) (Fig. 6i), and P. yadongensis with three or five (Fig. 6m, n). Species belonging to sect. Chenella usually had the highest number of vascular bundles. Ptilagrostis duthiei had nine (less frequently 7, 8 or 11; Fig. 6a, b), while the specimens of P. contracta had 11-14 vascular bundles (Fig. 6c, d). The only exception in section Chenella was P. chingii with five (rarely three) vascular bundles (Fig. 6g, h). Among the core Ptilagrostis species (sect. Ptilagrostis), P. malyschevii (Fig. 6e) and P. alpina (Fig. 6f) have the largest number of vascular bundles. Seven (rarely five) vascular bundles were recorded in specimens of P. malyschevii (Fig. 6e). In contrast, five vascular bundles were most common in P. alpina, with seven occurring occasionally (Fig. 6f). The leaves of P. malyschevii are noticeably wider in comparison with the remaining species of sect. Ptilagrostis. Ptilagrostis concinna (Fig. 6l), P. arcuata (Fig. 6k) and P. junatovii (Fig. 6j) are characterised by having a very similar pattern of leaf cross-sections with the presence of three to five vascular bundles, however, having three is more common in P. arcuata than in the cases of the other species mentioned. Three vascular bundles were present in both *P. mongholica* subsp. *mongholica* (Fig. 6p) and *P. mongholica* subsp. porteri. Ptilagrostis dichotoma (Fig. 6r) had three or rarely five vascular bundles. Almost all specimens of P. glabrifolia (Fig. 6t) had five vascular bundles with one exception that had three. Specimens of P. tibetica mostly had three (rarely 5) vascular bundles (Fig. 6s), whereas P. luquensis had three or five vascular bundles (Fig. 6o). In all studied species, short hairs or prickle-hairs were observed on the adaxial surface of leaves (on the ribs), while the abaxial surface was covered usually by more or less densely distributed prickles and thus, they were scabrous. Some specimens of P. chingii occur with glabrous or almost glabrous abaxial surface of leaves (as opposed to normally being scabrous).



**Figure 4.** SEM morphology of the floret of *Ptilagrostis tibetica* (a-c), *P. bhutanica* (d-f), *P. chingii* (g-i), *P. contracta* (j-l), *P. duthiei* (m-o) and *P. yadongensis* (p-s). Callus and the lower part of the lemma (a, d, g, j, m, p), epidermal pattern of the middle part of lemma (b, e, h, k, n, r), top of lemma (c, f, i, l, o, s). Abbreviations: c = cork cell, h = hook, l = long cell, s = silica body, m = macro-hair.



Figure 5. SEM morphology of the floret of *Ptilagrostis concinna* (a-c), *P. dichotoma* (d-f), *P. junatovii* (g-i), *P. luquensis* (j-l), *P. mongholica* subsp. *mongholica* (m-o) and *P. mongholica* subsp. *porteri* (p-s). Callus and the lower part of the lemma (a, d, g, j, m, p), epidermal pattern of the middle part of lemma (b, e, h, k, n, r), top of lemma (c, f, i, l, o, s). Abbreviations: c = cork cell, h = hook, l = long cell, s = silica body, m = macro-hair.



**Figure 6.** Cross-sections through vegetative leaves of *P. duthiei* (**a**, **b**), *P. contracta* (**c**, **d**), *P. malyschevii* (**e**), *P. alpina* (**f**), *P. chingii* (**g**, **h**), *P. bhutanica* (**i**), *P. junatovii* (**j**), *P. arcuata* (**k**), *P. concinna* (**l**), *P. yadongensis* (**m**, **n**), *P. luquensis* (**o**), *P. mon-gholica* subsp. mongholica (**p**), *P. dichotoma* (**r**), *P. tibetica* (**s**), *P. glabrifolia* (**t**). Abbreviations: (1) sclerenchyma strand, (2) ribs, (3) vascular bundles, (4) prickle-hairs. Scale bars: 100 µm. List of studied specimens is presented in Suppl. material 1.

*Ptilagrostis junatovii* and *P. luquensis* usually has glabrous leaves or rarely slightly scabrous. Whereas *P. concinna*, *P. glabrifolia* and *P. contracta*, have always glabrous and smooth leaves.

The widest leaves were noted in *P. contracta* and *P. duthiei* (0.6–1.2 mm; Table 5), while the narrowest in *P. arcuata*, *P. chingii*, *P. dichotoma*, *P. glabrifolia*, *P. luquensis*, *P. mongholica* subsp. *mongholica*, *P. mongholica* subsp. *porteri*, *P. tibetica*, and *P. yadongensis* (0.2–0.5 mm). In the remaining analysed species, the width of leaves ranged from 0.3 to 0.7 mm (Table 5).

Most of the studied species had discontinuous sclerenchyma strands (Fig. 6a, b, e–n). Occasionally, an almost continuous, thin layer of sclerenchyma was observed in *P. concinna*. The only species consistently with a continuous or slightly discontinuous (torn) layer of sclerenchyma was *P. contracta* (Fig. 6c, d). A somewhat similar sclerenchyma layer was observed in *P. malyschevii*, which also has a thick layer of it, but it never merges into a continuous ring (Fig. 6e). The main morphological differences in leaves' cross-sections between all *Ptilagrostis* species are summarised in Table 5.

Species	Width of the leaf (mm)	Number of vascular bundles	Character of abaxial leaf surface	Character of sclerenchyma layer	
P. alpina	0.3-0.5	5(-7)	slightly scabrous	discontinuous	
P. arcuata	0.2-0.3	3-5	scabrous	discontinuous	
P. bhutanica	0.4-0.6(-0.7)	(5–)7	scabrous or rarely glabrous	discontinuous	
P. chingii	(0.2-)0.3-0.4 (-0.5)	3-5	scabrous or rarely glabrous	discontinuous	
P. concinna	0.3-0.6	(3–)5	glabrous and smooth	discontinuous or occasionally continuous	
P. contracta	(0.6–)0.7–1.2	11–14	glabrous and smooth	continuous or slightly discontinuous (torn)	
P. dichotoma	(0.2-)0.3-0.4	3(-5)	scabrous rarely slightly scaberulous to almost glabrous (but not smooth)	discontinuous	
P. duthiei	(0.5-)0.6-1.0	(7–)9–11	glabrous or less frequently minutely scabrous	discontinuous	
P. glabrifolia	0.25-0.4	(3–)5	glabrous and smooth	discontinuous	
P. junatovii	0.3-0.7	(3–)5	glabrous, rarely somewhat scaberulous	discontinuous	
P. luquensis	0.2-0.4	3(-5)	glabrous, rarely somewhat scaberulous	discontinuous	
P. malyschevii	0.4-0.6(-0.7)	(5–)7	scabrous	discontinuous	
P. mongholica subsp. mongholica	0.3-0.5	3	scabrous	discontinuous	
P. mongholica subsp. porteri	0.3-0.5	3	scabrous	discontinuous	
P. tibetica	0.2-0.35	3(-5)	scabrous	discontinuous	
P. yadongensis	0.3-0.4(-0.5)	3-5	scabrous or rarely glabrous	discontinuous	

Table 5. Morphological characters of leaves of the analysed Ptilagrostis species.

#### Phylogenic analyses

The Maximum Likelihood analyses based on SNPs derived from DArT sequencing revealed the arrangement of analysed representatives of Ptilagrostis into three distinct clades with high (98-100%) bootstrap support (Fig. 7). The first clade consists of two well supported (100% bootstrap) sister subclades representing Ptilagrostis bhutanica and P. yadongensis, which belong to the section Barkworthia. These species differ from all the remaining members of Ptilagrostis by having paleae distinctly shorter than lemmas. All other species of Ptilagrostis are grouped within large clade, divided in two sister subclades 'Chenella' and 'Ptilagrostis'. The first consists of three taxa: P. contracta, P. duthiei and P. chingii, characterised by having awns minutely pilose on setas and the robust, usually over 60 cm tall culms (sect. Chenella). Within this subclade, specimens of P. chingii are well separated from the remaining samples, representing P. duthiei and P. contracta. Based on our results, the last two species are grouped together and cannot be clearly distinguished. The subclade 'Ptilagrostis' consists of nine species, P. malyschevii, P. alpina, P. mongholica, P. luquensis, P. concinna, P. arcuata, P. junatovii, P. dichotoma, and P. glabrifolia. Species of sect. Ptilagrostis are arranged into three well-supported (bootstrap > 96%) subclades.

#### Discussion

*Ptilagrostis* is a genus with Tertiary (Miocene-Pliocene) origin, distributed almost exclusively in central and north-eastern Asia, from the Himalayas to northeastern Siberia (Tzvelev 1976, 1977; Freitag 1985; Peterson et al. 2005; Wu and Phillips 2006; Nobis and Nobis 2013; Romaschenko et al. 2014), with



0,050

**Figure 7.** Maximum Likelihood tree based on 12,502 SNP markers derived from DArT sequencing of 16 taxa, including 15 taxa of *Ptilagrostis* and an outgroup (*Ptilagrostiella kingii*). Numbers on branches are bootstrap values. Abbreviations: *Ptilagrostis alpina* – P. alp, *P. arcuata* – P. arc, *P. bhutanica* – P. bhut, *P. chingii* – P. chin, *P. concinna* – P. conc, *P. contracta* – P. cont, *P. dichotoma* var. *dichotoma* – P. dich, *P. dichotoma* var. *roshevitsiana* – P. ros, *P. duthiei* – P. dut, *P. glabrifolia* – P. gla, *P. junatovii* – P. jun, *Ptilagrostiella kingii* – Pt. kin, *P. luquensis* – P. luq, *P. malyschevii* – P. mal, *P. mongholica* – P. mon, *P. yadongensis* – P. yad. List of samples is given in Suppl. material 1.

the Qinghai-Tibet plateau being the centre of its diversity (Zhang et al. 2017). Additionally, specimens representing *Ptilagrostis* migrated similarly as some other representatives of grasses (e.g. *Patis* Ohwi or *Eremopyrum* (Ledeb.) Jaub. & Spach) from eastern Asia via a land bridge across the Bering Strait during the Pleistocene glaciations to central North America (Johnston 2006; Romaschenko et al. 2014). Currently, only one taxon of *Ptilagrostis*, *P. mon-gholica* subsp. *porteri* (*=P. porteri*), is mentioned in the North American flora (Peterson et al. 2019). The second one, namely *P. kingii*, has been recently

transferred to *Ptilagrostiella* based on morphological and molecular evidence (Romaschenko et al. 2014, Peterson et al. 2019).

Some species within Ptilagrostis are relatively similar to each other, resulting in a limited number of morphological characters to distinguish among them. The key character most often used to differentiate particular species is the presence or absence of a tuft of hairs at the apex of anthers. However, a survey of the large number of materials has shown that specimens with glabrous anthers are occasionally also observed within specimens representing 'bearded-anthers'-species (Zhang and Chen 2024; see also comments in the Synopsis, below). There are five species groups within Ptilagrostis in which particular species are morphologically similar. However, most of the species representing particular groups are well separated geographically. Examples of such geographical vicariants are P. alpina-P. malyschevii; P. concinna-P. junatovii, P. mongholica-P. dichotoma within different areas of Asia, and P. mongholica subsp. mongholica-P. mongholica subsp. porteri between Asia and North America. The ranges of the mentioned above high-mountain and/or cold-adapted pair of species are currently well separated due to the contraction caused by the long-term warming in the Holocene. However, it seems that local secondary contact between selected Ptilagrostis members was possible during glaciations, similarly as it took place in other plant species within Central Asia (Nobis et al. 2023; Vintsek et al. 2022; Wróbel et al. 2024a, 2024b), and occasional gene exchange between them could have occurred. Nevertheless, to confirm this hypothesis within particular species of Ptilagrostis, further studies are required.

Currently, the factor highly contributing to the species-differentiation within co-occurring species of the genus is hybridisation, which is generally a common phenomenon in plants, and often observed within grasses (Nobis et al. 2016, 2019c, 2020, 2022; Baiakhmetov et al. 2020, 2021; Sinaga et al. 2024, Wróbel at al. 2024a). Due to the generally numerous populations and the relatively wide distribution ranges for some species of *Ptilagrostis*, gene exchange among selected species has a high likelihood of occurrence. One example of such an interspecific gene flow may be *P. arcuata* (a putative hybrid species from a cross among *P. concinna* and either *P. dichotoma* or *P. luquensis*). Specimens that are possibly hybrids between *P. dichotoma* and *P. tibetica* have also been examined (e.g. *B. Dickoré 9758* and 10819, see comments below in Synopsis). However, no hybridisation events in *Ptilagrostis* have been detected or confirmed using the molecular approach so far. This challenge will be an area of focus for our future studies.

The taxonomic and phylogenetic importance of lemma micromorphology within the Stipeae has been confirmed by many studies (Barkworth and Everett 1987; Brito 2005; Romaschenko et al. 2012; Nobis et al. 2019a, 2020; Tkach et al. 2021), and micromorphological patterns of the lemma are considered as conservative and significant, particularly at the level of genus. All of the *Ptilagrostis* species studied are characterised by having a saw-like pattern (Romaschenko et al. 2012), and more precisely *Ptilagrostis*-like LEP (Nobis et al. 2019a). In the middle part of the lemma, they have abundant silica-bodies with sinuate side-walls and numerous cork cells, however with sparse or more often without hooks (Nobis et al. 2019a, 2019b). Based on the result of studies of lemma epidermal patterns, generic affiliation of some species previously included in *Stipa* or *Achnatcherum* have been established (Nobis et al. 2016,

2019b, 2020). The epidermal patterns described above has been shown to be phylogenetically much more important than characters of the awn that were previously thought to be the key character (Nobis et al. 2019a).

Leaf cross-sections provide valuable taxonomic insights and help to differentiate species, especially those in some of the most taxonomically difficult genera, such as e.g. Festuca, Stipa, Muhlenbergia Schreb., and others. (Peterson et al. 1989; Martínez-Sagarra et al. 2017; Gudkova et al. 2013, 2023). However, these data were usually omitted in Ptilagrostis species descriptions and identification keys or mentioned only exceptionally (Malyschev 1965; Tzvelev 1974; Barkworth 1983). We found that leaf anatomy is one of the most important characters for proper identification of the species representing the sect. Barkworthia and sect. Chenella. All Ptilagrostis species are characterised by leaves with convoluted blades. The sclerenchyma layer and number of vascular bundles are the most distinctive characters of leaf anatomy in false needlegrasses. The sclerenchyma layer forms a continuous ring only in P. contracta and occasionally in P. concinna, while in other species it is disrupted. The highest number of vascular bundles, from (7-)9 to 11(-14), were found in *P. contracta* and P. duthiei. Among the remaining species, the number of vascular bundles was lower ranging from three or five. The exceptions were P. bhutanica and P. malyschevii that almost always have seven bundles.

Phylogenetic analyses revealed that Ptilagrostis is a well-distinguished and strongly supported clade within the Stipeae, closely related to Neotrinia and Orthoraphium (Romaschenko et al. 2012, 2014; Hamasha et al. 2012; Nobis et al. 2019a, 2020). However, both of these genera distinctly differ morphologically from Ptilagrostis representatives (Nobis et al. 2019a, 2019b). Neorinia splendens (Trin.) M. Nobis, P.D. Gudkova & A. Nowak is distinguished by the general habit of the plant with dense tufts and tall stems, long and rigid leaves and elongated panicle with numerous flowers. Whereas Orthoraphium roylei Nees possesses a unique lemma with deflexed, apical prickles (Nobis et al. 2019b). Moreover, previously performed molecular analyses of cpDNA and ITS sequences clearly separated these genera from Ptilagrostis (Romaschenko et al. 2008, 2012, 2014; Hamasha et al. 2012; Zhang et al. 2017). In our molecular analyses, including 14 of 15 currently distinguished false needlegrasses, the examined species were organised in three well-distinguishable clades that correspond to the three sections, Barkworthia, Chenella and Ptilagrostis. The species making up the clade of sect. Barkworthia (P. bhutanica and P. yadongensis) are well distinguished both morphologically and molecularly from the remaining Ptilagrostis species. Both aforementioned taxa have narrow panicles and paleas much shorter than lemmas.

A second clade (corresponding to sect. *Chenella*) included *P. duthiei*, *P. chingii* and *P. contracta*. Both *P. duthiei* and *P. chingii* have initially been described as representatives of *Stipa* (Hooker 1897; Hitchcock 1930). However, based on the morphology of the awn, they were later transferred to *Achnatherum* (Keng 1976; Kuo and Lu 1987). According to Wu and Phillips (2006), *P. duthiei* and *P. chingii* are morphologically fairly similar, and the only feature to distinguish them from each other is the length of the anthers (2.8–4.5 vs. 2–2.5 mm respectively). However, our studies revealed that anther lengths overlap one another. Anther length of *P. duthiei* is 3–4 mm, while anther length of *P. chingii* is 2.0–3.3 mm. Our data has shown that the best characters to distinguish these species are

the width of vegetative leaves and number of vascular bundles on the crosssections of leaves. In *P. duthiei*, leaves are noticeably wider and have more vascular bundles than in *P. chingii*. However, the species descriptions by Wu and Phillips (2006) indicated that leaf width is 0.5–1 mm in both species resulted in frequent misidentification of the two species. The third species of sect. *Chenella*, *P. contracta*, was recently described (Zhang et al. 2017) and is a strongly supported member within sect. *Chenella* most similar to *P. duthiei*. Our studies show that these two closely related species are differentiated by a contracted (*P. contracta*) vs. open panicle (*P. duthiei*). In comparison to *P. duthiei*, *P. contracta* also has slightly wider vegetative leaves, 0.5–1.2 mm wide, with 9–14 vascular bundles visible on the cross-section, and continuous or slightly discontinuous (torn) ring of sclerenchyma. Our molecular analyses reveal that our one representative of *P. contracta* is paraphyletic within a clade with representatives of *P. duthiei*. Further analysis with additional representatives of *P. contracta* are needed to more clearly define the phylogenetic relationships between the two species.

The third clade included all the remaining species belonging to the sect. Ptilagrostis. Within this clade, three further subclades comprising morphologically similar species can be distinguished. The first one, Ptilagrostis alpina-P. malyschevii subclade, consists of a pair of morphologically very similar species. Both of them have open panicles, fairly broad leaves with five to seven vascular bundles. Another subclade consists of P. dichotoma and P. glabrifolia (and *P. tibetica*, absent in our molecular, but present in morphological analysis) comprising morphologically very similar species, characterised by having open panicles, and filiform leaves with three or five vascular bundles. Central, the most numerous in species subclade includes: P. concinna, P. junatovii, P. arcuata, P. luquensis and P. mongholica. The species organisation within this subclade is somewhat puzzling. Ptilagrostis concinna, P. junatovii and P. arcuata are characterised by clearly contracted panicles, which enables them to be easily distinguished from the other species of this subclade. The presence of putative hybrids (P. arcuata) and possible gene flow events among species of this subclade seems to be the most probable explanation for this arrangement. However, further analyses on the population level using tools of integrative taxonomy are needed to reveal the evolutionary history of this group of taxa.

#### An identification key to species of Ptilagrostis

4	Lemma and palea distinctly unequal, lemma 0.5–2.0 mm longer than palea; awn upper segment with 0.3–0.9 mm long hairs (sect. <i>Barkworthia</i> )
-	Lemma and palea equal or subequal; awn upper segment with 1–3 mm
5	Awn upper segment near geniculation with hairs 0.6–0.9 mm long; glumes distinctly unequal, lower glume 1.8–2.5 mm longer than the upper glume; lemma 0.7–1.3 mm longer than palea; leaf vascular bundles 3–5
-	Awn upper segment near geniculation with hairs $0.3-0.5$ mm long; glumes subequal, the lower $0.2-0.5(-0.8)$ mm longer than the upper), lemma $1.2-2.0$ mm longer than palea; leaf vascular bundles 7 <b>P. bhutanica</b>
6	Glumes $2.6-3.5(-4.0)$ mm long; floret (lemma+callus) $2.2-2.7(-3.0)$ mm long; awn 7-13 mm long; anthers $1.0-1.4$ mm long, glabrous at the apex <b>P. luguensis</b>
-	Glumes 4.0–12.5 mm long; floret 3–8 mm long; awn 7–52 mm long; an- thers 1.2–4.0 mm long, glabrous or hairy at the apex
7	Panicle contracted, 1–2 cm wide; panicle branches 0.3–2.8 cm long, suberect or narrowly ascending
- 8	Panicle open, 3–10 cm wide; panicle branches 2–6 cm long, spreading <b>10</b> Leaves filiform, 0.2–0.3 mm in wide, slightly scabrous; awn 8–14 mm long, without or with hardly visible geniculation; upper segment of the awn (seta) usually arcuate <b>P. arcuata</b>
-	Leaves 0.3–0.7 mm in wide, glabrous and smooth; awn 9–20 mm long, ge- niculate upper segment of the awn (seta) straight arcuate or flexuous
9	Membranous bracts at base of panicle present and persistent; awn 8–15 mm long
-	Membranous bracts at base of panicle absent, impermanent, caducous or sporadically residual; awn 12–20 mm long
10 -	Lemma covered by scattered long hairs throughout11 Lemma covered by long hairs basally, glabrous in the middle, and pilose or scabrous apically12
11	Leaves distinctly scabrous; leaf vascular bundles in cross-section 3 (rarely 5); glumes purple near base, straw coloured near apex; anthers glabrous. <i>P. tibetica</i>
-	Leaves glabrous and smooth; leaf vascular bundles in cross-section 5 (rarely 3); glumes dark purple almost throughout; anthers with a tuft of bairs at apex (rarely glabrous)
12	Anthers apex glabrous (plants of north-central Asia and North America)
– 13	Anthers apex with a tuft of hairs (plants of south-central Asia)
-	(plants from Asia) <i>P. mongholica</i> subsp. mongholica Awn 10–20 mm long; floret 3.0–4.0(–4.7) mm long (plants from N America) <i>P. mongholica</i> subsp. porteri
14	Vegetative leaves $(0.4-)0.5-0.6(-0.7)$ mm in diameter; leaf vascular bundles 5-7; awn 15-52 mm long15
-	Vegetative leaves 0.2–0.3(–0.4) mm in diameter, filiform; leaf vascular bundles 3 (rarely 5); awn 9–20 mm long <b>P. dichotoma</b>

- Panicle usually with 15–25 spikelets; panicle branches glabrous; awn (20–)28–52 mm long; floret 4.0–5.2 mm long; glumes (4.5–)5.0–6.5(–7.0) mm long; leaf vascular bundles 7 (rarely 5)...... *P. malyschevii*

### Synopsis of Ptilagrostis

#### Ptilagrostis Griseb., Flora Rossica 4(13): 447. 1852.

**Type.** *Ptilagrostis mongholica* (Turcz. ex Trin.) Griseb. [basionym: *Stipa mon-gholica* Turcz. ex Trin.].

# Ptilagrostis sect. Ptilagrostis

Type. P. mongholica (Turcz. ex Trin.) Griseb.

**Description.** Species belonging to this section are characterised by equal or subequal glumes, lemmas and paleas comparable in length, and awns covered by hairs > 1 mm long (usually 1–3 mm long).

# 1. *Ptilagrostis alpina* (F. Schmidt) Sipliv., Spisok Rastenij Gerbarija Flory SSSR 18: 60. 1970.

Suppl. material 2: fig. S2

Lasiagrostis alpina F. Schmidt, Reisen im Amur-Lande 73. 1868. Basionym.

- Stipa alpina (F. Schmidt) Roshev., Izvestiya Imperatorskogo Botanicheskogo Sada Petra Velikago 14(Suppl. 2): 50. 1915.
- ≡ Stipa mongholica fo. alpina (F. Schmidt) Roshev., Flora Aziatskoi Rossii 1(12): 132. 1916.
- ≡ *Stipa alpina* (F. Schmidt) Petrov, Flora lakutiae 1: 136. 1930.

**Type.** 'in protologue: Auf dem kahlen Berge an der Burej-Quelle, 3 Juli'. Lectotype designated by Tzvelev 1976: 566, Ad fluv. Amur, fontes Burejae, 4 July 1859, *F. Schmidt* s.n. (LE01009402!).

**Description.** *Perennial plants*, densely tufted, with a few culms and numerous vegetative shoots; culms 15–35 cm tall. *Leaves of vegetative shoots*: sheaths glabrous; *ligules* lanceolate, the longest 0.8–1.9 mm; *blades* slightly scabrous, filiform, convolute, green, pale green to greyish, 0.3–0.5 mm in diameter, with 5(–7) vascular bundles. *Cauline leaves*: sheaths glabrous or minutely scabrous; *ligules* on the lower sheaths lanceolate. *Panicle* open, (3–)5–12(–15) cm long and 3–8 cm wide, with 7–13 spikelets; *branches* scabrous. *Glumes* subequal, purple, (3.6–)4.2–5.2 mm long, lanceolate. *Floret* (=anthecium, =lemma + callus) 3.4–4.5 mm long. *Callus* 0.3–0.5 mm long, densely pilose; callus base obtuse. *Lemma* coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the midlength and with hairs at apex; *lemma apex* with two lobes. *Awn* 15–28(–30) mm



Figure 8. Ptilagrostis alpina, general habit.

long, unigeniculate; **the lower segment of the awn (column)** 5–10 mm long, twisted, with 1.2–1.6 mm long hairs; **terminal segment of the awn (seta)** straight, 10– 17 mm long, with 1.2–1.6 mm long hairs, gradually decreasing in length towards the apex. **Anthers** 1.3–2.5 mm long, with a tuft of hairs at the apex. **Phenology.** Flowering from July to August. Figures. Figs 6f, 8, https://www.gbif.org/species/4149846.

**Distribution.** Eastern Asia: Russia: eastern Siberia nad Russin Far East, NE China?, Japan? (Tzvelev 1976; Probatova 1985; Probatova et al. 2006; Tzvelev and Probatova 2019).

Habitat. Grasslands and stony slopes, at 900-2200 m elev.

**Selected studied specimens of** *P. alpina.* RUSSIA • Ad fluv. Amur, fontes Burejae; 4 July 1862 [1859?]; *F. Schmidt* s.n. (syntype: LE01009403) • Khabarovskiy territory, Ayano-Mayskiy district; headwater of river Magej (180 km to S of village Nelkan); valley, edge of the seasonal melted glacier, conspicuous; 900 m elev.; 10 Aug 1978; *S. Kharkevich, T. Buch 1089* (MO-4886943, NY) • Buryatiya, Verkhovya reki, Barguzin, Goltsy k yugo-zapadu ot ozera Balan-tomur; 1 Aug 1964; *V. Siplivinskiy* s.n. (COLO195597) • Sakhalin, Vostochno-Sakhalinskie gory, gornyi massiv g. Vodorazdelnaya (1428.7 m) - g. Nevelskogo (1397.8 m); 19 Jul 1988; *I.B. Vyshin, V.J. Barkalov* s.n. (LE) • Tsentral. Sakhalin, yug Vostochno-Sakhalinskogo Khrebta, izvestnyakovaya g. Vaida, v verkh. r. Vitnitsy; travyanistyi krutoi sklon pod skalami; 20 Aug.2006; *V.J. Barkalov*, *V.V. Yakubov* s.n. (LE).

#### **2.** *Ptilagrostis arcuata* **Z.S.** Zhang & W.L. Chen, Phytotaxa 269(2): 232. 2016. Suppl. material 2: fig. S3

=? P. pugeensis X.Y Zhang & W.L. Chen, Botanical Journal of the Linnean Society 206: 76. 2024. Type: China. Sichuan: Puge County, 267 Luoji Mountain, 27.579°N, 102.361°E, 4017 m, 1 Oct 2014, Z.S. Zhang & L.L. Li 380-1 (holotype: PE).

**Type.** CHINA, Yunnan: Dêqên, Bai-Ma mountain, 4366 m, 15 Sep 2014, *Z.S. Zhang & L.L. Li 217* (holotype PE; isotype: US, K1222374!, K1222375!)

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms 15-50(-60) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades scabrous, filiform, convolute, green, pale green to greyish, 0.2-0.3 mm in diameter, with 3-5 vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle contracted, 5.5-11 cm long and 1-2 cm wide; branches glabrous. Glumes subequal, purple, 5.0-6.6 mm long, lanceolate. Floret (lemma + callus) (4.0-)4.5-5.7 mm long. Callus 0.4-0.5 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn 8-14 mm long, unigeniculate; the lower segment of the awn (column) 4-5 mm long, twisted, with 1.2-2.0 mm long hairs; terminal segment of the awn (seta) straight, 7-9 mm long, with 1.2-1.9 mm long hairs, gradually decreasing in length towards the apex. Anthers 1.2-2.1 mm long, with a tuft of hairs or sporadically glabrous at the apex.

Phenology. Flowering from August to October.

**Figures.** Fig. 6k; additional figures in Zhang et al. (2016a: figs 1, 2); https:// powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77157281-1.

**Distribution.** China: Sichuan, Yunnan, Xizang and Nepal (Zhang et al. 2016a; Zhang and Chen 2024).

Habitat. Alpine meadows, thickets, grassy mountain slopes, moors by the river, at 3900-4600(-4900) m elev.

Note. It is not excluded that P. arcuata is a product of spontaneous hybridisation between P. concinna and P. luquensis or P. dichotoma. The origin of this taxon needs further study. Recently Zhang and Chen (2024) described a new taxon, P. pugeensis, morphologically similar to P. arcuata, and known from only one locality. The distinctiveness of this taxon requires molecular confirmation.

Selected studied specimens of P. arcuata. CHINA · SE Tibet, Xizang: Nyaingentanglha Shan. Yangbajain - Damxung, NW of Lhasa, Valley SE of Nyaingentanglha Feng, Kobresia spp. - moor by the river, alpine, elev. 4880 m, 30°18'N, 90°38'E, 11 Aug 1989, B. Dickoré 3761 (MSB-152861) · Namchabarwa NW slope, Village "Pei No. 4" - Nam La Tso, lower alp. moist Kobresia meadow and Rhododendron dwarf-scrub among gneiss boulders, elev. 4430 m, 29°35'N, 94°59'E, 15 Sep 1989, B. Dickoré 5352 (MSB-152913).

# 3. Ptilagrostis concinna (Hook. f.) Roshev., Flora Unionis Rerumpublicarum Sovieticarum Socialisticarum 2: 75. 1934. Suppl. material 2: fig. S4

Stipa concinna Hook. f., The Flora of British India 7(22): 230. 1897(1896). Basionym

Type. Sikkim Himalaya, in the Tibetan region, 14-16000 ft, 11 Sept. 1849, J.D. Hooker s.n. (holotype: K!; isotypes: LE00009287!, CAL 2351!, GOET006941!, W!, P02240692)

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (5-)10-30(-40) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades glabrous and smooth, convolute, green, pale green to greyish, 0.3-0.6 mm in diameter, with (3-)5 vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle contracted, 2-6 cm long and 1-2 cm wide; branches glabrous, lowest branches enclosed by a sheathing lanceolate membranous bract. Glumes subequal, purple, 4.0-7.7 mm long, lanceolate. Floret (lemma + callus) 3.3-4.5(-5.0) mm long. Callus 0.3-0.6 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn (8-)10-13(-15) mm long, unigeniculate; the lower segment of the awn (column) 3.0-5.0(-6.5) mm long, twisted, with 1.0-1.9 mm long hairs; terminal segment of the awn (seta) straight, 7.0-8.0(-9.5) mm long, with 1.0-1.9 mm long hairs, gradually decreasing in length towards the apex. Anthers 1.5–2.5 mm long, with a tuft of hairs or rarely glabrous at the apex.

Phenology. Flowering from July to September.

Figures. Figs 5a-c, 6l, 9; additional figures in Wu et al. (2007: fig. 279); https://www.gbif.org/species/4149833, https://powo.science.kew.org/taxon/ urn:lsid:ipni.org:names:418941-1, http://www.efloras.org/object\_page.aspx?object\_id=95534&flora\_id=2.



Figure 9. Ptilagrostis concinna, general habit.

Distribution. South and south-eastern Central Asia, in China, India and Nepal (Tzvelev 1968; Freitag 1985; Wu and Phillips 2006; Nobis et al. 2019a).
Habitat. It grows on alpine mats, meadows, moist grassy places, swamps, shrubs and *Kobresia* moors, at 3500–5500 m elev.

Note. Within the range of P. concinna, specimens with anthers glabrous at the apex are sporadically noted. Sometimes, specimens with glabrous and bearded anthers co-occur in the same locality or even in the same population. Specimens with glabrous anthers are observed also within other Ptialgrostis species that are characterised by having anthers bearded at the apex such as P. concinna, P. junatovii or P. glabrifolia. Tzvelev (1974) was the first who distinguished specimens with glabrous anthers as Ptilagrostis concinna subsp. schischkinii. Later on, this taxon was raised to the rank of species by Czerepanov (1981). Further molecular studies are required to determine the systematic position of these 'glabrous-anthers' specimens. Until then, such specimens may be distinguished, in our opinion, at most in the rank of variety. It must be mentioned, however, that after careful examination of the specimen, described by Tzvelev (1974) as Ptilagrostis concinna subsp. schischkinii, we found that it has no bracts at the bottom of the panicle, thus morphologically it is more similar to P. junatovii than to P. concinna. Its occurrence (northern Central Tian Shan) is also more closely located to the range of P. junatovii rather than P. concinna. Thus, we here decided to transfer it to the previous species in the rank of variety (see below). In the meantime, during the revision of the specimens of P. concinna, we found within the range of this species, the specimens with glabrous anthers, and well-developed bracts at the bottom of panicle. This morphotype is here recognised as a variety of P. concinna:

# *Ptilagrostis concinna var. xizangensis* M.Nobis & Krzempek, var. nov. urn:lsid:ipni.org:names:77351829-1

**Diagnosis.** From the nominal variety it differs in having anthers glabrous at the apex (instead of bearded).

**Type.** CHINA • Xizang, SE.Tibet, Nyaingentanglha Shan. Yangbajain – Damxung, NW of Lhasa, S slope of Nyainqentanglha Feng, high alpine *Kobresia pygmaea*-turf on steep S-facing slope, elev. 5290 m, 30°20'N, 90°34'E, 13.08.1989. *B. Dickoré 3931* (holotype: MSB-152847, Suppl. material 2: fig. S5, isotypes: KRA528809, MSB-152860 – specimen mounted in the upper-left corner of the sheet).

Selected studied specimens of *P. concinna* var. *concinna*. CHINA • Sichuan, reg. bor.: Dongrergo; in prato alpino aperto; elev. ca. 4800 m; 2 Jul 1922; *H. Smith 3728* (BM001031161) • Sikang, Kangting (Tachienlu) distr., Tapaoshan; ad rupes; elev. 4600–4800 m; 22 Aug 1934; *H. Smith 11474* (V-038934) • Sichuan, Sikang, Taofu (Dawo) distr., Haitzeshan; in rupibus; elev. 4700 m; 31 Aug 1934; *H. Smith 11687* (V-047430) • Xinjiang, Kun-lun, Kashgarya, morenovyi vodoazdel mezhdy r. r. Atraknr i Tyuzytek; mkhovaya tundra; na vysote 4500–5000 m; 20 Jul.1942; *V.I. Serpukhov 5454* (LE) • Kun-lun, verkhovya r. Tuznaf, v 3–4 km vost. per. Sarnk (217 km Tibetskoi shoce); 4800 m; 4 Jun 1959; *A.A. Yunatov, Yuan I-fen 320* (LE) • Tibet (Xizang), Bassein Yan-tsy-tszyana (r. Goluboi), pereval Cha-mu-bug-la; 15700 ft; 26 Jul 1900; *V.O. Ladygin* s.n. (LE). – INDIA • Kashmir, Apharwat; elev. 13,300 ft.; 12 Aug 1956; *O. Polunin 56/207* (BM001191546). Additional specimens studied are listed in Suppl. material 1.

**4.** *Ptilagrostis dichotoma* Keng ex Tzvelev, Rasteniia Tsentral'noi Azii 4: 43. 1968. Suppl. material 2: fig. S6

= Ptilagrostis dichotoma Keng, Claves Generum et Specierum Graminearum Primarum Sinicarum Appendice Nomenclatione Systematica 213. 1957 [nom. inval., without Latin description].

**Type.** CHINA: Gansu and Qinghai border [in regione opp. Labrang], Y.C. Wu 478 (holotype: NJ, isotype: LE!)

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (12-)15-50(-74) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades scabrous rarely slightly scaberulous to almost glabrous (but not smooth), filiform, convolute, green, pale green to greyish, (0.2-)0.3-0.4 mm in diameter, with 3(-5) vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle open, 4-18 cm long and 3-10 cm wide; branches glabrous or rarely scabrous. Glumes subequal, purple, (3.5-)4.0-5.5(-7.0) mm long, lanceolate. Floret (lemma + callus) 3.5-5.2 mm long. Callus 0.3-0.6 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn (9.0-)11.0-15.5(-20.0) mm long, unigeniculate; the lower segment of the awn (column) (3.0-)4.0-6.5(-8.0) mm long, twisted, with (1.3-)1.7-2.4(-3.0) mm long hairs; terminal segment of the awn (seta) straight, 7-10 mm long, with 1.4-2.0(-3.0) mm long hairs, gradually decreasing in length towards the apex. Anthers 1.3–2 mm long, with a tuft of hairs at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 5d-f, 6r, 10; additional figures in Wu et al. (2007: fig. 280); https://www.gbif.org/species/7325743, http://www.efloras.org/object\_page. aspx?object\_id=95535&flora\_id=2.

**Distribution.** The species occurs in southern Central Asia, in mountain areas of China, India, Nepal, Bhutan, and Myanmar (Wu and Phillips 2006; Nobis et al. 2019a).

**Habitat.** Alpine meadows, bogs, mats, under shrubs, and forests, at 3000–5000 m elev.

**Note.** This taxon is very similar to *P. mongholica* in having very narrow, filiform leaves. However, they differ from each other in length of awns (shorter in *P. dichotoma*) and characters of anthers (bearded at the top in *P. dichotoma* and glabrous in *P. mongholica*). Within the northern part of the range of *P. dichotoma*, specimens with slightly scabrous panicle branches (not glabrous as in typical specimens of nominal variety *P. dichotoma* var. *dichotoma*) were described by Tzvelev (1968) as:

*Ptilagrostis dichotoma var. roshevitsiana Tzvelev*, Rastenia Tsentral'noi Azii 4: 43. 1968. [≡ *Ptilagrostis roshevitsiana* (Tzvelev) L.B. Cai, Acta Phytotaxonomica Sinica 43(1): 65–67. 2005]. **Type.** China occidentalis: prov. Kansu: in regione media sylvarum jugi Austro-Tetungensis, ca. 2800 m, 24 July 1880, *N.M. Przewalski* s.n. (holotype: LE!, Suppl. material 2: fig. S7). However, besides character of panicle branches (glabrous vs. scabrous), the two varieties mentioned above do not differ in any additional morphological character.



Figure 10. Ptilagrostis dichotoma, general habit.

**Selected studied specimens of** *P. dichotoma* var. *dichotoma*. BHUTAN • Upper Mo Chu, E bank of Tharizam Chu; shady ground by stream under *Juniperus & Salix*; elev. 4080 m; 28°01'N, 89°35'E; 25 Sep 1984; *I. W. J. Sinclair & D. G. Long 5323* (E00619019). – CHINA • Qinghai, Maqin (Maqên) Xian, Dawu Xiang, SE of Maqin (Maqên); on slopes, alpine meadow with Hedysarum and N facing slope with very dense turf and frequent dwarf shrubs, mostly *Potentilla*; elev. 3920
m; 34°24'11"N, 100°23'34"E; 29 Aug 1993; *T. N. Ho, B. Bartholomew, M. Gilbert 527* (MO) • Maqin (Maqên) Xian, Xihalong Guo, between Jungong (Gyumgo) and Maqin (Maqên) on S side of the Huang He; among shrubs in NW facing *Picea* woodland, *Picea* forest on N & E slopes, *Juniperus* forest on W facing slopes, alpine meadow along the valley between and at top of altitude range covered; elev. 3500–3600 m; 34°38'56"N, 100°36'38"E; 23 Jul 1993; *T. N. Ho, B. Bartholomew, M. Gilbert 265* (E00619219) • Yunnan, Baima Snow Mountain, Diqing; hillside meadow; elev. 4600 m; 25 Sep 1986; *H. Sun, Z. Qian 0809* (KUN0323197) • Xizang, Cona County, Mountain Pass; elev. 4500 m; 18 Jul 1975; *C. Zhengyi, Q. Du 75-997* (KUN0323186) • Sichuan, reg. bor.-occid.: ca. 45 km ad bor.-orient. versus a Sankar-von-ma; in uliginosis; elev. ca. 4100 m; 4 Sep 1922; *H. Smith 4338* (MO-4366934) • Xisheng, Baizhu, Chayu County; elev. 3600–3700 m; 8 Sep 1982 (KUN0382062). Additional specimens studied are listed in Suppl. material 1.

**Selected studied specimens of** *P. dichotoma* var. *roshevitsiana*. CHINA • Qinghai, Huang-yuan Hsien, O-Yo; expanded northern slope; 8 Aug 1944; *Keng 5194* (H2012/01581 3) • Maqin (Maqên) Xian, Xihalong Guo, between Jungong (Gyumgo) and Maqin (Maqên) on S side of the Huang He; among shrubs in NW facing *Picea* woodland, *Picea* forest on N & E slopes, *Juniperus* forest on W facing slopes, alpine meadow along valley between and at top of altitude range covered; elev. 3500–3600 m; 34°38'56"N, 100°36'38"E; 23 Jul 1993; *T. N. Ho, B. Bartholomew, M. Gilbert 265* (MO-4648135) • Sichuan, reg. bor.: Dongrergo; in prato herboso-fruticoso; elev. 4300–4400 m; 20 Jul 1922; *H. Smith 3798* (MO-4366943, V-038623).

# 5. *Ptilagrostis glabrifolia* X.Y. Zhang & W.L. Chen, Botanical Journal of the Linnean Society 206: 79. 2024.

**Type.** CHINA. Xizang: Markam 341 County, Lawu Mountain, 4326 m, 16 Sep 2021, *X.Y. Zhang & W.H. Li* 395 (holotype: PE).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms 20-50 cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades glabrous and smooth, filiform, convolute, green, pale green to greyish, 0.25-0.4 mm in diameter, with (3-)5 vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle open, 5-11 cm long and 4-8 mm wide; branches glabrous. Glumes subequal, purple, 5.2-7.0 mm long, lanceolate. Floret (lemma + callus) (3.5-)4.6-5.7 mm long. Callus 0.4-0.6 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; *lemma apex* with two lobes. Awn (10.0-)12.0-17.5(-20.0) mm long, unigeniculate; the lower segment of the awn (column) 6-7(-8) mm long, twisted, with 1.2-2.1 mm long hairs; terminal segment of the awn (seta) straight, 10.0-13.0(-14.0) mm long, with 1.0-1.7 mm long hairs, gradually decreasing in length towards the apex. Anthers 1.5-2.1 mm long, with a tuft of hairs or glabrous at the apex.

Phenology. Flowering from July to September.

Figures. Fig. 6t; additional figures in Zhang and Chen (2024: fig. 4).

**Distribution.** China: Sichuan, Yunnan, Xizang, Nepal (Zhang and Chen 2024). **Habitat.** Alpine meadows, alpine Rhododendron thickets, at 3400–4400 m elev.

**Note.** *Ptilagrostis glabrifolia* is the most similar to *P. tibetica*; both species have lemmas pubescent throughout, but *P. glabrifolia* has completely glabrous leaves of vegetative shoots (instead of distinctly scabrous as in *P. tibetica*), glumes almost up to the apex dark purple vs. glumes purple only in the lower half and light brown to straw-coloured in the upper half, respectively. In contrast to *P. tibetica*, *P. glabrifolia* has anthers bearded on the apex or there are at least solitary hairs (Zhang and Chen 2024). However, during our studies, we found two sheets with specimens of *Ptilagrostis* morphologically corresponding to the description of *P. glabrifolia*, but differing in having anthers completely glabrous on the apex. This morphotype is here recognised as a variety of *P. glabrifolia*:

## *Ptilagrostis glabrifolia* var. *himalayensis* M.Nobis & Krzempek, var. nov. urn:lsid:ipni.org:names:77351830-1

**Diagnosis.** The new variety differs from *P. glabrifolia* var. *glabrifolia* in having anthers glabrous at the apex.

**Type.** NEPAL • East of Chalike Pahar, elev. 13,500 ft., 3 Aug 1954, *Stainton, Sykes & Williams 3737* (holotype: K – H2012/0158/5, Fig. 11). Paratype: CHINA: Yunnan, Deqin, east slope of Baima Mountain, elev. 4300–4500 m, 14 Jul 1981, *Green Team 3019* (KUN319284).

**Selected studied specimens of** *P. glabrifolia* var. *glabrifolia*. NEPAL • Maharigaon; growing on grass slopes among Scripus on south aspect; elev. 15,000 ft.; 20 Jul 1952; *O. Polinin, W. R. Sykes, L. H. J. Williams 226* (H2012/0158/7) • East of Chalike Pahar; elev. 13,500 ft.; 3 Aug 1954; *Stainton, Sykes & Williams 3737* (E00619018) • Ganja La (N side); foot of scree, tussock grass growing among moss-covered boulders, with *Potentilla fruticosa, Rhodiola & Kobresia* spp.; elev. 4300 m; 19 Jul 1986; G. & S. Miehe 5924 (H2012/0158/6).

## 6. *Ptilagrostis junatovii* Grubov, Botanicheskie Materialy Gerbariia Botanicheskogo Instituta Imeni V.L. Komarova Akademii Nauk SSSR 17: 3–4. 1955.

Suppl. material 2: fig. S8

**Type.** MONGOLIA: Changai, jugum Tarbagatai, Dzagastuin-Daba, in partis subalpinis cariceto-cobresietis, inter fruticulos *Betula rotundifolia* (MNR, Arachangayskii aimak, Tsakhir somon, khr. Tarbagatai, pereval Tszagastuin daba, vysokogornyi poyas, zarosli *Betula rotundifolia*, po protalinam zanyatym osokovo-kobrezievym lugom, 8 Aug 1951; *A.A. Yunatov* s.n. (holotype LE!, isotype LE!).

**Description.** *Perennial plants*, densely tufted, with a few culms and numerous vegetative shoots; culms (10-)15-25(-30) cm tall. *Leaves of vegetative shoots:* sheaths glabrous; *ligules* lanceolate; *blades* glabrous and smooth rarely somewhat scaberulous, convolute, green, pale green to greyish, 0.3–0.7 mm in diameter, with (3–)5 vascular bundles. *Cauline leaves:* sheaths glabrous or



Figure 11. The holotype of *Ptilagrostis glabrifolia* var. *himalayensis*.

minutely scabrous; *ligules* on the lower sheaths lanceolate. *Panicle* contracted, 4-10 cm long and 1-2 cm wide; *branches* glabrous. *Glumes* subequal, purple, (5.0-)6.0-7.5 mm long, lanceolate. *Floret* (lemma + callus) (4.0-)4.5-6 mm long. *Callus* 0.4-0.7 mm long, densely pilose; callus base obtuse. *Lemma* coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; *lemma apex* with two lobes. *Awn* (12-)14-20 mm long, unigeniculate; *the lower segment of the awn* (*column*) 5.0-7.0 mm long, twisted, with 1.0-1.5 mm long hairs; *terminal segment of the awn* (*seta*)

straight, 7.0–11.0 mm long, with 1.0–1.3 mm long hairs, gradually decreasing in length towards the apex. *Anthers* ca. 1.3–2.2 mm long, with a tuft of hairs or rarely glabrous at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 5g-i, 6j, Fig. 12; additional figures in Wu et al. (2007: fig. 279); https://www.gbif.org/species/4149793, http://www.efloras.org/object\_page. aspx?object\_id=95534&flora\_id=2.

**Distribution.** The species occurs in alpine habitats in the mountain areas of North Central Asia, in Russia, Kazakhstan, Mongolia and northern China (Tzvelev 1976, Tzvelev and Probatova 2019; Wu and Phillips 2006).

Habitat. Alpine mats, stony slopes, gravels, at 2200-3500(-4500) m elev.

**Note.** In Tian Shan Mts, within the south-western part of the range of *P. junatovii*, specimens with glabrous anthers are sporadically noted. Nevertheless, besides this character, these specimens (which also do not have membranaceus bracts at the bottom of the panicle) do not differ from the typical representatives of *P. junatovii* in any additional morphological character (see also note under description of *P. concinna*). Such specimens are distinguished here as a distinct variety of *P. junatovii*.

# *Ptilagrostis junatovii* var. *schischkinii* (Tzvelev) M.Nobis & Krzempek, comb. et stat. nov.

urn:lsid:ipni.org:names:77351831-1 Suppl. material 2: fig. S9

- *Ptilagrostis concinna* subsp. *schischkinii* Tzvelev, Novosti Sistematiki Vysshchikh Rastenii 11: 8. 1974. **Basionym.**
- = Ptilagrostis schischkinii (Tzvelev) Czerep., Sosudistye Rasteniia SSSR 379. 1981.

**Type.** TIEN SHAN: Semirechensk distr., Przhevalskii post, Kokdzhar River, subalpine meadows and slopes, 2700 m, 26 Jul 1912, *B. Sapozhnikov & B. Shishkin* s.n. (holotype: LE!, with Tzvelev's notes: '*Ptilagrostis concinna* (Hook f.) Roshev. subsp. *tianschanica* m. subsp. nova! Typus! N. Tsvelev, 1972' and '*Ptilagrostis concinna* (Hook f.) Roshev. subsp. *schischkinii* Tzvel. subsp. nova, Typus! VI.1972, N. Tzvelev').

**Selected studied specimens of** *P. junatovii* var. *junatovii*. RUSSIA · Zapadnyy Altay, khr. Ivanovskiy, ver. Vyshe Ivanovskaya; kamenistaya tundra; elev. 2600 m; 10 Jul 1981; *Yu.A. Kotukhov* s.n. (LE) · Montes Sajanenses, Orientales, Jugum Kitojense, fl. Saghan-Sajr, prope ostium fontis Zmeevikovyi; in rupibus siccis cum Kobresia in subalpinis; elev. 2400 m; 28 Jul 1958; *L. Malyschev* 4352 (NY) · Gorna Altaisk Auton. Oblast. Kuraisky Range, northeast of Ak-Tash; alpine cirque basin and screes, above mercury mine area; elev. 2400–3000 m; 15 Jul 1987; *D. Murray, W.A. Weber, I. Krasnoborov* 394 (COLO434876). – MONGOLIA · Zap. Prikhubs., pravoberezhe Khomon-gola, v 25 km k cev. ot Rinchin-Lkhuzhby, zap. sklon Sula-Uly, lug, 2600–2700 m, 8 Aug 1972, *V. Grubov et al.* 825 (LE). Arkhaigaiskii aimak, g. Khan-undur; yuzhnui sklon, kobreznik; 9 Aug 1974; *Baizra*[?]... *et al.* 6160 (LE). Additional specimens studied are listed in Suppl. material 1.



**Selected studied specimens of** *P. junatovii* var. schischkinii. CHINA • Eastern Tian-Shan, N slope, upper Danu-gol [Danugou] river, W of Manas River, 7–8 km S of Danugou Pass; elev. 3450 m; *Kobresia-meadow*; 22 Jul 1957; *Guan 507* (LE, KRA634251).

## 7. *Ptilagrostis luquensis* P.M. Peterson, Soreng & Z.L. Wu, Sida 21(3): 1356, 1358, f. 1. 2005.

**Type.** CHINA • Gansu Prov: Luqu Co.: Ca. 30–40 km N of Gansu/Sichuan border on road from Chengdu to Lanzhou, ca. 20 km S of Waxu and 10 km E of Gahai, ca. 230 km SSW of Lanzhou at km post 394, 34°24'N, 102°17'E, 3440 m, 18 Sep 1997, *R. J. Soreng, P. M. Peterson & H. Sun 5383* (holotype: US; isotypes: HNWP, K!, KUN!, MO, PE).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms 5-23 cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades abaxially glabrous and smooth (somewhat scaberulous only along keel), filiform, convolute, green, pale green to greyish, 0.2-0.4 mm in diameter, with 3(-5) vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle loosely contracted to open, 2-6 cm long and 2-4 cm wide; branches glabrous and smooth. Glumes subequal, whitish with the base purplish, 2.6-3.5(-4.0) mm long, lanceolate. Floret (lemma + callus) 2.2-2.7(-3.0) mm long. Callus 0.2-0.3 mm long, densely pilose; callus base obtuse. Lemma coriaceous, palegreen, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes, 0.4-0.6 mm long. Awn 6.0-10.0(-13.0) mm long, unigeniculate; the lower segment of the awn (column) 2.0-4.0(-5.0) mm long, twisted, with 1.5-1.9 mm long hairs; terminal segment of the awn (seta) straight, 5.0–8.0 mm long, with 1.0–1.5(–1.7) mm long hairs, gradually decreasing in length towards the apex. Anthers 1.0-1.4 mm long, glabrous at the apex.

Phenology. Flowering from August to September.

**Figures.** Figs 5–I, 6o, 13; additional figures in Peterson et al. (2005: fig. 1); https://www.gbif.org/occurrence/3946801294.

**Distribution.** Mountain areas of Central Asia: China: Gansu, Qinghai, Sichuan, Xizang (Peterson et al. 2005; Wu and Phillips 2006).

Habitat. Alpine meadows, at 3300-4800 m elev.

Selected studied specimens of P. luquensis. CHINA · Gansu, Luqu Co., ca. 30-40 km N of Gansu-Sichuan boarder on rd. from Chengdu to Lanzhou, ca. 20 km S of Waxu and 10 km E of Gahai, ca. 230 km SSW Lanzhou, k.p. 394; elev. 3440 m; 34°10'N, 102°25'E; Grassy nearly level plains surrounded by low grassy hills, in dense thatch, mollisol soils, with Poa, Calamagrostis, Koeleria, Roegneria, Stipa, Ptilagrostis; 18 Sep 1997; R.J. Soreng, P.M. Peterson, Sun Hang 5383 (KUN0096489 - isotype) • Qinghai, Maqin (Maqên) Xian, Muchang, Dawu Xiang, SE of Magin (Magên); wet area with pool and hummock complex and stream flowing through, embankment of road; elev. 3980 m; 34°20'3"N, 100°30'34"E; 5 Aug 1993; T.N. Ho, B. Bartholomew, M. Gilbert 790 (BM000580229, KRA628866) Magin (Magên) Xian, Dawu Xiang, along the Deleni He, S of Magin (Magên); steep slope with semi-consolidated scree and alpine meadow in alternating strips, on grassy slope; elev. 3050 m; 34°21'54"N, 100°11'34"E; 6 Aug 1993; T.N. Ho, B. Bartholomew, M. Gilbert 810 (BM000577764) • Huang-yuan Hsien, Hara Kutur, 14 Aug 1944, Keng 5468 (K H2012/01581 4) • Sichuan; elev. 4000 m; 27 Aug 1987; Qinghai-Tibet Team 4390 (KUN0319289).



Figure 13. Ptilagrostis luquensis, general habit.

# 8. *Ptilagrostis malyschevii* Tzvelev, Novosti Sistematiki Vysshchikh Rastenii 11: 7. 1974.

Suppl. material 2: fig. S10

- = Stipa mongholica var. barbellata Roshev., Flora Azyatskoi Rossii 12: 132. 1916 (lectotype of this taxon was selected and distinguished by Tzvelev in October 1972 as the holotype of *P. malyschevii*).
- ≡ Ptilagrostis mongholica var. barbellata (Roshev) Roshev., Flora SSSR 2: 75. 1934.

**Type.** KYRGYZSTAN, Tsentralnyi Tian-Shan, Semirechenskaya obl., Pishpekskii u., severn. sklony u r. Buzulgan, 18 July 1908, *R.J. Roshevits 1244* (holotype: LE! with note '*Ptilagrostis malyschevii* m. sp. nova! Typus! X.1972, N. Tzvelev; isotype LE!).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (10-)15-45(-70) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate, the longest 2.3-4.5; blades scabrous, convolute, green, pale green to greyish, 0.4-0.6(-0.7) mm in diameter, with (5-)7 vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle open, 5-15 cm long and 4–10 cm wide, with 15–25 spikelets (in young individuals fewer than 15); branches glabrous. Glumes subequal, purple, 4.4-5.0(-6.0) mm long, lanceolate. Floret (lemma + callus) 3.8-4.6(-5.3) mm long. Callus 0.3-0.7 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn (20-)28-45(-52) mm long, unigeniculate; the lower segment of the awn (column) (6-)10-17(-20) mm long, twisted, with 1.2-2.0 mm long hairs; terminal segment of the awn (seta) straight, (10-)15-25(-32) mm long, with 1.0-1.9 mm long hairs, gradually decreasing in length towards the apex. Anthers 2-3 mm long, with a tuft of hairs at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 6e, 14; additional figures in Tzvelev et al. (1974: fig. 1); Qin et al. (2004: fig. 118); https://www.gbif.org/species/4149793, http://www.efloras. org/object\_page.aspx?object\_id=95534&flora\_id=2.

**Distribution.** Mountain areas of north-central Central Asia, in Kyrgyzstan, Tajikistan, Kazakhstan, Russia, and China (Tzvelev 1974, 1976; Tzvelev and Probatova 2019).

**Habitat.** Alpine mats, gravels and stony slopes, at (2600–)2900–4500(– 5000) m elev.

Selected studied specimens of *P. malyschevii*. CHINA • Jilin, Highland Bai Shan; elev. 2600 m; 27 Jul 1963 (KRA528840, KRA528837, IFP15852001a0021, IFP15852001a0004) • Sichuan, grasslands between Labrang and Yellow River near camp, Wanrgon valley; among willow bushes; elev. 12200 ft.; 29 Jul 1926; *J.F. Rock 14530* (E00690600, NY). – KAZAKHSTAN • Semirechensk, obl. Dzharkentsk. u. Gory Ketmen-tau. Yuzhnyy sklon, r. Kessyk ak-tas; zona alpíyskaya, syrovatyye luga; vys. 1400 sazh; 14 Aug 1917; *R. Abolino 5345* (TASH015592) • Semirechensk, obl. Dzharkentsk, u. Gory Ketmen-tau Pereval Ak-tas; zona alpíyskaya, kamenistyy lug; vys. 1500 sazh; 13 Aug 1917; *R. Abolino 3766* (TASH015593). – KYRGYZSTAN • Issyk-Kul Region, Central Tian Shan, ca. 133.5 km ESE of Balykchy, ca. 25 km S of Barskoon; alpine grasslands; elev. 2790 m;



Figure 14. Ptilagrostis malyschevii, general habit.

41.924582°N, 77.641257°E; 5 Jul 2022; *M. Nobis, E. Klichowska, A. Wróbel 1547* (KRA628874) • Issyk-kul Region, ca. 96 km to the W from the Issyk-kul Lake and ca. 51 to the SE from Przewalsk, near the road A364; over the mountain pass; elev. 3491 m; 42°21'58.54"N, 79°1'27.63"E; 2 Aug 2016; *M. Nobis, A. Nobis 838* (KRA487167) • Naryn Region, Central Tian Shan, ca. 87.5 km SW of Naryn, ca. 58.8 km SW of At-Bashy; alpine grasslands; elev. 3112 m; 10 Jul 2022; 40.822641°N, 75.289191°E; *M. Nobis, E. Klichowska, A. Wróbel 1580* 

(KRA628893, KRA628894, KRA628895) • Central Tien Shan, ca. 76.5 km E of Kyzyl Suu, ca. 192 km SE of Almaty; alpine meadow; elev. 2810 m; 42°25'35.14"N, 78°56'53.41"E; 09 Jul 2015; M. Nobis, *A. Nowak 632* (KRA476208) • Central Tien Shan, ca. 52 km E of Przewalsk, ca. 197 km SE of Almaty; alpine grassland; elev. 3428 m; 42°25'37.73"N, 79°1'41.85"E; 09 Jul 2015; *M. Nobis, A. Nowak 636* (KRA481613) • Central Tian-Shan, ca. 18 km NE of Songköl, ca. 40.5 km SW of Kochkor; steppe; elev. 2892 m; 41°56'55.56"N, 75°25'46.59"E; 31 Jul 2016; leg. *M. Nobis, A. Nobis 829* (KRA522756). Additional specimens studied are listed in Suppl. material 1.

**Note.** During the revision of herbarium materials in Shenyang (IFP), we found specimens collected in NE China that are morphologically intermediate between *P. malyschevii* and *P. alpina*. These specimens have short ligules, scabrous panicle branches and fewer flowers in the panicle that are typical for *P. alpina*, while other features such as long awns, large lemmas and glumes are characteristic for *P. malyschevii*. It is possible that *P. alpina* is also present in the area and hybridisation between the two species occurs there. Further studies are required to verify this hypothesis.

Selected specimens characterised by intermediate characters between *P. malyschevii* and *P. alpina* studied. CHINA • Antu County, Changbai Mountain reserve; 23 Jul 1986 (KRA528839, IFP15852001a0026) • 29 Jul 1975 (KRA528838, IFP15852001a0024).

# 9. *Ptilagrostis mongholica* (Turcz. ex Trin.) Griseb., Flora Rossica 4(13): 447. 1852.

Suppl. material 2: fig. S11

- Stipa mongholica Turcz. ex Trin., Mémoires de l'Académie Impériale des Sciences de Saint-Pétersbourg. Sixième Série. Sciences Mathématiques, Physiques et Naturelles. Seconde Partie: Sciences Naturelles 4,2(1): 42. 1836. **Basionym.**
- = Achnatherum mongholicum (Turcz. ex Trin.) Ohwi, Journal of Japanese Botany 17(7): 403. 1941.
- = Lasiagrostis mongholica (Turcz. ex Trin.) Trin. & Rupr., Species Graminum Stipaceorum 87. 1842.
- = Oryzopsis mongolica (Turcz. ex Trin.) Beal, Botanical Gazette 15(5): 111.
   1890.

**Type.** In pratis humidis torrentem Dschiginai in Okam influentem [E Sayan], 1830, *Turcz[aninov]* s.n. (lectotype: LE01009420!, designated [as holotype] by Tzvelev 1976: 556; syntypes: H, K, KFTA, LE (12 sheets!), LECB, US, W).

#### 9a. Ptilagrostis mongholica subsp. mongholica

Stipa czekanovskii Petrov, Flora lakutiae 1: 136, f. 42. 1930; = Ptilagrostis czekanowskii (Petrov) Sipliv., Spisok Rastenij Gerbarija Flory SSSR 18: 60. 1970. Type: Sibiria orient. ad fl. Olenek, inter Majgada superiorem et ostium fl. Alakit, 11 Jul 1874 [fr.], A. Czekanowski et F. Muller s.n. (lectotype, designated here, LE 01009404!; isolectotypes: LE 01009405 and 01009406);

Stipa mongholica var. minutiflora V.S. Titov ex Roshev., Flora Aziatskoi Rossii 1(12): 131–132. 1916; ≡ Ptilagrostis mongholica subsp. minutiflora (V.S. Titov ex Roshev.) Tzvelev, Novosti Sistematiki Vysshchikh Rastenii 11: 7. 1974; ≡ Ptilagrostis minutiflora (V.S. Titov ex Roshev.) Czerep., Sosudistye Rasteniia SSSR 379. 1981]. Type: Russia: [Siberia], Enis. gub. Minus., y. Abakanskaya inorodnaya uprava, dol. rr. Ulenya i Karo, bolotnistyi lug, 1–3 Aug 1909, V. Titov s.n. (lectotype, **designated here**, LE01009407!, isolectotype: LE 01009408!, TK (2 sheets!, including one with original, hand-written label; syntypes LE01009409! and LE01009410!

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (10-)20-50(-60) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades scabrous, convolute, green, pale green to greyish, 0.3–0.5 mm in diameter, with 3 vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle open, 12-17 cm long and 5-9 cm wide; branches glabrous. *Glumes* subequal, purple, (4.5–)5.0–7.0(–7.8) mm long, lanceolate. Floret (lemma + callus) (3.5-)4.0-5.5(-6.0) mm long. Callus 0.3-0.7 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn (13-)15-26(-33) mm long, unigeniculate; the lower segment of the awn (column) 5.0-13.0(-15.0) mm long, twisted, with 1.0-2.0 mm long hairs; terminal segment of the awn (seta) straight, 8.0-16.0(-22.0) mm long, with 1.2-1.6 mm long hairs, gradually decreasing in length towards the apex. Anthers 1.8–3 mm long, glabrous at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 5m-o, 6p, 15; additional figures in Wu et al. (2007: fig. 280); https://www.gbif.org/species/2703369, http://www.efloras.org/object\_page. aspx?object\_id=95537&flora\_id=2.

**Distribution.** Alpine areas of north-central Central Asia, in Kazakhstan, Russia, China, and an island locality in Afghanistan (Tzvelev 1974, 1976; Wu and Phillips 2006; Tzvelev and Probatova 2019).

**Habitat.** *Ptilagrostis mongholica* subsp. *mongholica* has wide ecological amplitude of habitat preferences. It grows on alpine mats, steppes, bogs, wet and dry meadows, rocky grasslands, at 2000–4800 m elev.

**Note.** Within the taxon, a variety characterised by the presence of smaller glumes, smaller florets, and a greater number of flowers in a panicle (20–30) was distinguished by Titov ex Roshevitz (1916) and named *S. mongholica* var. *minutiflora*. Later, it was raised to the rank of subspecies (Tzvelev 1974) and then to the rank of species (Czerepanov 1981; Tzvelev and Probatova 2019). This taxon was treated as endemic to Khakasia, however later there were confirmed collections also from Altai in Russia and Kazakhstan (Tzvelev and Probatova 2019). Bearing in mind that the morphological variability of *P. minutiflora* is located entirely within the smaller variability range of *P. mongholica* (number of flowers 15–28 vs. 8–22; glumes length 4.5–5 vs. 4.6–7.8 mm; floret length 3.3–4.0 vs. 3.8–6.0; awn length 13–20 vs. 15–33 mm, respectively), the independence of this taxon needs molecular confirmation (and comparison with representatives of *P. mongholica* s. *str.* at the population level). Based on our biometrical studies



Figure 15. Ptilagrostis mongholica subsp. mongholica, general habit.

on the representatives of both taxa (including typical specimens), we consider *S. mongholica* var. *minutiflora* to be conspecific with the nominal species. The first lectotypification of *Stipa mongholica* var. *minutiflora* was made by Tzvelev (1976: 556), however, there was no precise designation of the lectotype in the publication. On both sheets with specimens of *S. mongholica* var. *minutiflora* at LE, there are Tzvelev's labels stating 'Ptilagrostis minutiflora (Titov ex Roshev.) Czer. 1981, Sosud. Rast SSSR: 379, Lectotypus!, V.1991, N. Tzvelev'.

Selected studied specimens of P. mongholica subsp. mongholica. AFGHANISTAN • prov. Badakhshan, Wakhan, Darya-e Birgula-e Jelga Chelab Tal, Nw des Kol-e Chaqmaqtin; elev. 4200-4400 m; 74°06'N, 37°15'E; 20 Jul 1971; O. Anders 7578 (MSB-186166). - CHINA · Hebei, Chili, Hsiao-wu-tai-shan, Tienlin-ssü; in prato alpino; elev. 2800 m; 22 Jul 1921; H. Smith 1236 (V-038624) · Sichuan, reg. bor.: Dongrergo (Hsioeh-pau-ting); in silva mixta; elev. 4000 m; 20 Jun 1922; H. Smith 3797 (V-040980). - KAZAKHSTAN • Gorno-Altayskaya a. o., khr. Terektinskiy, verkh. r. Karakol, r. Arykhem; lishaynikovaya tundra; 12 Jul 1983; A. S. & T. S. Revushkiny, S. N. Vydrina, V. F. Balashova, N. I. Gordeyeva s.n. (KRA455209). - MONGOLIA · Ajmak Bajanchongor, Somon Galuut, Changaj Mountains, Sant Valley (side from Cagan-Turutuingol); mountain steppe at the bottom of the valley; elev. 2600 m; 16 Jul 1974; A. Pacyna s.n. (KRA101114) · Ajmak Bajanchongor, Somon Galuut, Changai mountains, Olon-Nur valley; alpine grassland on the watershed above the valley; elev. 2640 m; 6 Jul 1974; A. Pacyna s.n. (KRA101112) · Mungun-Moritu, Centr somona: Khentej, Dund-Bajdakagiju-Gol catchment; in NNEE part; valley depression, wet meadow; elev. 1650 m; 25 Jul 1978; F. Święs s.n. (KRA1011093). - Russia · Gorno-Altayskaya a.o., Kosh-Agachskiy r-n, Mokhro-Oyuk; kobrezyvaya tundra; 18–22 Jul 1986; A. S. Revushkin, S. N. Vydring, A. V. Rakitin, N. Sergeyeva, S. A. Pshevorskaya, S. A. Pul'kina s.n. (KRA451213) · Gorno-Altaisk Autonomous Oblast, wet sedge-willow meadow between Tenga and Yabogan Pass; elev. 1100 m; 30 Aug 1978; T.S. Elias, W. Weber, C.S. Tomb 4828 (NY) • Burjatia, distr. Bargusin, jugum Jushno-Mujsky ad fontes fl. Bargusin, in glareosis ripa sinistra fluminis Bargusin prope lac. Balan-Tomur; 7 Aug 1964; V. Siplivinsky s.n. (NY) · Altai, prope pug. Eschtu-kol; 27 Jul 1924; B. Schischkin s.n. (NY). Additional specimens studied are listed in Suppl. material 1.

# 9b. *Ptilagrostis mongholica* subsp. *porteri* (Rydb.) Barkworth, Systematic Botany 8(4): 417. 1983.

Stipa porteri Rydb., Bulletin of the Torrey Botanical Club 32(11): 599. 1905. Basionym.

= Ptilagrostis porteri (Rydb.) W.A. Weber, University of Colorado Studies: Series in Biology 23: 2. 1966.

**Type.** USA: Rocky Mountains, *Hall & Harbour 648* [error for 646] (lectotype designated by Barkworth 1983: 714 in PH; isolectotypes: GH, MO-3055595, MO-3055594, MO-5472475, MO-3055593, NY-431562, US-992164, US-992165, US-907470).

**Description.** *Perennial plants*, densely tufted, with a few culms and numerous vegetative shoots; culms 20–50 cm tall. *Leaves of vegetative shoots:* sheaths glabrous; *ligules* lanceolate; *blades* scabrous, convolute, green, pale green to greyish, 0.3–0.5 mm in diameter, with 3 vascular bundles. *Cauline leaves:* sheaths glabrous or minutely scabrous; *ligules* on the lower sheaths lanceolate. *Panicle* open, rarely loosely contracted, 5–12 cm long and 2–6 cm wide; *branches* glabrous. *Glumes* subequal, purple, 4.5–6.0 mm long, lanceolate. *Floret* (lemma + callus) 2.5–4.0 mm long. *Callus* 0.2–0.5 mm long, densely pilose; callus base obtuse. *Lemma* coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed



Figure 16. Ptilagrostis mongholica subsp. porteri, general habit.

hairs, hairless in the mid-length and with hairs at apex; *lemma apex* with two lobes. *Awn* 10–23 mm long, unigeniculate; *the lower segment of the awn* (*column*) 4.0–6.0 mm long, twisted, with 1.0–1.7 mm long hairs; *terminal segment of the awn* (*seta*) straight, 8.0–12.0 mm long, with 1.2–1.6 mm long hairs, gradually decreasing in length towards the apex. *Anthers* 1.5–3.0 mm long, glabrous at the apex.

Phenology. Flowering from July to August.

**Figures.** Figs 5p-s, 16; additional figures in Barkworth (1983), Barkworth (2007: 144); Johnston (2006: 18–19); https://www.gbif.org/species/2703367.

**Distribution.** North America: Colorado, New Mexico (Barkworth 1983; Soreng 2003; Johnston 2006).

**Habitat.** Alpine habitats, in poorly-drained wetlands and wet meadows, at 2700–3650 m elev.

Note. The taxon is the most similar to P. mongholica, however, it differs in having smaller glumes, lemmas and awns as well as by the general distribution range. Based on morphology (and also habitat preferences), P. mongholica subsp. porteri is the taxon most similar to S. mongholica var. minutiflora that was described 11 years later by Roshevitz (1916). We treat it here as conspecific with P. mongholica. However, further studies with the involvement of molecular methods are needed to reconstruct the phylogenetic relationships within this complex of taxa. Weber (2003) stressed that P. mongholica subsp. porteri differs from the nominal subspecies in habitat requirements, and the first taxon is restricted to montane fens and willow carrs, while subsp. mongholica is a taxon of rocky sites, mountain grasslands, and alpine meadows. Based on our observations and analysis of the habitat descriptions on herbarium labels, P. mongholica subsp. mongholica can grow in both dry and wet habitats (see above); thus, the habitat parameter does not seem to be a good enough character for the differentiation of these two taxa. Johnston (2006) mentioned that some populations of P. mongholica subsp. porteri have open panicles, while the other is contracted. Because individuals with open and contracted panicles can be found, sometimes even in the same populations of *P. mongholica* subsp. porteri, their taxonomic status should be a subject of further research.

Selected studied specimens of P. mongholica subsp. porteri. UNITED STATES • Park Co., South Park, 4 miles W of Jefferson, near Fremont's Knoll (see Pike Nat. Forest map); elev. 10,000 ft.; on peat hummocks in rich calcareous fen; 27 Jul 1989; D.J. Cooper 1682 (COLO00769711) • Park Co., Long Gulch, north of the road to Lost Park, 11.3 mi E of Jefferson; SW¼ SW¼ S13 T8S R74W; elev. 10020-10030 ft.; 4 Oct 1982; B.C. Johnston, L. Hendzel 2655 (COL000571570, COLO00571588) • Park Co., South Park. Hummocky area on edge of rich fen, Forest Service land ¼ mi NW of Silverheels Ranch house; 6 Jul 1990; D.J. Cooper 1788 (COL000571455) • Park Co., Lost Park, bottom just S of Lost Park Campground,;NW1/4 NW1/4 S12 T9S R73W; elev. 9840 ft.; 4 Oct 1982; B.C. Johnston, L. Hendzel 2658 (COL0571471) • Park Co., Geneva Park, Sec. 13; T.6S., R. 75W.; elev. 9,700 ft.; on hummocks in meadow; 26 Jul 1966; R. Gierisch, W.C. Hickey 3102 (COLO00571513) • Park Co., South Park, High Creek Fen, 10 mi. S of Fairplay; elev. 9300 ft.; on hummocks in moist part of calcareous fen, with Salix candida, S. brachycarpa, Pentaphylloides floribunda, Carex scirpoidea, Parnassia parviflora; 12 Aug 1996; N. Lederer, W. Jennings, W. Marotti, P. Murphy 96-HC-1 (COL000571497) • Park Co., South Park, on peat hummocks in rich fen, Albert Wahl Ranch at base of Kenosha Pass, 6 Sep 1990, D.J. Cooper 1942 (COLO00571604). Park Co., just northeast of junction of Hooper Trail and road to Lost Park, ca. 13 mi east of Jefferson; SW¼ SW¼ S13 T8S R74W; tops of peat- willow hummocks; elev. 10040 ft.; 13 Aug 1981; B.C. Johnston 2497 (COLO00571620) • Park Co., 11.3 mi. SE of Jefferson on road to Lost Park; on peaty hummocks of willow streamside, with Salix, Betula glandulosa and Potentilla fruticosa; 25 Sep 1966; W.A. Weber 12984 (COLO00571521).

**10.** *Ptilagrostis tibetica* (Mez) Tzvelev, Rasteniia Tsentral'noi Azii 4: 45. 1968. Suppl. material 2: fig. S12

Stipa tibetica Mez, Repertorium Specierum Novarum Regni Vegetabilis 17(13– 18): 207. 1921. **Basionym.** 

**Type.** Tibet Occ. Regio alp., Lasiag[rostis] Mongholica Trin., 14,000 ft., *Hooker fil. & Thomson* s.n. (holotype: B destroyed; lectotype K – H2012/0158/8! (Herbarium Hookerianum (1867) – specimen in the middle part of the sheet) **designated here**, isolectotype LE00009272).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms 20-45(-70.0) cm tall. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate; blades scabrous, filiform, convolute, green, pale green to greyish, 0.20-0.35 mm in diameter, with 3(-5) vascular bundles. Cauline leaves: sheaths glabrous or minutely scabrous; ligules on the lower sheaths lanceolate. Panicle open, 8-16 cm long and 5-10 cm wide; branches glabrous. Glumes subequal, purple, (4.5-)5.0-6.5 mm long, lanceolate. Floret (lemma + callus) 3.6-5.0 mm long. Callus 0.3-0.5 mm long, densely pilose; callus base obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs, hairless in the mid-length and with hairs at apex; lemma apex with two lobes. Awn 10-16 mm long, unigeniculate; the lower segment of the awn (column) 5-7 mm long, twisted, with 1.2-2.0 mm long hairs; terminal segment of the awn (seta) straight, 7-10 mm long, with 1.2-1.6 mm long hairs, gradually decreasing in length towards the apex. Anthers 2-3 mm long, glabrous at the apex.

Phenology. Flowering from August to September.

Figures. Figs 4a-c, 6s, 17.

**Distribution**. Mountain areas of south and south-western Central Asia, in India, Nepal and China (Zhang and Chen 2024).

Habitat. Alpine meadows and cryophilous steppes, at 4200-4800 m elev.

**Selected studied specimens of** *P. tibetica*. INDIA • Tibet Occ. Subchilum Kumaon; elev. 14500 ft.; *R. Strachey, J.E. Winterbottom* s.n. (H2012/0158/8) • NW India, Jammu and Kashmir State, Ladakh, Indus Vy: Zhung (Leh), Shagrot to confluence of Purang and Kyammar Lungpa; elev. 4370 m; 33°36,4'N, 77°45,8'E; 4 Sep 2001; *L. Klimeš 1533* (KRA479095) • Ladakh, Rupshu, Tso Moriri, Zerlung Marlung; elev. 4540 m; 32°53'N, 78°16,5'E; 25 Aug 1999; *L. Klimeš 658* (KRA479075, KRA479096) • Shushal, Ladak; among drama along stream; elev. 14,200 ft.; 26 Jul 1931; *W. Koelz 2445* (NY) • Tsakzhun Tso, Ladak; along stream; elev. 15,000 ft.; 21 Jul 1931; *W. Koelz 2401* (NY). – NEPAL • Dolpo, Mahajung Khola, 6 miles E of Tingkyu; elev. 4800 m; 4 Aug 1973; *Grayhilson, Phillips 489* (H2012/0158/9).

**Note.** Since *P. tibetica* grows together or in neighbouring localities with *P. dichotoma*, gene flow among these two taxa may sometimes occur. The result of such hybridisation can be specimens collected by Dickoré from Tibet, which are characterised by lemmas covered up to 2/3 by long hairs and long prickles above.

Studied specimens of potential hybrids *P. tibetica* × *P. dichotoma*. CHINA • S Tibet, Xizang: Tibetan Himalaya N of Bhutan, Kuru Chu, Hill SW of Lhozak



Figure 17. Ptilagrostis tibetica, general habit.

Vy. junction; subalp, moist meadow; 28°18'N, 90°51' E; elev. 4200 m; 22 Jul 1994; *B. Dickoré* 9758 (MSB-152874) • Tsangpo tributary, Nangxian - Mainling, Lilung Chu Eastern branch (High Camp); alp. moist turf spots, screes and boulder fields, gneiss; 29°0'N, 93°59'E; elev. 4820 m; 11 Aug 1994; *B. Dickoré 10819* (MSB-152848).

#### Ptilagrostis sect. Barkworthia M. Nobis, A. Nobis & A. Nowak

#### Type. P. yadongensis Keng & Tang

**Description.** Species belonging to this section are characterised by clearly unequal lemmas and paleas (lemma 0.7-2.0 mm longer than palea), awns with hair on the upper segment < 1 mm long (usually 0.2-1.0 mm long) and 2-3 times shorter than on column, and the glumes unequal.

# 11. *Ptilagrostis yadongensis* Keng & Tang, Journal of Southwest Agricultural University 4: 44. 1985.

- = Ptilagrostis macrospicula Cai, Acta Botanica Boreali-Occidentalia Sinica 23(11): 2018. 2003. superfl. name.
- Stipa milleri Noltie, Edinburgh Journal of Botany 56(2): 288. 1999; = Ptilagrostis milleri (Noltie) M. Nobis & A. Nobis, Nordic Journal of Botany 31: 623. 2013.
   Type: India, Sikkim, Goichang, Lasha Chhu valley, 27°55'52"N, 88°36'17"E, 4555 m a.s.l., 19 Jul 1996, EENS 349 (holotype: E!, isotype: BSHC).

**Type:** CHINA, Xizang: Yadong, 14 Sept. 1974, *Qinghai-Xizang Exped.* 74–2496 (lapsus calami as 74–2469; holotype: HNWP, isotype: PE).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (7-)8-15(-20) cm tall, 1-2-noded distributed in the lower part of the culm and usually hidden within the leaf-sheath. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate, on the external sheaths (1.0-(1.3-1.5(-1.6)) mm long, whereas on the internal sheaths, (1.3-)(1.6-2.0(-4.0))mm long; blades filiform, convolute, green to pale green or greyish, (5.7–)7.0–9.0 cm long, 0.3-0.4(-0.5) mm in diameter, with 3-5 vascular bundles, adaxial (upper) surface covered by 0.05-0.1 mm long hairs, abaxial surface scabrous or less frequently (some leaves) glabrous. Cauline leaves: sheaths glabrous; ligules on the lower sheaths lanceolate (1.2-)1.4-1.7(-1.8) mm long, on the middle and upper sheaths (1.5-)1.7-2.6(-3.0) and (1.7-)2.0-3.7(-4.0) respectively; blades convolute, green, pale green or greyish, adaxial (upper) surface covered with 0.05-0.1 mm long hairs, abaxial (lower) surface scabrous. Panicle contracted, (3.5-)3.9-4.9(-5.3) cm long, at base enclosed by the sheath of the uppermost leaf; branches ascending, scabrous or almost so, single or paired, lower branch (1.9-)2.1-2.9(-3.5) cm long. *Glumes* unequal, the lower (1.5-)1.8-2.5(-2.7) mm longer than the upper, brownish or purplish, lower glume (10.0-)11.0-11.5(-12.3) mm long, upper glume (7.5–)8.5–10.0(–11.0) mm long, lanceolate. *Floret* (=anthecium, =lemma + callus) 5.0-6.3(-6.6) mm long. Callus (0.4-)0.5-0.6 mm long, densely pilose, on ventral part with hairs 0.4-0.5 mm long, on dorsal with 0.4 mm long hairs; callus base 0.4-0.5 mm long and 0.3-0.4 mm in diameter, obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs 0.4-0.5 mm long, hairless in the mid-length and with hairs at apex; lemma apex with unequal hairs (0.4-)0.6-0.8 mm long and with two apical lobes (0.3-)0.4-0.5(-0.6) mm long. Palea in (0.3–)0.7–1.3(–1.5) mm shorter than lemma in length. Awn (13– )15-17(-18) mm long, unigeniculate; the lower segment of the awn (column) (5-)6-7(-8) mm long, twisted, with (1.2-)1.4-1.7(-1.8) mm long hairs; terminal **segment of the awn (seta)** straight, 8–10 mm long with hairs shorter than those on columns, (0.6–)0.7–0.9 mm long, gradually decreasing in length towards the apex. **Anthers** ca. 0.7–1.4 mm long, glabrous at the apex.

**Phenology.** Flowering from July to September.

**Figures.** Figs 4p-s, 6m, n, 18; additional figures in Noltie (1999: 286) and Cai (2003).

**Distribution.** Bhutan, China: Xizang, Nepal (Noltie 1999; Wu and Phillips 2006; Nobis and Nobis 2013; Nobis et al. 2015, 2019b; Zhang et al. 2016b).

**Habitat.** Alpine grasslands, open moraine screes, rocks and sands near lakesides, at 3500–4900 m elev.

Selected studied specimens of P. yadongensis. BHUTAN . Thimphu (Upper Mo Chu), Lemcheng; herbaceous vegetation regenerating after fire on S-facing slop; elev. 4550 m; 27°58'N, 89°30'E; 16 Jul 2000; G. & S. Miehe 00-211-11 (E00180489). - CHINA · Xizang: S Tibet, Tibetan Himalaya, Everest E, Kama Chu, Pethang Ringmo Up, Kangchung Gl; 27°59'N, 87°6'E; elev. 4770 m; alp. dry Kobresia pygmaea-Stipa turf, cushions and open moraine scree, gneiss; 13 Oct 1989; B. Dickoré 6361 (KRA528812, MSB-152846). - NEPAL • Pandang Keipo (Upper Langtang); elev. 4600 m; Kobresia nepalensis mat with Gremineae, S-facing slope, grazed seasonally; 30 Sep 1986; G & S. Miehe 13090 (KRA528815) • Eastern Development Region, Sagarmatha, Solu Khumbu, Chola Tsho, north side of lake; 27°55'18"N, 86°47'50"E; elev. 4500 m; SE facing slope, rocks and sand near lakeside, Juniperus indica dwarf scrubland with Rhododendron setosum and Potentilla ruticose; 21 Sep 2005; M.F. Watson, K.R. Rajbhandari, K.K. Shrestha, D. Knott, C.A. Pendry, S.K. Acharya, U. Koirala, L.N. Mandar, N. McCheyne, R.C. Poudel, S. Rajbhandary, S. Vaidya DNEP3 AX98 (E00270142). Additional specimens studied are listed in Suppl. material 1.

#### 12. Ptilagrostis bhutanica (Noltie) M. Nobis, PhytoKeys 128: 109. 2019.

Stipa bhutanica Noltie, Edinburgh Journal of Botany 56(2): 289. 1999. Basionym.

**Type:** Bhutan. Ha: W side of Chelai La, 29 Sept. 1998, H.J. Noltie, N. Pradhan, Sherub & T. Wangdi 349 (holotype: E!, isotype: THIM).

**Description.** *Perennial plants*, densely tufted, with a few culms and numerous vegetative shoots; culms (6-)8-41(-54) cm tall, 2-noded distributed in the below the middle of the culm length, the upper one usually visible the lower hidden within the leaf-sheath. *Leaves of vegetative shoots:* sheaths glabrous or less frequently scabrous; *ligules* lanceolate, on the external sheaths (0.9-)1.2-1.8(-2.3) mm long, whereas on the internal sheaths, (1.3-)1.6-2.5(-3.5) mm long; *blades* filiform, convolute, green, pale green to greyish, (6.1-)6.5-16.0(-25.5) cm long, 0.4-0.6(-0.7) mm in diameter, with (5-)7 vascular bundles, adaxial (upper) surface covered by 0.05-0.1 mm long hairs, abaxial (lower) surface scabrous (some leaves can be glabrous). *Cauline leaves:* sheaths glabrous; *ligules* on the lower sheaths lanceolate, (1.0-)1.5-2.0 mm long, on the middle and upper sheaths (1.5-)1.8-2.5(-3.5) and (2.0-)2.4-3.0(-3.7) respectively; *blades* convolute, green, pale green or greyish, adaxial surface covered with short hairs, abaxial surface scabrous. *Panicle* contracted, (3.6-)5.0-9.5(-13.0) cm long; *branches* ascending, scabrous, single or paired, lower



Figure 18. Ptilagrostis yadongensis, general habit.

branch (1.8-)2.7-5.0(-6.0) cm long. *Glumes* subequal, the lower 0.2-0.5(-0.8) mm longer than the upper, dark purple, lower glume 7.2-9.4(-12.0) mm long, upper glume (7.0-)7.3-9.0(-11.5) mm long, lanceolate. *Floret* (lemma + callus) (5.8-)6.1-7.5(-8.4) mm long. *Callus* 0.4-0.6(-0.7) mm long, densely

pilose, on ventral part with hairs 0.3-0.5(-0.7) mm long, on dorsal with (0.3-)0.4-0.5 mm long hairs; callus base 0.4-0.5(-0.6) mm long and 0.3-0.4 mm in diameter, obtuse. *Lemma* coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs 0.3-0.5(-0.7) mm long, hairless in the mid-length and with hairs at apex; *lemma apex* with unequal hairs (0.3-)0.5-1.2(-1.8) mm long and without apical lobes. *Palea* (1.0-)1.2-2.0(-2.3) mm shorter lemma in length. *Awn* (12.0-)13.5-15.0(-17.0) mm long, unigeniculate; *the lower segment of the awn* (column) (4.5-)5.0-6.0(-7.0) mm long, twisted, with (0.5-)0.6-0.9 mm long hairs; *terminal segment of the awn* (seta) straight, (7-)8-9(-10) mm long, with hairs shorter than those on columns, 0.3-0.5 mm long, gradually decreasing in length towards the apex. *Anthers* ca. 0.8-2.0 mm long, glabrous at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 4d–f, 6i, 19; additional figures in Noltie (1999: 286); https:// www.gbif.org/species/12192155; https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77211695-1.

**Distribution.** Bhutan, China: Sichuan, Yunnan, Xizang (Noltie 1999; Nobis et al. 2016, 2020; Zhang et al. 2016b).

**Habitat.** Alpine grasslands, pastures, thickets and scrubs, forests at tree-line, at 3500–4500 m elev.

Selected studied specimens of P. bhutanica. BHUTAN • Thimphu District: Mountain E of Thimphu. Dry; open, grassy mountain top; elev. 3500 m; 24 Jul 1988; J.R.I. Wood 6504 (E00690620) • Gasa (Upper Mo Chu), Rodophu; forbrich pasture encroached by Rhododendron lepidotum on SE-facing slope; elev. 4330 m; 28°2'N, 89°47'E; 16 Aug 2000; G. & S. Miehe 00-289-31 (E00180486) · Gasa (Upper Pho Chu), Tarina Camp; Abies densa forest on S-facing lower slope; elev. 4040 m; 28°3'N, 89°57'E; 1 Sep 2001; G. & S. Miehe 00-342-07 (E00180448). - CHINA · Sichuan: Hanyuan County, Shuajingsi Town northeast mountain; subalpine environment; Z.X. Tang 1486 (KRA628871) · Kangding County, Jianguan camp; subalpine meadow; X.W. Tian 165(6) (KRA628872, KRA628873) · Litang County, Pingdi; environment alpine; elev. 4300 m (KRA528813) • Litang County; alpine meadow, south hillside; X.W. Tian 149 (KRA628870) • Xizang: E Tibet, Ningjing Shan, Mekong (Lancang) tributary, W of Markham (Gartog); 29°41'N, 98°30'E; elev. 4300 m; subalp.-lower alp. turf, Rhododendron dwarf-scrub, Picea forest at tree-line; 1 Jul 1994; B. Dickoré 8628 (MSB-152870, E00132107).

## *Ptilagrostis* sect. *Chenella* M.Nobis, Krzempek & Klichowska, sect. nov. urn:lsid:ipni.org:names:77351832-1

#### Type. Ptilagrostis duthiei (Hook.f.) M.Nobis & P.D.Gudkova.

**Description.** Species belonging to this section are characterised by having upper awn segments scabrous, culms (40-)50-100 cm tall, leaves of the vegetative shoots with 7–11 veins.

**Etymology.** The name of the section honours the eminent botanist Professor Wen-Li Chen (Chinese Academy of Sciences, Beijing, China), for her contribution to the knowledge of grasses.



Figure 19. Ptilagrostis bhutanica, general habit.

### 13. Ptilagrostis chingii (Hitchc.) M.Nobis & Krzempek, comb. nov.

urn:lsid:ipni.org:names:77351833-1 Suppl. material 2: fig. S13

*Stipa chingii* Hitchc., Proceedings of the Biological Society of Washington 1930, xliii. 94. 1930. **Basionym.** 

- Achnatherum chingii (Hitchc.) Keng, Claves Generum et Specierum Graminearum Primarum Sinicorum 213. 1957; nom. inval.
- = Achnatherum chingii (Hitchc.) Keng, Flora Tsinlingensis 1(1): 152. 1976.

**Type.** CHINA, Kansu Province, vicinity of Labrang, [collected in open woods, 4000 m] up to 3 ft., elev. 3000 to 4000 m, 17–20 Aug 1923, *R.C. Ching 785* (holotype: US-1245799; isotype E00890601!).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (35-)50-70(-95) cm tall, 2-3-noded distributed usually in the middle part of the culm, exerted from the leaf-sheaths. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate, on the external sheaths (0.4-)1.0-1.8(-2.5) mm long, whereas on the internal sheaths, (0.8-)1.3-2.0(-2.5) mm long; blades filiform, convolute, green to pale green, (15.4-)23.0-28.9(-35.3) cm long, (0.2-)0.3-0.4(-0.5) mm in diameter, with 3-5 vascular bundles, adaxial surface covered by 0.1-0.2 mm long hairs, abaxial surface scabrous or rarely glabrous. Cauline leaves: sheaths glabrous or less frequently minutely scabrous; ligules on the lower sheaths lanceolate (1.1-)2.0-2.7(-3.3) mm long, on the middle and upper sheaths (1.8-)2.5-3.8(-4.8) and (2.3-)3.0-4.3(-5.1) respectively; blades convolute, green, pale green or greyish, adaxial surface covered with short hairs, abaxial surface scabrous or glabrous. Panicle contracted to loosely contracted (sporadically open in var. laxum), (7.5-)12.0-19.5(-25.0) cm long; branches ascending, usually scabrous, single or paired, lower branch (2.1-)4.7-7.8(-11.3) cm long. *Glumes* subequal, the lower 0.1-0.3(-0.6) mm longer than the upper, yellowish, brown, green or purple, lower glume (5.3-)6.4-9.0(-10.2) mm long, upper glume (5.3-)6.4-8.5(-10.2) mm long, lanceolate. Floret (lemma + callus) (5.2-)5.9-6.7(-7.2) mm long. Callus (0.4-)0.5-0.7(-0.8) mm long, densely pilose, on ventral part with hairs (0.3-)0.4-0.6(-0.7) mm long, on dorsal with (0.3-)0.4-0.6(-0.8) mm long hairs; callus base 0.4-0.5(-0.8)0.6) mm long and 0.3-0.4(-0.5) mm in diameter, obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs (0.3-)0.4-0.5(-0.6) mm long, hairless in the mid-length and with hairs at apex; lemma apex with unequal hairs (0.2-)0.3-0.4(-0.5) mm long and with two apical lobes (0.5-)0.6-0.9(-1.2) mm long. Palea equal or slightly, in 0.2-0.4 mm shorter than lemma. Awn (11-)12-14(-16) mm long, 1-geniculate; the lower segment of the awn (column) (4.0-)5.0-6.0(-7.0) mm long, twisted, with (0.4-)0.5-0.6(-0.7) mm long hairs; terminal segment of the awn (seta) straight, (6-)7-9(-10) mm long, scabrous, at base with 0.2-0.3 mm long hairs, gradually decreasing in length towards the apex. Anthers ca. 2.0-3.3 mm long, bearded (occasionally glabrous) at the apex.

Phenology. Flowering from July to September.

**Figures.** Figs 4g-i, 6g, h, 20; additional figures in Wu et al. (2007: fig. 286); http://www.efloras.org/object\_page.aspx?object\_id=94971&flora\_id=2; https:// www.gbif.org/tools/zoom/simple.html?src=//api.gbif.org/v1/image/cache/ occurrence/1018988494/media/46df1686e91fd3599853d46b42f1d539.

**Distribution.** Bhutan, China: Gansu, Qinghai, Shaanxi, Shanxi, Sichuan, Xizang, Yunnan, India (Wu and Phillips 2006).

**Habitat.** Alpine grasslands, pastures, steep rocky, dry slopes, thickets, coniferous and mixed forests, at 2000–4100 m elev.

Selected studied specimens of *P. chingii*. BHUTAN • Gasa (Upper Mo Chu), Thangkaphu Chu/Tsharijathang junction; open *Abies-Betula* forest on NE-facing slop, Takin pasture and rest places; elev. 4070 m; 27°59'N, 89°32'E; 23 Jul 2000; *G.*& *S.Miehe* 00-237-03 (E00180485). – CHINA • Kansu: vicinity of Labrang; elev. 3000 to 4000 m; 17–20 Aug 1923; *R.C. Ching* 785 (E00690601) • Qinghai: N of Hushu, Twelve



Figure 20. Ptilagrostis chingii, general habit.

Windings Slopes; mountain slope with *Rhododendron, Berberis* and *Potentilla*; 37°01'43.39"N, 102°14'52.93"E; 3064 m; 28 Jul 2010; *B. Paszko 557* (KRA634202) • wild hillside forest; elev. 3800 m; 9 Aug 1983 (KUN0234291) • Shaanxi: Taibai Mountain; hillside wetland; elev. 2400 m; 1 Aug 1956 (KUN0319602) • Xiaoshi Cave, Dongbao, Ningwu; elev. 2000 m; 8 Aug 1984 (MO4741165) • Beside the village of Majia, Ningwu; hillside grassland; 26 Jul 1957; *Shanxi Team* s.n. (MO4486351)

· Sichuan: Sikang: Taofu (Dawo) distr., Mt Yara, NW slopes; in silva Larcin; elev. 4000 m; 29 Aug 1934; H. Smith 11894 (V-040409, MO4365633) • Sikang: Taofu (Dawo) distr., valley west of Mt Yara; in prato herboso-fruticoso; elev. 3900 m; 1 Sep 1934; H. Smith 11791 (V-040410, MO4365640) • Ch'un-ch'e; reg. bor, in silva abietina; elev. 3200 m; 1 Aug 1922; H. Smith 4129 (V-038567) • inter Mergo et Sankar; reg. bor.-occid. in prato aprico; elev. 3500 m; 3 Sep 1922; H. Smith 4215 (MO4366937) • Xiangcheng Xian, Reda: Vicinity of the town of Reda; 99°37'55"N, 29°6'11"E; elev. 3450-3650 m; dry slopes with cut over Quercus, Pinus, Berberis, Cotoneaster, around Quercus in scrubby area; 16 Jul 1998; D.E. Boufford, B. Bartholomew, C.Y. Chen, M.J. Donoghue, R.H. Ree, H. Sun & S.K. Wu 28773 (M05308755; E00293189; NY) • Kangding Bridge; hilly terrain; elev. 3600 m; 20 Jul 1963; K. Guan, W. Wang s.n. (MO4711607) • Xiangcheng County, Wuming Mountain; elev. 3850 m; 17 Aug 1981 (KUN0234200) • elev. 3500-3600 m; 21 Aug 1985 (KUN0234292) • elev. 4000 m; 31 Aug 1962 (KUN0234195) • elev. 3700-3900 m; 7 Sep 1953 (KUN0234286) • Xizang: Nanggên Xian, Bêca Xiang: along the Ba Qu towards the Xizang border from Bêca Forest Station, SE of Bêcaka; elev. 3600 m; 31°50'N, 96°33'E; Picea likiangensis forest, mostly on steep rocky, moss-covered slopes; some areas partially felled, flat areas near river with Salix bushland and open disturbed areas, growing among mosses at valley bottom under large Salix shrubs; 8 Sep 1996; T.N. Ho, B. Bartholomew, M. Watson, M. Gilbert 2980 (E00059739) • Yushu Xian: just E of Jiangxi Forest Station on E side of the Zi Qu, SE of Mozhong; elev. 3540 m; 32°5'N, 97°1'E; growing under Picea; 27 Aug 1996; T.N. Ho, B. Bartholomew, M. Watson, M. Gilbert 2479 (E00061514, MO5094288). - INDIA · Kashmir: above Gulmarg; elev. 12000 ft.; Aug 1926; R.R. Stewart 8788 (NY).

**Note.** During the revision of the herbarium materials, we found two specimens of *P. chingii* collected from Bhutan that were identified as *P. bhutanica*. Similar misidentification was mentioned by Zhang et al. (2016b) in the case of *P. bhutanica* collected in China (but misidentified as *P. chingii*). The two species, however, differ in the ratio of lemma to palea length, a difference that is apparently more pronounced in *P. bhutanica*. Other features to distinguish among the two species are the number of vascular bundles present in leaf cross-section, where *P. chingii* usually has 5, while *P. bhutanica* 7 and the length of hairs on the seta being 0.2–0.3 and 0.3–0.5 mm, respectively.

Within *P. chingii*, specimens with panicles contracted to loosely contracted and with panicles open are observed. Specimens with contracted to loosely contracted panicles represent the typical variety *P. chingii* var. *chingii*, whereas the second one, with open panicles (Suppl. material 2: fig. S14), is here recognised as a distinct variety.

### *Ptilagrostis chingii* var. *laxum* (S.L.Lu) M.Nobis & Krzempek, comb. nov. urn:lsid:ipni.org:names:77351834-1

- Achnatherum chingii var. laxum S.L. Lu, Acta Biologica Plateau Sinica 2: 19. 1984. **Basionym.**
- Achnatherum chingii var. laxum P. C. Kuo & S. L. Lu, Flora Xizangica 5: 257. 1987. nomen illeg. homonym.

Type. CHINA: Sichuan: Sertara, 10 Sept. 1961, Q.L. Zhang 350163 (holotype: NJU).

**Selected specimens studied of** *P. chingii* var. *laxum*. CHINA • Sichuan: Prov. Sze-ch'uan; reg. bor.-occid.: inter Mergo et Sankar in prato apricot, reg. bor.-occid; elev. 3500 m; 3 Sep 1922; *H. Smith* 4215 (MO4366937) • Xizang: Yushu Xian: just E of Jiangxi Forest Station on E side of the Zi Qu, SE of Mozhong; elev. 3540 m; 32°5'N, 97°1'E; growing under *Picea*; 27 Aug 1996; *T.N. Ho, B. Bartholomew, M. Watson, M. Gilbert* 2479 (E00061514, MO5094288).

# 14. *Ptilagrostis duthiei* (Hook.f.) M. Nobis & P.D. Gudkova, PhytoKeys 128: 107. 2019.

Stipa duthiei Hook.f., Flora of British India 7: 232. 1896. Basionym.

■ Achnatherum duthiei (Hook.f.) Kuo & Lu, Flora Reipublicae Popularis Sinicae 9(3): 322, pl. 80, f. 9–14. 1987.

**Type.** [India] Tehri Garwhal, Lekhus, below Srikanta, 12000–13000 ft, 11 Aug. 1853, *Duthie 273* (holotype: K 32097!, isotype CAL 2350!).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms (40-)60-100(-110) cm tall, 3-noded, distributed below the middle of the culm, exerted from leaf-sheaths. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate, on the external sheaths (0.5-)1.0-1.4(-1.5) mm long, whereas on the internal sheaths, (0.5-)0.9-1.7 mm long; blades convolute, green, pale green to greyish, (17.4-)31.7-41.4(-52.5) cm long, (0.5-)0.6-1.0 mm in diameter, with (7-)9-11 vascular bundles, adaxial surface covered by 0.15–0.25 mm long hairs, abaxial surface glabrous or less frequently minutely scabrous. Cauline leaves: sheaths glabrous or less often slightly scabrous; ligules on the lower sheaths lanceolate (0.8-)1.5-2.0(-2.6) mm long, on the middle and upper sheaths (1.9-)2.0-2.2 and 2.2-2.9(-3.9), respectively; blades convolute, green, pale green or greyish, adaxial surface covered with short hairs, abaxial surface glabrous or less frequently scabrous. Panicle open, 20.3-22.5(-25.6) cm long; branches ascending, usually scabrous, single or paired, lower branch (4.8-)5.7-9.0(-10.7) cm long. Glumes subequal, the lower 0.2–0.5 mm longer than the upper, yellowish, brown, green or purple, lower glume (9.3-)9.5-11.0(-11.8) mm long, upper glume 9.0-11.0(-11.6) mm long, lanceolate. *Floret* (lemma + callus) (6.2-)6.5-7.2(-7.5) mm long. Callus 0.5-0.6(-0.7) mm long, densely pilose, on ventral part with hairs (0.3-)0.4-0.6(-0,8) mm long, on dorsal with (0.3-)0.4-0.5 mm long hairs; callus base (0.3-)0.4-0.6 mm long and 0.3-0.4(-0.5) mm in diameter, obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs 0.3-0.4(-0.5) mm long, hairless in the mid-length and with hairs at apex; lemma apex with unequal hairs 0.2-0.3(-0.4) mm long and with two minute apical lobes 0.2-0.3(-0.5)mm long. Palea equal or slightly, 0.1–0.3 mm shorter than lemma in length. Awn (12-)14-16(-17) mm long, unigeniculate; the lower segment of the awn (column) (4-)5-6(-7) mm long, twisted, with (0.4-)0.5-0.6 mm long hairs; terminal segment of the awn (seta) straight, (8-)9-10(-11) mm long, scabrous, at base with hairs 0.2–0.3 mm long, gradually decreasing in length towards the apex. Anthers ca. 3-4 mm long, bearded at the apex.

Phenology. Flowering from July to September.

Plilagnostis Suthiei [Hod.] MAG. St. M. Nobis 2019 Supa duthiei Ktock f. Dar. 2011 1357 19 57 En North of Base M. 13,000 ltone 14/8/1954 Anny dwaf Redolendron Inflorescence purple-green. No. 3844 Coll. Stainton, Sykes & William

Figure 21. Ptilagrostis duthiei, general habit.

**Figures.** Figs 4m-o, 6a, b, 21; additional figures in Wu et al. (2007: fig. 281); http://www.efloras.org/object\_page.aspx?object\_id=94972&flora\_id=2; https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77200949-1; https://www.gbif.org/species/10596562.

**Distribution.** Bhutan, India, Nepal, China: Qinghai, Shaanxi, Sichuan, Xizang, Yunnan (Freitag 1985; Noltie 2000; Wu and Phillips 2006; Nobis et al. 2019b).

**Habitat.** Alpine grasslands, shrublands, coniferous and mixed forests, at (2500–)3500–4000(–4500) m elev.

**Selected studied specimens of** *P. duthiei*. CHINA • Shaanxi; elev. 3650 m; 16 Jul 1963 (KUN0323199) • Sichuan; E Tibet, Litang - Batang, Jinsha (Yangtse) tributary, E of Yidun/Yarw; 30°15' N, 99°25'E; mont. moist *Salix* scrub by stream; elev. 3640 m; 25 Jun 1994; *B. Dickoré 8343* (MSB-152907) • Sikang, between Taining (Ngata) and Taofu (Dawo), Sunglingk; in silva muscosa abietina; elev. 3900 m; 11 Sep 1934; *H. Smith 12034* (V-040408, MO4365639) • Sikang, Kangting (Tachienlu) distr., Chungo valley, Mt Yara, NE slope; in silva mixta; elev. 3900 m; 18 Aug 1934; *H. Smith 11145* (V-040407) • Sze-ch'uan, reg. bor., Dongrergo (Hsioeh-pau-ting); in silva mixta; elev. 4000 m; 20 Jul 1922; *H. Smith 3797* (V-040980) • elev. 3650 m; 16 Jul 1963 (KUN0323199) • Sikang, Kangting (Tachienlu) distr.: Chungo valley: in jugo bor.-orient. montis Yara; in silva mixta; elev. 3900 m; 18 Aug 1934; *H. Smith 11145* (MO4365638). – NEPAL • North of Barse; among dwarf *Rhododendron*; elev. 13,000 ft.; 14 Aug 1954; *Stainton*, *Sykes & Williams 3844* (BM001191540, E00690624).

# 15. *Ptilagrostis contracta* Z.S. Zhang & W.L. Chen, PlosOne, 12(1): e0166603: 3-4. 2017.

**Type.** CHINA. Sichuan: Litang, elev. 3701 m, 26 Sep 2014, *Z.S. Zhang & L.L. Li* 341 (holotype: PE).

Description. Perennial plants, densely tufted, with a few culms and numerous vegetative shoots; culms 57-105 cm tall, 3-noded distributed below the middle of the culm, exerted or hidden by the leaf-sheaths. Leaves of vegetative shoots: sheaths glabrous; ligules lanceolate, on the external sheaths 0.5-0.6 mm long, whereas on the internal sheaths, 1.0-2.1 mm long; blades convolute, green, pale green to greyish, 27.2-30.3 cm long, (0.6-)0.7-1.2 mm in diameter, with 11-14 vascular bundles, adaxial surface covered by 0.15-0.25 mm long hairs, abaxial surface glabrous and smooth. Cauline leaves: sheaths glabrous; ligules on the lower sheaths lanceolate 0.6-1.1 mm long, on the middle and upper sheaths 1.5–1.6 and 1.8–2.1, respectively; **blades** convolute, green, pale green or greyish, adaxial surface covered with short hairs, abaxial surface glabrous. Panicle contracted, 13-31 cm long; branches ascending, glabrous, single or paired, lower branch 2.7-4.5 cm long. Glumes subequal, the lower slightly 0.2-0.4 mm longer than the upper, yellowish, brown, green or purple, lower glume 10.0-14.0 mm long, upper glume 10.0-14.0 mm long, lanceolate. Floret (lemma + callus) 7.0-8.3 mm long. Callus 0.7 mm long, densely pilose, on ventral part with hairs 0.3-0.5 mm long, on dorsal with 0.4 mm long hairs; callus base 0.5 mm long and 0.3-0.5 mm in diameter, obtuse. Lemma coriaceous, pale-green, purplish or brownish, covered from the bottom up to 1/3 of its length, by dense ascending to appressed hairs 0.4–0.5 mm long, hairless in the mid-length and with hairs at apex; lemma apex with unequal hairs 0.5-0.6 mm long and with two apical lobes 0.5-0.6 mm long. Palea slightly, 0.1–0.2 mm shorter lemma in length. Awn 12–16(–20) mm long, unigeniculate; the lower segment of the awn (column) 4–7 mm long, twisted, with 0.4–0.6 mm long hairs; terminal segment of the awn (seta) straight, 7–9 mm long, scabrous, at base with 0.2(-0.3) mm long, gradually decreasing in length towards the apex. Anthers 2.5-3.0 mm long, bearded at the apex.





Phenology. Flowering from July to September.
Figures. Figs 4j–l, 6c, d, 22; additional figures in Zhang et al. (2017: fig. 1, 3).
Distribution. China: Sichuan (Zhang et al. 2017).
Habitat. Alpine grasslands, thickets and forests, at 3500–4300 m elev.

Selected studied specimens of *P. contracta*. CHINA • Sichuan: Sikang: Taofu (Dawo) distr., Haitzeshan; in prato herboso-fruticoso; elev. 3900 m; 26 Aug 1934; *H. Smith 11589* (V-040415) • Sikang: Taofu (Dawo) distr., Mt Yara, NW slopes; in silva *Larcina*; elev. 4000 m; 29 Aug 1934; *H. Smith 11607* (V-038902) • Hei-tze-shan; in the lake side; elev. 4600 m; 29 Aug 1934; *C.S. Liu* s.n. (PE 00052432, PE00052433) • Rangtang County, Peng Du; subalpine shrub meadow in the middle of the valley; elev. 4100 m; 17 Jul 1975 (PE00052429).

### Species excluded from Ptilagrostis

- *Ptilagrostis kingii* (Bol.) Barkworth = *Ptilagrostiella kingii* (Bol.) Romasch. [basionym *Stipa kingii* Bol.; ≡ *Oryzopsis kingii* (Bol.) Beal; Peterson et al. 2019].
- *Ptilagrostis purpurea* (Griseb.) Roshev. = *Stipa purpurea* Griseb. [Nobis et al. 2020, 2022].
- Ptilagrostis pelliotii (Danguy) Grubov = **Achnatherum pelliotii** (Danguy) Röser & H.R. Hamasha [basionym *Stipa pelliotii* Danguy; Hamasha et al. 2012].
- Ptilagrostis semenovi Krasn. = **Stipa tremula** (Rupr.) M. Nobis [=Stipa semanowii Krassn.; Nobis et al. 2022].
- *Ptilagrostis subsessiliflora* (Rupr.) Roshev. = *Stipa subsessiliflora* (Rupr.) Roshev. [Tzvelev 1976, Freitag 1985, Nobis et al. 2020].

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## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

M.K. revision of the herbarium materials, field studies, macro- and micromorphological analyses, leaf cross-section analysis with the input of MN, molecular analyses, inter-

pretation and visualisation of the results; E.K. field studies, molecular analyses with the input of M.N. and interpretation and visualisation of the results; M.N. idea of studies and project coordination, revision of the herbarium materials, field studies, SEM studies, interpretation and visualisation of the results; M.K, M.N. writing the manuscript with the input of EK. All authors read and approved the final version of the manuscript.

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#### **Data availability**

The SNP dataset derived from the DArTseq pipeline in the genlight format is available via Figshare repository, https://figshare.com/s/2f39354c7ead1305e705.

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## Supplementary material 1

## Insights to the taxonomy and phylogeny of the genus *Ptilagrostis* worldwide (Poaceae, Stipeae) with a key to species identification, checklist and outlines for further studies

Authors: Marta Krzempek, Ewelina Klichowska, Marcin Nobis Data type: xlsx

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Link: https://doi.org/10.3897/phytokeys.249.128729.suppl1

## Supplementary material 2

#### Fourteen additional images

Authors: Marta Krzempek, Ewelina Klichowska, Marcin Nobis Data type: docx

- Explanation note: fig. S1. Cluster analysis (UPGMA) of all examined specimens of all Ptilagrostis species. List of specimens examined is presented in Suppl. material 1.
  fig. S2. The holotype of *Ptilagrostis alpina* (LE). fig. S3. The isotype of *Ptilagrostis arcuata* (K). fig. S4. The isotype of *Ptilagrostis concinna* (LE). fig. S5. The holotype of *Ptilagrostis concinna* var. *xizangensis* (MSB). fig. S6. The isotype of *Ptilagrostis dichotoma* (LE). fig. S7. The holotype of *Ptilagrostis dichotoma* var. *roshevitsiana* (LE).
  fig. S8. The holotype of *Ptilagrostis junatovii* (LE). fig. S9. The holotype of *Ptilagrostis concinna* subsp. *schischkinii* (LE). fig. S10. The holotype of *Ptilagrostis malyschevii* (LE). fig. S12. The lectotype of *Ptilagrostis tibetica* (K). fig. S13. The isotype of *Ptilagrostis chingii* (E). fig. S14. *Ptilagrostis chingii* var. *laxum*, general habitat (E).
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## PhytoKeys

#### **Research Article**

# *Leontopodium nyingchiense* (Asteraceae), a new species from Xizang (Tibet), China

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#### Abstract

Leontopodium nyingchiense, a new species of Asteraceae from the Xizang (Tibet) Autonomous Region of China, is described and illustrated here. Morphologically, it is most similar to *L. lingianum* but can be distinguished by the combination of monoecious and dioecious individuals, involucral bracts arranged in 3–5 series (with outer series herbaceous and middle to inner series membranous), fimbriate apices on female florets, rough-edged lobes on male florets, and achenes lacking costae. Phylogenetic analyses further support the separation of this new species from related taxa. Finally, we characterize this new species through both morphological comparisons and molecular analyses.

**Key words:** *Leontopodium*, morphological analysis, new species, Nyingchi, phylogenetic analysis



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#### Introduction

Leontopodium R.Br. ex Cass. belongs to the Asteraceae, Gnaphalieae, Filagininae (Smissen et al. 2020). It comprises perennial herbs or subshrubs with approximately 60 species worldwide, primarily distributed in mountainous regions of Asia and Eurasia. In China, there are 37 species and 2 varieties of *Leontopodium*, with 16 species being endemic to China (Chen and Bayer 2011). This genus is a common component of alpine meadows and slopes in the western and southwestern regions of China, with the Tibetan Plateau being the center of its species diversity (Safer et al. 2011; Lee et al. 2016; Zhao 2021) and 21 species distributed in Xizang (Chen et al. 2023). During the fourth national survey of traditional Chinese medicine resources conducted from 2013 to 2015, an unidentified species of Asteraceae was discovered in Lulang Town, Nyingchi City, Xizang (Tibet) Autonomous Region. In August 2024, this species was re-investigated, and additional specimens and molecular materials from leaves were collected. This species is morphologically very similar to *L. lingianum* (Y.L.Chen) Dickoré, but it differs from the latter in several characters. Its involucral bracts are arranged in 3–5 series, with the outer series being herbaceous and the inner and middle series having a membranous texture. The species can be either monoecious or dioecious. The achenes are without costa, puberulous, and papillose at the apex. The female floret corolla has fimbriate apices, and the male floret corolla has lobes that are rough and granulate at the edges. Based on morphological comparisons and phylogenetic analyses, we have identified this taxon as a new species.

#### Materials and methods

#### Morphological assessment

Three sets of specimens (a total of 12 specimens), collected from different individuals in 2015 and 2024 in Lulang Town, Nyingchi City, Xizang (Tibet) Autonomous Region, were examined. Based on a thorough review of the literature and detailed comparisons of specimens, we carefully analyzed the morphological differences in the involucre, florets, and achenes between the new species and *Leontopodium lingianum*.

#### **Phylogenetic analysis**

Based on the literature (Blöch et al. 2010; Xu et al. 2023), 24 ETS sequences were downloaded from GenBank for phylogenetic analysis, including 37 from Leontopodium and 3 from Gamochaeta as an outgroup (Table 1). DNA extraction from the leaf tissue of the new species was performed using the CTAB method, and ETS primers were designed using SnapGene v6.02 (Table 2). PCR amplification was then carried out, and the amplified products were detected by agarose gel electrophoresis. Following detection, the PCR products were submitted to Sangon Biotech (Shanghai) Co., Ltd. (https://store.sangon.com/) for sequencing. All sequences were aligned using MUSCLE v3.8.31 (Edgar 2004) and manually adjusted with PhyDE-1 v0.9971 (Müller 2005). The best nucleotide substitution model was determined using Modeltest v3.7 (Posada and Crandall 1998) based on the Akaike Information Criterion (AIC), with the HKY model selected as the best fit. Maximum likelihood (ML) analyses were inferred with RAxML 7.2.6 (Stamatakis 2006). A Bayesian phylogenetic tree was constructed using MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001). The Bayesian analysis started from a random tree and ran four Markov chain Monte Carlo (MCMC) algorithms, sampling every 100 generations for a total of 1,000,000 generations. The tree visualization was performed using Figtree v1.4.4 (Rambaut 2008).

Species	Voucher	GenBank accession
Leontopodium alpinum Colmeiro ex Willk. & Lange	Wiedermann, 9282 WU	FJ639981
Leontopodium andersonii C. B. Clarke	Dickoré, 14068 WU	FJ640006
Leontopodium artemisiifolium Beauverd	Dickoré, 14574 WU	FJ640009
Leontopodium caespitosum Beauverd	Dickoré, 14040 WU	FJ640010
Leontopodium calocephalum Beauverd	Dobner & Xiao, D01-2395 WU	FJ640012
Leontopodium campestre (Ledeb.) HandMazz.	_	OP946418
Leontopodium conglobatum (Turcz.) HandMazz.	_	OP946419
Leontopodium dedekensii Beauverd	Dobner & al., MD01-2358 WU	FJ640001
Leontopodium delavayanum HandMazz.	_	OP946421
Leontopodium discolor Beauverd	CCCP (US)	KT865349
Leontopodium fangingense Y.Ling	_	OP946422
Leontopodium forrestianum HandMazz. ex W.W.Sm. & al.	_	OP946423
Leontopodium franchetii Beauverd	Dickoré, 14499 WU	FJ639983
Leontopodium giraldii Diels	_	OP946426
Leontopodium haastioides HandMazz.	Dickoré, 9892 WU	FJ640013
Leontopodium himalayanum DC.	Dickoré, 5228 WU	FJ640004
Leontopodium jacotianum Beauverd	Dobner & al., Md01-2445 WU	FJ639995
Leontopodium japonicum Miq.	Togasi, 1228W	FJ639984
Leontopodium japonicum var. saxatile Y.S.Chen	-	OP946432
Leontopodium junpeianum Kitam.	-	OP946435
Leontopodium leontopodioides (Willd.) Beauverd	Narantuja, S-070800 BM	FJ640014
Leontopodium lingianum (Y.L.Chen) Dickoré	Dickoré, 10836 WU	FJ639988
Leontopodium microphyllum Hayata	Hörandl, 9549 WU	FJ640015
Leontopodium muscoides HandMazz.	_	OP946437
Leontopodium nanum (Hook.f. & Thomson ex C.B.Clarke) HandMazz.	Dickoré, 9539 WU	FJ640005
Leontopodium nivale (Ten.) A.Huet ex HandMazz.	Schneeweiss & Schönswetter, 8926 WU	FJ640017
Leontopodium niveum HandMazz.	Nie 1087 (KUN)	KT865359
Leontopodium ochroleucum Beauverd	Klimes, 03-21-30 WU	FJ639997
Leontopodium pusillum (Beauverd) HandMazz.	Miehe & al., 98-35212 B	FJ640018
Leontopodium sinense Hemsl.	Dobner & al., MD01-2397 WU	FJ639999
Leontopodium smithianum HandMazz.	_	OP946443
Leontopodium souliei Beauverd	Dobner & al., MD01-2404 WU	FJ639998
Leontopodium stoechas HandMazz.	Nie 2443 (KUN)	KT865363
Leontopodium stracheyi C.B.Clarke ex Hemsl.	Miehe & Miehe, 98-09509 WU	FJ640020
Leontopodium cf. stracheyi C.B.Clarke ex Hemsl.	Dickoré, 10529 WU	FJ640022
Leontopodium subulatum (Franch.) Beauverd	Nie 1074 (KUN)	KT865365
Leontopodium wilsonii Beauverd	-	OP946446
Gamochaeta norvegica (Gunnerus) Y.S.Chen & R.J.Bayer	-	OP946406
Gamochaeta pensylvanica (Willd.) Cabrera	-	OP946407
Gamochaeta sylvatica (L.) Fourr.	-	OP946409

 Table 1. GenBank accession numbers and vouchers for the samples used in this study.

 Table 2. ETS primer sequences and PCR reaction conditions.

DNA fragment Primer		Primer sequences	PCR reaction conditions		
ETS	ETS-F	GCGCAACAACTTCCACC	94 °C 5 min; 94 °C 1 min, 54 °C 45s, 72 °C 1 min,		
	ETS-R	GGCAGGATCAACCAGGT	30 cycles; 72 °C 5 min		

#### Results

#### **Taxonomic treatment**

*Leontopodium nyingchiense* X.Z.Lan, W.L.Zheng & W.Q.He, sp. nov. urn:lsid:ipni.org:names:77351835-1

**Type.** CHINA • Xizang (Tibet) Autonomous Region, Nyingchi city, Lulang town, alt. ca. 4440 m, 29°40.82'N, 94°46.71'E (DDM), 24 July 2015, *Xiao-Zhong Lan, Lian-Qiang Li 542621150724809LY* (holotype: XZE!; isotype: KUN! barcode 1628268) (Figs 1–5, Table 3).

**Diagnosis.** Leontopodium nyingchiense is morphologically most similar to *L. lingianum* but can be distinguished by several features. The species includes monoecious and dioecious individuals, with some plants having both male and female flowers in the same individual. Its involucral bracts are in 3–5 series; the outermost series is herbaceous, while the middle and innermost series are membranous. The female floret corolla has brown, fimbriate apices and a fringed, ragged tip, while the male floret corolla has lobes with rough, granulate edges. Additionally, the achenes lack costae.

Description. Perennial, pulvinate, caespitose herb, monoecious or dioecious, less than 10 cm tall. Leaves alternate or verticillate, imbricate, sessile, nearly sheathless at the base, elliptic, entire; apex herbaceous, both surfaces silky lanate; lower part membranous and glabrous, white with a reddish tint when young, turning brownish upon maturity, 3-12 mm × 1.6-2.5 mm. Capitula with uniform florets, either all male or all female, or bisexual florets, solitary at the apex of stems and branches, nearly sessile, immersed among leaves or slightly extending beyond them; ebracteate. Involucre hemispherical, 3-5 mm in diameter; phyllaries imbricate, in 3 series in male or monoecious capitula, in 5 series in female capitula; outermost bracts spatulate, entire, herbaceous, covered on both surfaces with lanate indumentum; middle and inner bracts 3-6 mm × 0.8-3 mm, lanceolate or linear, with fimbriate, lacerate margins, membranous, covered on the abaxial surface with lanate indumentum. Receptacle alveolate, with irregularly edged pits. Female florets fertile; corolla tubular, 2.5-3 mm long, 4-lobed at the apex, with brown fimbriate, lacerate margins. Male florets (or bisexual florets) do not produce viable seeds, with a non-functional ovary; corolla tubular, 1-3 mm long, 5-lobed at the apex, with short triangular lobes and rough-edged margins. Anthers linear, tailed at the base, without appendages at the apex; filaments free; stigma apex blunt. Achenes obovate-oblong, puberulous, ca. 1 mm long; sterile ovary ca. half the size of the achene, glabrous. Pappus in one layer, persistent, white, often serrulate, the upper part slightly thickened in male florets.

**Phenology.** Flowering from July to early August, fruiting from late August to mid-September.

**Etymology.** The epithet indicates the type locality, i.e. Nyingchi area, Xizang, China.

Vernacular name. lín zhī huǒ róng cǎo (Chinese pronuciation); 林芝火绒草 (Chinese name).

**Distribution and habitat.** *Leontopodium nyingchiense* was discovered in Lulang Town, Nyingchi City, Xizang, China. This new species grows in alpine meadows at elevations of approximately 4400–4800 meters.



Figure 1. Leontopodium nyingchiense X.Z.Lan, W.L.Zheng & W.Q.He A habitat B, C morphology in the field D, E whole plant.

Characters	Leontopodium nyingchiense	Leontopodium lingianum			
Plant	monoecious or dioecious	dioecious			
Involucre	involucral bracts in 3–5 series: the outer layer herbaceous, the middle and inner series membranous	involucral bracts multi-layered, densely imbricate, similar in shape to the leaves			
Floret	apex of the female floret corolla brown, with fimbriate and lacerate margins; lobes of the male floret corolla with rough-edged margins	upper part of the corolla densely covered with white hairs			
Achene	without a costa	with a costa			

Table 3. Morphological comparisons between Leontopodium nyingchiense and L. lingianum.

**Conservation status.** The species appears to be narrowly distributed, currently known only from alpine meadows near Dongbazai Village in Lulang Town, Nyingchi City, with approximately 300–400 individuals observed (a total of less than 1,000 individuals). The habitat of the Nyingchi Edelweiss is susceptible to disturbance or degradation. Further field investigations are needed to assess the precise distribution of the species, and it is possible that other populations might be found in similar habitats such as the Sejila Mountains. Therefore, we provisionally classify this species as Data Deficient (DD) according to the International Union for Conservation of Nature (IUCN 2024) criteria.

Additional specimens examined (paratypes). CHINA • Xizang (Tibet) Autonomous Region, Nyingchi city, Lulang town, Dongbazai Village, alt. ca. 4650 m, 29°40.08'N, 94°47.08'E (DDM), 10 Aug. 2024, *Wen-qi He, Lian-Qiang Li & Guo-Zhu Chai 542621240810002LY* (KUN!, barcode 1628269, ♂) • alt. ca. 4601 m, 29°40.07'N, 94°47.07'E (DDM), 10 Aug. 2024, *Wen-qi He, Lian-Qiang Li & Guo-Zhu Chai 542621240810032LY* (XZE!, ♀).



Figure 2. Leontopodium nyingchiense X.Z.Lan, W.L.Zheng & W.Q.He A male inflorescence B female inflorescence C dissected male inflorescence with female florets D dissected floret E hermaphroditic floret F dissected sterile branch G outer and inner involucral bracts H leaf I–J involucre.



**Figure 3.** *Leontopodium nyingchiense* X.Z.Lan, W.L.Zheng & W.Q.He **A** female herbarium specimen **B** male herbarium specimens (holotype and isotype).



Figure 4. Leontopodium nyingchiense X.Z.Lan, W.L.Zheng & W.Q.He A whole plant B male inflorescence C female inflorescence D male floret E dissected male floret F stamen G female floret H dissected female floret I leaf J outer involucral bracts (drawing by Wenqi He).

#### Molecular phylogeny

Bayesian analysis (Bayes) and Maximum Likelihood (ML) analysis yielded similar phylogenetic trees (Fig. 6), with each branch annotated with the corresponding Bayesian posterior probabilities and ML bootstrap values. The phylogenetic results indicate that the four samples of the new taxon form a well-supported



Figure 5. Leontopodium nyingchiense X.Z.Lan, W.L.Zheng & W.Q.He A–C morphological structure of pollen under SEM D–F morphological structure of seeds under SEM.

group (posterior probability = 98%, ML bootstrap = 100%). These samples are closely related to *Leontopodium calocephalum*, a relationship that is further supported by the phylogenetic tree constructed in this study. Previous studies by Blöch et al. (2010) and Xu et al. (2023) demonstrated that *Leontopodium* is a monophyletic group, a finding that is consistent with our results.

#### Discussion

According to the phylogenetic analysis, *Leontopodium nyingchiense* and *L. calocephalum* are very closely related, though they can be easily distinguished morphologically. The primary morphological distinction is that *L. nyingchiense* is a dwarf cushion plant and lacks involucral bracts. Morphologically, *L. nyingchiense* is most similar to *L. lingianum*, followed by *L. haastioides*. All three species are dwarf cushion plants and also lack involucral bracts, though *L. ny-ingchiense* differs from *L. haastioides* in that it does not produce rhizomes.

Sinoleontopodium was first described as a new genus by Chen (1985), but it was merged into the genus *Leontopodium* by Dickoré (Blöch et al. 2010), a classification accepted by POWO (Plants of the World Online https://powo.science.kew. org/). This classification was supported by our phylogenetic analysis using ITS and ETS sequences, which positioned this genus within *Leontopodium*. However, morphologically *L. lingianum*, *L. nyingchiense* and two species in the dense cushion group, *L. haastioides* and *L. aurantiacum*, are distinctly different from the other species in the genus due to the typical character of *Leontopodium*: prominent involucral bracts. This morphology suggests that *L. lingianum*, *L. nyingchiense* and the dense cushion group may warrant classification as a separate genus or subgenus. However, the phylogenetic results presented in this study indicate that, except for *L. aurantiacum* (for which molecular data is unavailable), the remaining three species are only distantly related. Therefore, with more molecular data, the classification of these species within the genus may need to be reassessed.



**Figure 6.** Bayesian consensus tree of *Leontopodium nyingchiense* and related species based on ETS sequences. The tree is constructed using the ETS sequences. Numbers below the branches represent maximum likelihood bootstrap support (ML), and numbers above the branches represent Bayesian posterior probabilities. *Leontopodium nyingchiense* is highlighted in blue.

Field observations of *L. nyingchiense* revealed both monoecious and dioecious individuals. Typically, monoecious individuals have one male floret in the center of the female inflorescence, with the male floret being much smaller than the female florets. In dioecious individuals, male inflorescences are slightly larger

than female ones, with the male florets being concealed among the leaves, while female inflorescences are located above the leaves. However, our dissection of samples in the laboratory revealed that in male plants, a small number of female florets occur within male inflorescences without any discernible pattern. These female florets are morphologically identical to those on female plants, possessing only a pistil with no stamens, and develop normally. In contrast, the male florets are functionally bisexual, with the pistil showing abnormal development, the ovary being sterile, and possessing five stamens. Therefore, in functional terms, this species lacks true male florets, possessing only bisexual and female florets. Under natural conditions, some fertile bisexual florets may occur, but the sample size in this study was insufficient, and further research is required.

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#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### **Author contributions**

Investigation: JQT, BXZ, FYZ, WQH, FYZ, ZHL, GZC. Methodology: WLZ, FYZ. Software: KHZ, ZFC. Writing - original draft: WQH. Writing - review and editing: XZL, WLZ.

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#### **Data availability**

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

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### PhytoKeys

**Research Article** 

## Resurrection of *Eurya degeneri* (Pentaphylacaceae), endemic to Kaua'i, Hawaiian Islands

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#### Abstract

*Eurya degeneri* Kobuski (Pentaphylacaceae), a dioecious shrub endemic to Kaua'i, is resurrected from synonymy with *E. sandwicensis* A. Gray. *Eurya degeneri* is distributed in montane mesic to wet forests in central and northwestern Kaua'i. It can be distinguished from *E. sandwicensis* by its glabrous young stems, leaves and pedicels, linear-elliptic to ovate-elliptic leaves, longer pedicels (5–11 mm long), and shorter petals (4.5–7 mm long). A key to Hawaiian *Eurya* is provided. Two lectotypes are here assigned. *Eurya degeneri* is preliminarily designated as Critically Endangered according to the IUCN Red List guidelines.

**Key words:** Critically endangered, dioecious, *Eurya*, Hawaiian Islands, Kaua'i, Pentaphylacaceae, resurrection

#### Introduction

Eurya Thunb. (Pentaphylacaceae) is a genus of approximately 130 species of dioecious shrubs and trees distributed across Asia and the Pacific (Wang et al. 2013). In the Hawaiian Islands Eurya has been documented from mesic to wet forests on the islands of Kaua'i, O'ahu, Moloka'i, Maui, and Hawai'i (Wagner et al. 1999). Kobuski (1935) recognized two species and three infraspecific taxa of Hawaiian Eurya. The two species were differentiated by leaf shape: E. degeneri with cuneate leaf bases and acute apices and E. sandwicensis with subcordate to truncate leaf bases and obtuse or rounded apices. Wagner et al. (1999) placed all taxa described by Kobuski into synonymy with E. sandwicensis. Over the past several decades the National Tropical Botanical Garden (NTBG) Science staff have rigorously studied and documented the distribution and abundance of Hawaiian Eurya. Careful examination of stems, leaves, and floral structures from these more recent collections reaffirm Kobuski's (1935) designation of E. degeneri as a species and have revealed additional characters that further distinguish species of Hawaiian Eurya. In addition to the leaf shape characteristics described by Kobuski (1935), E. degeneri differs from E. sandwicensis in possessing glabrous young stems, leaves, and pedicels. Flowers in E. degeneri also have longer pedicels and shorter petals than E. sandwicensis. To date, no plants fitting the description of E. degeneri have been documented outside of Kaua'i.



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#### Methods

Herbarium specimens from PTBG were examined for the description of species. Specimens were selected to demonstrate the range of observed morphological variation and geographic distribution of *Eurya* across Kaua'i. Measurements for vegetative traits were made to the nearest mm. Both male and female flowers were selected for examination across both species. Flowers were rehydrated by boiling and measurements for reproductive traits were made to the nearest 0.5 mm. Herbarium specimens and label data were referenced for estimation of flowering phenology of *E. degeneri* and *E. sandwicensis* on Kaua'i. Diacritical marks following Pukui et al. (1974) were added to place names in the Specimens Examined sections. The proposed conservation status articulated here for *E. degeneri* follows the IUCN Categories and Criteria (IUCN 2012) and guidelines of the IUCN (IUCN 2024).

#### **Taxonomic treatment**

1. Eurya degeneri Kobuski, J. Arnold Arbor. 16: 351. 1935.

Figs 1, 3A

*Eurya sandwicensis* A. Gray forma *grandifolia* Wawra, Flora. 56: 168. 1873. Type: Hawaiian Islands. Kaua'i: *Wawra 2025* (lectotype, designated here: W [W0221862!]; isolectotype: W [W0221863!]).

*Eurya degeneri* Kobuski forma *grandifolia* (Wawra) Kobuski, J. Arnold Arbor. 16: 352. 1935. Type: Based on *E. sandwicensis* A. Gray var. *grandifolia* Wawra.

*Eurya degeneri* Kobuski forma *stenophylla* Kobuski, J. Arnold Arbor. 16: 352. 1935. Type: Kaua'i, *Rock 17274* (holotype: P [P00641578!]).

**Type material. USA · Hawaiian Islands.** Kaua'i: Open forest, Waineke swamp Koke'e, 1 Jul, 1926, *O. Degener 8675* (holotype: A [00024935!]; isotypes: BISH [BISH1014632!, BISH1014633!], NY [NY0035824!], WIS [0255833!]).

**Description.** Densely branched shrubs or trees 1.5–5(–7) m tall, young stems glabrous, rarely glabrate. Leaves closely spaced; blades coriaceous, linear-elliptic, rarely oblong-elliptic, (1.5-) 2.2-5.0(-9.0) cm long, 0.7-2.4(-3.0) cm wide, midrib tinged reddish, secondary veins yellow-green, lower surface conspicuously reticulate, glabrous, upper surface glabrous, margins weakly revolute, crenulate with black inflexed mucronate teeth, apex acute to narrowly acute, occasionally acuminate, base acute to cuneate, rarely rounded, petioles 2-4 mm long. Flowers 1, rarely 2 in the leaf axils, erect to occasionally nodding; pedicels 5-11 mm long, glabrous, rarely glabrate; bracteoles 2, minute; sepals 5, purplish brown, thick-coriaceous, suborbicular, unequal, 3-6 mm long, glabrous, rarely glabrate, margins scarious, persistent, enlarging up to 6 mm in fruit; petals 5, pale yellow to cream, somewhat fleshy, obovate, 4.5-7 mm long, connate at base; staminate flowers with 10 stamens, filaments distinct, 1.5 mm long, adnate to base of petals, ca. 1/2 as long as anthers, anthers opening by longitudinal slits; pistillate flowers with 8-10 staminodes, staminodes 2-3.5 mm long, ovary 3-celled, styles 3, 1.5-2 mm long. Fruit a globose berry, dark bluish black, 7 mm in diameter. Seeds numerous.



Figure 1. Eurya degeneri Kobuski A habit B leafy twig C flower D fruits. Photos by K.R. Wood. A, B, D Wood, Heintzman & Deans 18866 (PTBG) C Wood & Perlman 17935 (PTBG).

**Etymology.** The specific epithet was selected by Kobuski (1935) to honor Otto Degener, whose collections in *Eurya* informed taxonomic work in the genus. **Phenology.** *Eurya degeneri* has been collected with buds, flowers, and fruit from February through October.

**Conservation status.** IUCN Red List Category. *Eurya degeneri* demonstrates an Extent of Occurrence (EOO) of ca. 80 km<sup>2</sup>, an Area of Occupancy (AOO) of 34 km<sup>2</sup>, and a population of ca. 217 mature individuals fragmented across 10 subpopulations. National Tropical Botanical Garden researchers have observed declines in EOO, AOO, habitat quality, and number of individuals in

subpopulations of *E. degeneri*. When these population characteristics are evaluated using the World Conservation Union's IUCN (2012) Red List Categories and Criteria *Eurya degeneri* is preliminarily designated as: CR B1ab(i,ii,iii,iv,v)+ C2a(i). This Critically Endangered (CR) category designates the species as facing a very high risk of extinction in the wild. Relevant threats to both species of Hawaiian *Eurya* are elaborated upon in the "Conservation" section below.

Specimens examined. USA · Hawaiian Islands. Kaua'i: Alaka'i bog near Kilohana lookout, 1210 m, 13 Feb 2004, K.R. Wood & T. Menard 10574 (PTBG, BISH, US) · Hanākapī'ai headwaters, along crown ridge between Pihea and Wainiha, 1250 m, 22 Jul 2008, K.R. Wood 13187 (PTBG, BISH, US) · Awa'awapuhi, above stream and south of Kainamanu, 1088 m, 7 Feb 1995, K.R. Wood & D. Boynton 4022 (PTBG) • Kalalau Rim, North, below Puu o Kila, 950-1150 m, 6 Jul 1991, K.R. Wood 1022 (PTBG) • Limahuli Preserve, rim of Wainiha between Hono o Nā Pali and Pali 'Ele'ele, 975 m, 14 Oct 2007, K.R. Wood & N. Tangalin 12585 (PTBG, BISH) Limahuli Valley, east side of ridge separating Limahuli and Hanākapī'ai Valleys, above major waterfall, 770 m, 6 Aug 1991, K.R. Wood et al. 1109 (PTBG, MO, F) • Ridge below upper weather port, Hanākapī'ai side, 888 m, 30 Jun 2015, S. Walsh & M. Edmonds SKW78 (PTBG) • Upper Limahuli Preserve, Hanākapī'ai side in upper back bowl, 951 m, 19 Nov 2011, N. Tangalin 2898 (PTBG, NY, UC) • Hono O Nā Pali NAR, Pōhākea region, steep ridge above Hanākapī'ai, 914 m, 28 Jul 2011, K.R. Wood & M. Query 14733 (PTBG, BISH, MO, NY, US) • Hanākapī'ai, 1100 m, 14 Jan 2011, K.R. Wood et al. 18866 (PTBG) · 'Ohi'a/'Olapa Forest, 968 m, 5 Mar 2019, A.M Williams AMW559 (PTBG) • Kalalau valley, back of valley below Pihea area, 762 m, 15 Jun 2000, K.R. Wood & D. Boynton 8541 (PTBG) • Ridge down valley from near Pihea, 1054 m, 23 Jul 1992, S. Perlman and K.R. Wood 12905 (PTBG) Kokee, Hono O Nā Pali NAR, Pihea Trail to Pihea Vista and out east along ridge, 1321 m, 27 Sep 2010, N. Tangalin & K.R. Wood 2403 (PTBG, BISH, NY) • Lā'au Ridge, plateau region north of Pu'u Kamana and south of Kamakeanu, 1250 m, 17 Feb 2000, K.R. Wood et al. 8242 (PTBG) • Limahuli, upper east drainage between Pali 'Ele'ele and Hono O Nā Pali, 914 m, 9 Oct 1996, K.R. Wood & S. Perlman 5696 (PTBG, BISH, US) • North Bog, Rim of Wainiha, 1189 m, 29 Feb 2000, K.R. Wood et al. 8285 (PTBG) • Waikoali stream, Alaka'i Swamp, stream banks, 1127 m, 12 Jun 1996, S. Perlman & K.R. Wood 15400 (PTBG) • Waimea District, Koke'e State Park, along Kawaikoī Stream Trail, about 0.5 km northeast of intersection with Pihea Trail, 1010 m, 8 Sep 1988, D.H. Lorence et al. 6273 (PTBG) • Waikoali Bogs, Circle Bog, and scattered bogs to NE, 1219–1280 m, 21 Nov 1994, K.R. Wood et. al 3777 (PTBG) • Mohihi, 1183 m, 20 Sep 2018, K.R. Wood & S. Perlman 17935 (PTBG).

#### **2.** *Eurya sandwicensis* **A.** Gray, Bot. U.S. Expl. Exped. 1839–1842. 1: 209. 1854. Figs 2, 3B

*Ternstroemiopsis sandwicensis* (A. Gray) Urb., Ber. Deutsch. Bot. Ges. 14: 49. 1896. Based on *Eurya sandwicensis* A. Gray.

Eurya sandwicensis A. Gray var. prostrata Kobuski, J. Arnold Arbor. 16: 350.
1935. Type: Hawaiian Islands. Moloka'i, Degener 8676 (lectotype (designated here): A [00024940!]; isolectotype: A [00024939!]; isotypes: BISH [BISH1014635, BISH1014636!], G [G00354796!], MASS [00320484!], NY [00353842!], US [00435645!], WIS [0255835!]).



Figure 2. Eurya sandwicensis A. Gray A habit B leafy twig C flower D fruits. Photos by K.R. Wood A Wood, Query & Perlman 16077 (PTBG) B Wood, Query & Perlman 16187 (PTBG) C, D Wood, Heintzman & Deans 18839 (PTBG).

**Type. USA · Hawaiian Islands.** O'ahu: *W. Rich s.n.* (lectotype GH [00024938!], designated by St. John (1985: 569); isolectotypes: NY [00353841!], P [P00641573!], US [00113988!].

**Description.** Moderately branched shrubs or trees 1.5-4(-6) m tall, young stems sericeous to strigose, rarely glabrate, hairs golden yellow. *Leaves* closely spaced; blades coriaceous, ovate to oblong, rarely obovate, (2.5-)3.0-6.5(-9) cm long, (1.2-)1.5-3.0(-4) cm wide, midrib tinged reddish, secondary veins yellow-green, lower surface conspicuously reticulate, strigose along midrib, sparsely



Figure 3. Leaf and stem comparison of Kaua'i species of *Eurya* **A** *Eurya degeneri* **B** *Eurya sandwicensis*. Photos by Neil Brosnahan **A** *Wood*, *Perlman* & *Mehrhoff* 3777 (PTBG) **B** *Wood*, *Kirkpatrick* & *Perlman* 15599 (PTBG).

so on secondary veins, upper surface glabrous to glabrate, margins weakly revolute, crenulate with black inflexed mucronate teeth, apex obtuse to rounded, rarely acute or emarginate, base subcordate to truncate, rarely cuneate; petioles 1-3(-4) mm long. *Flowers* 1, rarely 2 in the leaf axils, nodding to suberect; pedicels 3-5(-7) mm long,  $\pm$  strigose with golden hairs; bracteoles 2, minute; sepals 5, purplish brown, thick-coriaceous, suborbicular, unequal, 3-7 mm long,  $\pm$  strigose, margins scarious, persistent, enlarging up to ca. 8 mm long in fruit; petals 5, pale yellow to cream, somewhat fleshy, obovate, (5-)8.0-10.5 mm long, connate at base; staminate flowers with (10-)15-16 stamens, filaments distinct, 1-2 mm long, adnate to base of petals, ca. 1/2 as long as anthers, anthers opening by longitudinal slits; pistillate flowers with (5-)8-10 staminodes, staminodes 2-3.5 mm long, ovary 3- or rarely 4-celled, styles 3 or rarely 4, 1.5-2 mm long. *Fruit* a globose berry, dark bluish black, 7-10 mm in diameter. Seeds numerous.

**Etymology.** The specific epithet was selected by Asa Gray (1854) to acknowledge the former European name of the Hawaiian Islands.

**Phenology.** On Kaua'i, *Eurya sandwicensis* has been collected with flowers from January through September and with fruit from April through December.

**Conservation status.** The conservation status of *Eurya sandwicensis* was last assessed in 1998. At that time, it was listed as Vulnerable (World Conservation Monitoring Centre 1998).

Specimens examined. USA · Hawaiian Islands. Kaua'i: Alaka'i, east of the Sincock bog system, wet forest near Kapoki, 1426 m, 8 May 2007, K.R. Wood & J. Fafson 12371 (PTBG) • Alaka'i Swamp, Kaua'i Forest Bird Survey, transect 3 between Halepaakai and Halehaha streams, 14 Feb 1989, S. Perlman et al. 10630 (PTBG, US, BISH, MO) · Along ridge between Kāhili and Kawaikini, back of Hanapēpē valley on one side and 'lole on the other, 1116-1189 m, 19 Sept 1994, S. Perlman & K.R. Wood 14389 (PTBG) • Hanalei District, Alakai, 1459 m, 8 Feb 2012, K.R. Wood 14878 (PTBG, UC) • Summit of Nāmolokama, 1265 m, 1 Feb 2000, K.R. Wood 8171 (PTBG) • 'lole headwaters, 991 m, 10 Jan 2012, K.R. Wood 14824 (PTBG) • loc. cit., 853 m, 28 Aug 2013, K.R. Wood et al. 15653 (PTBG, BISH, CAS) • loc.cit., 870 m, 8 Aug 2013, K.R. Wood et al. 15599 (PTBG, BISH) • loc.cit., 890 m, 29 Jul 2021, K.R. Wood et al. 18789 (PTBG) • loc.cit., 890 m, 20 Oct 2021, K.R. Wood et al. 18839 (PTBG) • Kamo'oloa headwater drainage below Kapalaoa, [853–975 m], 4 Oct 1996, K.R. Wood 5669 (AD, PTBG) • Wahiawa headwaters, SE below Kapalaoa, 863 m, 22 Dec 2010, K.R. Wood & N. Tangalin 14442 (PTBG) • NE Alaka'i, 1400 m, 13 Aug 2018, K.R. Wood 17905 (PTBG, BISH, CAS) • Ridge north of Kāhili, between Kapalaoa and Kawaikini, 963 m, 20 Sept 1994, S. Perlman & K.R. Wood 14395 (PTBG, HAST) • Ridge south of Kawaikini, 1400 m, 18 Sep 2014, K.R. Wood, M. Query, & S. Perlman 16077 (PTBG) • Ridge south of Kawaikini, 1341 m, 7 Jan 2015, K.R. Wood & M. Query 16187 (PTBG) • Wahiawa Mtns, on the ridge between Mt. Kāhili and the relay towers, 30 Aug 1984, T. Flynn 937 (PTBG) • Waimea Canyon, 792 m, 11 Apr 2010, K.R. Wood 14182 (PTBG, BISH, US).

#### Key to the Hawaiian species of Eurya

Distinguishing features of the two species of Hawaiian *Eurya* are summarized in Table 1. The species can be identified with the following key.

#### Relationships within Hawaiian Eurya

Although morphologically distinct and with different distributions, *E. degeneri* and *E. sandwicensis* share similar habitats, conservation threats, and reproductive traits. We describe and compare morphology, distribution, ecology, conservation, and reproductive attributes for the two Hawaiian *Eurya* species in the subsequent passages.

	1				
Character	E. degeneri	E. sandwicensis			
Habit	densely branched shrub or tree	moderately branched shrub or tree			
Young stems	glabrous	sericeous to strigose			
Leaf blade	linear-elliptic to ovate-elliptic	ovate to oblong			
Lower leaf surface	glabrous	strigose along midrib, sparsely so on secondary veins			
Leaf apex	acute to narrowly acute	obtuse to rounded			
Leaf base	acute to cuneate	subcordate to truncate			
Pedicels	5–11 mm long, glabrous	3–5 mm long, strigose			
Petals	4.5-7.0 mm long	(5–)8.0–10.5 mm long			
Sepals	glabrous, rarely glabrate	strigose			

#### Table 1. Comparison of morphological characters for Hawaiian species of Eurya.

#### Morphology

*Eurya degeneri* can be distinguished from *E. sandwicensis* by its densely branched habit (*vs* moderately branched); having young stems glabrous (*vs* sericeous to strigose); leaf blades linear-elliptic to ovate-elliptic (*vs* ovate to oblong); lower leaf surface glabrous (*vs* strigose along midrib, sparsely so on secondary veins); leaf apex acute to narrowly acute (*vs* obtuse to rounded); leaf base acute to cuneate (*vs* subcordate to truncate); pedicels 5–11 mm long, glabrous (*vs* 3–5 mm long, strigose); and petals 4.5–7.0 mm long (*vs* (5–)8.0–10.5 mm long in *E. sandwicensis*) (Table 1).

#### Distribution

*Eurya degeneri* is known from ca. 217 individuals distributed around the central to northwestern regions of Kaua'i. They occur in ten fragmented sub-populations, having an extent of occurrence (EOO) of ca. 80 km<sup>2</sup>, an area of occupancy (AOO) of ca. 34 km<sup>2</sup>, with an elevational range of 762–1585 m (Fig. 4). *Eurya sandwicensis* has a multi-island distribution, including the islands of Kaua'i, O'ahu, Moloka'i, Maui, and Hawai'i. *Eurya sandwicensis* is only known from 600–700 individuals on Kaua'i and is even rarer on the other islands. Although *E. sandwicensis* and *E. degeneri* overlap in their distribution in the central and northwestern regions of Kaua'i, the two taxa have never been observed growing together. Colonies of *E. sandwicensis* also extend down along the northern and southern windward ridges of Kaua'i where *E. degeneri* is not found. The constrained range of *E. degeneri* suggests that this taxon has a more restricted range of ecological parameters to which it is suited.

#### Ecology

Both *Eurya degeneri* and *E. sandwicensis* predominantly occur in *Metrosider*os Banks ex Gaertn. (Myrtaceae) / *Cheirodendron* Nutt. ex Seem. (Araliaceae) montane wet forests and are often associated with a mix of other tree genera such as *Polyscias* J.R. Forst. & G. Forst. (Araliaceae), *Dubautia* Gaudich. (Asteraceae), *Perrottetia* Kunth (Dipentodontaceae), *Cyrtandra* J.R. Forst. & G. Forst. (Gesneriaceae), *Antidesma* L. (Phyllanthaceae), *Hydrangea* Gronov.



#### Kaua`i, Hawaiian Islands

**Figure 4.** Distribution map of known locations of *Eurya degeneri* and *E. sandwicensis* on Kaua'i.

(Hydrangeaceae), *Syzygium* Gaertn. (Myrtaceae), *Bobea* Gaudich., *Coprosma* J.R. Forst. & G. Forst., *Kadua* Cham. & Schltdl., *Psychotria* L. (all Rubiaceae), and *Melicope* J.R. Forst. & G. Forst. (Rutaceae). Terrestrial matting ferns such as *Dicranopteris* Bernh., *Diplopterygium* (Diels) Nakai, and *Sticherus* C. Presl (all Gleicheniaceae) are also common components along with sedges such as *Gahnia* J.R. Forst. & G. Forst., *Carex* L., and *Machaerina* Nees (all Cyperaceae). Both species of *Eurya* on Kaua'i are found growing in highly weathered soils having low fertility (Deenik and McCellan 2007).

#### Conservation

Relevant threats to the habitat of both species of *Eurya* on Kaua'i include feral pigs (*Sus scrofa*), rats (*Rattus* spp.), and occasional feral goats (*Capra hircus*) and black-tailed deer (*Odocoileus hemionus*). Landslides after heavy rains can also be a very serious threat, especially the steeper habitats along the windswept ridges and bases of cliffs. The most serious invasive non-native plant species include *Miconia crenata* (Vahl.) Michelang. (Melastomataceae), *Sphaeropteris cooperi* (Hook. ex F. Muell.) R.M. Tryon (Cyatheaceae), *Buddleja asiatica* Lour. (Scrophulariaceae), *Psidium cattleyanum* Sabine (Myrtaceae), *Cyperus meyenianus* Kunth (Cyperaceae), and *Hedychium gardnerianum* Sphep. ex Ker Gawl. (Zingiberaceae).

#### Reproductive biology

Although the genus Eurya is described as dioecious or rarely monoecious (Wagner et al. 1999; Min and Bartholomew 2007), Wang et al. (2013) report that hermaphroditic flowers were observed on E. obtusifolia H.T. Chang, thereby expanding our understanding of the breeding systems of Eurya. For Hawaiian Eurya, most flowers we examined were unisexual with plants dioecious, but it should be noted that several flowering voucher specimens in this study appeared to have perfect flowers (i.e., E. sandwicensis (Perlman et al. 10630 (PTBG, US), Wood 14878 (PTBG, UC) and E. degeneri (i.e., Wood & Boynton 4022 (PTBG)). In addition, some E. degeneri specimens in northwestern Kaua'i demonstrate leaf characteristics that are large for the species (i.e. Perlman & Wood 12905 (PTBG), Walsh & Edmonds SKW78 (PTBG, MBK), and Wood et al. 1109 (PTBG, MO, F)). This large-leaved morphotype was the basis for an infraspecific taxon (E. degeneri f. grandifolia (Wawra) Kobuski), but as these individuals are distributed in the same geographic region as E. sandwicensis, they may represent hybrids between the two species. Further research is needed to determine if some flowers on Hawaiian Eurya can be truly perfect with functional stamens and pistils and if hybridization can occur between the two taxa on Kaua'i.

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#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Conceptualization: KRW, JCH. Formal analysis: DHL, JCH, KRW. Investigation: DHL, KRW. Writing - review and editing: JCH, KRW, DHL

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#### Data availability

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

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### PhytoKeys

Research Article

### Taxonomic studies on the genus *Sanicula* (Apiaceae) from China (III): The morphology and distribution of *S. caerulescens* (Apiaceae), with *S. oviformis* reduced to a new synonym

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#### Abstract

In the present study, we examined the morphological variations within *Sanicula caerulescens* and determined the identity of *S. oviformis* through observations of herbarium specimens (including type material) and field studies of plants in their type locality. Our findings revealed that *S. oviformis*, originally described from Nanchuan County in southern Chongqing, is conspecific with *S. caerulescens*. This species is mainly distributed in Chongqing, Guizhou, western Hunan, Sichuan and north-eastern Yunnan in China, as well as in Hà Giang in Vietnam. Key morphological characters of leaves, inflorescences and fruits confirm this synonymy. Based on these findings, we propose reducing *S. oviformis* to a synonym of *S. caerulescens*. Additionally, the geographical distribution of *S. caerulescens* is clarified.

Key words: Chongqing, morphology, Sanicula, taxonomy, Umbelliferae

#### Introduction

Sanicula caerulescens Franchet was described on the basis of one gathering, *M. Delavay 456* (K000697287, P00835131, P00835132; Fig. 1A–C), from Chengfeng Shan (also referred to Tchen-fong-chan) in Shuifu, Zhaotong City, located in north-eastern Yunnan Province, southwest China. In the protologue, Franchet (1894) emphasised two distinguishing features of *S. caerulescens* in comparison with *S. orthacantha* Moore – foliar morphology and arrangement of its inflorescences. Specifically, the leaves of *S. caerulescens* have petiolate segments, with lateral segments being either entire or slightly lobed. The inflorescences of this species are characterised by a floral branch that may be either simple or irregularly bipartite, with each arrangement supporting three or four sessile umbels. Additionally, the lower peduncle of the floral branch, as well as the sessile umbels, is typically accompanied by a small leaf, which lacks the subulate bracts that are uniquely present at the base of the pedicels. This particular inflorescence structure is markedly different from that of *S. orthacantha*.

Shan and Constance (1951) recognised this species and noted that its original dichasial branching has been lost, leading to the formation of a pseudoracemose



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**Copyright:** <sup>©</sup> Hui-min Li et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). inflorescence. They subsequently reduced *S. dielsiana* Wolff and *S. stapfiana* Wolff as synonyms of *S. caerulescens*. Hiroe (1979) accepted their treatment.

Since its original description, *S. caerulescens* has consistently been regarded as a distinct species by numerous authors, including Diels (1901), Boissieu (1906), Wolff (1913), Shan and Constance (1951), Hiroe (1979), Liou (1979), Wu (1984), Yang (1989), Liu (1997), Sheh and Phillippe (2005) and Pimenov (2017). The species has been recorded in south-western China (Chongqing, Guizhou, Hunan, Sichuan and Yunnan) and north-eastern Vietnam (Liou 1979; Wu 1984; Yang 1989; Liu 1997; Sheh and Phillippe 2005; Sun 2010; Pimenov 2017).

Sanicula dielsiana Wolff was described based on a single specimen, C. Bock & A.V. Rosthorn 114 (O-V2290040; Fig. 1D), collected from Mount Jinfo (also referred to as Chin fu shan) in Nanchuan County, located in southern Chongqing Municipality (a directly-administered municipality formerly belonging to Sichuan Province) (Wolff 1910). Later, Wolff (1913) recognised this species and noted that Diels (1901) had initially cited its three collections under S. orthacantha. These collections included C. Bock & A. v. Rosthorn 114 (Fig. 1D), along with two additional specimens: C. Maries s.n. (K; Fig. 2A) from Ichang and A. Henry 3526 (P03226670, US03074343; Fig. 2B; only P03226670 is shown here) from Ichang and its surrounding areas in Hubei Province. However, our preliminary examination indicated that the latter two collections were misidentified and actually belong to S. lamelligera. Meanwhile, Wolff (1913) described S. stapfiana Wolff, based on E. Faber 887 (K000697292, M0215722; Fig. 3A; only K000697292 is shown here) from Mount Emei (also known as Mount Omei) in south-western Sichuan. Shan and Constance (1951) later concluded that both S. dielsiana and S. stapfiana were described from the same region and likely represent minor variants of S. caerulescens. Thus, they treated both as synonyms of S. caerulescens. This treatment has been accepted by Hiroe (1979), Liou (1979), Liu (1997), Sheh and Phillippe (2005) and Pimenov (2017).

Sanicula erythrophylla Bobrov was described based on a single collection, *G.N. Potanin s.n.* (LE01029607; Fig. 3B) from the Ya River Valley, situated below the City of Ya'an (also known as Ya Chou) in western Sichuan (Bobrov 1950). In the protologue, Bobrov highlighted two features that set this species apart from other members of *S.* ser. *Orthacantha* Wolff – its aphyllous stems and the lilac-purple undersides of its tripartite leaves. Despite this detailed description, the species received little attention in subsequent taxonomic treatments until Liou (1979) synonymised it with *S. caerulescens* without offering a rationale. This synonymisation was later endorsed by Liu (1997), Sheh and Phillippe (2005) and Pimenov (2017). The latter author also designated the P00835131 sheet as the lectotype of *S. caerulescens* (Fig. 1A).

Sanicula oviformis X.T. Liu & Z.Y. Liu was described from a single collection, *M.L. Sheh 83646* (NAS00028684, NAS00028685, NAS00082799; Fig. 3C, D; NAS00028684 and NAS00082799 are shown here) from Mount Jinfo (Sheh et al. 1991). In the protologue, the authors indicated that *S. oviformis* is closely related to *S. lamelligera* Hance, sharing traits such as generally long and thin stems (vs. short and erect), palmately trisect leaves with ovate or obovate segments and slightly crenate or nearly entire margins (vs. medium segments slightly trisect, lateral segments bipartite or lobate, with erose serrate margins) and fruit spines lacking base lamellae (vs. spines with base lamellae). Although only known from the type material, *S. oviformis* has been consistently



Figure 1. Lectotype (A) and isolectotype (B, C) sheets of Sanicula caerulescens, alongside the holotype (D) sheet of S. dielsiana.



Figure 2. Specimens (A, B) of *Sanicula lamelligera* from Ichang and neighbouring areas in Hubei Province, all previously misidentified and cited by Diels (1901) as *S. orthacantha* and by Wolff (1913) as *S. dielsiana* A *C. Maries s.n.* (K) B *A. Henry* 3526 (P).

recognised as a distinct, endemic species restricted to its type locality by subsequent authors, including Sheh and Phillippe (2005) and Pimenov (2017).

The aim of this study is to elucidate the morphological variation of *S. caerulescens* and to determine the identity of *S. oviformis*, based on observations of herbarium specimens (including type material) and living plants in the field.

#### Materials and methods

For morphological comparisons, we conducted a thorough examination of specimens or high-resolution images of related *Sanicula* L. from the following herbaria: A, AU, BM, CDBI, CDCM, CSFI, CSH, E, FJIDC, FJSI, GNUG, GXMG, GXMI, GYBG, GZAC, GZTM, HAST, HGAS, HHE, HUFD, HX, HZ, IBK, JIU, JJF, JSPC, K, KUN, L, LBG, LE, MO, N, NAS, NTUF, NY, O, P, PE, PEY, QNUN, SM, SYS, SZG, TAI, TAIF, TI, TIE, US, WCSBG, WUK, ZM and ZY. Field observations were conducted across six populations in Chongqing, Hubei, Sichuan and Yunnan Provinces. Of these, four key populations are highlighted: one from Mount Jinfo in Nanchuan County, southern Chongqing, the type locality of *S. dielsiana* and *S. oviformis*; one from Shizhu County in south-eastern Chongqing; one from Mount Emei in south-western Sichuan, the type locality of *S. stapfiana*; and one from Yongshan County in



Zhaotong, north-eastern Yunnan, the type locality of *S. caerulescens*. The morphological comparisons presented are the result of a comprehensive analysis of both herbarium specimens and fresh materials collected during our fieldwork.

Figure 3. Holotype (A) sheet of *Sanicula stapfiana*, holotype (B) sheet of *S. erythrophylla* and holotype (C) and isotype (D) sheets of *S. oviformis*.

#### **Results and discussion**

The type material of Sanicula caerulescens (Fig. 1A-C) has 2-7 stems, approximately 10-30 cm long. The basal leaves are numerous, cordate-ovate in shape and vary in texture from chartaceous to subcoriaceous. These leaves follow a subternate to trifoliate pattern, with distinctly petiolulate segments. The median segment is obovate to ovate-lanceolate, measuring 1.0-4.0 cm in length and 0.7-3.0 cm in width, with a cuneate base tapering into a petiolule and an obtusely trilobed apex. The lateral segments are obliquely ovate, shallowly bilobed and feature crenate-serrate margins with spinose teeth. All segments are supported by petioles several times longer than the leaf blades. The upper cauline leaves are considerably reduced, forming small sheaths 2.0-5.0 mm long, with pinnately lobed blades. The inflorescence has a pseudoracemose structure, with lower umbels either sessile or borne on short peduncles along the main branches of the stem. The fruits are covered with short spicules, which are either erect or slightly curved. These spicules occasionally feature membranous bases or remain underdeveloped along the fruit's furrows. These morphological features were corroborated through field observations of living plants in Yongshan County, Zhaotong, north-eastern Yunnan (Fig. 4), the type locality of S. caerulescens. Furthermore, the lectotype designated by Pimenov (2017), i.e. M. Delavay 456 (P00835131; Fig. 1A), aligned with the original Latin description precisely and is well-preserved, reaffirming the validity of the lectotypification, which we hereby accept.

The type specimens of *Sanicula dielsiana* (Fig. 1D) have 1–2 stems 12–30 cm long. The basal and upper cauline leaves are similar to those of *S. caerulescens*, characterised by slight and continuous variations in both length and width. The median segments of the basal leaves measure 2.0–6.5 cm in length and 2.0–5.0 cm in width, whereas the upper cauline leaves range from 2.0 to 8.0 mm in length. No significant differences were noted in the characters of the inflorescence or fruit. These observations were further supported by field studies conducted at the type locality, Mount Jinfo, located in Nanchuan County, southern Chongqing (Fig. 5).

Sanicula oviformis, described from the same locality as S. dielsiana (Mount Jinfo, Nanchuan County, southern Chongging), was found to have been based on a rather depauperate type specimen with a significantly damaged inflorescence and flowers. Amongst the examined sheets, only the holotype sheet NAS00082799 has a pseudoracemose inflorescence, with the lower umbels being either sessile or short-pedunculate and the fruits covered with spicules (Fig. 3C). The stem length is approximately 30 cm. The basal leaves are numerous, cordate-ovate in shape and have a subcoriaceous texture. These leaves follow a trifoliate pattern, with distinctly petiolulate segments. The median segment is broadly ovate, measuring 0.8-3.0 cm in length and 0.6-2.0 cm in width, with a broadly cuneate base narrowing into a petiolule and an obtusely trilobed apex. The lateral segments are obliquely ovate, shallowly bilobed and feature crenate-serrate margins with spinose teeth. All segments are supported by petioles that are several times longer than the leaf blades. The upper cauline leaves are significantly reduced, forming small sheaths measuring 1.5-4.0 mm in length, with pinnately lobed blades. Although the type specimens reveal that S. oviformis has relatively diminutive leaves, our discovery of a population in Shizhu County, south-eastern Chongqing, near Nanchuan County (Fig. 6), demonstrated a significant variability in leaf size both within and between populations.



Figure 4. Sanicula caerulescens in the wild (China, Yunnan, Zhaotong, the type locality of *S. caerulescens*) A habitat B habit C leaf (left: adaxial surface; right: abaxial surface) D portion of inflorescence (side view) E portion of inflorescence, with the arrow indicating upper reduced and sheathing cauline leaves; G umbellule (side view; a involucellate bracteoles, b calyx teeth) H staminate flowers (side view) I fertile flower with fruit, stamens, petals and calyx teeth J mericarps. Photographed by Hui-Min Li.

Thus, the small leaves of *S. oviformis* were considered to exhibit a considerable morphological variability.

As previously noted, *S. oviformis* was compared with *S. lamelligera* by Sheh et al. (1991), with the latter distinguished by a range of morphological characters. In a subsequent study, we conducted a more detailed examination of *S. lamelligera* (Li and Song 2022). Notably, the sheets of *Z.Y. Liu* 15276 (E00088651, HAST070536, K, KUN0465625, P03226696, PE00754708; Fig. 7; only E00088651, K, KUN0465625, and PE00754708 are shown here) from Mount Jinfo, Nanchuan, Chongqing Municipality, were misidentified as *S. lamelligera* by Z.Y. Liu, a co-author of *S. oviformis*. Additionally, two gatherings collected along the Furong River



Figure 5. Sanicula caerulescens in the wild (China, Chongqing, Nanchuan, Mount Jinfo, the type locality of *S. dielsiana* and *S. oviformis*) **A** habitat **B** habit **C** leaf (left: adaxial surface; right: abaxial surface) **D** portion of inflorescence (side view) **E** portion of inflorescence (top view) **F** portion of inflorescence, with the arrow indicating upper reduced and sheathing cauline leaves **G** umbellule (side view; **a** involucellate bracteoles, **b** calyx teeth) **H** staminate flowers (side view) **I** fertile flower with fruit, stamens, style, petals and calyx teeth **J** mericarps. Photographed by Hui-Min Li.

in Wulong County (a district neighbouring Nanchuan) were labelled by Liu as distinct species on their determination slips, i.e. *Z.Y. Liu 182290* (PE01989764; Fig. 8A) and *Z.Y. Liu 182499* (PE1989767; Fig. 8B). The former was labelled as either *S. caerulescens* or *S. pengshuiensis* – which, according to Li and Song (2022), should now be treated under *S. lamelligera*. We identified the sheet PE1989767 as *S. caerulescens*. These discrepancies suggest that the authors may not have fully grasped the extent of morphological variability within *S. lamelligera* or *S. caerulescens*. A detailed morphological comparison between the two species is provided in Table 1. Additionally, it is noteworthy that no additional specimens of *S. oviformis* have been recorded since its original description.



Figure 6. Sanicula caerulescens in the wild (China, Chongqing, Shizhu) A habitat B habit C leaf (left: adaxial surface; right: abaxial surface) D portion of inflorescence (side view), with the arrow indicating upper reduced and sheathing cauline leaves E portion of inflorescence (top view) F umbellule (side view; a involucellate bracteoles, b calyx teeth) G fertile flower with fruit, style and calyx teeth. Photographed by Hui-Min Li.



**Figure 7.** Specimens of *Sanicula caerulescens* collected from Chongqing, Nanchuan. All misidentified as *S. lamelligera* by Z.Y. Liu, a co-author of *S. oviformis* **A** *Z.Y. Liu* 15276 (E) **B** *Z.Y. Liu* 15276 (K) **C** *Z.Y. Liu* 15276 (KUN) **D** *Z.Y. Liu* 15276 (PE; the arrow indicating the spinous fruit).



**Figure 8**. Specimens of *Sanicula caerulescens* collected from Chongqing, Wulong **A***Z*.Y. *Liu* 182290 (PE) **B***Z*.Y. *Liu* 182499 (PE). All arrows indicating the spinous fruits.

	0 Januar Hinana	0
	S. lamelligera	S. caerulescens
Roots	rhizome short, woody, bearing fibrous roots, occasionally with nodes	rhizome, rarely stoloniferous, oblique rootstock, bearing fibrous roots
Stems	slender and erect, few branches above the middle, 8–40 cm tall	slender and erect, several branches above the middle, 5–40 cm tall
Basal leaves	cordate suborbicular, ternate, segments distinctly petiolate; median segments cuneate-obovate to rhombic, obtuse to acute and more or less trilobed at the apex; lateral segments oblique, bilobed to the middle or only notched or rarely entire; margins crenate-serrate with spinose teeth.	cordate-ovate or ovate, sub-ternate to trifoliate; median segments obovate to ovate-lanceolate, distinctly shallowly trilobed, petiolate; lateral segments obliquely ovate, shallowly bilobed; margins irregularly crenulate- serrate with spinose teeth
Cauline leaves	reduced, trisect or undivided, bract-like, linear- lanceolate to obovate-lanceolate, ca. 5 mm long, shortly petiolate or sessile	significantly reduced, degenerating into sheathing, trisect or undivided, bract-like, ca. 2.0 mm long, subsessile or sessile
Inflorescence	1- or 2- to several branches	pseudo-racemose, sometimes the lower umbels in fascicles, sessile or short-pedunculate
Involucrate bracts	trisect or linear-lanceolate, ca. 2 mm long	lanceolate to ovate, acuminate, ca. 1.6 mm long
Rays of umbels	1–7, ca. 3.5 mm long	terminal umbels pedunculate, 3- to 12-radiate, 2.5– 7.0 mm long
Involucellate bracteoles	4–6, linear to lanceolate, ca. 0.6 mm long	4–6(–8), linear to lanceolate, ca. 1 mm long
Umbellules	4-7-flowered	5-7-flowered
Staminate flower	3–6 per umbellule; pedicels ca. 1.5 mm long; petals white, pinkish or bluish-purple	4–6 per umbellule; pedicels 2.1–4.0 mm long; petals mainly blue to purple
Fertile flower	1 per umbellule, sessile	1 per umbellule, sessile
Mericarps	variably spiculate-lamellate, comb-like	covered with short and straight spinous bristles, usually fused at the base, forming a thin tier
Vittae	5	5

Table	1. Mor	pholoaid	cal com	parisons	between	Sanicula	lamelligera	and S.	caerulescens
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In our examination of the type specimens of *Sanicula stapfiana* (Fig. 3A) and *S. erythrophylla* (Fig. 3B), particularly concerning critical diagnostic characters, such as leaves, inflorescence and fruits, no significant differences were observed when compared to *S. caerulescens*. These observations were further corroborated by field studies conducted at Mount Emei, the type locality for *S. stapfiana*, located in south-western Sichuan (Fig. 9).

In conclusion, *Sanicula dielsiana*, *S. erythrophylla*, *S. stapfiana* and *S. oviformis* are morphologically indistinguishable from *S. caerulescens*. Therefore, we endorse the treatments by Shan and Constance (1951) and Liou (1979), reducing *S. dielsiana*, *S. stapfiana* and *S. erythrophylla* to synonyms of *S. caerulescens*. Additionally, we also propose to synonymise *S. oviformis* with *S. caerulescens*.



Figure 9. Sanicula caerulescens in wild (China, Emei Shan, the type locality of *S. stapfiana*) **A** habitat **B** habit **C** leaf (left: adaxial surface; right: abaxial surface) **D** portion of inflorescence (side view), with the arrow indicating upper reduced and sheathing cauline leaves **E** portion of inflorescence (top view) **F** umbellule (side view; **a** involucellate bracteoles, **b** calyx teeth) **G** staminate flowers (side view) **H** fertile flower with fruit, style and calyx teeth I mericarps. Photographed by Hui-Min Li.
#### **Taxonomic treatments**

# Sanicula caerulescens Franch., Bull. Soc. Philom. Paris 8 (6): 109. 1984. Figs 1, 3–9

- Sanicula dielsiana Wolff, Repert. Sp. Nov. Regni Veg. 8: 524. 1910. Туре: Сніма. Chongqing, Nanchuan, Mount Jinfo (= Chin fu shan), 28 July 1891, *C.* Bock & A.V. Rosthorn 114 (holotype: O-V2290040!).
- = Sanicula stapfiana Wolff in Engler, Pflanzenr. 4 (228): 58. 1913. Type: CHINA. Sichuan, Mount Emei (= Omei), alt. 3000 ft = 915 m, December 1887, E. Faber 887 (holotype: K000697292!; isotype: M0215722!).
- = Sanicula erythrophylla Bobrov, Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 13: 167. 1950. Type: CHINA. Sichuan, Ya'an (= Ya Chou), the valley of Ya River, 4 April 1893, G.N. Potanin s.n. (holotype: LE01029607!).
- Sanicula oviformis X.T. Liu & Z.Y. Liu, Acta Phytotax. Sin. 29 (5): 471. 1991, syn. nov. Type: CHINA. Chongqing, Nanchuan, Mount Jinfo, alt. 650 m, 28 June 1983, *M.L. Sheh 83646* (holotype: NAS00082799!; isotypes: NAS00028684!, NAS00028685!).

**Type.** CHINA. Yunnan, Zhaotong, Shuifu, Chengfeng Shan (= Tchen-fong-chan), May 1882, *M. Delavay* 456 [lectotype: P00835131! designated by Pimenov (2017); isolectotypes: K000697287!, P00835132!]. Fig. 1A–C.

Description. Perennial. Rhizome, rarely stoloniferous, oblique rootstock, bearing fibrous roots. Stems 2-7, slender and erect, several branches above the middle, 5-40 cm tall. Basal leaves 2-14(-23), long petiolate; petioles 2-17 cm long; blade glabrous adaxially and abaxially, usually purplish-red on the back, 1-7 cm long, (2.2–)4.5–14 cm wide, cordate-ovate or ovate, sub-ternate to trifoliate, the median segment obovate to ovate-lanceolate, distinctly shallowly trilobed, petiolate, the lateral segments obliquely ovate, shallowly bilobed, the margins irregularly crenulate-serrate with spinose teeth. Cauline leaves significantly reduced, sheathing, subsessile or sessile, with pinnately lobed blades, resembling involucrate bract, ca. 2.0 mm long. Inflorescence pseudo-racemose, sometimes the lower umbels in fascicles, sessile or short-pedunculate; involucrate bract lanceolate to ovate, acuminate, ca. 1.6 mm long; terminal umbels pedunculate, 3- to 12-radiate, 2.5-7.0 mm long; involucellate bracteoles 4-6(-8), linear to lanceolate, ca. 1 mm long. Umbellules 5-7-flowered, staminate flowers 4-6 per umbellule, pedicels 2.1-4.0 mm long, petals mainly blue to purple. Fertile flowers 1 per umbellule, sessile; calyx teeth linear to lanceolate, acute, ca. 0.8 mm long; styles ca. 2.0 mm long, recurved. Mericarps globose to ellipsoid, 2.0-3.0 mm long, 1.0-1.5 mm broad, covered with short and straight spinous bristles usually fused at the base forming a thin tier; mericarp flattened dorsally, orbiculate in cross-section. Vittae 5.

**Distribution.** Sanicula caerulescens is widely distributed in China (Chongqing, northern Guangxi, Guizhou, Hubei, western Hunan, Sichuan and Yunnan) and Vietnam (Hà Giang) (Fig. 10).

**Habitat.** It grows on mountain slopes under forest or along ravine streams or at forest margins at elevations of 400–1700 m above sea level.

Phenology. Flowering and fruiting from March to July.

**Etymology.** The epithet *caerulescens* is derived from the Latin term referring to the blue hue of the petals in the flowers.

Additional specimens examined. CHINA. Chongqing Municipality • Beibei District, 25 March 1934, T.T. Yu 2835 (NAS, PE) • Beibei District, Jinyun Mountain, 5 July 1956, Anonymous 326 (SM) • Beibei District, Jinyun Mountain, 5 May 1956, M.L. Li s.n. (SM) • Beibei District, Jinyun Mountain, 12 July 1983, M.L. Sheh 83674 (NAS) • Beibei District, Jinyun Mountain, alt. 480 m, 29 March 1964, Sichuan Exped. 0060 (CDBI, PEY) • Beibei District, Jinyun Mountain, alt. 700 m, 12 May 1943, S.J. Wang 1059 (NAS, PE) • Beibei District, Jinyun Mountain, alt. 740 m, 21 April 1956, Sichuan & Guizhou Exped. 114 (PE) • Beibei District, Jinyun Mountain, alt. 800 m, 28 March 2013, Z.F. Xu & W. Qin G0013 (KUN) · Beibei District, Jinyun Mountain, April 1939, R.H. Shan 1166 (NAS) · Beibei District, Jinyun Mountain, May 1949, Y.W. Law 286 (NAS) • Beibei District, Jinyun Mountain, R.H. Shan 1297 (NAS) • Beibei District, Jinyun Mountain, S.J. Wang 1502 (PE) • Bishan District, 29 May 1978, Bishan Exped. 0129 (SM) • Dazu County, Yulong Town, alt. 400 m, 14 June 1978, Dazu Exped. 0240 (SM) • Fuling District, Lidu District, alt. 900 m, 10 April 1979, Fuling Exped. 0002 (SM) • Nanchuan County, 1932, T.H. Tu 2848 (IBK, NAS) · Nanchuan County, 1955, SM Exped. 2785 (SM) · Nanchuan County, 11 March 1957, S.G. Tang 1722 (SM) • Nanchuan County, alt. 1300 m, 4 June 1962, Anonymous 95748 (SM) • Nanchuan County, alt. 500 m, 5 April 1979, Anonymous 0027 (SM) • Nanchuan County, alt. 820 m, 1 April 1957, C.H. Hsiung & T.L. Chou 90051 (PE) • Nanchuan County, Bailuping, alt. 840 m, 17 April 1957, K.F. Li 60545 (KUN, PE) · Nanchuan County, Bailuping, alt. 970 m, 12 April 1957, K.F. Li 60437 (KUN, PE) • Nanchuan County, Dashi Village, alt. 500-550 m, 7 April 1979, Anonymous 0081 (SM) • Nanchuan County, Honghegou, alt. 700 m, 1 July 1983, M.L. Sheh 83653 (NAS) • Nanchuan County, Mount Jinfo, 8 June 1935, S.P. Chang 123 (NAS) • Nanchuan County, Mount Jinfo, alt. 1200 m, 2 June 1935, K.L. Chu 1053 (NAS, PE) • Nanchuan County, Mount Jinfo, Daheba, 29°4'42.39"N, 107°12'9.15"E, alt. 666–688 m, 9 May 2021, H.M. Li, Y.S. Zhang & X. Zhang LHM1139 (NAS) • Nanchuan County, Mount Jinfo, Daheba, 29°5'46.81"N, 107°12'0.5"E, alt. 704 m, 8 May 2021, H.M. Li, Y.S. Zhang & X. Zhang LHM1132 (NAS) • Nanchuan County. Mount Jinfo, Daheba, alt. 650 m, 28 June 1983, M.L. Sheh 83645 (NAS) • Nanchuan County, Mount Jinfo, Daheba, alt. 690 m, 26 April 1962, Anonymous 95548 (SM) • Nanchuan County, Mount Jinfo, Daheba, alt. 785 m, 20 March 1957, K.F. Li 60107 (PE) • Nanchuan County, Mount Jinfo, Daheba, alt. 880 m, April 1932, T.H. Tu 2775 (IBK, NAS, PE) • Nanchuan County, Mount Jinfo, Daheba, alt. 1000 m, 7 April 1957, K.F. Li 60356 (KUN, PE) • Nanchuan County, Mount Jinfo, Daheba, alt. 1300 m, 4 June 1962, Anonymous 95748 (SM) · Nanchuan County, Mount Jinfo, Daheba, alt. 1550 m, 30 June 1957, K.F. Li 62459 (PE) • Nanchuan County, Nanping Town, alt. 1000 m, 1 July 1957, C.H. Hsiung & B.Q. Li 95026 (NAS, SM) . Nanchuan County, Sanjianghonghegou, alt. 700 m, 1 July 1983, M.L. Sheh 83654 (NAS) · Nanchuan County, Sanguan Town, 29°4'47.28"N, 107°12'6.48"E, alt. 661 m, 26 May 2020, W. Zhou & H.M. Li LHM1016 (NAS) • Nanchuan County, Sanguan Town, alt. 640 m, 7 March 1996, Z.Y. Liu 15276 (E, HAST, K, KUN, P, PE) • Pengshui County, Lianhe Village, alt. 430 m, 12 May 2000, Anonymous 0133B (SM) · Rongchang District, Shuanghe Town, 24 April 1979, Anonymous 619 (SM) · Shizhu County, Huangshui District, alt. 1400 m, 18 May 1978, W.H. Wang 289 (CDBI) • Shizhu County, Shiliu Village, 29°44'35.96"N, 108°16'9.2"E, alt. 1219 m, 6 May 2021, H.M. Li., Y.S. Zhang & X. Zhang LHM1125 (NAS) • Shizhu County, Xituo Town, alt. 150 m, 5 April 1979, Shizhu Exped. 0040 (SM) • Wulong County, Furong River, alt. 300 m, 27 April 2008, Z.Y. Liu 182499 (PE) · Wulong County, Furong

River, alt. 350 m, 25 April 2008, Z.Y. Liu 182290 (PE) • Yongchuan District, Bangiao Town, alt. 350 m, 23 April 1978, Yongchuan Exped. 52 (SM) · Yongchuan District, Jiulong Village, alt. 500 m, 18 May 1978, Yongchuan Exped. 248 (SM). Guangxi Province · Huanjiang County, Mulun National Nature Reserve, 107°58'41.85"N, 25°8'7.43"E, alt. 502 m, 15 May 2013, Huanjiang Exped. 451226130315013LY (GXMG, IBK) • Huangjiang County, Mulun National Nature Reserve, alt. 570 m, 16 April 2012, R.C. Peng & L.F. Fu ML0955 (IBK) • Huangjiang County, Mulun National Nature Reserve, alt. 670 m, 19 April 2012, Y.S. Huang, Y.B. Liao & M.Q. Han Y1288 (IBK) • Huanjiang County, Mulun National Nature Reserve, alt. 750 m, 28 February 2011, W.B. Xu & L. Wu 11095 (IBK). Guizhou Province · Chishui City, 29 April 1965, Guizhou Exped. 125 (HGAS) • Chishui City, Jinshagou, 20 May 2019, L. Liu 201905203 (GNUG) · Libo County, Maolan Scenic Spot, alt. 340 m, 24 April 2018, T. Jiang 20181675 (QNUN) · Libo County, Maolan Scenic Spot, alt. 420 m, 23 April 2018, Z.H. Li 201814397 (QNUN) • Libo County, Maolan Scenic Spot, alt. 459 m, 19 April 2018, F. Li 20185229 (QNUN) · Libo County, Maolan Scenic Spot, alt. 475 m, 17 April 2018, X.J. Ma 20188141 (QNUN) • Libo County, Maolan Scenic Spot, alt. 493 m, 20 April 2018, H.T. Long 20185417 (QNUN) · Libo County, Maolan Scenic Spot, alt. 498 m, 21 April 2018, M.J. He 20185370 (QNUN) · Libo County, Maolan Scenic Spot, alt. 567 m, 21 April 2018, K.Q. Zhang 20184413 (QNUN) • Suiyang County, Kuankuoshui, 12 May 2008, S.Q. Zhang S4073 (ZY) • Suiyang County, Yinjianggou, 22 May 2014, D. Hu XS14054478 (ZY) • Suiyang County, alt. 1050 m, 5 April 1990, K.M. Lan 90-0006 (GZAC) • Suiyang County, 20 May 1986, Q.S. Xie 201 (ZY) • Xishui City, Shaguangou, alt. 1055 m, 21 May 2015, Q.Q. Ran SCH1503252 (ZY) • Xishui City, Shaguangou, alt. 978 m, 20 May 2015, T. Xu SCH1503270 (ZY) • Xishui City, Shaguangou, alt. 987 m, 20 May 2015, Y.L. Peng SCH1503246 (ZY) • Xishui City, Shaguangou, 15 May 2012, Q. Li SCH1202116 (ZY) • Xishui City, Yinjianggou, alt. 978 m, 19 May 2015, W.Y. Wu SCH1503057 (ZY) · Xishui City, Yong'an Town, 106°24'49.23"N, 28°12'16.12"E, alt. 1236.7 m, 29 March 2014, M.C. Wang GYZ1403290916 (GYBG) • Zunyi City, Hejiagou, alt. 910 m, 20 May 2014, M. Xie XS14054040 (ZY) • Zunyi City, Lengshui River, 5 March 2013, J.H. Liu LSH031 (ZY) • Zunyi City, Shanpen District, Dingcun Village, 4 April 1959, N Guizhou Exped. 0022 (HGAS, IBK). Hubei Province • Enshi City, Pingbaying National Forest Park, 108°57'46.67"N, 29°22'55.32"E, alt. 1404 m, 21 May 2021, Y.S. Zhang LHM1120 (NAS). Hunan Province · Baojing County, Baiyunshan, 18 July 2009, D.G. Zhang 080308007 (JIU) • Huayuan County, Gumiao River, alt. 200 m, 16 April 2008, Y.G. Qu 080416062 (JIU) • Jishou City, Dehang Scenic Spot, 6 June 1993, G.X. Chen 574 (JIU) • Yongshun County, Qingping Village, Dongba River, 110°11'10.7"N, 29°02'13.7"E, alt. 332 m, 23 March 2015, K.D. Lei 4331271503231562 (JIU). Sichuan Province · Anyue County, Kongque Village, alt. 490 m, 10 April 1964, Anonymous 366 (CDBI) Anyue County, Shiyang Town, 6 May 1978, Anonymous 1299 (SM) 
 Bazhong City, Bazhou District Zaolin Town, alt. 420 m, 14 April 1979, Bazhong Exped. 1004 (SM) • Dazhou District, Xuanhan County, alt. 850 m, 6 May 1978, Xuanhan Exped. 0167 (SM) • Dazhu County, Jixing Village, alt. 850 m, 16 May 1978, Dazu Exped. 0125 (SM) • Dujiangyan City, Lingyan Mountain, 6 June 1959, S. Jiang & C.L. Jin 549 (KUN) • Dujiangyan City, alt. 700 m, 17 April 1997, D.Z. Lu 199704 (BJFC) • Ebian Yi autonomous county, 25 May 1959, C.H. Hsiung & B.Q. Li 95026 (SM) • Emei Shan city,1939, G. Zhang 1907 (NAS) • Emei Shan City, 103°17'30.55"N, 29°35'12.17"E, alt. 1344.73 m, 2 June 2024, C.F. Song, H.M. Li & J.W. Zhu LHM1557

(NAS) • Emei Shan city, Dacheng Temple Qingyin Pavilion, alt. 900 m, 27 March 1959, Sichuan Econ. Plant Exped. 0209 (NAS, PE, SM) • Emei Shan City, Ereshan, 10 March 1949, Chow & Hsie 784 (NAS) • Emei Shan City, Shuangfu Town, Yuelian Village, 18 May 1978, Emei Exped. 122 (SM) · Emei Shan City, 1 April 1942, W.P. Fang 18263 (TIE) • Emei Shan City, 27 March 1959, Anonymous 404 (PE, SM) • Emei Shan City, Sichuan Univ. Biol. Depart. Exped. 53902 (WUK) • Guang'an City, Jincheng Mountain, alt. 570 m, 29 April 1959, Anonymous 00101 (SM) • Guangyuan City, Chaotian District, alt. 1750 m, 18 September 1972, NE Yunnan Exped. 769 (KUN) • Hongya County, Wawu Mountain, 23 July 1950, C.W. Yao 2440 (NAS, PE) · Jiajiang County, Huatou District, Menkanlin, 11 May 1959, Sichuan Econ. Plant Exped. 8015 (PE, SM) · Junlian County, Haoba Town, Fujiagou, alt. 1200 m, 10 July 1977, Anonymous 0539 (SM) • Kaijiang County, Nandinggou, alt. 1050-1100 m, 18 June 1978, Kaijiang Exped. 0248 (SM) · Lu County, 10 May 1959, Anonymous 5155 (SM) • Pingchang county, alt. 400 m, 15 June 1978, Pingchang Exped. 384 (SM) · Qionglai City, alt. 800 m, 6 April 1979, Anonymous 0079 (SM) · Songpan County, Huaying Mountain Huanglong Temple, 14 March 1941, Y.C. Yong 4090 (NAS, PE) • Tianguan County, Yongxing District, alt. 910 m, 1 June 1982, D.Y. Peng 45446 (CDBI) · Xuyong County, Shuiwei Town, Huagaoxi National Nature Reserve, 105°32'8"N, 28°16'36"E, alt. 419 m, 12 March 2013, X.F. Gao, Z.M. Zhu & X.L. Zhao HGX11087 (CDBI) • Xuyong County, Shuiwei Town, Huagaoxi National Nature Reserve, alt. 1050-1150 m, 15 April 2012, X.F. Gao, Z.M. Zhu & X.L. Zhao HGX10084 (CDBI) • Yibin City, Changning County, Wanling Town, alt. 560 m, 12 June 1977, Anonymous 0044 (SM) · Zizhong County, Xinqiao Town, alt. 600 m, 28 March 1979, Zizhong Exped. 86 (SM). Yunnan Province · de kan-tse-pin, E.E. Maire s.n. (P) • Long-ki,1894, M. Delavay 4912 (P) • Long-ki,1894, M. Delavay 4934 (P) · Long-ki, 1894, M. Delavay s.n (P, US) · Long-ki, 22 March 1894, M. Delavay s.n. (PE) • Long-ki, May 1894, M. Delavay 4976 (P) • Zhaotong City, Shuifu County, Chenfengshan, 20 May 1901, F. Ducloux 2130 (P) • Zhaotong City, Suijiang County, alt. 1307 m, 27 March 2023, C.F. Song, H.M. Li & J.W. Zhu SCF0097 (NAS) • Zhaotong City, Suijiang County, alt. 1700 m, 4 July 1973, Anonymous s.n (HITBC) · Zhaotong City, Yongshan County, alt. 1513 m, 27 March 2023, C.F. Song, H.M. Li & J.W. Zhu SCF0101 (NAS) · Zhaotong City, Yongshan County, alt. 1538 m, 27 March 2023, C.F. Song, H.M. Li & J.W. Zhu SCF0103 (NAS).

VIETNAM. Hà Giang Province • Quan Ba District, Tung Vai Commune, Thang Village, 104°51'48.8"N, 23°03'13.4"E, alt. 1050–1150 m, 21 April 2018, *L. Averyanov et al. VR 546* (LE) • Quan Ba District, Tung Vai Commune, Thang Village, 104°50'41.6"N, 23°03'41.5"E, alt. 1200–1400 m, 22 April 2018, *L. Averyanov et al. VR 629* (LE).

**Note.** Sanicula caerulescens has been recorded in Chongqing, Guizhou, western Hunan, Sichuan and north-eastern Yunnan in China, as well as Hà Giang in Vietnam (Diels 1901; Boissieu 1906; Wolff 1913; Shan and Constance 1951; Hiroe 1979; Liou 1979; Wu 1984; Yang 1989; Liu 1997; Sheh and Phillippe 2005; Sun 2010). Pimenov (2017) also noted its presence in Hubei Province, although no specific specimens were cited. During our fieldwork in 2021, we were delighted to discover a population [*Y.S. Zhang LHM1120* (NAS)] in Enshi, Hubei. Furthermore, a critical examination of specimens under *Sanicula* L. confirmed that *S. caerulescens* is also distributed in northern Guangxi, as well as in all adjacent regions. Therefore, we clarify that the distribution of *S. caerulescens* extends across China (Chongqing, northern Guangxi, Guizhou, Hubei, western Hunan, Sichuan and Yunnan) and Vietnam (Hà Giang).



Figure 10. Distribution of Sanicula caerulescens (black circle).

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# Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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### Author contributions

Investigation: WZ. Methodology: WJZ. Writing – original draft: MHL. Writing – review and editing: FCS.

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#### **Data availability**

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

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# PhytoKeys

**Research Article** 

# The Araceae of Sumatra I: A new species of *Alocasia* from Aceh, Indonesia

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#### Abstract

*Alocasia roseus* is described as a new species from Aceh Besar Regency, Sumatra (Indonesia). The plants found produce stunning inflorescence, leading to over-collecting in the wild. The new species is morphologically similar to *A. flemingiana* but differs by its greyish green adaxial leaves and pale reddish to greenish purple or pale brownish green abaxial leaves, lacking interprimary veins and sinus not naked, thecae overtopped by synconnective, and a pale pink appendix. The new species is also similar to *A. arifolia*, from which it differs by the glabrous and pale dull green petiole, the colour of abaxial and adaxial leaves, not forming interprimary vein, sinus not naked, and pale pink appendix. The new species is compared with other similar Indonesian taxa and an identification key to the species of *Alocasia* in Sumatra, supplemented with photographs, are provided.

Key words: Aceh Besar, Alocasia roseus, Indonesian flora, new taxon

#### Introduction

The genus *Alocasia* (Schott) G.Don (Araceae Juss.) consists of 100 species (Promprom et al. 2024) but current research suggest that there may be an additional 41 undescribed species (Boyce and Croat 2023). This genus is distributed in tropical and subtropical Asia ranging from the Malesian region toward Oceania and to mainland Australia (Nauheimer et al. 2012; POWO 2024; Promprom et al. 2024). Borneo is considered to bear the richest diversity and endemism of *Alocasia* (Hay 1998). However, the diversity and distribution of *Alocasia* are poorly understood in the Indonesian archipelago, with about 27 known species (Asih and Lestari 2022; Asih et al. 2022). Prior to this study, there were seven species of *Alocasia* recognised in Sumatra (Hay 1998; Erlinawati 2011; Kurniawan et al. 2013; POWO 2024).

Our knowledge of *Alocasia* in the Sumatra is currently inadequate and, consequently, further exploration and collection of plants is necessary. The under-collection of *Alocasia* from Sumatra is reflected in the few specimens held at Herbarium Bogoriense (BO) and other international herbaria. Furthermore, the last taxonomic revision of *Alocasia* was done over twenty-five years ago (Hay 1998) who recognised six Sumatran *Alocasia* taxa (*Alocasia alba* Schott, *A. arifolia* Hallier f., *A. inornata* Hallier f., *A. longiloba* Miq., *A. kerinciensis* A.Hay,



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**Copyright:** © Ni Putu Sri Asih et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). and A. *puber* (Hassk.) Schott). *Alocasia macrorrhizos* (L.) G.Don is listed as an introduced species to Sumatra (POWO 2024).

Based on Praetorius s.n. (L.1415481), *A. puber* was thought to occur in Sumatra (Hay 1998), but is here now regarded as *A. alba. A. puber* is only known from Jawa. Erlinawati (2011) recorded *A. flemingiana* Yuzammi & A.Hay from Siberut Island (a small island off the western coast of Sumatra), a species that was previously only known from Jawa (Hay 1998). A recent study by Mustaqim and Setiawan (2019) provided an update on the distribution of *A. alba* upon finding the species in Tanggamus Regency, Lampung Province, Sumatra.

Since *Alocasia* has been known as one of the most popular ornamental plants, sought for and traded by enthusiasts and the general public, many species are collected directly from the forest and traded illegally. One of the species, traded by people from Aceh, is here considered as the new species *Alocasia roseus*. The work is part of an ongoing study of Araceae family in Sumatra region.

# Material and methods

Plants were collected from the forest near Kueh Kemukiman Keude Bieng, Kueh Village, Aceh Besar Regency, Aceh Province, Sumatra (Fig. 1), by people who live nearby the forest. It was then cultivated at a nursery, from which we obtained some plants. Pertinent literature was considered for the taxonomic investigation (e.g. Hallier 1901; Hay 1998) Photographs of herbarium specimens held at L were included in this study.



Figure 1. Distribution map of *Alocasia roseus* Asih & Yuzammi, sp. nov. showing the location where the new species was discovered in Aceh Besar (red shading). Inserts: overview of Indonesian archipelago (right) and Aceh Province (left). Map by Rani Yudarwati.

# **Taxonomic treatment**

*Alocasia roseus* Asih & Yuzammi, sp. nov. urn:lsid:ipni.org:names:77346080-1 Fig. 2, Table 1

**Type (prepared from a cultivated plant in a private nursery).** INDONESIA • Aceh, Aceh Besar, Lhoknga, Desa Kueh, Kueh Kemukiman Keude Bieng, *PSA 395* (holo-type BO! [dried specimens and inflorescences in spirit]; isotype ANDA!) (Fig. 2).

**Diagnosis (Table 1).** Alocasia roseus is morphologically similar to *A*. *flemingiana* and *A*. *arifolia* from which it differs by having greyish green adaxial leaves and pale reddish to greenish purple or pale brownish green abaxial leaves, interprimary vein absent, sinus not naked, and pale pink appendix (vs. mid-green to dark green adaxial and green-yellowish/paler abaxial leaves, forming poorly to conspicuous interprimary vein, sinus naked up to 3 cm, and cream/pale apricot appendix).

**Description.** Small herb c. 55 cm tall; *rhizome* erect; *leaves* several together; petiole 34–38 cm long, pale dull green, glabrous, faintly mottled greenish, sheathing in the lower about 1/3 of its length, pale dull green; *blades* leathery, sagittate, greyish green adaxially, reddish to greenish purple to pale brown greenish abaxially, margin entire; *anterior lobe* 12.8–14.4 cm long, 9.7–11.6 cm wide, the widest is base of anterior lobe, tip acuminate, 7 mm long; anterior costa with 4–5 primary lateral veins on each side, proximal ones diverging at 76–96° on each side then running to submarginal vein, distal primary veins diverging at 40–45° on each side, primary vein prominent adaxially then forward to marginal becoming flush to lamina, prominent abaxially, with inconspicuous axillary glands, secondary and tertiary venation flush to lamina adaxially, rather prominent and conspicuous abaxially then running to conspicuous submarginal vein inserted c. 1.5 mm from margin, interprimary collective veins absent; *posterior costae* diverging at 60–80°,

Diagnostic characters	Alocasia roseus	Alocasia flemingiana	Alocasia arifolia
Blade	leathery, greyish green adaxially, reddish to greenish purple to pale brown greenish abaxially	membranous, mid-green adaxially, yellowish green abaxially	membranous to thinly coriaceous, somewhat glossy, dark/mid-green adaxially, paler abaxially
Venation	not forming interprimary collective veins	interprimary veins absent or poorly differentiated	forming undulating to zig-zag interprimary collective veins
Glands	inconspicuous axillary glands	distinct small flat glands	inconspicuous axillary glands
Sinus	not naked	naked for up to 1 cm	naked in the sinus for up to 3 cm
Constriction	at base of male zone or to half way along male zone	at top of sterile zone of spadix	at sterile interstice
Spadix	shorter than spathe, shortly stipitate for 2–4 mm long	somewhat shorter to almost as long as spathe, stipitate for c. 5 mm	almost as long as spathe, shortly stipitate, c. 3 mm long
Thecae	overtopped by synconnective, thecae opening by apical pores	somewhat displaced to overtopped by synconnective, thecae opening through apical slits	overtopped by synconnective, thecae opening by apical pores
Appendix	pale pink, gradually tapering to a blunt point, faintly irregularly channeled	Cream-coloured appendix, tapering	pale apricot-coloured, somewhat constricted at base, slightly narrower than male zone, tapering toward a point

Table 1. Comparison of important diagnostic characters of Alocasia roseus, A. flemingiana and A. arifolia.



**Figure 2.** Alocasia roseus: **A** habit **B** adaxial leaf **C** abaxial leaf **D** petiole **E** inflorescence with spathe **F** inflorescence with the spathe removed showing the zonation of reproductive organs **G** female zone ( $\mathcal{Q}$ ) **H** sterile interstice zone (s) **I** male zone ( $\mathcal{J}$ ) **J** appendix (a). (Photographed and edited by Ni Putu Sri Asih and Julisasi Tri Hadiah).

not naked in the sinus; **posterior lobes** acute, 6–7.2 cm long; **inflorescences** soliter or in pair, subtended by green cataphylls and then dried at flower anthesis; **peduncle** to c. 14.6 cm long, resembling petioles in colour and faintly mottled; **spathe** c. 9.7 cm long; **lower spathe** ovoid, yellowish green, c. 2.3 cm long, c. 1.8 cm diam; **limb** lanceolate, yellow to greenish, erect then tilted 45° after 3 days, separated from the lower spathe by a constriction at the base of male flowers or at top sterile interstice (to the midpoint of the male zone); **spadix** shorter than the spathe, c. 7 cm long, shortly stipitate, 2–4 mm, whitish-green, cylindric; **female zone** cylindric, 0.9–1 cm long, 0.9 cm wide; **ovaries** subglobose, green, stigma raised on a style 0.5–1 mm, conspicuously, (2–)3–4-lobed, pale yellow; **sterile interstice** cream-coloured, not attenuate, 0.4–0.5 cm long, narrower than male zone, with 4–5 whorls of rhomboid synandrodia; **male zone** cylindric, cream-coloured, 1.1-1.2 cm long; synandria rhombohexagonal to somewhat irregular, with the synconnective overtopped the thecae; thecae opening by apical pores; **appendix** pale pink, gradually tapering to a blunt point, faintly irregularly channeled, **c**. 3.5–4.2 cm long, slightly wider than the male zone; **fruit** unknown.

**Distribution and habitat.** The species is only known from Aceh Besar, Sumatra Island. It grows on the hillside of the forest, in shade and humid areas.

**Etymology.** The specific epithet, *roseus*, is based on the pale pink appendix of the staminodes found in this new species. This color of the appendix is rarely found in the genus.

**Conservation status.** *Alocasia roseus* is known only from a single locality in the forest near Kueh Kemukiman Keude Bieng, Kueh Village, Aceh Besar Regency, Aceh Province, Indonesia. Since further populations could occur, we prefer to assess these species as Data Deficient (DD) according to the IUCN Red List criteria (2022).

**Notes.** *Alocasia roseus* belongs to the informal group "Macrorrhizos" (*sensu* Hay 1998) in view of the following showed characteristics: inflorescences in pairs, the spathe constriction aligning with the sterile interstice of the spadix, and the synconnective overtopping the thecae. This species is the second, besides *A. balgooyi*, in the Macrorrhizos group with no naked sinus in their posterior lobe. *Alocasia roseus* also has an appendix color that differs from other species in the Macrorrhizos group. Pink being a rare colour of appendix that is found only in *A. melo* and *A. princeps* (Hay 1998).

#### Key of Alocasia Species in Sumatra

1	Leaf blades shallowly to completely peltate in mature plant2
-	Leaf blades not peltate in mature plant
2	Peltate leaf with posterior lobes almost fully fused; appendix white
	Alocasia kerinciensis A. Hay
_	Peltate leaf with posterior lobes never almost fully fused; appendix very
	pale orange to bright yellowAlocasia longiloba Miq.
3	Robust to massive plant4
_	Medium to small plant6
4	Interprimary collective vein well-defined Alocasia alba Schott
_	Interprimary collective vein poorly defined5
5	Inflorescences paired among lead base; peduncle exceeding length of
	cataphylls at anthesis; petiole glabrous Alocasia macrorrhizos (L.) G.Don
-	Inflorescences many, clustered together; peduncle short, mostly hidden
	with leaf sheath and cataphylls; petiole glabrous or minutely pubescent
	Alocasia inornata Haillier f.
6	Intermarginal vein not formed; petiole puberulent or glabrous
	Alocasia arifolia Hallier f.
_	Intermarginal vein conspicuous; petiole glabrous7
7	Abaxial leaf reddish to greenish purple to pale brown, with greenish tinge;
	appendix pale pink Alocasia roseus Asih & Yuzammi
_	Abaxial leaf yellowish green; appendix cream-colored
	Δlocasia flemingiana Yuzammi & Δ Hav

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# Additional information

# **Conflict of interest**

The authors have declared that no competing interests exist.

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# Author contributions

NPSA: plant identification, description, writing-first draft. Y: description, writing-review and editing. IE: writing-review and editing. JTH: critical review and editing.

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# Data availability

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

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**Research Article** 

# *Cladopus yangjiangensis* (Podostemaceae), a new species from Guangdong, South China, redefining the phylogenetic relationships within *Cladopus*

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#### Abstract

This paper introduces *Cladopus yangjiangensis*, a newly identified species that enriches our understanding of the diversity of the Podostemaceae in East Asia. Distinctive in its morphological traits, this species is characterized by the region's longest flowering shoots and exhibits a high number of elongated leaves per cluster, along with relatively slender roots. Phylogenetic analyses using Maximum Likelihood and Bayesian Inference methods on plastome and *matK* sequences confirm *C. yangjiangensis* as a distinct species. It forms a clade with *C. fukienensis*, its closest relative, together branching off from *C. austrosinensis*. The plastome of *C. yangjiangensis* is 132,818 bp in length, comprising two inverted repeat regions of 20,881 bp, which are separated by large and small single-copy regions of 78,713 and 12,343 bp, respectively. Genetic analysis reveals the extensive loss of the *ycf1* and *ycf2* genes in the chloroplast genome, a trait common to the Podostemaceae, suggesting adaptations to environmental conditions or gene transfers to nuclear or mitochondrial genomes. This study improves the clarity of phylogenetic relationships in previous studies and underscores the importance of continued taxonomic and phylogenetic research.

Key words: Biodiversity, chloroplast genome, morphology, phylogeny, Podostemaceae

# Introduction

The Podostemaceae family is a distinctive group within the angiosperms, notable for its species richness among aquatic flowering plants. These plants primarily inhabit tropical and subtropical regions, with some species extending into temperate zones (Cook 1996; Kato 2016). Podostemaceae species are uniquely adapted to rocky habitats within waterfalls or rapids. During high water levels in summer, they grow vigorously in their vegetative form submerged in water. As water levels recede in autumn and winter, these plants emerge to



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**Copyright:** <sup>©</sup> Miao Zhang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). flower, fruit, and complete their life cycle (Koi and Kato 2012; Kato et al. 2019). Despite the similarity in habitats worldwide, the morphology of Podostemaceae is notably unique and diverse (Koi and Kato 2012). A striking feature is their roots, which function primarily as adhesive organs, contrasting with the anchoring and absorptive roots of terrestrial plants. The stems, leaves, and flowers are also determined by the root system (Koi and Kato 2020).

The Podostemaceae family is composed of three subfamilies: Tristichoideae, Weddellinoideae, and Podostemoideae, encompassing approximately 51 genera and 350 species (Kita and Kato 2001; Koi et al. 2022). Tristichoideae and Weddellinoideae are relatively smaller groups, whereas Podostemoideae is the most species-rich and widely distributed subfamily (Costa et al. 2011; Katayama et al. 2022). Tristichoideae are characterized by their prominent perianth, while Podostemoideae are distinguished by their very small, scalelike or filamentous perianth and membranous spathe covering the bud (Kita and Kato 2001).

Within the Podostemoideae subfamily, the genus *Cladopus* is notable for its flat, nearly cylindrical or strap-like roots, digitate and rough-surfaced bracts that are 4–7-lobed, one or rarely two stamens, and smooth-surfaced capsules (Kato 2006, 2008; Koi and Kato 2012). Phylogenetically, *Cladopus* is closely related to *Paracladopus*, sharing a common ancestor with *Hanseniella*, *Hydrodiscus*, and *Thawatchaia* (Koi and Kato 2012; Koi et al. 2012). Geographically, *Cladopus* species are found in Southeast Asia, East Asia, Malaysia, and Australia (Kato et al. 2019). In China, *Cladopus* species have been reported in Fujian, Guangdong, Hainan, Guangxi, and Hong Kong (Kato et al. 2017; Li et al. 2024).

There have likely been a total of 10 species of *Cladopus* plants worldwide, including: *C. austrosinensis*, *C. doianus*, *C. fukienensis*, *C. fallax*, *C. javanicus*, *C. nymanii*, *C. pierrei*, *C. queenslandicus*, *C. taiensis* and *C. yinggelingensis* (Kato 2008; Lin et al. 2016). Molecular phylogenies of the *Cladopus* section reveal two major clades: one consisting of *C. fallax*, *C. javanicus*, *C. nymanii*, *C. queenslandicus* and *C. taiensis*, and another including the Chinese and Japanese species (Kita and Kato 2001, 2004; Kato and Kita 2003; Kato et al. 2019). Currently, it is generally accepted that five species of *Cladopus* are distributed in China: *C. austrosinensis*, *C. doianus*, *C. fukienensis*, *C. pierrei* and *C. yinggelingensis* (Li et al. 2024).

During a field survey in Yangjiang City, Guangdong Province, China, in February 2024, we encountered a plant in a stream that initially appeared similar to members of the *Cladopus* genus. However, upon more thorough examination, unique features were revealed, suggesting its potential as a previously unknown species. Subsequent comprehensive morphological and molecular systematic analyses confirmed that our collected specimens represent a new species, which we have named *Cladopus yangjiangensis*.

#### Materials and methods

#### Morphological description

The morphological description of the new species was based on the study of specimens collected in a variety of spots in 2024. 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.

#### DNA extraction, amplification and sequencing

In this study, total DNA was extracted from fresh leaves of the new species using a Quick DNA Isolation Kit (Huayueyang, Beijing). The phylogenetic position of the new species was determined by *matK* and whole plastome sequences. The partial plastid *matK* regions (PQ497705, PQ497706) were amplified via polymerase chain reaction (PCR) using TaKaRa Ex Taq polymerase (TaKaRa, Tokyo, Japan) under the following conditions: 3 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 90 s at 72 °C; and 7 min at 72 °C (Koi et al. 2012). The PCR products were treated with Mag-MK 96 Well PCR Products Purification Kit (Sangon Biotech, Shanghai) to remove the extra primers. Sequencing was conducted using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI 3130xl Genetic Analyser (Applied Biosystems). The primers used for the DNA amplification and the cycle sequencing are listed in Suppl. material 1: table S1. Other parts of 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.

#### Genome sequencing, assembly, annotation and analysis

Purified total DNA of *Cladopus yangjiangensis* was fragmented, genome skimming was performed using next-generation sequencing technologies on the Illumina Novaseq 6000 platform with 150 bp paired-end reads and 400 bp insert size by Berry Genomics Co. Ltd. (Beijing, China), and 10 GB of reads was obtained.

The paired-end reads were filtered and assembled into complete plastome using a GetOrganelle v1.7.5.0 (Jin et al. 2020) with appropriate parameters, with K-merset "21,45,65,85,105", the word size is 0.6. Following previous studies, our workflow includes five key steps: 1. Mapping reads to seed and assembling seed-mapped reads for parameter estimation; 2. Recruiting more target-associated reads through extending iterations; 3. Conducting de novo assembly; 4. Roughly filtering for target-like contigs; 5. Identifying target contigs and exporting all configurations (Camacho et al. 2009; Bankevich et al. 2012; Langmead and Salzberg 2012; Jin et al. 2020). Graphs of the final assembly were visualized by Bandage (Wick et al. 2015) to assess their completeness. Gene annotation was performed using CPGAVAS2 (Shi et al. 2019) and PGA (Qu et al. 2019). The different annotations of protein coding sequences were confirmed using BLASTx. The tRNAs were checked with tRNAscan-SE v2.0.3. Final chloroplast genome maps were created using OGDRAW.

#### **Phylogenetic analysis**

The phylogenetic relationship was constructed using Maximum likelihood (ML) analyses with the *matK* sequence. In total, 29 samples (Suppl. material 1: table S2) of *Cladopus* were included in our analysis. Two species of *Paracladopus* were used as outgroups. Each individual sequence was aligned using MAFFT

7.310 (Katoh and Standley 2013) with default settings. All missing data were treated as gaps. The best nucleotide substitution model according to the Bayesian Information Criterion (BIC) was K3Pu+F+R2, which was selected by ModelFinder (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 2000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under K3Pu+F+R2 model (2 parallel runs, 2000000 generations).

To construct a phylogenetic tree based on plastome sequences, a total of 29 plastome sequences of *Cladopus, Terniopsis, Polypleurum, Paracladopus, Marathrum, Hydrobryum, Cratoxylum*, and *Apinagia* were included in our analysis (Suppl. material 1: table S3). *Cratoxylum cochinchinense* was used as outgroup. Each individual locus was aligned using MAFFT 7.310 (Katoh and Standley 2013) with default settings. The best nucleotide substitution model according to the Bayesian Information Criterion (BIC) was TVM+F+R4, which was selected by ModelFinder (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 2000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under the GTR+F+I+G4 model (2 parallel runs, 2000000 generations), in which the initial 25% of sampled data were discarded as burn-in. Phylograms were visualized in ChiPlot (Xie et al. 2023).

# Results

#### Characteristics of Cladopus yangjiangensis plastome

The chloroplast genome of *Cladopus yangjiangensis* is 132,818 bp in length (Fig. 1), which exhibits a typical quadripartite structure, comprising a pair of IR regions (20,881 bp) divided by an SSC region (12,343 bp) and an LSC (78,713 bp) region. The overall GC content of the genome was 35.14%, while the GC content of LSC, SSC, and IR regions were 31.99%, 27.93%, and 42.35%, respectively. A total of 108 unique genes were identified in the plastome, it contains 74 protein-coding genes, 30 tRNAs, and 4 rRNAs. A total of 16 genes were duplicated in the IR regions, including *ndhB*, *rpl2*, *rps7*, *rps12*, *rps15*, *rrn4.5S*, *rrn5S*, *rrn16S*, *rrn23S*, *trnA-UGC*, *trnI-GAU*, *trnI-CAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, *trnV-GAC* (Table 1). There were six genes lost, including *rpl23*, *trnT-CGU*, *infA*, *ycf*15, and uncommon losses of *ycf1* and *ycf2*. The annotated plastome was documented in GenBank (PQ510206).

#### Comparative analysis of the plastomes

A comparative analysis was conducted on the plastid genomes of all six known species within the genus *Cladopus* found in China (Table 2). The plastid genome sizes varied slightly, ranging from 132,046 bp in *C. austrosinensis* to 132,907 bp in *C. yinggelingensis*, with an overall average size of 132,739 bp. Each plastid genome displayed the typical quadripartite structure, consisting of a large single-co-



**Figure 1.** Circular gene map of the plastid genome of *Cladopus yangjiangensis*. 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.

py (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions. The LSC region was the largest, comprising approximately 59.1% to 59.4% of the total plastid genome length, followed by the IR regions at 31.4% to 31.6%, and the SSC region at 9.2% to 9.3%. This consistent structural organization across species underscores the conserved nature of the plastid genome in *Cladopus*.

The GC content was consistent across the species, ranging from 34.88% to 35.39%, with a mean value of 35.12%. This homogeneity in GC content suggests a stable evolutionary trajectory with limited genomic rearrangements or substitutions. The number of protein-coding genes varied slightly, ranging from 73 to 75. The number of transfer RNA genes was either 29 or 30, while the number of ribosomal RNA genes remained consistently four in each species. These gene counts reflect the core functionality of the plastid genome, which is primarily involved in photosynthesis and genetic expression.

Category, Group of Genes	Gene Names
Photosynthesis:	
Subunits of photosystem I	psaA, psaB, psaC, psaI, psaJ
Subunits of photosystem II	psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ
Subunits of NADH dehydrogenase	ndhA*, ndhB*(2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK
Subunits of cytochrome b/f complex	petA, petB*, petD*, petG, petL, petN
Subunits of ATP synthase	atpA, atpB, atpE, atpF*, atpH, atpI
Large subunit of rubisco	rbcL
Self-replication:	
Subunits of RNA polymerase	rpoA, rpoB, rpoC1*, rpoC2
Proteins of large ribosomal subunit	rpl14, rpl16*, rpl2*(2), rpl20, rpl22, rpl32, rpl33, rpl36
Proteins of small ribosomal subunit	rps11, rps12*(2), rps14, rps15(2), rps18, rps19, rps2, rps3, rps4, rps7(2), rps8
Transfer RNAs	trnA-UGC*(2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC*, trnH-GUG, trnI- CAU(2), trnI-GAU*(2), trnK-UUU*, trnL-CAA(2), trnL-UAA*, trnL-UAG, trnM-CAU, trnN-GUU(2), trnP-UGG, trnQ-UUG, trnR-ACG(2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC(2), trnV-UAC*, trnW-CCA, trnY-GUA, trnfM-CAU
Ribosomal RNAs	rrn16S(2), rrn23S(2), rrn4.5S(2), rrn5S(2)
Other genes:	
Maturase	matK
Protease	clpP
Envelope membrane protein	cemA
c-type cytochrome synthesis gene	ccsA
Acetyl-CoA carboxylase	accD
Unknown function:	
Conserved open reading frames	ycf3*, ycf4

Table 1. Gene contents of the plastid genome of Cladopus yangjiangensis.

Notes: \*gene with one introns; Gene (2): Number of copies of multi-copy genes.

Species	Accession	Num	ber of G	enes		L	_ength (bp)		GC content
Species	no.	PCGs	tRNA	rRNA	Total	LSC	SSC	IR	(%)
C. austrosinensis	PQ510207	73	29	4	132,046	77,993 (~59.1%)	12,295 (~9.3%)	20,879 × 2 (~31.6%)	34.98
C. doianus	PQ510208	75	30	4	132,896	78,967 (~59.4%)	12,211 (~9.2%)	20,859 × 2 (~31.4%)	34.88
C. fukienensis	NC_082923.1	74	30	4	132,834	78,741 (~59.3%)	12,331 (~9.3%)	20,881 × 2 (~31.4%)	35.39
C. pierrei	NC_082924.1	74	30	4	132,893	78,865 (~59.3%)	12304 (~9.3%)	20,862 × 2 (~31.4%)	34.92
C. yinggelingensis	NC_082925.1	74	30	4	132,907	78,878 (~59.3%)	12,311 (~9.3%)	20,859 × 2 (~31.4%)	35.36
C. yangjiangensis	PQ510206	74	30	4	132,818	78,713 (~59.3%)	12,343 (~9.3%)	20,881 × 2 (~31.4%)	35.14

Table 2. Basic features of the plastid genomes of all known species within the genus Cladopus in China.

#### **Phylogenetic analysis**

The present study confirms *Cladopus yangjiangensis* as a new species based on phylogenetic analysis, using Maximum likelihood (ML) and Bayesian Inference (BI) methods on plastome data and the *matK* sequence. The phylogenetic tree based on plastome data includes seven genera from the subfamily Podoste-moideae and *Terniopsis* from the subfamily Tristichoideae (Fig. 2), thereby explicitly illustrating the relationships among these taxa. *Cladopus* is shown to be a monophyletic group within Podostemoideae, comprising two subclades. The new species is clearly distinctly separated from the most closely related species, *C. fukienensis*, with strong support (BP = 100, PP = 1.00), and is positioned within a clade that also contains *C. austrosinensis*. The same pattern was found in the



**Figure 2.** Phylogenetic consensus tree of 29 complete plastid sequences derived from the Podostemaceae species in the genera *Cladopus, Terniopsis, Polypleurum, Paracladopus, Marathrum, Hydrobryum,* and *Apinagia.* Numbers above and below branches indicate RAxML (left) bootstrap probabilities (BP) and Bayesian (right) posterior probabilities (PP), respectively. Cratoxylum cochinchinense was included as an outgroup. \* indicates bootstrap probabilities (BP) = 100 and Bayesian posterior probabilities (PP) = 1.00, unless otherwise indicated at nodes.

*matK* tree (Fig. 3), constructed from 29 samples of the of the *Cladopus* species, providing a comprehensive view of the phylogenetic relationship within the genus.

# **Taxonomic treatment**

*Cladopus yangjiangensis* X.T.Liu, G.Di Chen & B.Hua Chen, sp. nov. urn:lsid:ipni.org:names:77352702-1 Figs 4–8

**Diagnosis.** *Cladopus yangjiangensis* shares certain similarities with *C. fukienensis* and *C. austrosinensis*, such as comparable ovary lengths, and analogous stigma and capsule shapes. However, it is distinguished by several unique traits. The leaves of *C. yangjiangensis* are markedly elongated, measuring 18.7–26.7 mm, which is substantially longer than those of *C. fukienensis* (1.3–5.0 mm) and *C. austrosinensis* (up to 6 mm). The flowering shoots of *C. yangjiangensis* also exceed those of other two species, ranging from 4.2 to 13.1 mm, compared to 3.5–6.0 mm in *C. fukienensis* and 1.6–3.5 mm in *C. austrosinensis*. Additionally, *C. yangjiangensis* produces a greater number of bracts, with counts ranging from 20 to 54, in contrast to 12–36 in *C. fukienensis* and 8–14 in *C. austrosinensis*. Finally, the root width of *C. yangjiangensis* is notably narrower, at about 0.4 mm, compared to 0.4–1.3 mm in *C. fukienensis* and 0.5–1.3 mm in *C. austrosinensis* (Table 3).



**Figure 3.** Phylogenetic tree of 29 sequences based on *matK* genes. The left tree is Maximum likelihood tree and the number above branches indicate RAxML bootstrap probabilities (BP). The right tree is MrBayes tree and the number above branches indicate Bayesian posterior probabilities (PP), respectively. *Paracladopus* were outgroups of the trees.

Characteristics	C. austrosinensis	C. fukienensis	C. yangjiangensis
Root width (mm)	0.5-1.0(-1.3)	0.4-1.3	ca. 0.4
The number of leaves	to 5	2-5	3-8
Leaves length (mm)	to 6.0	1.3-5.0	18.7-26.7
Flowering shoots length (mm)	1.6-3.0(-3.5)	3.5-6.0	4.2-13.1
The number of bracts	8-12(-14)	12-19(-36)	20-54
Finger-like lobes	3-9	3-7	3-5
The size of spathella (mm)	/	1.3-1.9 × 0.9-1.4	1.7 × 0.3
The morphology and length of tepals (mm)	linear, 1.0–1.5	linear or subulate, 0.6–0.7	broadly linear, acuminate at end, 0.7–1.1
Stamen length (mm)	ca. 1.5	ca. 1.3	1.2-2.4
Ovary length (mm)	1.0-1.5	1.1-1.5	1.2-1.7
Stigma length (mm)	ca. 0.6	0.4-0.5	0.3-0.4
Ovules locule	25-34	25-35	30-45
Length of stamens vs. length of ovaries	Equal	shorter or equal	longer
Capsule stalk length (mm)	1.2-1.7	(0.5-)1.2-2.8	1.2-1.3(-1.7)
The size of capsule (mm)	ca. 1.5	1.0-1.3 × 0.8-1.3	1.1-1.4 × 0.9-1.2
The size of seed (mm)	0.2-0.3 × 0.1-0.2	0.3-0.5 × 0.2-0.3	0.3-0.5 × 0.1-0.3
Distribution	South China	South & southeast China	South China

Table 3. Comparison of three congeneric species of *Cladopus*.



Figure 4. Cladopus yangjiangensis **A** habitat (Photographed by Guo-Di Chen) **B** root with tufts of leaves, leaves linear, brick red in color **C** plants in bud adhering to rock surface **D** flowering shoot **E** top view of flower (red arrow pointing to the stamen) **F** mature fruits.

**Type.** CHINA • Guangdong: Yangjiang City Yangxi County, Tangkou Town, Tongyou village, elevation 200 m, 21°49'N, 111°28'E, 24 December 2023, *XT Liu* & *GD Chen 0001* (*Holotype* FNU! barcode FNU0041314; isotype FNU! barcode FNU0041315).

Description. Perennial aquatic herb; roots narrowly ribbon-like, compressed, dorsiventral, succulent, ca. 0.4 mm width, 0.1-0.2 mm thick, adhere to the surface of underwater rocks, brick-red during winter, dark green during reproduction; stem short, arising from root branch axils (Fig. 4); flowering shoots obpyramidal, solitary, erect, 4.2-13.1 mm tall; leaves linear, in rosette on vegetative shoots, 3-8 in number, 18.7-26.7 mm long, deciduous at flowering; leaves on reproductive shoots palmate (also known as bracts), lobes 3-5 digitate, central lobe long, columns two, opposite, overlapping, bracts 20-54, upper leaves larger, diminishing towards apex, 1.5-1.9 × 1.1-2.0 mm, lobes rigid and coarse after water loss; flowers bisexual, solitary at fertile branch apex, enclosed in pale red spathe during early development; spathella globose, acumen short, 1.7 × 0.3 mm; tepals two, broadly linear, acuminate, 0.7-1.1 mm long; stamen single, 1.2-2.4 mm long; filament nearly cylindrical, slightly flattened, 0.9-1.3 mm long; anthers two, elliptical, yellow, ~ 0.6-0.9 mm long; Ovary single, pale yellow-green, ellipsoid, two-chambered, 1.2-1.7 × 0.9-1.1 mm; ovules ovate, 15-23 per chamber, attached to entire placenta (Figs 5, 6); capsule brownish, globose, smooth, 1.1–1.4 mm long;



Figure 5. *Cladopus yangjiangensis* **A** flowering shoot on roots, inverted tower shape (reproductive leaves (a.k.a. bracts) imbricate, finger-like) a flower with 2 stigmas, a stamen (stamen clearly longer than ovary) **B** stamens, arrow points to two tepals, fused to ovary **C** gynoecia, arrow points to reddish, ribbon-shaped stigmas **D** stamen and tepals (tepals attached to filament bases on either side) **E** stamen **F** gynoecium with ellipsoid ovary, no bracts **G** longitudinal ovary section **H** ovules. Scale bars: 2 mm (**A**); 500 μm (**A**, **B**, **C**, **D**, **E**, **F**, **G**); 200 μm (**H**).

fruit stalk 1.2–1.7 mm long; seeds small, yellow, narrowly ovoid,  $0.3-0.5 \times 0.1-0.3$  mm (Fig. 7).

**Distribution and habitat.** Many other plants grow in the surrounding habitat, whose tree layer includes *Archidendron clypearia* (Fabaceae), *Engelhardia roxburghiana* (Juglandaceae), *Aporosa dioica* (Phyllanthaceae), *Zanthoxylum avicennae* (Rutaceae), *Sterculia lanceolata* (Malvaceae) and others; the shrub layer includes *Acronychia pedunculata* (Rutaceae), *Rhaphiolepis indica* (Rosaceae), *Rubus leucanthus* (Rosaceae, *Ficus pyriformis* (Moraceae), *Glochidion lanceolarium* (Phyllanthaceae), *Garcinia oblongifolia* (Clusiaceae), *Melastoma sanguineum* (Melastomataceae), *Saurauia tristyla* (Actinidiaceae), *Adina pilulifera* (Rubiaceae), *Pavetta hongkongensis* (Rubiaceae), and others; the vegetation layer includes *Blechnopsis orientalis* (Blechnaceae), *Plenasium vachellii* (Osmundaceae), *Acorus gramineus* (Acoraceae), *Pandanus austrosinensis* 



Figure 6. *Cladopus yangjiangensis* **A** vegetative shoot **B** tufts of leaves on root (clustered, roots subterete, linear leaves) **C** abaxial and adaxial views of the upper leaves of reproductive shoots **D** leaves with some lobes markedly long at the base of vegetative shoots. Scale bars: 5 mm (**A**, **B**); 1 mm (**C**, **D**).

(Pandanaceae), Alpinia hainanensis (Zingiberaceae), Thysanolaena latifolia (Poaceae), Miscanthus floridulus (Poaceae), Pentasachme caudata (Apocynaceae) and others; and some exotic plants include Stauntonia obovatifoliola (Lardizabalaceae), Phanera erythropoda (Fabaceae), Rourea microphylla (Connaraceae) and others.

**Phynology.** Flowering and fruiting season in November to February of the following year.

**Etymology.** The Yang Jiang Chuan Tai Cao (阳江川苔草). The epithet *yang-jiangensis* (阳江) refers to Yangjiang City, Guangdong Province, South China, where this new species was found.

**Conservation status.** According to our investigation, *Cladopus yangjiangensis* was only found in a stream in Yangjiang City, Guangdong 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 also be included on the national secondary protection list during the upcoming revision process.



Figure 7. *Cladopus yangjiangensis* **A** fruiting shoots on root **B** stalked capsule, tan, globose, smooth **C** fertile seeds and ovules in a mature fruit **D** fertile seeds **E**, **F** adaxial and lateral views of placenta. Scale bars: 2 mm (**A**); 400 µm (**B**); 200 µm (**C**, **D**, **E**, **F**).

# Discussion

The discovery of *Cladopus yangjiangensis* enriches our understanding of the diversity of the Podostemaceae in East Asia, showcasing unique morphological and genetic characteristics that distinguish it from other species in the genus. Currently, five species of *Cladopus* are recognized in China: *C. austrosinensis*, *C. doianus*, *C. fukienensis*, *C. pierrei*, and *C. yinggelingensis*. Among these, *C. austrosinensis* is found in Hainan and Guangdong, while *C. fukienensis* has a wider distribution, occurring in Fujian, Guangdong, Hong Kong, and other regions.

Previous phylogenetic analyses demonstrated that sequences identified as *Cladopus austrosinensis* (accession numbers AB104560.1, AB104559.1, and LC 144911.1) are paraphyletic and interspersed with sequences from *C. fukienensis*, hinting at unrecognized species diversity within this group (Kita and Kato 2004; Kato et al. 2017). Our recent *matK* gene sequencing of specimens collected from Boluo County, near the original discovery site in Huizhou, east-central Guangdong, confirmed their identity as genuine *C. austrosinensis*. Subsequent



Figure 8. Illustration of *Cladopus yangjiangensis* **A** tufts of leaves on root **B** flowering shoot on roots **C** leaves with some lobes markedly long at the base of vegetative shoots **D** abaxial and adaxial views of leaves on the upper part **E** flower with 2 stigmas, a stamen and 2 tepals **F** gynoecium without bracts, ovary ellipsoid **G** stamen **H** longitudinal section of the ovary **I** adaxial view of the placenta **J** stalked capsule, globose, smooth **K** seeds.

fieldwork in Yangjiang City, southern Guangdong, revealed a *Cladopus* species with distinct morphological characteristics, which are different from *C. austros-inensis*. Genetic analyses align this population with the southern Guangdong samples (accession no. AB104559.1), forming a monophyletic group. This supports the identification of the Yangjiang population as a distinct species, previously misidentified as *C. austrosinensis*. We propose the designation of this population as a new species, *Cladopus yangjiangensis*, thereby resolving the phylogenetic discrepancies highlighted in earlier studies (Kita and Kato 2004; Kato et al. 2017).

Cladopus yangjiangensis exhibits several unique morphological features that distinguish it within the East Asian Cladopus species. Notably, it possesses exceptionally long flowering shoots, reaching up to 13.1 mm, the longest recorded among Cladopus species in East Asia, although elsewhere species such as C. javanicus and C. gueenslandicus have longer flowering shoots that can reach lengths of 30-70 mm and 30-90 mm, respectively (Kato et al. 2019). It also has extremely long leaves in the vegetative state (18.7-26.7 mm), the longest among all Cladopus species in East Asia (Lin et al. 2016; Kato et al. 2017; Werukamkul et al. 2018). Other distinctive features include its inverted tower-shaped flowering shoots, distinct bracts, roots, stamens, and stigmas, as confirmed through detailed microscopic anatomical observations. Furthermore, the roots of C. yangjiangensis, like other Podostemaceae, deviate from the typical radially symmetric cylindrical roots of most angiosperms. They are dorsiventral and range from compressed subcylindrical to ribbon-like forms (Cusset 1992; Kato and Hambali 2001; Rutishauser and Pfeifer 2002). Specifically, C. yangjiangensis is notable for having thinner roots, adding to its distinctive profile within the genus.

Genetic analysis further supports the distinctiveness of C. yangjiangensis. The chloroplast genomes of Cladopus species, including C. yangjiangensis, are characterized by the extensive loss of the ycf1 and ycf2 genes, a phenomenon observed across the Podostemaceae family. These genes, among the longest in the chloroplast genome (Ren et al. 2020), are typically about 20 kb in length (Dong et al. 2015; De Santana Lopes et al. 2018), and their loss may contribute to the reduced chloroplast genome size in this family. While gene loss in chloroplast genomes is common, the absence of ycf1 and ycf2 genes, which encoded essential cellular functions, suggests potential gene transfer to nuclear or mitochondrial genomes (Cauz-Santos et al. 2017). However, homologs have yet to be identified in the nuclear genome (Drescher et al. 2000). Previous studies have indicated that plants in moist, shady environments often lack the ycf1 gene (Wen et al. 2022), suggesting a similar environmental influence on Podostemaceae. Interestingly, ycf1 gene loss is more commonly associated with parasitic and heterotrophic plants (Li et al. 2020; Yudina et al. 2021), making its occurrence in autotrophic Cladopus species noteworthy. Additional chloroplast genome data from similar habitats are needed to validate the correlation between ycf gene presence and environmental conditions.

The discovery of *C. yangjiangensis* and recent findings of new and newly recorded Podostemaceae species in China, such as *Polypleurum chinense* (Chen et al. 2022), *Paracladopus chiangmaiensis* (Wu et al. 2022), and *Terniopsis yongtaiensis* (Zhang et al. 2022), suggest that the wild resources of Podostemaceae in China have been significantly underestimated. It is anticipated that further in-depth investigations will uncover additional distribution sites of Podostemaceae within China, enhancing our understanding of this unique and diverse family.

# Key to the species of Cladopus

I	Flowering shoots 25.0–90.0 mm long2
-	Flowering shoots 1.0–13.1 mm long3
2	Bracts digitate; roots 2.0–4.0 mm wide; ca. 100 ovules per ovary
	Cladopus javanicus
-	Bract trilobed; roots 0.5–2.0 mm wide; ca. 50–80 ovules per ovary
3	Roots > 2.0 mm wide
-	Roots 0.2–2.0 mm wide
4	Bracts 15–20 Cladopus yinggelingensis
-	Bracts 4–125
5	Stamens distinctly longer than ovary Cladopus nymanii
-	Stamens as long as or slightly longer than ovary6
6	Stigmas narrowly fan-shaped with dilate apex, obovate-spatulate
	Cladopus doianus
	•
-	Stigmas linear
– 7	Stigmas linear
- 7 -	Stigmas linear
- 7 - 8	Stigmas linear
- 7 - 8 -	Stigmas linear
- 7 - 8 - 9	Stigmas linear
- 7 - 8 - 9	Stigmas linear
- 7 - 8 - 9	Stigmas linear.7Flowering shoots ca. 6.0 mm long; bracts 8–10.Cladopus pierreiFlowering shoots 1.0–2.0 mm long; bracts 4–6.Cladopus taiensisStamens 0.3–0.6 mm longCladopus fallaxStamens 1.5–2.4 mm long9Flowering shoots 1.6–3.0(–3.5) mm long; bracts 8–12.9Flowering shoots 3.5–13.1 mm long; bracts 12–54.10
- 7 - 8 - 9 - 10	Stigmas linear
- 7 - 8 - 9 - 10	Stigmas linear.7Flowering shoots ca. 6.0 mm long; bracts 8–10.Cladopus pierreiFlowering shoots 1.0–2.0 mm long; bracts 4–6.Cladopus taiensisStamens 0.3–0.6 mm longCladopus fallaxStamens 1.5–2.4 mm long9Flowering shoots 1.6–3.0(–3.5) mm long; bracts 8–12.9Flowering shoots 3.5–13.1 mm long; bracts 12–54.10Leaves 1.3–5.0 mm long; stamens as long as or slightly shorter thanovary.Cladopus fukienensis
- 7 - 8 - 9 - 10	Stigmas linear.7Flowering shoots ca. 6.0 mm long; bracts 8–10.Cladopus pierreiFlowering shoots 1.0–2.0 mm long; bracts 4–6Cladopus taiensisStamens 0.3–0.6 mm longCladopus fallaxStamens 1.5–2.4 mm long9Flowering shoots 1.6–3.0(–3.5) mm long; bracts 8–12.9Flowering shoots 3.5–13.1 mm long; bracts 12–54.10Leaves 1.3–5.0 mm long; stamens as long as or slightly shorter than ovary.Cladopus fukienensisLeaves 18.7–26.7 mm long; stamens distinctly longer than ovaryCladopus fukienensis

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# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Conceptualization: BHC, GDC, XTL. Formal analysis: ZXC, YLH. Funding acquisition: BHC. Investigation: BHC, YLH, XTL, MT, GDC, ZXC. Methodology: MT, BHC, MZ, ZXC, YLH. Supervision: BHC. Writing - original draft: MZ. Writing - review and editing: MZ, MT, BHC.

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#### Data availability

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

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# **Supplementary material 1**

#### Supplementary data

Authors: Miao Zhang, Xiu-Ting Liu, Min Tian, Zhang-Xue Chen, Ying-Lin Huang, Guo-Di Chen, Bing-Hua Chen

Data type: doc

- Explanation note: **table S1.** Primers used in this study. **table S2.** The information of phylogenetic analysis for the species used in the present study (*matK*). **table S3.** The information of phylogenetic analysis for the species used in the present study (plastid genome).
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# PhytoKeys

**Research Article** 

# Rubus tingzhouensis (Rosaceae), a new species from Fujian, China

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#### Abstract

*Rubus tingzhouensis* C.An & G.C.Lin, a newly-defined species within the family Rosaceae from Fujian Province, China, is formally described and illustrated here. *R. tingzhouensis* is morphologically similar to *R. swinhoei*, but can be distinguished by several key characteristics, such as long, reddish-purple stipitate glands, soft bristles, light yellow short trichomes (vs. shortly grey tomentose at the early stage, glabrescent) and scattered epidermal prickles (vs. few to many curved prickles). Furthermore, the stipules are pinnately deeply laciniate, measuring 1–1.5 cm (vs. ovate-oblong to ovate-lanceolate, 0.5–0.8 cm). Phylogenetic analyses, based on partial sequences and the complete plastome data, provide robust support for a close relationship between *R. tingzhouensis* and *R. swinhoei*, while also highlighting distinct genetic differentiation between these two species. The chloroplast genome of *R. tingzhouensis* is 156,311 bp in length and comprises 132 unique genes, including 86 protein-coding genes, 37 transfer RNAs, eight ribosomal RNAs and one pseudogene.

Key words: Biodiversity, classification, floristic survey, morphology, new taxon

# Introduction

The genus *Rubus* L. (Rosaceae), established by Carl Linnaeus in 1753, initially comprised only ten species. Over the course of more than 270 years of botanical exploration and taxonomic refinement, *Rubus* has expanded to compass nearly 700 species (Gao et al. 2023; Huang et al. 2023). This expansion has made it one of the most speciose genera amongst angiosperms. This genus exhibits a broad distribution, predominantly spanning temperate and subtropical regions of the Northern Hemisphere. In China alone, over 208 species were documented, of which 139 are considered endemic (Lu and Boufford 2003). The high number of endemic species highlights China's unique biodiversity and emphasises the importance of conservation efforts to protect these species, which are often vulnerable to habitat loss and other environmental pressures.



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*Rubus* is distinguished by its diverse morphological characteristics, which include variations in leaf morphology, inflorescence structure, reproductive patterns, fruit colour and size, stem armature and other distinguishing traits (Woznicki et al. 2016; Xiong et al. 2019). The plants are typically characterised by the presence of bristles, prickles or glandular hairs and their leaves can be simple, palmate or pinnate. The floral structures are generally pentamerous and predominantly bisexual. The achenes are aggregated drupelets or drupaceous fruits borne on a peduncle, with fruit shapes varying from semi-spherical to conical or cylindrical (Yu et al. 2022).

The taxonomy of *Rubus* remains particularly challenging due to the extensive morphological variations exhibited by species within the genus. This complexity is further exacerbated by apomixis, polyploidy and frequent hybridisation events (Thompson 1997; Alice and Campbell 1999). Given its considerable significance in ornamental and medicinal value, there has been a growing interest amongst Chinese botanists in exploring the taxonomy of *Rubus*, including the description of new species and the identification of novel geographical distributions.

Plastome-based phylogenetic inference has emerged as a robust method for resolving relationships and identifying lineages within the family Rosaceae. This approach has been particularly effective in elucidating both shallow and deep phylogenies, as evidenced by several recent studies (Zhang et al. 2017; Liu et al. 2019; Liu et al. 2020a, 2020b; Liu et al. 2021; Su et al. 2021; Liu et al. 2022; Jin et al. 2023; Liu et al. 2023a, 2023b; Xu et al. 2023; Jin et al. 2024a, 2024b; Wang et al. 2024; Xue et al. 2024). These studies indicated that the plastome data could provide high-resolution insights into species boundaries, divergence times and the historical biogeography of the family, contributing significantly to the current understanding of both inter- and intra-generic relationships. In this study, we performed next-generation sequencing (NGS) for this potential new species and assembled the whole plastome to estimate its phylogenetic position in *Rubus*.

An unusual *Rubus* population was collected during a field expedition in April 2023 in Xuancheng Town, Changting County, Fujian Province, China. These plants had ovate to oblong-lanceolate leaves and racemose inflorescences, either terminal or axillary, similar to *R. swinhoei* Hance. However, they are distinct due to the dense covering of long, reddish-purple stipitate glands, soft bristles and short, light yellow trichomes, interspersed with scattered epidermal prickles on the stems. To clarify the taxonomic status of this population, we carried out molecular analyses to reconstruct phylogenetic trees involving 46 *Rubus* species. This approach provided insights into their phylogenetic relationships. The results confirmed that the collected population represents a new species. By comparing morphological and molecular evidence, we concluded that this species is indeed new and we provide its formal description and illustration here.

#### Material and methods

#### Morphological observations

We conducted extensive field surveys and detailed observation studies to assess the population status and phenological traits of the species. Specimens were collected during peak flowering periods, allowing for precise
measurements of leaves, inflorescence and fruit structures, including their dimensions, features and colours. High-resolution photographs documented key characteristics and floral structures of the fully opened flowers were examined using a Leica M205a microscope. All specimens were deposited in the South China Botanical Garden Herbarium, Chinese Academy of Sciences (IBSC) and the China National Herbarium (PE) at the Institute of Botany, Chinese Academy of Sciences.

#### **DNA extraction and sequencing**

Whole genomic DNA was extracted from fresh leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) and shipped to Jisi Huiyuan Biotechnology Co., Ltd. (Nanjing, Jiangsu) for sequencing. Quality assessment involved checking DNA integrity and concentration via gel electrophoresis and spectrophotometry. DNA was then fragmented by ultrasonication, purified and subjected to end repair, 3' end adenylation, adapter ligation and gel electrophoresis for size selection. PCR amplification was used to generate sequencing libraries and only high-quality libraries were selected for paired-end (PE) sequencing on the Illumina NovaSeq 6000 platform, with a read length of 150 bp.

# Genome assembly, annotation and analysis

The sequencing data generated 19.82 GB of raw data. Bowtie2 v.2.2.4 (Langmead and Salzberg 2012) was used to align the reads against a custom chloroplast genome database for assembly. Annotation was performed using two complementary methods to enhance accuracy, verify results and improve reliability. Prodigal (https://github.com/hyattpd/Prodigal) was used for annotating chloroplast coding sequences (CDS), while hmmer v.3.4 and aragorn v.1.2.36 were used for predicting rRNA and tRNA genes (Laslett and Canback 2004; Potter et al. 2018). Additionally, gene sequences from related species available on NCBI were aligned against the assembled sequences using BLAST v.2.6 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for alternative annotations. A comparative analysis between the two annotation sets was conducted and discrepancies were resolved manually to ensure accuracy and eliminate redundancies. Finally, OrganellarGenomeDRAW (OGDRAW) was used to generate the chloroplast genome map (Greiner et al. 2019).

# **Phylogenetic study**

To determine the phylogenetic position of the new species within *Rubus*, chloroplast genome sequences of 48 species, including *Rubus* species, were retrieved from the NCBI database (Suppl. material 2), with *Rosa laevigata* Michx. and *Pygeum topengii* Merr. serving as outgroups. The sequences were aligned using MAFFT v.7.310 with default parameters (Katoh and Standley 2013) and Maximum Likelihood phylogenetic inference was performed with RAxML-NG (Kozlov et al. 2019) using the GTRGAMMA model, with a rapid Bootstrap analysis of 1000 replicates. The resulting phylogenetic tree was visualised using the ChiPlot online programme (Xie et al. 2023).

## Results

#### Morphological characteristics of Rubus tingzhouensis

Specimens of *Rubus tingzhuoensis* were compared with type specimens of several closely-related species within *Rubus*, revealing significant morphological differences, particularly in leaf (e.g. unique shape patterns), indumentum texture (e.g. density and colour of trichomes) and stipules (e.g. shape and size) (Suppl. material 1). To provide a comprehensive description, we measured and documented features such as branchlets, leaves, and floral structures, including indumentum, prickles, stipules and inflorescences. These morphological traits were compared with those of *R. tingzhouensis*, *R. swinhoei* and *R. amphidasys* Focke (Table 1). Notable differences in the indumentum, as well as distinct variations in stipules and stamens, strongly support the classification of *R. tingzhouensis* as a new species.

Differentiatin	g characters	Rubus amphidasys	Rubus swinhoei	Rubus tingzhouensis	
Habit		trailing shrubs	climbing shrubs	climbing shrubs	
Branchlets		Reddish-brown brown to purplish-brown		Reddish-brown	
Indumentum		dense reddish-brown long stipitate glands, soft bristles and long yellowish hairs	shortly grey tomentose at first, glabrescent	densely covered with reddish- purple long stipitate glands, soft bristles and light-yellow short hairs, with scattered epidermal prickles	
Prickles		usually unarmed	with few to many curved prickles	sparsely prickles	
Stipules	shape & size	deeply laciniate, 0.8–1.5 cm	ovate-oblong to ovate- lanceolate,0.5–0.8 cm	deeply laciniate, 1–1.5 cm	
	indumentum	with long glandular hairs, villous		with long glandular hairs, villous	
Leaves	petioles	20-55 mm	5-10(15) mm	15-20 mm	
	shape	broadly to narrowly ovate	ovate to oblong-lanceolate,	ovate to oblong-lanceolate,	
	size	5-11 × 3.5-9 cm	5−11 × 2.5−5 cm	8-16 × 3.5-6 cm	
	indumentum	both surfaces villous	adaxially glabrous, abaxially grey tomentose, or subglabrous	adaxially hirsutullous, purple long stipitate glands along veins, abaxially densely yellowish-brown tomentose and pubescent	
	base, margin and apex	base cordate, margin 3–5-lobed, terminal lobe much larger and longer than lateral lobes, apex shortly acuminate, rarely acute, lateral lobes obtuse, unevenly sharply serrate.	base rounded or truncate to shallowly cordate, margin unevenly serrate to doubly serrate, rarely incised, apex acuminate.	base cordate, margin unevenly serrate to doubly serrate, apex acuminate to acute.	
Inflorescences	position	osition terminal or axillary terminal		terminal or axillary	
Flower	number and size	5–12, 1–1.5 cm	5–6, 1–1.5 cm	5–10, 2–2.5 cm	
	corolla	Petals white, broadly ovate to oblong, 4–7 × 3–5 mm, base barely clawed, margin premorse or coarsely serrate	Petals white, broadly obovate to suborbicular, 5–7 × 4–6 mm, both surfaces thinly pubescent, base shortly clawed, apex obtuse, undulate.	Petals white, broadly ovate to oblong, 5–6 × 4–5 mm, base barely clawed, apex slightly concave.	

Table 1. Morphological comparison between R. tingzhouensis and its allied species.

\*: *R. amphidasys* exhibits significant differences in leaf morphology and growth form compared to the newly-described species; however, it possesses similar indumentum characteristics, warranting its inclusion in the comparative analysis.

#### **Phylogenetic analysis**

Building on previous molecular studies (Wang et al. 2016; Xu et al. 2024), our dataset included 46 *Rubus* species and two outgroup species. Phylogenetic analysis of complete and partial chloroplast genome sequences confirmed that *R. tingzhouensis* represents a distinct species, supported by a high bootstrap value and unique clade positioning within sect. *Malachobatus* (Fig. 1). The chloroplast genome tree positioned *R. tingzhouensis* within the sect. *Malachobatus* Focke, forming a clade with four other members of this section, with a strong bootstrap value (BP = 91). Specifically, *R. tephrodes* Hance and *R. hunanensis* Hand.-Mazz. formed a sister group, consistent with previous findings (Yu et al. 2022), while *R. kawakamii* Hayata and *R. swinhoei* exhibited a closer phylogenetic relationship. Within this clade, *R. tingzhouensis* forms a distinct lineage.



**Figure 1.** Phylogenetic trees of *Rubus* reconstructed separately based on (A) complete plastome DNA sequences and (B) plastid fragments (*rbcL*, *rpl20-rps12* and *trnG-trnS*). Numbers at nodes represent Maximum Likelihood bootstrap percentages (BP). The new species sequenced in this study are highlighted in red font.

#### The chloroplast genome features of Rubus changii

The complete annotated chloroplast genome of *R. tingzhouensis* is a double-stranded circular DNA with a length of 156,311 bp (Fig. 2). This genome length is comparable to that of other species within the Rubus genus, indicating a conserved genomic structure typical of the family Rosaceae. The chloroplast genome consists of two inverted repeats (IR) regions (IRA and IRB, each 25,801 bp), a large single-copy (LSC) region (85,842 bp) and a small single-copy (SSC) region (18,867 bp). The LSC, SSC and IR regions account for 55.4%, 12.3% and 32.3% of the total length, respectively. These proportions are typical for chloroplast genomes in *Ru*-



**Figure 2.** Gene map of the *R. tingzhouensis* plastome. Two purple scalloped areas identify the inverted repeat regions (IRa and IRb). Genes drawn inside and outside of the map are transcribed in clockwise and counter-clockwise directions, respectively. The dark grey bar graphs inner circle shows the GC content.

*bus* and contribute to genome stability and gene expression regulation, consistent with related species in the Rosaceae family. The overall GC content is 37.18%. The *R. tingzhouensis* chloroplast genome contains 132 unique genes, including 86 protein-coding genes, 37 transfer RNAs, eight ribosomal RNAs and one pseudogene.

Introns are present in several coding genes, which is consistent with other chloroplast genomes of flowering plants. Of the 86 protein-coding genes, 16 contain introns (Table 2). Two genes, *clpP* and *rps*12 have two introns each, while 12 additional genes (*ndhA*, *ndhB*, *petB*, *petD*, *rpl*16, *rpl2*, *rps*16, *trnA*-UGC, *trnG*-UCC, *trnI*-GAU, *trnK*-UUU, *trnL*-UAA and *trnV*-UAC) contain a single intron each.

# **Taxonomic treatment**

*Rubus tingzhouensis* C.An & G.C.Lin, sp. nov. urn:lsid:ipni.org:names:77352705-1

Figs 3, 4 Chinese name: 红毛木莓

**Type.** China • Fujian: Longyan City, Changting County, Xuancheng Town, Xiashe Village, 25°24'06"N, 116°22'34"E, forests on mountain slopes, alt. ca. 351 m, 18 April 2024, *C. An & G.C. Lin. 240418* (holotype: IBSC [barcode 1021457!]; isotypes: PE [barcode 02468523!, 02468524!]).

Category	Gene group	Gene name		
Photosynthesis	Subunits of photosystem I	psaA, psaB, psaC, psaI, psaJ		
	Subunits of photosystem II	psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL psbM, psbN, psbT, psbZ		
	Subunits of NADH dehydrogenase	ndhA*, ndhB*(2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ		
	Subunits of cytochrome b/f complex	petA, petB*, petD*, petG, petL, petN		
	Subunits of ATP synthase	atpA, atpB, atpE, atpF, atpH, atpI		
	Large subunit of rubisco	rbcL		
	Subunits photochlorophyllide reductase	_		
Self-replication	Proteins of large ribosomal subunit	rpl14, rpl16*, rpl2*(2), rpl20, rpl22, rpl23(2), rpl32, rpl33, rpl36		
	Proteins of small ribosomal subunit	rps11, rps12**(2), rps14, rps15, rps16*, rps18, rps19, rps2, rps3, rps4, rps7(2), rps8		
	Subunits of RNA polymerase	rpoA, rpoB, rpoC1, rpoC2		
	Ribosomal RNAs	rrn16(2), rrn23(2), rrn4.5(2), rrn5(2)		
	Transfer RNAs	trnA-UGC*(2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC*, trnH-GUG, trnI-CAU(2), trnI-GAU*(2), trnK-UUU*, trnL- CAA(2), trnL-UAA*, trnL-UAG, trnM-CAU, trnN-GUU(2), trnP-UGG, trnQ- UUG, trnR-ACG(2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC(2), trnV-UAC*, trnW-CCA, trnY-GUA, trnfM-CAU		
Other genes	Maturase	matK		
	Protease	clpP**		
	Envelope membrane protein	cemA		
	Acetyl-CoA carboxylase	accD		
	c-type cytochrome synthesis gene	-		
	Translation initiation factor	_		
	other	_		
Genes of unknown function	Conserved hypothetical chloroplast ORE	#vcf1 vcf1 vcf15(2) vcf2(2) vcf3** vcf4 vcf68(2)		

Table 2. Gene composition in the chloroplast genome of *R. tingzhouensis*.



Figure 3. *Rubus tingzhouensis* C. An & G. C. Lin **A** plant in natural habitat **B** leaf (adaxial surface) **C** leaf (abaxial surface) **D**, **E**, **F** flower and inflorescence **G** androecium **H** stamen **I** stigma **J** petal **K** stipule.

**Diagnosis.** This species is similar to *R. swinhoei* in its growth habit, with ovate to oblong-lanceolate leaf blades and botryoid inflorescences that may be terminal or axillary. However, *R. tingzhouensis* can be distinguished by its dense indumentum of long, reddish-purple stipitate glands, soft setae and light yellow short trichomes on the plant surface. It also has scattered epidermal prickles, adding to its distinct appearance. Additionally, it has deeply laciniate stipules measuring 1–1.5 cm in length, which are significantly more divided than those of related species, making them a key distinguishing feature.

**Description. Vines, lianas and shrubs.** *Stems* cylindrical, greyish-brown, with dense, reddish-purple long glandular hairs, soft bristles, short yellowish hairs and sparse prickles, apically rooting. *Leaves* simple; blades ovate to oblong-lanceolate, herbaceous,  $8-16 \times 3.5-6$  cm, apex acuminate to acute, base cordate; adaxially flat, hirsutullous with long, purple stipitate glands along veins, abaxially densely yellowish-brown tomentose and pubescent, with long soft hairs along mid-ribs;



Figure 4. Illustration of *Rubus tingzhouensis* C. An & G. C. Lin **A** habit **B** petal **C** longitudinal section of flower **D** free lobes of calyx **E** stamen **F** pistil **G** flower **H** stipule **I** bract **J** margin and trichome of leaf **K** trichome of abaxial leaf **L** section of leafed stem. Drawn by Yunxiao Liu.

principal veins sparsely retrorsely aculeolate, margin unevenly serrate to doubly serrate, apex acuminate to acute, lateral veins 9 to 10 pairs; petiole 1.5–2 cm, with dense, long, purplish-red glandular hairs and soft bristles; stipules caducous, free, pinnatipartite, lobes narrowly elliptic or lanceolate, densely covered with long

glandular hairs and tomentose-villous, 1-1.5 cm. *Inflorescences* terminal or axillary, short botryoid, 5-10 flowered; involucral bracts 6-9 mm, lobed, lobes linear or lanceolate, villous, rachis and pedicels with dense reddish-purple long glandular hairs and soft bristles; peduncle 5-10 cm, pedicels 1.5-2 cm; *Flowers* 2-2.5 cm. Sepals ovate-lanceolate, 5-6 mm, apex acuminate to caudate, outer sepals usually 2- or 3-laciniate; abaxially densely greyish-white pubescent, purplish-red long glandular hairs and soft bristles, adaxially densely greyish-white pubescent. Petals white, broadly ovate to oblong,  $5-6 \times 4-5$  mm, base barely clawed, slightly shorter than sepals. Stamens many; filaments linear, lower part slightly broader; anthers with few long hairs. Carpels many, style longer than stamens, glabrous.

Phenology. Flowering in March to May, fruits have not been seen yet.

**Etymology.** The specific epithet 'tingzhouensis' refers to the ancient region Tingzhou (汀州) in south-western Fujian, China, where this species was discovered. The Chinese name, "红毛木莓" (hong mao mu mei), reflects the plant's dense covering of reddish-purple long hairs.

**Distribution and ecology.** Currently, this species is only found in Changting County and Shanghang County, Fujian Province, China (Fig. 5). It is sporadically distributed in the understorey of the primary forest in mountain valleys at an altitude of 300–400 m. The habitat features a thick layer of dead branches and leaf litter, as well as a substantial amount of humus, supporting vigorous growth of herbaceous, shrubby and woody plants. Associated species include *Vernicia montana* Lour. and *Pterocarya stenoptera* C.D.C. in the tree layer; *R. corchorifolius* L.f., *Buddleja lind-leyana* Fortune, *Diplospora dubia* (Lindl.) Masam., *Callicarpa kochiana* Makino, *llex pubescens* Hook. & Arn., *Itea omeiensis* C.K.Schneid., *C. formosana* R.Br., *Cleroden-drum cyrtophyllum* Turcz., *Loropetalum chinense* (R.Br.) Oliv., *Mallotus apelta* (Lour.) Müll.Arg., *Trema tomentosa* (Roxb.) H.Hara, *Phyllanthus glaucus* Wall. ex Müll.Arg., *Melastoma malabathricum* L. and *R. reflexus* Ker Gawl. in the shrub layer; *Lysimachia alfredii* Hance, *Senecio scandens* Buch.-Ham. ex D.Don, *Blechnopsis orientalis* (L.) C.Presl and *Dicranopteris pedata* (Houtt.) Nakaike in the herbaceous layer.

**Conservation assessment.** *R. tingzhouensis* is documented in limited populations distributed within sparse forests on mountain slopes in Shanghang, Changting and Wuping County, Fujian Province or thrives along stream margins and under mixed forests. Notably, one population in Changting County is adjacent to a scenic locale, heightening its susceptibility to considerable anthropogenic disturbance. Furthermore, most of these populations are located outside designated conservation zones, making them vulnerable to ongoing exploitation of woodland resources by local residents. Thus, this newly-recognised species is assigned a preliminary status of Vulnerable (VU D2) according to the IUCN Red List Categories and Criteria (Standards and Group 2006; Betts et al. 2020), reflecting a population with a severely limited occupancy range (typically less than 20 km<sup>2</sup>) or few locations (typically five or fewer).

# Discussion

Based on the well-supported phylogenetic trees (Fig. 2), *Rubus tingzhouensis* is identified as a member of Sect. *Malachobatus* Focke. This species shares a close relationship with *R. swinhoei*, which aligns strongly with our morphological observations. Combining its distinct morphological differences with the high-support phylogenetic relationship, we confirm its designation as a new





species. Despite its morphological resemblance to *R. swinhoei*, this new species exhibits distinct differences in pubescence and stipule morphology; for instance, it is densely covered with long, reddish-brown stipitate glands, soft bristles and light-yellow short hairs, with scattered epidermal prickles and its stipules are pinnately deeply laciniate. These disparities robustly substantiate its status as a new species, a conclusion further supported by subsequent phylogenetic analyses. It is noteworthy that *R. swinhoei* is widely distributed globally. However, *R. tingzhouensis* is an endemic species found in the south-western mountainous regions of Fujian Province, typically occurring at altitudes around 300 m. Currently, the discovered population of this species is limited, warranting intensified efforts in population surveys and conservation endeavours.

Due to its straightforward and stable genetic architecture, coupled with its amenability to sequencing, the chloroplast genome has garnered increasing attention for applications in species identification, phylogenetic reconstruction, demographic history elucidation and species divergence investigations (Palmer 1987; Liu et al. 2020c). Nevertheless, genomic data pertaining to *Rubus*, especially complete chloroplast genomes within the NCBI database, remained notably scarce. In this present investigation, we conducted the sequencing and assembly of the entire chloroplast genome of the new *Rubus* species. Its structure resembles that of most angiosperms (Palmer and Stein 1986; Dobrogojski et al. 2020), comprising a circular double-stranded molecule spanning 156,311 bp in total length. In detail, it exhibits the characteristic quadripartite organisation consisting of a large single-copy region (LSC), a small single-copy region (SSC) and a pair of inverted repeat regions (IR), measuring 85,842, 18,867 and 25,801 bp, respectively. Furthermore, the GC content is 37.18%. This structure is consistent with the genomic organisation in reported *Rubus* species (Lu et al. 2024).

However, the plastid phylogeny only represented the maternally inherited phylogeny and cannot depict the accurate evolutionary history of *Rubus*. Looking ahead, the phylogenomic era, characterised by the analysis of hundreds or even thousands of single-copy nuclear genes (SCNs), is rapidly gaining momentum across the plant systematic community (Liu et al. 2021). The nuclear SCNsbased research promises to revolutionise our understanding of plant evolution, offering unprecedented insights into the genetic underpinnings of diverse plant groups and various fields (Jin et al. 2023; Xu et al. 2023). As we delve deeper into this new era, our study serves as a critical stepping-stone, providing a robust and comprehensive framework that will undoubtedly facilitate future research. By leveraging the power of plastome data and the emerging techniques in phylogenomic analysis, we are poised to uncover new layers of complexity and diversity within the plant kingdom, enriching our understanding of its evolutionary history.

#### Additional specimens examined

- Rubus swinhoei Hance. China Taiwan, Tamsui; Apr. 1864 (fl.); holotype: Richard Oldham 03341152 (P) • Guangdong, Lo-Fau-Shan; 2600 m alt.; May 1883 (fl.); isotype: James Alexander Calder 000946959 (BM) • Taiwan, Nan-To and mountains northwards; Oct 1887 (fl.); holotype: A. Henry 000737663 (K); syn. *R. hupehensis* • Fujian, Kuatun; May 1898 (fl.); holotype: M. de Latouche 00755178 (P); syn. *R. adenanthus* • Taiwan, Shichiseitonzan; Mar 1911 (fl.); holotype: Yaichi Shimada 12172 (TAIF); syn. *R. adenotrichopodus*.
- *R. amphidasys* Focke ex Diels—China Sichuan, Mont du College, Gorges Yangpa; aest. 1891 (fl.); holotype: Rosthorn 1172563 (B).
- R. doyonensis Hand. Mazz.-China · Yunnan, near the village of Bahan (Pehalo), in warm, mixed rainforests by the Nu-jiang (Salween) River; 2600 m alt.; 20 Jun 1916 (fl.); holotype: Heinrich Handel-Mazzetti 0059386 (WU).
- R. hanceanus Kuntze-China Guangdong, West River, near Foshing; 18 May 1882 (fl.); paratype: C. Ford 000737848 (K).

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# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Conceptualisation and Methodology: Chang An. Investigation: Gui-Can Lin, Cheng-Zi Yang. Data Curation: Yi-Xue Zhuang, Tao Wang. Software and Visualisation: Yi-Xin Yao, Yan-Xiang Lin. Writing-Original draft: Ming Chen, Chang An. Writing-Review and Editing: Chang An, Yan-Xiang Lin and Yuan Qin. All authors have read and approved the final manuscript.

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# **Data availability**

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

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# **Supplementary material 1**

#### The type specimens of closely-related species of Rubus tingzhouensis

Authors: Chang An

Data type: png

- Explanation note: A holotype of *R. swinhoei*, P03341152 B holotype of of *R. adenanthus*, P00755178 C holotype of *R. hupehensis*, K000737663 D holotype of *R. amphidasys*, B10 1172563 E holotype of *R. doyonensis*, WU0059386 F paratype of *R. hanceanus*, K000737848.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.249.138951.suppl1

# Supplementary material 2

# Taxa and GenBank accession numbers for plastome sequences used in this study

Authors: Chang An

Data type: xls

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# **Supplementary material 3**

#### Digital photos of plants R. amphidasys

Authors: Chang An

Data type: zip

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# Supplementary material 4

#### Digital photos of plants R. swinhoei

Authors: Chang An

Data type: zip

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Link: https://doi.org/10.3897/phytokeys.249.138951.suppl4

# **Supplementary material 5**

#### Digital photos of plants R. tingzhouensis

Authors: Chang An

Data type: zip

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Link: https://doi.org/10.3897/phytokeys.249.138951.suppl5

# PhytoKeys

**Research Article** 

# *Lysimachia pubiflora* (Primulaceae), a new species from Hubei, China

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#### Abstract

A new species, *Lysimachia pubiflora*, is described and illustrated from western Hubei Province, China. It is similar to *L. jinzhaiensis*, but differs in having flagelliform runners on the stems, indumentum on the plant and flowers, and the smaller calyx and anthers. The new species can be distinguished from all other species in the *Lysimachia* Subgen. *Lysimachia* Sect. *Nummularia* by its glandular-pubescent corolla.

Key words: Hubei, Lysimachia pubiflora, new species, taxonomy

# Introduction

*Lysimachia* L. belongs to the family Primulaceae (*sensu lato*) (Angiosperm Phylogeny Group IV 2016; Sennikov 2016; Larson et al. 2023). This genus comprises approximately 180 species, mainly distributed in the temperate and subtropical regions of the Northern Hemisphere, with a few species found in Africa, Latin America, and Oceania (Chen and Hu 1979; Chen et al. 1989; Hu and Kelso 1996; Hao and Hu 2001). The "Flora of China" records that there are 138 species in China (Hu and Kelso 1996), primarily distributed in the southwestern Karst regions (Chen and Hu 1979).

In the past two decades, about 20 new endemic species have been discovered in China, predominantly in the expansive mountainous regions south of the Huai River (Yan et al. 2023; Zhang et al. 2024), highlighting Central China as a hotspot for *Lysimachia* diversity (Yi 2020; Ke et al. 2021). In June 2014, during an expedition to survey wild plant resources in Fang County, western Hubei Province, Qi-Liang Gan encountered an interesting *Lysimachia* species that resembles *Lysimachia hemsleyana* Maxim. ex Oliv. in having stems with whip-like branches (vs. terete stems and scattered glandular punctations on the leaf, calyx and corolla in *L. hemsleyana*). Further study showed that the newly collected species is more similar to *L. jinzhaiensis* S. B. Zhou & Kun Liu (Liu et al. 2014) both in the quadrangular stems, and scattered glandular striate on the leaf, calyx and corolla (vs. glabrous plant, wingless petiole, and without whip-like branches in *L. hemsleyana*). The diagnostic features distinguishing



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**Copyright:** <sup>©</sup> Han Xu et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). *L. hemsleyana*, *L. jinzhaiensis*, and *L. pubiflora* are summarized in Table 1. Based on unique combinations of characters, we propose that the newly collected specimens should be placed in *Lysimachia* Subgen. *Lysimachia* Sect. *Nummularia* (Gilib.) Klatt (Chen et al. 1989), representing a species new to science.

# Materials and methods

Specimens were collected in Fangxian County, Hubei Province. Comparisons were made with specimens of closely related species from main herbaria of China, such as PE, IBSC, HIB, KUN and several online databases, including CVH, JSTOR, IPNI, POWO, K, GH, P, and A (Holmgren et al. 1990; Fu 1993; Qin et al. 2019). All morphological characters were observed and measured using dissecting microscopes and described using the terminology suggested by Harris and Harris (1994).

# **Taxonomic treatment**

# Lysimachia pubiflora Q.L.Gan, Z.Y.Li & H.Xu, sp. nov.

urn:lsid:ipni.org:names:77352827-1 Figs 1, 2

**Diagnosis.** *Lysimachia pubiflora* is most similar to *L. jinzhaiensis* in several characteristics, including the quadrangular stems, glandular striations on the leaves, calyx, and corolla, as well as the presence of axillary solitary flowers featuring unequal calyx lobes. It also shares a yellow corolla with an orange-red base. However, *L. pubiflora* can be distinguished from *L. jinzhaiensis* by the presence of flagelliform runners on the stems (vs. absent in *L. jinzhaiensis*), pilose young stems and calyx lobes (vs. glabrous in *L. jinzhaiensis*), calyx lobes 4.5–5 mm long (vs. 6–8.5 mm long), and anthers 1.1–1.3 mm long (versus ca. 1.5 mm long). The new species can easily be distinguished from all other species in Sect. *Nummularia* by its glandular-pubescent corolla.

**Type.** CHINA • Hubei Province, Fangxian County, Hongta Town, Nantang Village, alt. 712 m, 12 June 2024, *Qi-Liang Gan 4450* (holotype, PE !).

**Paratypes.** CHINA • Hubei: Fangxian County, Yerengu Town, Tanjiawan Village, alt. 697 m, 12 June 2024, Qi-Liang Gan 4447 (PE !) • Fangxian County, Yerengu Town, Tanjiawan Village, alt. 697 m, 12 June 2024, Qi-Liang Gan 4448 (PE !) • Fangxian County, Hongta Town, Nantang Village, alt. 712 m, 12 June 2024, Qi-Liang Gan 4449 (PE !).

**Description.** Herbs perennial. Rhizome horizontal, below-ground, 5–12 cm long, with adventitious roots at the nodes; stems usually 3–5 caespitose, 50– 120 cm long, quadrangular, pubescent, at least when young, with a spreading habit, distal parts of stems and branches usually slender and smaller leaved, forming the flagelliform runners that usually root at the 1–3 distal nodes. Leaves opposite, rarely 3-whorled in the upper section of stems; petioles 0.5– 2.5 cm long, adaxial sides shallowly grooved, abaxial sides rounded, narrowly winged, margins pilose, amplexicaul at the base; leaf blades broadly ovate to deltoid-ovate, 1.5-9.5 cm long, 1-6.5 cm wide, with acute or subobtuse apices, broadly cordate, subrounded or truncate, rarely cuneate at the base (on the runners), margins entire or slightly undulate, densely scattered with transparent glandular striations that sometimes turn purple when dry, glabrous adaxi

Characters	L. jinzhaiensis	L. pubiflora	L. hemsleyana
Stems	quadrangular, without flagelliform runners	quadrangular, with flagelliform runners	terete, with flagelliform runners
Indumentum on stems	glabrous, glandular on young part	pilose when young, otherwise glabrous	pilose
Petiole	narrowly winged, glabrous, not amplexicaul	narrowly winged, ciliate, amplexicaul	wingless, pilose, not amplexicaul
Leaf blade	1.5−5.5 × 1−4.5 cm, densely scattered glandular striate, both surfaces glabrous	1.5−9.5 × 1−6.5 cm, densely scattered glandular striate, adaxially glabrous, abaxially sparsely pubescent along the midrib when young	1.5−4 × 1.2−3 cm, densely scattered glandular punctate, adaxially densely, abaxially sparsely strigillose
Calyx lobes	narrowly ovate or elliptic-lanceolate, 6–8.5 × 3.5–4 mm, unequal, densely glandular striate, glabrous outside	elliptic or elliptic-lanceolate, 4.5–5 × 1.5–2 mm, unequal, densely glandular striate, sparsely pilose outside	narrowly lanceolate, 6.5–7.5 × 1–1.5 mm, subequal, densely glandular punctate, sparsely pubescent outside
Corolla	yellow, base orange-red; lobes elliptic, narrowly ovate to sublanceolate, 8–13 × 4–5.5 mm, densely glandular striate, glabrous	yellow, with an orange to orange-red base; lobes ovate-lanceolate, 10–12 × 2.5–3 mm, densely glandular striate, glandular- pubescent outside and along margins	yellow; lobes elliptic or elliptic- lanceolate, 4–6 × 3.5–4 mm, scattered glandular punctate, glabrous
Filaments	connate tube 3–4 mm long, free parts 3–5 mm long	connate tube ca. 2 mm long, free parts 4–6 mm long	connate tube ca. 2 mm long, free parts 3–5 mm long
Anthers	ca. 1.5 mm long	1.1–1.3 mm long	ca. 1.5 mm long

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ally, and abaxially sparsely pubescent along the midrib when young, becoming glabrate with age; lateral veins in 4-6 pairs, with the lowest 1-2 pairs arising from the base, the others alternating, midrib and lateral veins impressed adaxially, raised adaxially, veinlets inconspicuous. Flowers solitary in axils of leaves; pedicel 1.1-2.5 cm long, usually shorter than subtending leaves, sparsely pubescent. Calyx 5–5.5 mm long, 5–parted almost to the base, connate part ca. 0.5 mm, lobes elliptic or elliptic-lanceolate, unequal,  $4.5-5 \times 1.5-2$  mm, densely transparent glandular striate, the stripes becoming purple when dry, sparsely pilose outside; corolla yellow, with an orange or orange-red base, rotate, 2-2.3 cm in diam., 5-parted, tube 1-1.5 mm long, lobes narrowly lanceolate, 10-12 mm long, 2.5-3 mm wide, sparsely transparent glandular striate, the stripes becoming black-purple when dry, glabrous inside, glandular-pubescent outside and along margins; stamens 5, adnate to the base of the corolla tube, erect, yellow, glabrous, filaments basally connate into a tube ca. 2 mm long, free parts 4–6 mm long, anthers basifixed, oblong, 1.1–1.3 mm long, open by lateral slits; pistil glabrous, ovary globose, ca. 1.5 mm in diameter, the style filiform, 7-8 mm long, stigma obtuse, slightly wider than the style. Capsule subglobose, 3-4 mm in diam., glabrous. Seeds dark brown, rhombic, 0.5-1 mm long, 3-4 angled, glabrous.

**Phenology.** Flowering from late May to early July; fruiting from mid-July to late August.

**Distribution and habitat.** This species is endemic to two specific townships in Fang County, confined to a narrow limestone valley that lies between the neighboring villages of Tanjiawan and Nantang. It is observed along roadsides, within water ditches, in sparse shrublands on hillsides, and at the edges of sparse forests. The elevation of its habitat ranges from 690 to 712 meters.

The main companion plant species include trees such as *Populus adenop*oda Maxim., *Quercus serrate* var. *brevipetiolata* (A. DC.) Nakai, *Platycarya strobilacea* Sieb. & Zucc., *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Vernicia fordii* (Hemsl.) Airy Shaw, *Pinus massoniana* Lamb.; shrubs such as *Cotinus coggygria* 



Figure 1. Lysimachia pubiflora sp. nov. A plant at early flowering stage B mature individual C rhizome and adventitious roots D stem E cross section of stem F pubescent young stem G upper part of flagelliform runner, showing the distal nodes with adventitious roots H leaves I larger leaves with broad-cordate base J, K petioles L, M glandular stripes on leaf blades usually transparent (L when fresh, M dried).



Figure 2. Lysimachia pubiflora A plants at full-bloom B, C flowering branches D axillary flowers E flower bud F pedicel G calyx (adaxial view) H calyx (lateral view) I corolla J a lobe of corolla (abaxial view) K stamens L capsule with persistent calyx M seeds.

var. pubescens Engl., Mallotus apelta (Lour.) Müll. Arg., Buddleja officinalis Maxim., Pyracantha fortuneane (Maxim.) Li, Zanthoxylum armatum DC., Coriaria nepalensis Wall., Rhus chinensis Mill., Salix wallichiana Anderss., Indigofera bungeana Walp., Ficus heteromorpha Hemsl., Lindera glauca (Sieb. & Zucc.) Bl., Rosa banksiae var. normalis Regel. Herbaceous plants consist of Miscanthus floridulus (Lab.) Warb. ex Schum. & Laut., Anemone hupehensis Lem., Geum japonicum var. chinense F. Bolle, Agrimonia pilosa Ledeb., Duchesnea indica (Andr.) Focke, Aster albescens (DC.) Hand.-Mazz., Leersia japonica (Makino) Honda, Pteridium aquilinum var. latiusculum (Desv.) Underw. ex A. Heller, Pteris vittata L., Cyrtomium tsinglingense Ching & K. H. Shing ex K. H. Shing, and others. Vines include Clematis armandii Franch., Biancaea decapetala (Roth) O. Deg., Dalbergia mimosoides Franch., and Smilax glauco-china Warb.

**Etymology.** The epithet 'pubiflora' refers to the glandular-pubescent corolla. Vernacular name: Mao Hua Guo Lu Huang (Chinese).

**Conservation assessment.** This species inhabits a narrow limestone valley, extending approximately ten kilometers in straight-line distance between two villages. The region is characterized by significant limestone exposure and thin, infertile soil layers, reflecting a fragile natural ecosystem. This ecosystem is highly susceptible to human activities. Following its initial discovery in 2014, the species has shown significant population fragmentation due to road construction, deforestation for agriculture, and livestock grazing. The current population size is estimated to consist of around one thousand individuals. Based on the IUCN Guide-lines (Version 16) (IUCN 2024), the species may be classified as 'Endangered'.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### **Author contributions**

Han Xu and Song-zhi Xu have completed the initial drafting of the paper, Qi-liang Gan carried out specimen collection and prepared the figures, and Zhen-Yu Li wrote and edited the entire article.

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#### **Data availability**

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

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#### **Research Article**

# A new species in *Begonia* sect. *Diploclinium* (Begoniaceae) from Guangxi, South China

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#### Abstract

Although Guangxi represents one of the distribution centres of begonias in China, the sect. *Diploclinium* (Wright) A. DC is not well documented herein. In this article, we illustrate a new species belonging to this section, *Begonia fangchengensis* Y.N.Huang, X.X. Feng & R.K.Li, which has been discovered in southern Guangxi. *Begonia fangchengsis* mostly resembles *B. rotundilimba* in elliptic leaf blade, palmate veins, dichasial cyme, three styles, axile placentation, and unequally 3-winged capsule. On the other hand, *B. fangchengensis* is characterized by creeping rhizome, pilose stipule, red and densely pilose petiole, pubescent leaf blade and pilose inflorescence bract, sparsely pilose ovary and the zygomorphic androecium, differing from the erect stem at anthesis, the green and densely villous petiole, the glabrous stipule, leaf blade, inflorescence bract, ovary and actinomorphic androecium in *B. rotundilimba*. Considering its small population size and narrow distribution, its conservation status is categorized as 'Endangered (D)' according to the IUCN Red List Categories and Criteria.

Key words: Limestone karst, morphology, new taxon, south China, taxonomy



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# Introduction

As one of the fastest-evolving genera in vascular plants around the world, *Begonia* L. species are now 2,151 in number (Hughes et al. 2015) and these are mainly distributed in the humid tropical and subtropical regions of Asia, America, and Africa. Over 270 species of *Begonia* have been reported in China and the taxa are expected to exceed 300 species in the future (Tian et al. 2018). The begonias are primarily distributed in southern and central China, with Yunnan and Guangxi representing the centers of natural distribution.

Approximately 90 species of *Begonia* have been documented in Guangxi and new species have been reported frequently in recent years (Liu et al. 2020; Feng et al. 2021; 2022; 2023; Tian et al. 2021; Zhou et al. 2024). The begonias in Guangxi are dominated by *B*.sect. *Coelocentrum* taxa from karst

landforms. Only nine species belonging to the sect. *Diploclinium* are distributed in Guangxi, including: *Begonia bambusetorum* H. Q. Nguyen, Y. M. Shui & W. H. Chen, *Begonia fimbristipula* Hance, *Begonia gigabracteata* H. Z. Li & H. Ma, *Begonia glechomifolia* C. M. Hu ex C. Y. Wu & T. C. Ku., *Begonia* grandis Dryander, *Begonia grandis* subsp. *sinensis* (A. Candolle) Irmscher, *Begonia hymenocarpa* C. Y. Wu, *Begonia obsolescens* Irmscher and *Begonia sinovietnamica* C. Y. Wu.

In October 2021, an unknown begonia was discovered growing on the slope by a stream during fieldwork in the Shiwanshan Mountain, southern Guangxi. This taxon is distinct from the commonly karst distributed sect. *Coelocentrum* begonias in consideration of its ovary locule and placentae type in the field. Some individuals have been well cultivated in the nursery of Dongguan Botanical Garden and flower characters were observed in January 2022. Based on detailed morphological comparisons with similar species, this *Begonia* species is identified as a new species of the section *Diploclinium*.

# Materials and methods

Morphological characters were observed and measured from fresh samples in the field. Morphological comparisons with similar taxa were undertaken by consulting the literature, examining herbarium (**IBK** and **IBSC**) specimens and observing living collections cultivated in the nursery of Dongguan Botanical Garden. The specimens were deposited at the Herbarium of South China Botanical Garden (**IBSC**), CAS.

# Taxonomy

Begonia fangchengensis Y.N.Huang, X.X.Feng & R.K.Li, sp. nov. urn:lsid:ipni.org:names:77346002-1 Figs 1, 2 Chinese name: 防城秋海棠

**Type**. **CHINA** • Guangxi Zhuang Autonomous Region (广西壮族自治区), Fangchenggang City (防城港市), Fangcheng District (防城区), Shiwanshan Yao Autonomous Town (十万山瑶族乡), Bajiao Mountain, 21°56′54″N, 108°14′20″E, (Fig. 3), 198 m alt., on the rock or slope beside the stream, 18 October 2021, *Yu Ni Huang, Xin Xin Feng & Ren Kun Li* (holotype: 0925761, IBSC!).

**Diagnosis.** Begonia fangchengsis mostly resembles *B. rotundilimba* in its elliptic leaf blade, palmate veins, dichasial cyme, three styles, axile placentation, and unequally 3-winged capsule. However, there are creeping rhizome, pilose stipule, red and densely pilose petiole, pubescent leaf blade and pilose inflorescence bract, sparsely pilose ovary and the zygomorphic androecium in *B. fangchengensis*. These characters differ from the erect stem at anthesis, the green and densely villous petiole, the glabrous stipule, leaf blade, inflorescence bract, ovary and actinomorphic androecium in *B. rotundilimba*.

**Description.** Perennial evergreen herb, monoecious, 15–30 cm height.

**Rhizome** creeping, ca. 6–8 cm long and 8–10 mm in diameter, internode short. **Stipules** membranous, reddish-green, translucent, triangular,  $4.5-8 \times 4-5$  mm, pilose. **Leaves** all basal, petiole 10–15 cm long, red and densely



Figure 1. *Begonia fangchengensis* drawn by Yunxiao Liu A habitat B stipule C, D bracts of inflorescences E, F front and back view of staminate flower G androecium H stamens I, J pistillate flower with 3 or 5 tepals K styles L styles and ovary M–Q ovary section from upper to lower positions.



Figure 2. Habitat and morphology of *Begonia fangchengensis* **A** habitat **B** flowering plant **C** creeping rhizome **D** stipules **E** leaf blade (adaxial) **F**, **G** leaf blade (abaxial) **H** juvenile leaf **I** inflorescence **J** bracts **K**, **L** front and side views of staminate flower **M**, **N** front and side views of androecium **O** stamens **P** pistillate flower with 3 tepals **Q**, **R**, **S** pistillate flower with 5 tepals **T** stigmas and ovary **U** ovary sections from upper to lower positions **V** capsule (Photos by Z.X. Liu).



Figure 3. Distribution of B. fangchengensis, B. rotundilimba and B. sinovietnamica.

white pilose; leave blade asymmetric, widely ovate to elliptic, 12-17 × 8-10 cm; fleshy; adaxially bright green and smooth; abaxially pale-green, densely covered with white pilose along the veins; base oblique-cordate; margin irregularly denticulate; apex caudate acuminate; venation palmate, primary veins 8, adaxially slightly concave, abaxially convex. Inflorescences arising directly from rhizome, dichasial cymes, peduncle 6.5-8 cm long, pilose; flowers unisexual, 2-5 flowers per inflorescence; bracts membranous, pale green, triangular to subcircular, 2-3 lobed apically, margin serrate with cilia, 7-8 × 4-6 mm. Staminate flower tepals 4, white; outer 2 larger, widely ovate, 17-21 × 15-20 mm, abaxially sparsely pubescent; inner 2, lanceolate, margin entire, 10-12 × 4-6 mm, glabrous; androecium zygomorphic, sub-globose, ca. 6 mm in diameter; stamens numerous, 3-3.5 mm long, filaments fused at base; anthers yellow, ca. 1.5 mm long, cuneiform, apex slightly bent and retuse. Pistillate flower tepals 3-5, white, glabrous; outer 2, widely ovate to subcircular, margin entire, 11-12 × 9-10 mm; inner 1-3, widely ovate, 8-11 × 4-7 mm; ovary yellowish-green, trigonous-ellipsoid, 8.0-8.5 × 5.2-5.5 mm (wings excluded), sparsely pilose, 3-locular, upper 1 (abaxial wing side) degenerated occasionally; placentation axile, bilamellate. Styles 3, fused at base, yellow, ca. 3 mm in diameter, stigma spirally U-shaped twisted. Capsules nodding, trigonous-ellipsoid, 9-10 × 6.0-6.5 mm (wings excluded), yellowish-green, sparsely pilose, wings 3, unequal, 2-lateral wings smaller, crescent-shaped, abaxial wing semilune-shaped.

**Distribution and habitat.** Currently only known from one locality in Fangcheng District, Fangchenggang City, Guangxi, China (Fig. 3). It grows in shaded environments along the stream or near a waterfall under the broadleaved forest.

Phenology. November-December (Flowering), January- February (Fruiting).

**Etymology.** The specific epithet *"fangchengensis"* refers to the Fangcheng District, Guangxi where the species is distributed.

**Conservation.** Only two populations with approximately 200 individuals have been found in the type locality. According to the IUCN Red List Categories and Criteria (IUCN 2024), *B. fangchengensis* should be assessed as 'Endangered (D)' due to its small geographic distribution and low population size.

# Discussion

The putative new *Begonia* species is morphologically characterized by having three styles, 3-locular ovary with axile and bifid placentation, representing the typical circumstance of the *Begonia* section *Diploclinium* (Lindl. 1846: 319) de Candolle (1859: 129). A total of 53 begonias of this section have been reported in China and most of them occur in Yunnan and Tibet, southeast China.

The putative new *Begonia* species mostly resembles *B. rotundilimba* from Pingbian, Yunnan in its elliptic leaf blade, palmate veins, dichasial cyme, three styles, unequally 3-winged capsule (Table 1, Fig. 4; Huang and Shui (1994)). In *B. rotundilimba*, there are erect stem at anthesis, green and villous petiole, and the stipule, leaf blade and inflorescence bract are all glabrous, being different from the creeping rhizome, pilose stipule and petiole, pubescent leaf blade and pilose inflorescence bract of *B. fangchengensis*. Furthermore, morphological dissimilarities also occur in reproductive organs, like the glabrous ovary and actinomorphic androecium of *B. rotundilimba*, compared with sparsely pilose ovary and the zygomorphic androecium in *B. fangchengensis*.

Besides the similarity to *B. rotundilimba* in morphology, the putative new *Begonia* species is closest to *B. sinovietnamica* in geographic distribution and altitude (200–400 m) (Fig. 3). In *B. sinovietnamica*, leaf blade is adaxially hirsute, tepals

Character B. rotundilimba		B. sinovietnamica	B. fangchengensis			
Rhizome	creeping, erect stem at anthesis; internode 2-3 cm long	elongate; internode 0.3-0.5 cm long	creeping; internode 0.3-1 cm long			
Stipule	ıle ovato-triangular, glabrous oblong, p		triangular, pilose			
Petiole	green, densely villous	reddish brown, densely villous	red, densely pilose			
Leaves	blade adaxially green, glabrous	blade adaxially green, hirsute	blade adaxially bright green, smooth			
Inflorescence bract	bracts glabrous, apex acuminate	bracts glabrous, apex acuminate	bracts apex 2-3 lobed, both pilose			
Staminate flower	androecium actinomorphic, anthers oblong	anthers oblong, apex obtuse	androecium zygomorphic, anthers cuneiform, apex slightly bent and retuse			
Pistillate flower	tepals 5, styles 3, ovary pilose, unequally 3-winged	tepals 4, styles 3, ovary sparsely pilose, subequally 3-winged	tepals 3-5, styles 3, ovary sparsely pilose, unequally 3-winged			
Phenology (flowering; fruiting)	Apr; Jul	Jul; Aug	Nov; Jan			
Habitat	alt. 1600-1800 m, Yunnan	alt. 230-400 m, Guangxi	alt. 198 m, Guangxi			

Table 1. Morphological comparisons of *B. fangchengensis* and relevant taxa.



**Figure 4.** Habitat and morphology of *Begonia rotundilimba* **A** habitat **B** flowering plant with erect stem at anthesis **C** leaf blade (adaxial) **D** leaf blade (abaxial) **E** petiole **F**, **G** front and back views of staminate flower **H** androecium **I**, **J** front and lateral view of pistillate flower **K** ovary section (Photos by W. G. WANG).

of pistillate flower are 4 and the 3-winged ovary is subequal, in contrast with the adaxially smooth blade, 3–5 tepals of pistillate flower and unequally 3-winged ovary of the new species. Furthermore, the two species also differ from each other in phenology, including flowering and fruiting time (Table 1; Wu (1997)).

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# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Conceptualization: XXF. Data curation: MNZ, BXC. Formal analysis: SSH. Investigation: PTH, YNH, WGW. Resources: XXF, RKL.

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#### Data availability

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

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Research Article

# Phylogenetic and taxonomic studies of six recently-described Stellaria species (Caryophyllaceae) from China, with an additional new species, Stellaria longipedicellata, from Sichuan

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#### Abstract

The phylogenetic studies of the tribe Alsineae (Caryophyllaceae) have revealed a clearer boundary between the genus Stellaria and related genera, primarily relying on the morphological characteristics of style 3, stamens 10 and petals deeply bifid. However, the newly-published species in China, which have 5 styles or ten or more lobes per petal, challenge this boundary and necessitate further studies. In this paper, we reviewed six newly-published Chinese species of Stellaria, utilising both molecular phylogenetic evidence from nuclear ribosomal internal transcribed spacer (ITS) and four plastid regions (trnL-F, matK, rbcL, rps16) and morphological evidence. Our results demonstrated that the five new species (Stellaria abaensis, S. multipartita, S. pentastyla, S. procumbens and S. zhuxiensis) were nested within the genus Stellaria, but Stellaria motuoensis was sister to the genus Schizotechium. Herein, we accepted four new Stellaria species and proposed a new combination in Schizotechium and a new synonym in Stellaria. Additionally, we described a new species Stellaria longipedicellata from Sichuan Province, China, which was distinguished from the closely-related species Stellaria decumbens by its glabrous body, linear-lanceolate leaves, long pedicellate flowers, prostrate growth habit and flowers nearly equal to or slightly shorter than sepals. Both molecular and morphological evidence support the treatment of S. longipedicellata as a new species of the genus Stellaria.

Key words: Alsineae, new combination, new species, Stellaria

# Introduction

The classification of three subfamilies has been widely accepted in the family Caryophyllaceae (Lu et al. 2001). Since the establishment of phylogenetic tree of Caryophyllaceae, the classification of eleven tribes has been increasingly accepted (Harbaugh et al. 2010; Greenberg and Donoghue 2011). The tribe Alsineae, as a major branch of Caryophyllaceae with a great diversity of species, has attracted much attention. Recent studies on the tribe Alsineae have clarified the boundaries of its genera and have described the following new genera: *Engellaria* Iamonico, *Hartmaniella* M.L.Zhang & Rabeler, *Nubelaria* M.T.Sharples & E.A.Tripp, *Rabelera* M.T.Sharples & E.A.Tripp, *Shivparvatia* Pusalkar & D.K.Singh, *Hesperostellaria* 



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Gang Yao, B. Xue & Z.Q. Song, Reniostellaria Gang Yao, B. Xue & Z.Q. Song and Torreyostellaria Gang Yao, B. Xue & Z.Q. Song (Zhang et al. 2017; Sharples and Tripp 2019; Iamonico 2021; Pusalkar and Singh 2022; Xue et al. 2023). Stellaria L. is particularly noteworthy as one of the genera with a high species richness in the tribe Alsineae, mainly characterised by 4 or 5 petals, petals usually deeply bifid (rarely retuse or multilobed) and capsules opening by valves 2 times number of styles (Chen and Rabeler 2001). Despite the species richness of this genus, limited research has been conducted on it. Previous phylogenetic studies of the family Caryophyllaceae have proposed that the traditional Stellaria was paraphyletic (Harbaugh et al. 2010; Greenberg and Donoghue 2011; Dillenberger and Kadereit 2014; Zhang et al. 2017). Due to the global studies on Stellaria, its classification has been clarified and species with non-deeply-bifid petals or 2 styles have been assigned as the following new genera: Nubelaria, Rabelera, Hesperostellaria, Reniostellaria and Torreyostellaria (Sharples and Tripp 2019; Xue et al. 2023). Then the core Stellaria in a new circumscription can be clearly distinguished from other related genera in having deeply-bifid petals (sometimes absent) usually in combination with six-valved capsules (Sharples and Tripp 2019). However, there is still a lack of research on new species of Stellaria with distinctive petals found in China.

In recent publications, six new Stellaria species have been documented in China, described as Stellaria abaensis H.F. Xu & Z.H. Ma, S. motuoensis Meng Li & Y.F. Song, S. multipartita Bo Xu & Meng Li, S. pentastyla W.Q. Wang, H.F. Xu & Z.H. Ma, S. procumbens Huan C. Wang & Feng Yang and S. zhuxiensis Q.L. Gan & X.W. Li. (Gan and Li 2014; Xu and Ma 2018; Song et al. 2020; Wang et al. 2020; Yang et al. 2020; Li et al. 2022). Amongst them, only three species (S. motuoensis, S. multipartita and S. pentastyla) were supported by the phylogenetic study, while the precise phylogenetic position of the remaining species requires further investigation. Notably, four species exhibit distinct flower morphology compared to the core Stellaria species. For instance, S. motuoensis lacks petals and bears 5 stamens, S. multipartita displays ten or more lobes per petal, while S. pentastyla and S. procumbens have 5 styles. Furthermore, two species share similar morphological characteristics with related species, such as S. abaensis and S. petiolaris Hand.-Mazz., S. zhuxiensis and S. vestita Kurz. Further in-depth research is crucial for a comprehensive understanding of these newly-named Stellaria species.

In addition, during a field survey in Sichuan Province of China, we discovered an undescribed species whose morphology does not correspond to any known *Stellaria* species. Based on detailed morphological and molecular studies, we hereby describe it as a new species.

#### Materials and methods

#### Sample and morphology

In this study, we sampled major genera of the tribe Alsineae and major clades of *Stellaria* in order to accurately determine the phylogenetic position of the new species, with *Arenaria serpyllifolia* L. serving as the outgroup. Samples and accession numbers are listed in Table 1, Suppl. material 1. The morphological traits of the new species were examined by either the original specimens or their images and also by specimens collected through our own field surveys.
Taxon	Location	Latitude, Longitude	Collector and number	Herbarium
Stellaria abaensis H.F. Xu & Z.H. Ma	Tianquan, Sichuan	31.049472, 102.874177	H.F. Xu SC0037	GAUA
Stellaria amplexicaulis (HandMazz.) Huan C. Wang & Feng Yang	Luding, Sichuan	26.016249, 98.620941	H.F. Xu & G.F. Mou YN0002	GAUA
Stellaria procumbens Huan C. Wang & Feng Yang	Luding, Sichuan	29.851945, 102.286631	W.Q. Wang et al. QSC0007	GAUA
Stellaria radians L.	Yakeshi, Neimenggu	49.327739, 120.676409	W.Q. Wang & R. Wu NM0010	GAUA
Stellaria longipedicellata W.Q. Wang & Z.H. Ma	Luding, Sichuan	29.863690, 102.289755	W.Q. Wang et al. QSC0009	GAUA
Stellaria zhuxiensis Q.L.Gan & X.W.Li	Zhuxi, Hubei	32.436378, 109.561435	W.Q. Wang & Z. Xie HB0034	GAUA

Table 1. Taxa sampled and the vouchers.

#### Phylogenetic analysis

Total DNA was extracted from silica gel dried leaves by modified CTAB (Doyle and Doyle 1987). Subsequently, we performed PCR amplification of the following markers as cited, ITS (5F, 4R), matK (390F, 1440R), rbcL (1F, 724R), rps16 (F, R) and trnL-F (C, F) (White et al. 1990; Taberlet et al. 1991; Popp and Oxelman 2001; Smissen et al. 2002; Kress and Erickson 2007). The PCR products were then sequenced by the Beijing Genomics Institute (BGI). The obtained sequences were double-stranded spliced and checked using GENEIOUS v.11.0.4 (Kearse et al. 2012) and the phylogenetic trees were conducted using PHY-LOSUITE v.1.2.2 (Zhang et al. 2020). Initially, the sequences were aligned by MAFFT v.7.313 (Katoh and Standley 2013). Then we used PARTITIONFINDER v.2.1.1 (Lanfear et al. 2016) to determine the best model under the Akaike Information Criterion (AIC). The selected models were SYM+I+G for ITS, GTR+G for matK, trnL-F, and rps16 and HKY+I+G for rbcL. The Bayesian Inference (BI) trees were constructed using MrBayes 3.2.6 (Ronquist et al. 2012) with 2,000,000 generations and the tree sampled every 100 generations. The first 25% trees of each run were discarded as burn-in. To assess the chain convergence, it was verified that the average standard deviation (SD) of the split frequencies was below 0.01. Finally, Maximum Likelihood (ML) trees were constructed using the GTRGAMMA model with 1,000 bootstrap replicates and default values for the remaining parameters on the CIPRES Science Gateway (Miller et al. 2010). No notable incongruence was found between the nrITS phylogenetic tree and the plastid phylogenetic tree.

#### Results

In the phylogenetic tree of the tribe Alsineae (Fig. 1), *Stellaria* formed a single clade, except for *Stellaria motuoensis*. Our results revealed that five new species (*S. abaensis*, *S. multipartita*, *S. pentastyla*, *S. procumbens* and *S. zhuxiensis*) were nested within *Stellaria*, but *S. motuoensis* was sister to *Schizotechium* with high support (BS = 100%, PP = 1.00). *Stellaria procumbens* was nested within the Larbreae clade and identified as the sister to *S. pentastyla* (BS = 100%, PP = 1.00). Notably, *S. abaensis* and *S. zhuxiensis* fell into the Larbreae clade and were sister to each other. Then *S. multipartita* was clustered with *S. pubera* Michx. and *S. corei* Shinnersin, forming the *Insignes* clade. Finally, *S. longipedicellata*, a potential new species was sister to *S. decumbens* Edgew.



**Figure 1.** Maximum likelihood (ML) tree of Alsineae inferred from the Alsineae-wide dataset (including nrITS, *matK*, *rbcL*, *rps16* intron and *trnL-F* intergenic region). Posterior probability (PP) in Bayesian Inference (BI) and bootstrap (BS) value in ML analysis are indicated above and below the stem branch of each phylogenetic node, respectively. NP indicates the topology was not present in BI analysis. \* indicates that the PP or BS value is less than 0.5 or 50%.

#### Discussion

Sect. Schizothecium Fenzl, a traditional section of the genus Stellaria, is characteried by having 3 styles and 1-2 seeds. It includes S. delavayi Franch., S. monosperma Buch.-Ham. ex D. Don and S. ovatifolia (Mizushima) Mizushima (Wu and Ke 1996). However, recent studies proposed that sect. Schizothecium should be resurrected as the genus Schizotechium (Pusalkar and Srivastava 2016; Arabi et al. 2022; Wang et al. 2023; Xue et al. 2023). Consequently, Stellaria delavayi and S. monosperma of sect. Schizothecium were reclassified under the genus Schizotechium (Pusalkar and Srivastava 2016; Xue et al. 2023). Moreover, it has been suggested that S. ovatifolia should revert to its previous name Brachystemma ovatifolium M. Mizush. (Wang et al. 2023). When S. motuoensis was published, it exhibited morphological similarities with S. monosperma var. paniculata (Edgew.) Majumdar and was sister to S. monosperma in the phylogenetic tree (Li et al. 2022). However, authors still considered S. motuoensis as part of Stellaria due to uncertainty of the phylogenetic tree of the tribe Alsineae and its morphological differences with Schizotechium species (Li et al. 2022). Nonetheless, more recent studies on the tribe Alsineae have accepted the genus Schizotechium (Pusalkar and Srivastava 2016; Arabi et al. 2022; Wang et al. 2023; Xue et al. 2023). Our study also indicated that S. motuoensis was nested within Schizotechium and sister to Schizotechium monosperma with high support (BS = 100, PP = 1.00, Fig. 1), which is consistent with previous studies (Li et al. 2022). Importantly, S. motuoensis (with many-flowered compounds cymes, 5 stamens, 2-3 styles and 1-3 seeds; see Table 2) is morphologically more similar to Schizotechium (with many-flowered compounds cymes, 5 stamens, 2-3 styles and 1-3 seeds compound cymes, 5 or 10 stamens, 2-3 styles and 1-6 seeds; see Table 2), rather than the core Stellaria (with lax dichasial cymes, rarely solitary, 10 stamens, 3 or 5 styles and numerous seeds) (Pusalkar and Srivastava 2016; Arabi et al. 2022, Li et al. 2022). Based on the morphological and phylogenetic evidence, we propose a new combination for Stellaria motuoensis.

Presence of the stellate hair is a highly distinctive character in *Stellaria* including *S. vestita*, *S. infracta* Maximowicz and *S. amplexicaulis* (Hand.-Mazz.) Huan C.Wang & Feng Yang (Chen and Rabeler 2001; Yang et al. 2020). *Stellaria zhuxiensis* closely resembles *S. vestita* with its sessile leaf and dense stellate indumentum, but differs by its longer petals and ovate leaves (Gan and Li

	S. motuoensis	Schizotechium monosperma	S. procumbens	S. pentastyla	S. longipedicellata	S. decumbens
Petal	absent	2-lobed to middle	deeply bifid	deeply bifid	deeply bifid	deeply bifid
Stem	glabrous basally, pubescent in apical part	pubescent with 1 or 2 rows of glandular hairs above	glabrous	glabrous	glabrous	densely white pubescent
Style	2-4	3	3 or 5	5	3, rarely 4	3
Leaf	shortly petiolate, lamina ovate or oblong	short or long petiolate, lanceolate or oblong- lanceolate to elliptic	sessile, linear or acicular	sessile, linear	sessile, linear- lanceolate	sessile, oblong
Seed	1-3	1-2	numerous	numerous	numerous	numerous
Pedicel	pubescent	pubescent	glabrous	glabrous	glabrous	pubescent
Stamen	5	10	10	10	10	10

Table 2. Morphological comparisons of six species.

2014). Interestingly, *S. zhuxiensis* was sister to *S. abaensis*, not to any other of the stellate-haired species (Fig. 1). Our findings were consistent with the previous studies, which indicated that *S. vestita* and *S. infracta* with stellate hairs were not sister taxa on the phylogenetic tree (Sharples and Tripp 2019). It could be attributed to the parallel evolution of stellate trichomes (Sharples 2019).

The classification of ser. Petiolares is widely accepted within Stellaria, characterised by the presence of distantly petiolate leaves (Endlicher 1840; Schischkin 1970; Wu and Ke 1996). The latest Stellaria phylogenetic study also kept the classification of the Petiolares clade (Sharples and Tripp 2019). Stellaria abaensis with long petioles was closely similar to the Petiolares clade species (Xu and Ma 2018). However, it is intriguing to discover that S. abaensis nested in the Larbreae clade instead of being associated with the Petiolares clade (Fig. 1). Similarly, being consistent with the previous studies (Sharples and Tripp 2019), petiolate species such as S. vestita and S. petiolaris were placed in the Larbreae clade rather than the Petiolares clade (Fig. 1). Hence, the presence of petiolate or sessile traits may not be reliable indicators for classification within Stellaria. Additionally, it is worth noting that S. abaensis was often misidentified as S. petiolaris before its official publication. For instance, S. capillipes (Franch.) C. Y. Wu (the synonymy of S. petiolaris) referred numerous specimens of S. abaensis and the description and pictures of S. petiolaris in the Flora of Yunnan actually corresponded to S. abaensis (Wu 1995). However, S. abaensis can be easily distinguished from S. petiolaris due to its glabrous leaves and long petiole with ciliate hair (vs. leaves and short petiole densely covered with white villous hair), glabrous plant body (vs. plant body densely covered with white villous hair), petal lobes ovate-oblong (vs. lobes narrowly linear) and the capsule longer than persistent sepals (vs. capsule ca. 1/2 as long as persistent sepals) (Chen and Rabeler 2001; Xu and Ma 2018).

The traditional Stellaria did not include species with five styles, which is commonly found in the related genus Cerastium L. (Chen and Rabeler 2001). However, since S. aquatica (L.) Scop. (Myosoton aquaticum (L.) Moench) was included within Stellaria, the genus Stellaria recently also includes five-styled species (Sharples and Tripp 2019; Wang et al. 2020; Xu et al. 2020; Yao et al. 2021; Arabi et al. 2022; Xue et al. 2023). This study revealed that S. pentastyla and S. procumbens bearing 5 styles are not sister to the clade including S. aquatica (Petiolares clade), but constitute a distinct clade (Fig. 1). This suggests the existence of a new group within Stellaria. Notably, S. pentastyla and S. procumbens share morphological similarities (refer to Table 2) such as linear leaves, glabrous stems and 5 styles (Wang et al. 2020; Yang et al. 2020). Moreover, they have overlapping distribution (Lushui, Yunnan) and similar altitudinal distribution range (2100-3800 m). After comparing the field and specimen morphology of S. procumbens and S. pentastyla, it was found that there was no significant difference between the two. It indicates that S. procumbens and S. pentastyla are not two distinct species, but the same species. Furthermore, our phylogenetic result supports this conclusion that S. procumbens and S. pentastyla form a strongly-supported clade (BS = 100, PP = 1.00, Fig. 1). Considering the publication date of S. pentastyla (4 March 2020) preceding that of S. procumbens (9 March 2020), we propose that S. procumbens should be treated as a new synonym of S. pentastyla according to the Art. 11. 4 of the Shenzhen Code (Turland et al. 2018).

Having five or more lobes per petal is an exceptional character state in Stellaria, with Stellaria radians L. as the only species exhibiting this trait (Chen and Rabeler 2001). Despite its unique floral morphology, S. radians is widely recognised as a member of Stellaria (Schischkin 1970; Wu and Ke 1996; Chen and Rabeler 2001). Previous phylogenetic studies also indicated that it was nested in core Stellaria and formed an Insignes clade with other Stellaria species (Sharples and Tripp 2019). Our results revealed that S. multipartita is another species of Stellaria which possesses ten or more lobes per petal because it formed a clade with Stellaria pubera and S. corei (Fig. 1). The previous study indicated S. radians formed a clade with S. pubera, S. corei and S. sessiliflora Y.Yabe (Sharples and Tripp 2019). It implied the close relationship between S. radians and S. multipartita. While S. multipartita shares similar petals with S. radians, it differs significantly in terms of petal characteristics (10-12-cleft vs. 5-7-cleft in S. radians) and distribution (Chongqing vs. Hebei, Heilongjiang, Jilin, Liaoning and Neimenggu in S. radians). This difference, as well as our phylogenetic data, support the treatment of S. multipartita as a new Stellaria species.

#### **Taxonomic conclusions**

Stellaria pentastyla W.Qiao Wang, H.F.Xu & Z.H.Ma, Phytotaxa 435: 71. [4 March] 2020. Type: CHINA. Yunnan: Lushui, elev. ca. 3102 m, 25°58'26"N, 98°40'44"E, 8 June 2017, Xu & Mou YN0014 (holotype GAUA!, isotypes IBSC!)

= Stellaria procumbens Huan C.Wang & Feng Yang, Phytotaxa 435: 195. [9 March] 2020. Type: CHINA. Yunnan Province: Luquan County, Zhuanlong Town, Jiaozishan National Nature Reserve, 26°04'58"N, 102°51'04"E, elev. 3380 m, 12 July 2019, H. C. Wang et al. LQ 7217 (holotype YUKU!, isotypes YUKU!), syn. nov.

Schizotechium motuoensis (Li & Song) W.Q.Wang & Z.H.Ma, comb. nov. urn:lsid:ipni.org:names:77352937-1

Stellaria motuoensis Meng Li & Y.F.Song, Nordic J. Bot. 2022(9)-e03683: 2.
 2022. Type: China, Xizang, Motuo County, Mt Doxong La, Xiaoyandong, ca.
 2650 m a.s.l., 95°04'10.33"E, 29°24'51.13"N, 22 October 2021, Meng Li 3021 (holotype: NF, isotypes: CDBI).

#### Stellaria longipedicellata W.Q.Wang & Z.H.Ma, sp. nov.

urn:lsid:ipni.org:names:77352938-1 Fig. 2

**Type.** CHINA • Sichuan: Luding, growing on scree slopes, elev. ca. 2881 m, 29°51'49.2"N, 102°17'23.0"E, 8 July 2022, *W.Q. Wang et al. QSC0009* (holotype GAUA!).

**Diagnosis.** S. longipedicellata is similar to S. decumbens in having a prostrate life form and few-flowered cymes or solitary flowers, but differs from the latter by having glabrous stems (vs. densely pilose stems), linear-lanceolate leaves (vs. oblong leaves), 1.2–2 cm pedicel, longer than sepals (vs. ca. 4 mm



Figure 2. Morphology of *S. longipedicellata* **A** habitat **B** habit **C** habit **D** inflorescence (cymes) **E** stem **F** inflorescence (solitary) **G** leaf **H** flower (style 3, rarely 4) **I** capsule.

or less pedicel, shorter than or equalling sepals) and petals slightly shorter than or sub-equalling petals (vs. petals ca. 1/2 as long as sepals, in Table 2).

**Description.** Perennial herbs, whole plants glabrous. Stems, slender, prostrate or slightly ascending, much branched, 5–10 cm tall. Leaves sessile, green, linear-lanceolate, minute, 3–7 mm long, 0.6–1 mm broad, apex acute. Inflorescence axillary or terminal, 1–3-flowered. Pedicel 1.2–2 cm, slender. Bracts lanceolate, 3–4 mm, margin membranous. Sepals 5, glabrous, lanceolate, 4–5 mm long, 0.8–1 mm broad, margin membranous, apex acuminate. Petals 5, slightly shorter than or subequalling sepals, deeply bifid. Stamens 10, slightly shorter than or subequalling petals. Styles 3, rarely 4, filiform. Capsule ovoid-cylindrical, slightly longer than or subequalling persistent sepals. Seeds numerous, red-brown, ovoid, conspicuously rugulose.

Phenology. Flowering time June–July, fruiting time August–September.

**Distribution and ecology.** It is only known from the type locality, growing on scree slopes.

#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Data availability

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

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#### **Supplementary material 1**

### List of taxa sampled and GenBank accession numbers of sequences used in phylogenetic analyses of Alsineae

Authors: Wenqiao Wang, Zhonghui Ma

Data type: xlsx

- Explanation note: This is a table including GenBank accession numbers of sequences used in phylogenetic analyses of Alsineae.
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## PhytoKeys

**Research Article** 

# Two new species of *Salvia* (Lamiaceae) from the dry forests of Dominican Republic

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#### Abstract

We describe two new species of *Salvia* from the Antillean dry forests, belonging to *Salvia* section *Urbania*. These species' names honor two Latin American botanists who have advanced our understanding either of the Dominican flora or the mint family (Lamiaceae). *Salvia claseana* is found in the Sierra Martín García. It resembles *S. calaminthifolia* but differs in having strigose stems, rhombic to trullate leaves with a cuneate, decurrent base, and larger flowers. *Salvia martineziana* inhabits the Sierra de Bahoruco. It resembles *Salvia brachyphylla* but differs in having strigose stems with retrorse trichomes and flowers disposed in the axils of the distal leaves. We provide descriptions, photographs, a distribution map and an identification key for the species of *Salvia* section *Urbania* from the Dominican Republic. Additionally, we sequenced three molecular markers (nrITS, *trnL-trnF*, and *trnH-psbA*) for the new taxa and other Dominican and Haitian *Salvia* species to investigate their phylogenetic relationships.

#### Resumen

Describimos dos nuevas especies de *Salvia* provenientes de los bosques secos antillanos, pertenecientes a *Salvia* sección *Urbania*. Estas especies honran a dos botánicos latinoamericanos que han ampliado nuestro conocimiento ya sea sobre la flora dominicana o la familia de la menta (Lamiaceae). *Salvia claseana* se encuentra en la Sierra Martín García. Se asemeja a *S. calaminthifolia* pero se diferencia de ella por tener tallos estrigosos, hojas rómbicas a truladas con una base cuneada, decurrente, y flores más grandes. *Salvia martineziana* habita en la Sierra de Bahoruco. Es semejante a *Salvia brachyphylla* pero difiere en tener tallos estrigosos con tricomas retrorsos y las flores dispuestas en las axilas de las hojas distales. Presentamos descripciones, fotografías, mapas de distribución y una clave de identificación para las especies de *Salvia* sección *Urbania* de República Dominicana. Además, secuenciamos tres marcadores moleculares (nrITS, *trnL-trn*F y *trnH-psbA*) para los nuevos taxones y otras especies de *Salvia* dominicanas y haitianas, con el fin de explorar sus relaciones filogenéticas.

Key words: Antilles, endemism, Hispaniola, phylogeny, sages



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#### Introduction

The genus *Salvia* L. (sage) comprises more than 1000 species, which renders it the most diverse group in the mint family or Lamiaceae (Harley et al. 2004). Of these, more than half (580 spp.) belong to *Salvia* L. subgenus *Calosphace* (Benth.) Epling, a monophyletic group, that is endemic to the New World, distributed mainly in the Neotropics (González-Gallegos et al. 2020). The Antilles is one of the four diversity centers of *Salvia* subg. *Calosphace*, with ca. 45 species (Jenks et al. 2013). From the islands that form the Antilles, Hispaniola harbors the highest number of sages, with 36 species, 86% of them endemic (González-Gallegos et al. 2020).

Salvia subg. Calosphace section Urbania Epling is a group of 10 species endemic to Hispaniola, most of them distributed exclusively in Haiti (Epling 1939). These plants are characterized by a suffrutescent habit with intricate branching, small leaves, usually deltoid-ovate, obovate or flabelliform. The flowers grow in the leaf axils (1–3 per axil) or are rarely arranged in short racemes and are typically blue or violet in color. The calyx has 3-veined posterior lobes; the corolla is ventricose, invaginate and epapillate; the stamens are included in the galea, and the posterior stigmatic branch is longer than the anterior (Epling 1939).

Dominican Republic houses 21 species of *Salvia* (González-Gallegos et al. 2020), three of them from *Salvia* section *Urbania* (Liogier 1994). During November of 2016, a botanical expedition was carried out in Dominican Republic, visiting the montane temperate and arid zones to collect different species of *Salvia*. Among the collected material, we found specimens of two species from section *Urbania* that do not match any of the currently described taxa. In this study we describe both and compare them to the taxa that morphologically resemble them the most. Additionally, we provide an identification key for the Dominican species of the section. Finally, using DNA sequences of three molecular markers (nrITS, *trnH-psbA* intergenic spacer and *trnL-trnF* region) from most of the sages from Dominican Republic and a few Haitian *Urbania* species, we evaluate the phylogenetic position of the new taxa and test the monophyly of section *Urbania*.

#### Materials and methods

#### Fieldwork, species identification and conservation status assessment

Herbarium specimens were collected and processed, and the individuals were photographed *in situ*. Voucher specimens of all the collected taxa were deposited at the herbarium of the Jardín Botánico Nacional Dr. Rafael M. Moscoso (JBSD) and the duplicates (when available) were sent to MEXU and XAL herbaria in Mexico (Thiers 2023). Additionally, for each of the herbarium specimens collected, leaf samples were taken for DNA extraction and flowers were preserved in alcohol for posterior morphological analysis.

The identification of the specimens was based on the most comprehensive revision of *Salvia* subgenus *Calosphace* by Epling (1939), the revision of *Salvia* of Hispaniola (Liogier 1994) and the revision of sections *Ekmania* (Torke 2000), *Gardoquiiflorae* (Zona et al. 2016) and *Wrightiana* (Zona et al. 2011).

The distribution map was made with QGIS v. 3.30 (QGIS Development Team, 2023), using the distribution data from the herbarium specimen and iNaturalist

observations from one of the authors, these occurrences and coordinates are not provided due to the species global conservation status of Critically Endangered. The species' extent of occurrence (EOO) and species' area of occupancy (AOO) were calculated using GeoCAT (Bachman et al. 2011). Finally, the conservation status of the new taxa was assessed based on these results and considering the IUCN guidelines (2022).

#### Taxon sampling, DNA extraction, amplification and sequencing

To test the phylogenetic position of the new taxa, as well as other sage species from the Caribbean, we sampled most of the species from Dominican Republic and a few taxa from Haiti, mainly belonging to section *Urbania* (Table 1; Suppl. material 1). DNA was extracted either from herbarium specimens or Silica gel-dried tissue, using the 2 × CTAB method (Doyle and Doyle 1987). DNA amplification followed the profiles and primer combinations described by Fragoso-Martínez et al. (2018), the only modification made is that the PCR reactions were carried out in 15  $\mu$ L volumes. Finally, sequencing was performed at Macrogen, Inc.

 Table 1. Voucher information and GenBank accession codes for the sampled sage species in this study. Detailed specimen information can be found in Suppl. material 1.

Species	Vauahaninformation	GenBa	ank accession nu	mbers
Species	voucher mormation	ITS	trnH-psbA	trnL-trnF
S. arborescens Urb. & Ekman	I. Fragoso-Martínez et al. 459 (JBSD, MEXU)	PP905439	PP907529	PP907549
S. arduinervis Urb. & Ekman	E. Ekman 3168 (TEX)	PP905440	PP907530	PP907550
S. bahorucona Urb. & Ekman	I. Fragoso-Martínez et al. 517 (JBSD, MEXU)	PP905441	PP907531	PP907551
S. brachyloba Urb.	I. Fragoso-Martínez et al. 509 (JBSD, MEXU)	PP905442	PP907532	PP907552
*S. claseana Fragoso & Salazar	I. Fragoso-Martínez et al. 529 (JBSD, MEXU)	PP905459	PP907548	PP907568
*S. calaminthifolia Vahl	E. Ekman 9443 (TEX)	PP905443	PP907533	PP907553
S. caymanensis Millsp. & Uline	I. Fragoso-Martínez & R. Middleton 309, cultivated	PP905444	PP907534	PP907554
S. decumbens Alain	I. Fragoso-Martínez et al. 519 (JBSD, MEXU)	PP905445	PP907535	PP907555
S. foveolata Urb. & Ekman	I. Fragoso-Martínez et al. 508 (JBSD, MEXU)	PP905446	PP907536	PP907556
S. lachnaioclada Briq.	I. Fragoso-Martínez et al. 608 (JBSD, MEXU)	PP905447	PP907537	PP907557
S. lavendula Alain	T. Clase et al. 1059 (JBSD)	PP905448	PP907538	PP907558
*S. martineziana Fragoso & Martínez-Ambr.	I. Fragoso-Martínez et al. 497 (JBSD, MEXU)	PP905458	PP907547	PP907567
*S. montecristina Urb. & Ekman	I. Fragoso-Martínez et al. 527 (JBSD, MEXU)	PP905449	PP907539	PP907559
*S. praeterita Epling	I. Fragoso-Martínez et al. 607 (JBSD, MEXU)	PP905450	PP907540	PP907560
S. selleana Urb.	I. Fragoso-Martínez et al. 503 (JBSD, MEXU)	PP905451	PP907541	PP907561
S. serotina L.	I. Fragoso-Martínez et al. 506 (JBSD, MEXU)	PP905452	PP907542	PP907562
*S. subaequalis Epling	GenBank BioSample: SAMN22547053	PP905453	-	_
S. tenella Sw.	T. Clase et al. 8266 (JBSD)	PP905454	PP907543	PP907563
S. thormanii Urb.	T. Clase et al. 8059 (JBSD)	PP905455	PP907544	PP907564
S. tuerckheimii Urb.	I. Fragoso-Martínez et al. 607 (JBSD, MEXU)	PP905456	PP907545	PP907565
S. uncinata Urb.	I. Fragoso-Martínez et al. 575 (JBSD, MEXU)	PP905457	PP907546	PP907566
*Salvia section Urbania.				

#### Sequence edition and alignment

The sequences were edited and assembled using Geneious v.10.2.6 (http:// www.geneious.com, Kearse et al. 2012). For one species (*S. subaequalis* Epling), we downloaded the raw data from the GenBank BioSample SAMN22547053 (Rose et al. 2021), and we assembled the ITS region using the default settings for the internal transcribed spacers in GetOrganelle (Jin et al. 2020). The edited and assembled sequences were uploaded to Genbank (www.ncbi.nlm.nih.gov/Genbank) with the accession numbers shown in Table 1. To determine the phylogenetic position of the new taxa, as well as other sage species from the Antilles, we combined the newly sequenced data with the most comprehensive data matrix of *Salvia* subgenus *Calosphace* from Fragoso-Martínez et al. (2018). Additionally, we included sequences from posterior studies of the same research group (González-Gallegos et al. 2018; Martínez-Ambriz et al. 2019; Fragoso-Martínez et al. 2021). The matrices of each marker were aligned individually, using MAFFT (Katoh and Standley 2013).

The matrix comprising the three molecular markers included 288 taxa, of which 269 belong to *Salvia* subgenus *Calosphace*. The length of this matrix was 2,197 bp: 722 bp from the nrITS region, 505 bp from the *trn*H*psb*A intergenic spacer and 970 bp from the *trn*L*-trnF* region. Based on the results of the congruence test between plastid and nuclear data performed in previous phylogenetic studies of *Salvia* subgenus *Calosphace* (Fragoso-Martínez et al. 2018), and to include the most comprehensive dataset of the subgenus, we decided to concatenate the matrices and analyze them together. However, we are aware that a certain amount of discordance between these kinds of datasets is expected due to different phenomena (e.g., hybridization, introgression, chloroplast capture, etc.) or differences in the inheritance process between the plastid and nuclear DNA. Thus, the trees from the separate analyses (ITS and plastid) are provided as part of Suppl. material 2.

#### Model selection and phylogenetic analyses

The concatenated matrix included three partitions corresponding to each molecular marker. For each partition, we evaluated 88 molecular substitution models using ModelFinder (Kalyaanamoorthy et al. 2017). The selected substitution models according to the Bayesian Information Criterion (BIC) were: GTR+F+I+G4 for the ITS region, TVM+F+I+G4 for the *trn*H-*psb*A IGS and TIM+F+G4 for the *trn*L-*trnF* region.

The Maximum likelihood analysis of the concatenated matrix was performed using the IQ-TREE algorithm (Nguyen et al. 2015) in the W-IQ-TREE web server (Trifinopoulos et al. 2016). Due to the length of the resulting tree, only the Angulatae clade is depicted in Fig. 4 and the complete tree is provided as Suppl. material 2. For this figure, the Angulatae clade was extracted from the main tree using the phytools package (Revell 2012) in R (R Core Team 2016) and edited with FigTree version 1.4.4 (Rambaut 2018).

#### Results

#### **Taxonomic treatment**

Salvia claseana Fragoso & Salazar, sp. nov. urn:lsid:ipni.org:names:77352939-1 Fig. 1

**Diagnosis.** Similar to *Salvia calaminthifolia*, but with the stems strigose (vs. cinereous); the leaves rhombic to trullate with the base cuneate, decurrent, the margin crenate-serrate (vs. deltoid-ovate, base truncate, margin subentire); the flowers bigger, with the calyx 5.5–8 mm long and the corolla tube 7.7–9 mm long (vs. 5–5.5 mm long and 5–6 mm long, respectively), and the lower lip reclinate (vs. reflexed).

**Type.** DOMINICAN REPUBLIC. **Azua, Las Charcas** • Paraje Boquerón, cañada subiendo por el puente Juana Guayacán, a 4 km de la desviación de la carretera Sánchez (Bani-Azua); 18°21'0.9138"N, 70°31'51.8118"W; 307 m; 28 Nov 2016; *I. Fragoso-Martínez, G.A. Salazar & T. Clase 529* (holotype: JBSD 129667; isotypes: MEXU 1512146, XAL 0154233).

Description. Suffruticose herbs, ca. 0.5 m tall; stems strigose with antrorsely appressed trichomes, internodes 1.5-4 cm long. Leaves rhombic to trullate, 1.3-3.6 × 1−3 cm, base cuneate, decurrent, apex acute, margin crenate-serrate, ciliate; upper leaf surface bullate, glabrescent; lower leaf surface pale, densely pubescent, simple trichomes minute, white, with amber spherical glands; petioles 0.5-1.2 cm long. *Flowers* axillary, 4(-8) per node; pedicels 2.5-6 mm long, trichomes simple, adpressed. Calyx green, tubular-campanulate, 5.5-8 mm long, strigose, with simple and glandular trichomes; tube  $4.7-6.5 \times 3$  mm; lobes deltate, apex apiculate, upper lobe 1.3–1.8 mm long, straight, 3-veined, margin ciliate, lower lobes 1.3-2.5 mm long, straight. Corolla violet with white nectar guides in the lower lobe, ca. 1 cm long, tube 7.7–9 × 2.6–3 mm, ventricose, invaginate, internally epapillate; lobes unequal in length, upper lobe galeate, 3.8-6 mm long, densely pubescent, lower lobe 5-8 × 7.8-8.4 mm, tetralobate, reclinate. Stamens included in the upper corolla lobe, fused close to the corolla opening; filaments 1.5-2 mm long; connective 7-8 mm long, sparsely pilose, with a bilobed tooth close to the insertion with the filament, straight; upper arm of the connective shorter than the lower arm, 3-3.5 mm long, thecae 1-1.5 mm long; lower arm 4.5-5 mm long. Style 9-11 mm long, densely pubescent near the branches, upper branch longer than the lower one, lower branch spathulate. Nectary disc surface with spherical glands near the base of the mericarps, nectary horn ca. 2 mm long, oblong, laterally compressed. *Mericarps* ovoid, 1.6-2 × 0.7-0.9 mm, smooth.

**Phenology.** Flowers were documented from November to May. Fruits have been observed after this period.

**Etymology.** The epithet "*claseana*" honors the Dominican botanist Teodoro Clase, head of the Botany Department of the Jardín Botánico Nacional "Dr. Rafael M. Moscoso". His botanical expeditions have resulted in ca. 12,000 collected specimens and he has described ca. 12 new species of angiosperms from Hispaniola. These contributions are undoubtedly crucial to the knowledge of the flora of Dominican Republic.



Figure 1. Salvia claseana and the botanist that honors **A** flowering branch **B** leaves **C** flowers **D** Teodoro Clase at the JBSD herbarium **E** calyx **F** corolla **G** stamens **H** detail of the connective tooth (Specimen photographs taken from *I. Fragoso et al.* 529).

**Distribution, habitat and conservation status.** Endemic to the dry forests with limestone soils from the Sierra Martín García in Azua, Dominican Republic (Fig 3). *Salvia claseana* is represented by three collections, each from a different population. In the GeoCat (Bachman et al. 2011) analysis, the species' extent of occurrence (EEO) is 22.86 km<sup>2</sup> with an area of occupancy (AOO) of 12 km<sup>2</sup>. Considering these results, combined with the high level of endemism of the region, and taking into account the IUCN criteria (IUCN, 2022), we suggest that *S. claseana* is placed in the category of Critically Endangered (CR).

**Comments.** Phylogenetically, this species is closely related to *Salvia praeterita* (Fig. 4); however, the latter has the flowers arranged into racemes, while the new species produces flowers in the leaf axils. Morphologically, *Salvia claseana* resembles *Salvia calaminthifolia* the most but differs from it by a number of characters (Table 2), from which the most conspicuous are: the pubescence, which is strigose in the former and cinereus in the latter, the shape and base of the leaves (rhombic to trullate, cuneate vs. deloid-ovate, truncate) and the size of its flowers (bigger in the new species, Table 2). Additionally, *Salvia calaminthifolia* is not closely related to the new species (Fig. 4) and it seems to be only distributed in Haiti (Liogier, 1994), despite the locality cited in the type specimen (Santo Domingo). No distribution data or collections from Dominican Republic were found representing this

Table 2. Morphological comparison among the new taxa and their more similar species, based data from type specimens, Epling (1939) and Liogier (1994).

	S. claseana Fragoso & Salazar	S. calaminthifolia Vahl	S. praeterita Epling	S. <i>martineziana</i> Fragoso & Martínez-Ambr.	S. brachyphylla Urb.
STEM					
Pubescence	Strigose, trichomes antrorse	Cinereous, trichomes straight	Strigose, trichomes antrorse	Strigose, trichomes retrorse	Hispid, trichomes straight
LEAF				·	
Shape	Rhombic to trullate	Deltoid-ovate	Ovate to deltoid- ovate	Obovate to flabellate	Obovate to flabellate
Size (cm)	1.3−3.6 × 1−3	0.5-1.2 × 0.7-1.5	1-2×0.5-1	1-1.5 × 0.5-1	1.5-3 × 1-2.5
Base	Cuneate, decurrent	Truncate	Cuneate, decurrent	Cuneate, decurrent	Cuneate, decurrent
Margin	Crenate-serrate	Subentire	Crenate-serrate	Crenate-serrate	Crenate-serrate
Upper surface	Bullate	Smooth	Bullate	Bullate	Bullate
Lower surface	Densely pubescent	Cinereous, trichomes adpressed	Densely pubescent	Tomentulose	Hispid
INFLORESCENCE				·	·
Presence	Absent (axillary flowers)	Absent (axillary flowers)	Present, terminal racemes	Absent (axillary flowers)	Present, axillary and terminal racemes
Flowers per node	4(-8)	2-6	2-6	2	3
CALYX					
Shape	Tubular- campanulate	Tubular- campanulate	Tubular- campanulate	Campanulate	Tubular- campanulate
Length (mm)	5.5-8	5-5.5	5-7	5-6.8	5.5-6.5
COROLLA					
Tube length (mm)	7.7-9	5-6	8.5-9.5	7-9	7.5-9.5
Upper lip length (mm)	3.8-6	3-4.7	4-4.6	4.5-6.5	5.6-6
Lower lip length (mm)	5-8	3.5-5.5	5-6	7-7.5	ca. 6.6
Lower lip position	Reclinate	Reflexed	Reclinate	Reflexed	Reclinate

species either in the JBSD herbarium or global databases such as GBIF.org (GBIF Secretariat 2023).

Additional specimens examined. DOMINICAN REPUBLIC. Azua, Barreras • Sierra Martín García, en los alrededores de Barreras; 170 m; 11 Sep 1984; *M. Mejía et al. 1180* (JBSD). Las Charcas • Parque Nacional Francisco Alberto Caamaño, paraje Boquerón; 18° 21'38.4366"N, 70°31'51.24"W; 298 m; 9 May 2014; *T. Clase & R. Ovidio S. 8645* (JBSD, MEXU).

#### Salvia martineziana Fragoso & Martínez-Ambr., sp. nov.

urn:lsid:ipni.org:names:77352940-1 Fig. 2

**Diagnosis.** Similar to Salvia brachyphylla, but differing from it by the pubescence of the plant (strigose with retrorse trichomes vs. hispid with erect trichomes); having smaller leaves  $(1-1.5 \times 0.5-1 \text{ cm vs. } 1.5-3 \times 1-2.5)$  with a tomentulose lower leaf side (vs. hispid); flowers axillary (vs. forming racemes), calyx campanulate (vs. tubular-campanulate) and lower lobe of the corolla reflexed (vs. reclinate).

**Type.** DOMINICAN REPUBLIC. **Independencia, Duvergé** • Parque Nacional Sierra de Bahoruco, 6.8 km al S de Puerto Escondido por el camino a la Caseta 1; 18°17'10.4994"N, 71°34'6.3978"W; 965 m; 24 Nov 2016; *I. Fragoso-Martínez, G.A. Salazar & T. Clase 497* (holotype: JBSD 129573; isotypes: MEXU 1512142, XAL 0154234).

Description. Suffruticose herbs, 0.3-0.5 m tall; stems strigose with retrorsely appressed trichomes, internodes 0.8-2.4 cm long. Leaves obovate to flabellate, 1-1.5 × 0.5-1 cm, base cuneate, decurrent, apex acute to obtuse, margin crenate-serrate, ciliate; upper leaf surface bullate, densely hirsute; lower leaf surface whitish, tomentulose simple trichomes minute, curved, white, with yellow spherical glands; petioles 4-8 mm long. Flowers axillary, 2 per node; pedicels 1.5-4.5 mm long, hirsute. Calyx green, campanulate, 5–6.8 mm long, densely hirsute, with simple and glandular trichomes; tube 3-5 × 3 mm; lobes ovate-deltate, apex apiculate, upper lobe 2.5-4 mm long, curved backwards, 3-veined, margin ciliate, lower lobes 1.6-2.5 mm long, straight. Corolla violet with white nectar guides in the lower lobe, 1.2-1.4 cm long, tube 7-9 × 2.9 mm, ventricose, invaginate, internally epapillate, lobes unequal in length, upper lobe galeate, 4.5-6.5 mm long, densely pubescent, lower lobe 7-7.5 × 8.4 mm, tetralobate, reflexed. Stamens included in the upper corolla lobe, fused close to the corolla opening; filaments 1.5-2 mm long; connective 7.5-8.5 mm long, sparsely pilose, with an entire tooth close to the insertion with the filament, retrorse; upper arm of the connective slightly longer than the lower arm, 4-4.5 mm long, thecae 1-1.5 mm long; lower arm 3.5-4 mm long. Style 13-15 mm long, densely pubescent near the branches, with simple and capitate glandular trichomes, upper branch longer than the lower one, lower branch spathulate. Nectary disc surface with spherical glands near the base of the mericarps, nectary horn ca. 1 mm long, oblong, laterally compressed. *Mericarps* ovoid, 1.5-2 × 0.4-0.6 mm, smooth.

**Phenology.** Flowers were documented from November to May. Fruits have been observed after this period.



Figure 2. Salvia martineziana and the botanist that honors **A** flowering branch **B** leaves **C** flowers **D** Martha Martínez-Gordillo conducting fieldwork in Mexico **E** calyx **F** corolla **G** stamens **H** detail of the connective tooth (Specimen photographs taken from *I*. Fragoso et al. 497).

**Etymology.** The epithet "martineziana" honors the Mexican botanist Martha Martínez Gordillo, specialist of the Euphorbiaceae and Lamiaceae families. Dr. Martínez works at the FCME herbarium at the Universidad Nacional Autónoma de México. She has conducted fieldwork mainly in the states of Chiapas, Guerrero, Mexico and Oaxaca. Her study of the Mexican flora, particularly that of Guerrero, has led to the description of more than 30 species of angiosperms, many of them from the genus *Salvia*. Dr. Martínez has taught botany to numerous generations of Mexican biologists, and her exemplary professional and academic ethics, determination, kindness and generosity are an inspiration to her students.

**Distribution, habitat and conservation status.** Endemic to the dry forests with limestone soils from Sierra de Bahoruco in Independencia, Dominical Republic (Fig. 3). *Salvia martineziana* is represented by two collections, each seemingly belonging to different populations, both from the southern region of Puerto Escondido in the Sierra de Barohuco. In the GeoCat (Bachman et al. 2011) analysis, the species' extent of occurrence (EEO) is 0.09 km<sup>2</sup> with an area of occupancy (AOO) of 8 km<sup>2</sup>. Considering these results, combined with the high level of endemism of the region, and taking into account the IUCN criteria (IUCN 2022), we suggest that *S. martineziana* should be placed in the category of Critically Endangered (CR).

**Comments.** This species is sister to a clade formed by two other Dominican species of *Salvia* section *Urbania* (Fig. 4). It differs from *S. praeterita* by lacking flowers in racemes and from *S. claseana* by the size of the leaves (smaller in



**Figure 3.** Distribution map of the four taxa of *Salvia* section *Urbania* from Dominican Republic. *Salvia* calaminthifolia was excluded from the map due to the lack of distribution data in the country aside from the type specimen.

S. martineziana:  $1-1.5 \times 0.5-1$  cm vs.  $1.3-3.6 \times 1-3$  cm) and the pubescence of the lower side of the blades (tomentulose vs. densely pubescent). Morphologically, S. martineziana resembles S. brachyphylla the most, a Haitian species. However, it differs mainly by the lack of racemes, having the flowers distributed in the axils of the upper portion of the branches.

Additional specimens examined. DOMINICAN REPUBLIC. Independencia, Duvergé • 5.2 km al S de Puerto Escondido en camino a Acetillar, Sierra de Bahoruco; 170 m; 9 May 1985; *T. Zanoni et al. 34648* (JBSD).



**Figure 4.** Phylogenetic relationships of the Angulatae clade based on three molecular markers (nrITS, *trnH-psbA* and *trnL-trnF*) with an increased taxon sampling including the new species and other Hispaniolan taxa. Bootstrap values  $\geq$ 80% are shown above the branches. The names of the new species are written in purple, while the names in gray correspond to the sections of the taxa from the Hispaniolan subclade. An extended version of the phylogenetic tree is provided in Suppl. material 2.

#### Identification key for the species of Salvia section Urbania

1a	Flowers in racemes
2a	Leaves and calyces densely hispid S. brachyphylla
2b	Leaves and calyces strigose to glabrate
3a	Bracts persistent
4a	Bracts flabellate, surrounding the flowers almost completely S. saccifera
4b	Bracts ovate, not surrounding the flowers completely
3b	Bracts deciduous5
5a	Lower leaf surface greenish, glabrous; flowers 6–12 per node S. hotteana
5b	Lower leaf surface whitish, strigose; flowers 2-6 per node S. praeterita
1b	Flowers axillary, not forming racemes6
6a	Leaf margin subentire, entire or sinuate7
7a	Leaves coriaceous S. montecristina
7b	Leaves membranaceus
8a	Lower leaf surface incanous, grayish
8b	Lower leaf surface glabrate, greenish9
9a	Leaves oblong-elliptical
9b	Leaves deltoid-ovate
6b	Leaf margin crenate-serrate10
10a	Leaf base truncate
10b	Leaf base cuneate
11a	Leaves rhombic to trullate, 1-3 cm wide, lower leaf surface densely pu-
	bescent
11b	Leaves obovate to flabellate, 0.5-1 cm wide, lower leaf surface tomentu-
	loseS. martineziana

#### **Phylogenetic relationships**

The Hispaniolan species included in the phylogenetic analysis are distributed in four different clades. The species from Salvia section Micranthae (Benth.) Epling - Salvia serotina L. and S. tenella Sw.-, are part of a clade that also contains taxa from sections Bracteata Epling and Subrotundae (Epling) Epling (99% BS; Suppl. material 1). Salvia decumbens Alain is included in the Flocculosae clade, along with species from the Andean region such as S. discolor Kunth and S. leucocephala Kunth (100% BS; Suppl. material 1). Salvia occidentalis Sw. is part of the Uliginosae clade and it is closely related to other species of section Microsphace (Brig.) Benth. (100% BS; Suppl. material 1). Most of the taxa surveyed are found in the Angulatae clade (Fig. 4), forming a Hispaniolan subclade (100% Bootstrap Support; BS). The species from this subclade belong to four different sections (Table 3) and one is unassigned (Salvia lavendula Alain). From these, only sections Urbania Epling and Gardoquiiflorae Epling are monophyletic (both with 100% BS). However, the latter is nested in a clade formed by species of two sections: the paraphyletic Ekmania Epling and the polyphyletic Wrightiana (Fig. 4).

The new species described in the present study belong to the monophyletic section *Urbania*. Within this clade *Salvia montecristina* Urb. & Ekman, endemic to Dominican Republic, is sister to the remaining species (Fig. 4). These taxa

Epling's sections sampled	Taxa sampled from the total
Ekmania Epling	8 spp. (100%)
*Gardoquiiflorae Epling	2 spp. (66%)
Micranthae	4 spp. (57%)
*Urbania Epling	6 spp. (50%)
Wrightiana Epling	2 spp. (66%)

Table 3. Sections sampled and the percentage of taxa sequenced in this study.

are arranged into two subclades, one containing Haitian species (100% BS) and another one formed by Dominican taxa (99% BS). The latter subclade comprises the new species and *Salvia praeterita* Epling.

#### Discussion

The new taxa described here are an addition to the sage species of Dominican Republic, increasing its diversity to 23 species (González-Gallegos et al. 2020), five of them belonging to *Salvia* section *Urbania*. As depicted in Fig. 3, these taxa are microendemic and their range of distribution is very narrow. Consequently, the conservation status assessment for the new taxa placed them both in the critically endangered (CR) category, following the IUCN criteria (IUCN 2022). Future studies should address the state and characteristics of the populations, to provide further insight into their dynamics and expand information about their conservation status.

Both species described here fit the circumscription of *Salvia* section *Urbania* provided by Epling (1939). Moreover, this section was proved here to be monophyletic, rendering its phylogenetic relationships congruent with the morphology of the group. The clade formed by this section is related to other Hispaniolan taxa belonging to three other sections, only one of them monophyletic (Table 3). These results are consistent with the findings of Fragoso-Martínez et al. (2018), where many of the clades of the Neotropical sages show strong geographical structure instead of morphological congruence. Nevertheless, section *Urbania* shows both morphological and geographic structures. Only a few of the 41 non-monotypic sections sampled to date show the same pattern (e.g., *Lavanduloideae* Epling, *Membranaceae* (Benth.) Epling and *Sigmoideae* Epling); however, less than half of the species of *Salvia* subgenus *Calosphace* have been sequenced. Thus, there are still relationships to be explored or defined.

Zona et al. (2016) conducted the first phylogenetic analysis of Hispaniolan sages. Their study, based on five species, suggested that the island's sage diversity originated from at least two colonization events. Our expanded taxonomic sampling has now revealed two additional colonization events: one involving species from section *Micranthae* and another from section *Microsphace*.

Despite a recent surge in the discovery of new Neotropical sages, the Dominican Republic has remained relatively unexplored in this regard. The last new sage species from the country was described almost 40 years ago (Liogier 1988). This underscores the critical need for continued botanical expeditions and taxonomic research to broaden our understanding of plant diversity in this region. Furthermore, incorporating more species of *Salvia* subgenus *Calosphace* into phylogenetic analyses will provide valuable insights into the evolutionary history of this lineage, and will allow us to group taxa with their closest relatives.

These newly discovered taxa from Hispaniola, which are critically endangered, are the first species of *Salvia* section *Urbania* to be evaluated under the IUCN criteria. However, they mirror a pattern seen in other native sages on the island, where some species are vulnerable (*Salvia arborescens* Urb. & Ekman, and *S. decumbens* Alain) and others are critically endangered (*Salvia buchii* Urb., *S. haitiensis* Urb., *S. lachnaioclada* Briq. and *S. paryskii* Skean & Judd). These results highlight the need for a comprehensive assessment of all Hispaniolan sage species, to develop effective strategies for their protection.

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#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

IFM wrote the manuscript, EMA, GAS and MR provided feedback on the draft and made insightful suggestions to improve it. IFM and GAS conducted the field expedition in 2016 and MR provided them with information about the localities of the surveyed taxa. EMA extracted the DNA samples, amplified the molecular markers, made the map, assessed the conservation status of the new taxa and assembled all the figures of the manuscript. IFM processed the molecular data and performed phylogenetic analyses.

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#### Data availability

All the newly sequenced data was uploaded to the NCBI with the accession numbers provided in Table 1. The complete phylogenetic tree of the combined dataset is provided as Suppl. material 2.

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#### **Supplementary material 1**

#### Additional information of the sampled sage species in the study

Authors: Itzi Fragoso-Martínez, Gerardo A. Salazar, Emmanuel Martínez-Ambriz, Martin Reith Data type: pdf

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#### Supplementary material 2

#### Complete phylogenetic trees of the plastid, nuclear and combined dataset

Authors: Itzi Fragoso-Martínez, Gerardo A. Salazar, Emmanuel Martínez-Ambriz, Martin Reith Data type: pdf

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