PhytoKeys 204: 1–8 (2022)
doi: 10.3897/phytokeys.204.87654
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Paraphlomis jinggangshanensis (Lamiaceae), a new species from Jiangxi, China

Wan-Yi Zhao[1*], Zhong Zhang[2*], Qiang Fan[1], Chun-Quan Chen[3], Wen-Bo Liao[1], David E. Boufford[4]

1 *State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yan-Sen University, Guangzhou 510275, China* **2** *Changguling Forestry Farm, Jinggangshan Nature Reserve, Jinggangshan 343600, Jiangxi China* **3** *Ji 'an Forestry Bureau, Ji 'an 343000, China* **4** *Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138-2020, USA*

Corresponding authors: David E. Boufford (david_boufford@harvard.edu), Wen-Bo Liao (lsslwb@mail.sysu.edu.cn)

Academic editor: Eberhard Fischer | Received 11 June 2022 | Accepted 14 July 2022 | Published 2 August 2022

Citation: Zhao W-Y, Zhang Z, Fan Q, Chen C-Q, Liao W-B, Boufford DE (2022) *Paraphlomis jinggangshanensis* (Lamiaceae), a new species from Jiangxi, China. PhytoKeys 204: 1–8. https://doi.org/10.3897/phytokeys.204.87654

Abstract

Paraphlomis jinggangshanensis (Lamiaceae), a new species from Jiangxi Province, China, is described and illustrated. The new species is morphologically similar to *P. intermedia*, but can be easily distinguished from the latter by its cordate leaf base (*vs.* cuneate, decurrent), stem and calyx tube with glandular hairs (*vs.* short pubescent), and glabrous anthers (*vs.* ciliate anthers). A phylogenetic analysis, based on ITS regions, suggests that *P. jinggangshanensis* represents a separate branch in *Paraphlomis* and is closely related to Clade II. It is currently known only from Jinggangshan National Natural Reserve. Because of its limited distribution and small population size, the species was assessed as Near Threatened (NT) according to the IUCN Red List Categories and Criteria.

Keywords

IUCN, Jinggangshan, Paraphlomideae, phylogenetic

Introduction

Paraphlomis (Prain) Prain, a member of the tribe Paraphlomideae Bendiksby (Lamiaceae: Lamioideae) (Bendiksby et al. 2011; Li et al. 2016; Zhao et al. 2021), is characterized by its herbaceous habit, actinomorphic calyx with five lobes less than half as long

* These authors contributed equally to this work.

as the tube, corolla 2-lipped (1/3) with hairy upper lip but hardly bearded along the margin, included stamens and an apically truncate ovary (Wu and Li 1977; Bendiksby et al. 2011; Ko et al. 2014; Chen et al. 2021). *Paraphlomis* is endemic to eastern and southeastern Asia, including China, India, Indonesia, Korea, Laos, Myanmar, Thailand, and Vietnam (Li and Hedge 1994; Ko et al. 2014; Zhang et al. 2020; Chen et al. 2021).

China, with 23 species documented in the *Flora of China* (Li and Hedge 1994), is the distribution center of *Paraphlomis*. Recently, a number of new species and infraspecies of *Paraphlomis* were described in China, including *P. javanica* var. *pteropoda* D. Fang & K.J. Yan and *P. javanica* var. *angustifolia* f. *albinervia* D. Fang & K.J. Yan (Yan and Fang 2009); *P. breviflora* B.Y. Ding, Y.L. Xu & Z.H. Chen (Ding et al. 2019); *P. kuankuoshuiensis* R.B. Zhang, D. Tan & C.B. Ma (Zhang et al. 2020); *P. jiangyongensis* X.L. Yu & A. Liu and *P. coronata* (Vaniot) Y.P. Chen & C.L. Xiang (Chen et al. 2021); *P. nana* Y.P. Chen, C. Xiong & C.L. Xiang (Chen et al. 2022a); *P. longicalyx* Y.P. Chen & C.L. Xiang (Chen et al. 2022b).

During a botanical expedition to Jinggangshan National Nature Reserve, western Jiangxi Province in June 2013, David Boufford and Wen-Bo Liao discovered an unknown species of *Paraphlomis* in Xiangzhou village. Its stem and leaves were densely covered with glandular trichomes and the base of leaves was clearly cordate. Based on its morphological characteristics, which differed from other species of *Paraphlomis*, we suspected that it represented an undescribed species. After carefully comparing it with congeneric specimens, consulting the literature, observing its morphology over two years of additional field investigations (in 2020 and 2021), as well as conducting molecular studies, we confirmed that the species is new to science and formally describe it below.

Materials and methods

Morphological study

The flowering and fruiting plants of the putative new species were examined in the field and compared with herbarium specimens deposited in A, GH and SYS (herbarium acronyms as in Thiers 2022). All morphological characteristics were measured using dissecting microscopes. Morphological characteristics of similar species of *Paraphlomis* were further observed in digital images of specimens available online at A, GH, KUN, NAS, PE and SYS. Five main characters (habit, leaf shape, calyx, anthers and trichomes) of the putative new species and its most similar species, *Paraphlomis intermedia*, were thoroughly compared.

Phylogenetic analyses

The nuclear DNA Internal Transcribed Spacers (ITS) was used for reconstructing the phylogeny of the suspected new species and related taxa based on previous study (Chen et al. 2021; Chen et al. 2022a). Most sequences were downloaded from GenBank, except for the new species, which was newly sequenced in the present study. Genomic DNA of the suspected new species was extracted from silica-gel-dried leaves using the

modified 2 × CTAB procedure of Doyle and Doyle (1987). The ITS sequences were amplified with primer pairs ITS4/ITSA, with PCR amplification and sequencing following Chen et al. (2016). A total of 18 accessions, representing 17 species of *Paraphlomis* and one species (*Phlomoides bracteosa* (Royle ex Benth.) Kamelin & Makhm.) of a related genus were sampled in the phylogenetic study. *Phlomoides bracteosa* was selected as an outgroup. The GenBank accession numbers are listed in Table 1. Nucleotide sequences were aligned and cleaned using MAFFT 7 (Katoh and Standley 2013). The phylogenetic relationships were assessed using the Maximum Likelihood (ML) method, which was constructed using the program IQ-TREE (Nguyen et al. 2015) with the best-fitting models (TIM+F+G4) chosen according to Bayesian Information Criterion (BIC).

Table 1. GenBank accession numbers of the sampled species used in this study.

Species	Voucher	ITS
Paraphlomis albida	A. Liu et al. LK0841 (CSFI); Ningyuan, Hunan, China	MW602124
Paraphlomis brevifolia	L. Wu & W.B. Xu 10965 (IBK); Yangshuo, Guangxi, China	MW602142
Paraphlomis coronata	C.L. Xiang 358 (KUN); Jiangkou, Guizhou, China	MW602123
Paraphlomis formosana	Zhong 3676 (E); Taiwan, China	JN680356
Paraphlomis gracilis	A. Liu LK0931 (CSFI); Changsha, Hunan, China	MW602134
Paraphlomis hirsutissima	Fang091060 (KUN); Yunnan, China	EU827096
Paraphlomis hispida	X. Li LX200702 (GXF); Napo, Guangxi, China	MW602132
Paraphlomis intermedia	X. Zhong et al. ZX16823 (CSH); Suichang, Zhejiang, China	MW602135
Paraphlomis javanica var. *pteropoda*	X. Li 2020090501 (GXF); Jingxi, Guangxi, China	MW602140
Paraphlomis javanica	L.B. Jia et al. JLB0029 (KUN); Maguan, Yunnan, China	MW602143
Paraphlomis jiangyongensis	A. Liu et al. LK1104 (CSFI); Jiangyong, Hunan, China	MW602129
Paraphlomis jinggangshanensis	W.Y. Zhao, Z.C. Liu, Z. Zhang, X.J. Li, ZWY-2060(SYS); Jinggangshan, Jiangxi, China	ON960152
Paraphlomis kwangtungensis	Y.P. Chen & Y. Zhao EM1391 (KUN); Huaiji, Guangdong, China	MW602126
Paraphlomis lanceolata	C.Z. Huang s.n. (KUN); Guidong, Hunan, China	MW602145
Paraphlomis lancidentata	X. Zhong et al. ZX16824 (CSH); Suichang, Zhejiang, China	MW602136
Paraphlomis membranacea	Fang091057 (KUN); Yunnan, China	EU827094
Paraphlomis paucisetosa	X.X. Zhu s.n. (KUN); Malipo, Yunnan, China	MW602125
Paraphlomis paucisetosa	X. Li LX200704 (GXF); Napo, Guangxi, China	MW602133
Paraphlomis seticalyx	A. Liu et al. LK1088 (CSFI); Daoxian, Hunan, China	MW602127
Phlomoides bracteosa	Anders 11464 (M); Afghanistan, Kunar, Chapadarrah	JN680373

Results

Morphological comparison

In morphology, the putative new species was most similar to *Paraphlomis intermedia* C.Y. Wu & H.W. Li. A comparison of their morphological features is presented in Table 2. These two species share such features as rhizomes with dense fibrous roots, calyx tube obconical, calyx teeth broadly triangular to broadly ovoid triangular and corolla white. The new species, however, differs from *P. intermedia* by its cordate leaf base (*vs.* cuneate, decurrent), stem and calyx tube with glandular trichomes (*vs.* short

Table 2. Morphological comparison of *Paraphlomis jinggangshanensis* and *Paraphlomis intermedia*.

Characters	*Paraphlomis jinggangshanensis*	*Paraphlomis intermedia*
Habit	erect, stem solitary, unbranched	erect, stem with branches in upper part
Rhizome	transverse, internodes 1.5–4 cm	inconspicuous, not transverse
Trichomes on stem	puberulent, trichomes retrorse	glandular trichomes erect
Leaf base	Cordate	broadly cuneate, abruptly decurrent
Calyx	obconical, sparsely pubescent outside	tubular or obconical, with dense glandular trichomes outside
Anthers	ovoid, ciliate	ovoid, glabrous
Nutlets	sparsely pubescent	glabrous

pubescence), anthers glabrous (*vs.* ciliate). Furthermore, the rhizome of *P. intermedia* has internodes about 1.5–4 cm long (observed in the type specimen), while the rhizome of the putative new species is rather shorter.

Phylogenetic placement of the putative new species

The aligned sequences of ITS were 627 bp in length. The resulting phylogenetic tree of *Paraphlomis* in this study was similar to that in a previous study (Chen et al. 2021). Our putative new species formed a separate branch (Fig. 1: ML = 62) that was sister to the previously suggested clades II, and IV by Chen et al. (2021). Fruit morphology is the main factor to distinguish subordinate grades of *Paraphlomis*. Specifically, species of Clade II have glabrous nutlets included in the fruiting calyces, species of Clade III have hairy nutlets, and species of Clade IV share glabrous nutlets that are obviously inflated and exserted from the calyx (Chen et al. 2021). The putative new species was closest to Clade II since its glabrous nutlets were included within the fruiting calyx. However, the putative new species was easily distinguishable from other species in Clade II by being densely covered with glandular trichomes and by the cordate leaf base.

Taxonomic treatment

Paraphlomis jinggangshanensis **Boufford, W.B. Liao & W.Y. Zhao, sp. nov.**
urn:lsid:ipni.org:names:77302738-1
Fig. 2
井冈山假糙苏

Type. China. Jiangxi Province, Jinggangshan City, Jinggangshan National Natural Reserve, roadsides, 26°38'N, 114°15'E, 740 m alt., 10 September 2021, *Wan-Yi Zhao, Zhong-Cheng Liu,Zhong Zhang, XU-Jie Li*, *ZWY-2060* (holotype: SYS!; isotypes: A!, SYS!)

Diagnosis. *Paraphlomis jinggangshanensis* is morphologically similar to *P. intermedia*, but differs by its pubescence of glandular trichomes, cordate leaf base, many-branched stems and glabrous anthers.

Description. Herbs, perennial, 0.4–1.0 m tall. **Rhizomes** short (not transverse), taproot obscure; roots fibrous. **Stems** erect, simple or much branched above middle,

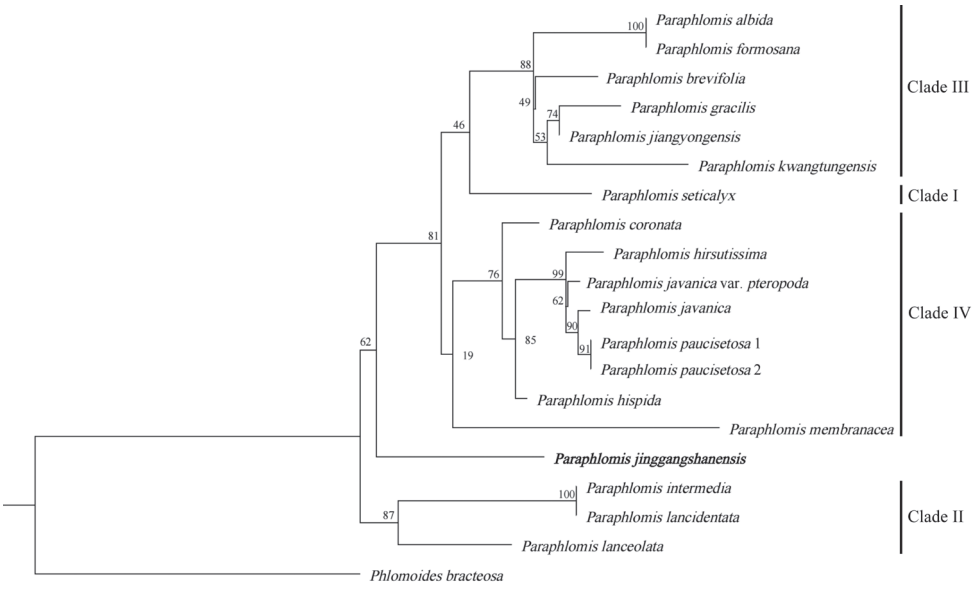


Figure 1. Phylogenetic relationships among 17 species of *Paraphlomis* and *Phlomoides bracteosa* based on ITS sequences. Numbers above branches indicate Maximum Likelihood bootstraps (ML). The new species described in this study is shown in bold.

4-angled, grooved, densely covered with short glandular trichomes. **Leaves** opposite; petiole to 9 cm long, with dense short glandular trichomes, green or purplish green; lamina ovate to ovate-oblong, papery, 4–10.2 × 2.5–6.5 cm, base cordate, margin crenate, apex acuminate; abaxially light green, covered with glandular trichomes (more densely so on veins), with glandular spots; adaxially green, densely covered with glandular trichomes, with glandular spots; lateral veins 4 or 5 pairs. **Verticillasters** 10–12 flowered, globose, 2.5–3.0 cm in diam; bracteoles few, ovate-triangular, apex obtuse, ca. 1 mm long, with short glandular trichomes, deciduous; pedicels 1.0–1.5 mm long, or obsolete. **Calyx** green, tubular-obconical, slight curving, ca. 7 mm long, with dense glandular trichomes outside, glabrous except for glandular trichomes on teeth inside, conspicuously 5-veined; teeth 5, subequal, triangular, ca. 1 mm long, apex acute. **Corolla** white, 1.2–1.6 cm long, with dense glandular trichomes outside, pilose annulate in throat inside; tube 8–10 mm long, straight, slightly dilated toward throat, obvious longer than calyx tube; corolla 2-lipped, upper lip oblong, margin entire, ca. 4 mm long, ca. 2.5 mm wide; lower lip 3-lobed, 4–5 mm long, dotted with red spots inside, middle lobe ovate to suborbicular, apex obtuse or retuse, lateral lobes obliquely oblong, apex obtuse. **Stamens** 4, inserted above middle of corolla tube, straight, included, filaments flat, sparsely puberulent-villous; anther cells 2, divergent, ovoid, glabrous. **Style** filiform, included, glabrous, apex subequally 2-lobed. **Ovary** 4-loculed, glabrous. **Nutlets** 4, triquetrous-obovoid, brown at maturity, ca. 2.2 mm long, apex rounded, glabrous. (Fig. 2)

Distribution and habitat. Based on our field observations, *Paraphlomis jinggangshanensis* is located only in Xiangzhou, in the Jinggangshan National Natural Reserve, Jiangxi Province.

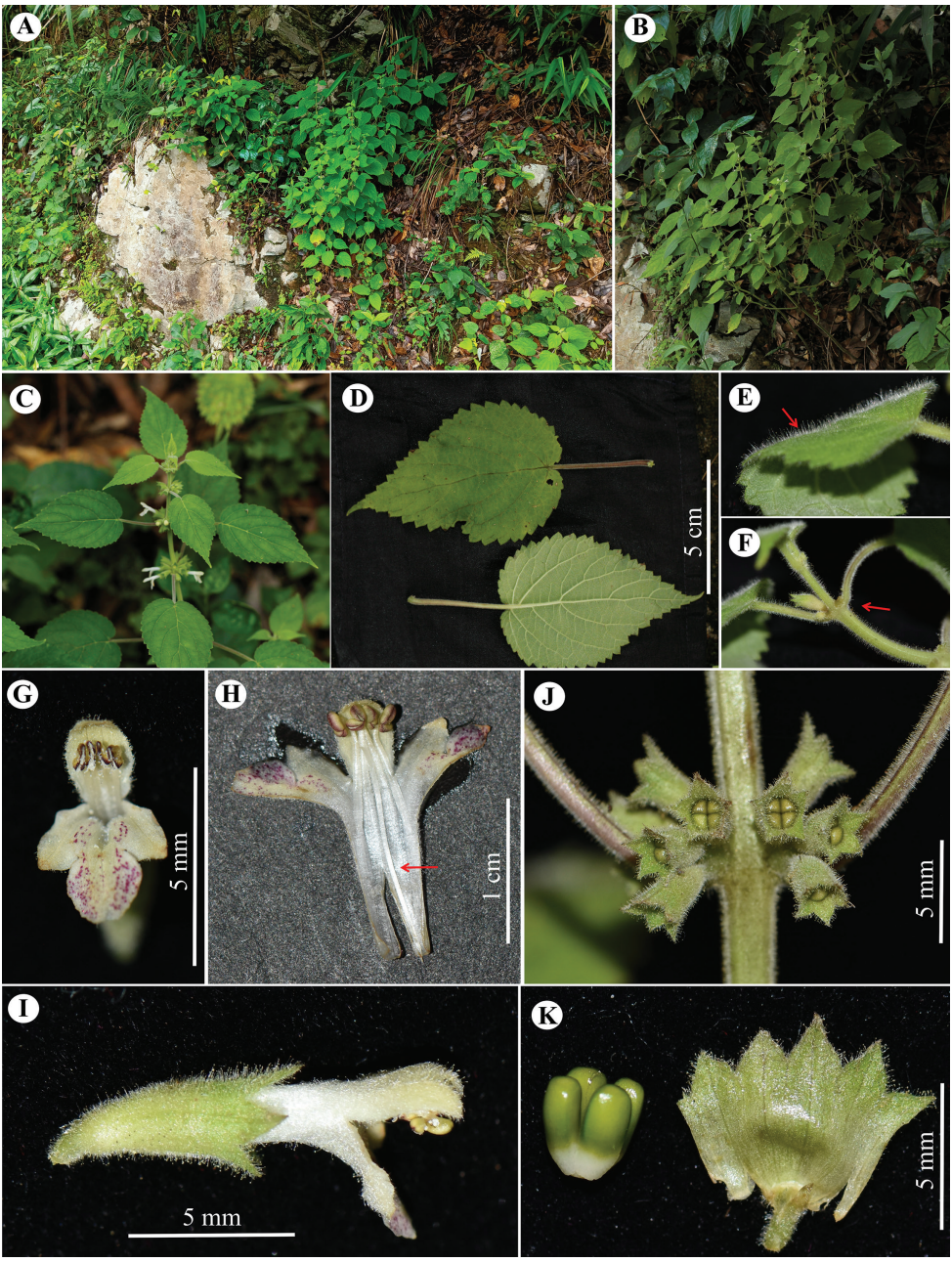


Figure 2. *Paraphlomis jinggangshanensis* **A** habit, growing on gravelly hillside **B** plant, stems much branched **C** flowering branch **D** leaves with long petiole, base cordate **E** both surfaces of leaf blade with dense glandular trichomes **F** stem, petiole, and calyx tube with dense glandular trichomes **G** front view of corolla, lower lip dotted with purplish red spots, throat villous annulate **H** inner view of corolla, filaments borne in middle of corolla tube; red arrow indicates glabrous style; anthers glabrous **I** lateral view of flower **J** inflorescence **K** fresh nutlets (glabrous) and inner view of calyx tube (**A–D** by Zhong Zhang **E–K** by Wan-Yi Zhao).

This area has been considered to be in the subtropical monsoon climate region. *Paraphlomis jinggangshanensis* often occurs in evergreen broadleaved forests along roads above valleys.

Conservation status. This species is currently known to occur only in the Jinggangshan National Natural Reserve in three populations numbering more than two thousand individuals. A road divides the distribution range of *P. jinggangshanensis*. Human activity (such as roadside weed removal) and exotic species have a negative effect on population regeneration. *Paraphlomis jinggangshanensis* is here suggested to be Near Threatened (NT) according to IUCN categories guidelines 10.1 (IUCN Standards and Petitions Subcommittee 2022).

Phenology. Flowering was observed from May to October, and fruiting from July to November.

Etymology. The specific epithet "jinggangshanensis" is derived from the type locality, Jinggangshan National Natural Reserve, Jiangxi Province, China.

Additional specimens examined (paratypes). China. Jiangxi: Jinggangshan City, Jinggangshan National Natural Nature Reserve; NE of the town of Ciping; vicinity of Xiangzhou, roadside, above valley, 26°37'49"N, 114°15'49"E, 545–575 m, 6 June 2013, *David E. Boufford*, *Wen-Bo Liao*, *Bao-Huan Wu*, *Hui-Min Xu & Tian-Tian Yuan 43074* (A); Jinggangshan National Natural Reserve, roadsides, 26°38'N, 114°15'E, 740 m alt., 18 June 2021, *Zhong Zhang Luofu-01* (A, SYS); *ibid.*, 15 July 2021, *Zhong Zhang Luofu-06* (A, SYS).

Acknowledgements

We thank Zhong-Cheng Liu, Xu-Jie Li for help with the field work and Xin-Jian Zhang, Qian-Yi Yin for providing images of herbarium specimen of *Paraphlomis* in KUN and PE. We thank Kai-Kai Meng and Yan-Shuang Huang for help with the phylogenetic analysis. This work was supported by the Basic Work Special Project of the National Ministry of Science and Technology of China (2013FY111500), and the National Natural Science Foundation of China (NSFC31970016).

References

Bendiksby M, Thorbek L, Scheen AC, Lindqvist C, Ryding O (2011) An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. Taxon 60(2): 471–484. https://doi.org/10.1002/tax.602015

Chen YP, Drew BT, Li B, Soltis DE, Soltis PS, Xiang CL (2016) Resolving the phylogenetic position of *Ombrocharis* (Lamiaceae), with reference to the molecular phylogeny of tribe Elsholtzieae. Taxon 65(1): 123–136. https://doi.org/10.12705/651.8

Chen YP, Liu A, Yu XL, Xiang CL (2021) A preliminary phylogenetic study of *Paraphlomis* (Lamiaceae) based on molecular and morphological evidence. Plant Diversity 43(3): 206–215. https://doi.org/10.1016/j.pld.2021.03.002

Chen YP, Xiong C, Zhou HL, Chen F, Xiang CL (2022a) *Paraphlomis nana* (Lamiaceae), a new species from Chongqing, China. Turkish Journal of Botany 46(2): 176–182. https://doi.org/10.55730/1300-008X.2680

Chen YP, Sun ZP, Xiao JF, Yan KJ, Xiang CL (2022b) *Paraphlomis longicalyx* (Lamiaceae), a new species from the Limestone Area of Guangxi and Guizhou Provinces, Southern China. Systematic Botany 47(1): 251–258. https://doi.org/10.1600/036364422X16442668423572

Ding BY, Chen ZH, Xu YL, Jin XF, Wu DF, Chen JB, Wu WJ (2019) New species and combination of Lamiaceae from Zhejiang, China. Guangxi Zhi Wu 39(1): 10–15.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

IUCN Standards and Petitions Subcommittee (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15. Prepared by the Standards and Petitions Subcommittee. https://www.iucnredlist.org/resources/redlistguidelines

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Ko SC, Lee YM, Chung KS, Son DC, Nam BM, Chung GY (2014) A new species of *Paraphlomis* (Lamiaceae) from Korea: An additional genus to the Korean flora. Phytotaxa 175(1): 51–54. https://doi.org/10.11646/phytotaxa.175.1.6

Li XW, Hedge IC (1994) Lamiaceae. In: Wu ZY, Raven PH (Eds) Flora of China, Vol. 17. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 50–299. http://www.iplant.cn/info/Lamiaceae?t=foc

Li B, Cantino PD, Olmstead RG, Bramley GLC, Xiang CL, Ma ZH, Tan YH, Zhang DX (2016) A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Scientific Reports 6(1): e34343. https://doi.org/10.1038/srep34343

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Thiers BM (2022) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 08.06.2022]

Wu CY, Li HW (1977) *Paraphlomis* Prain. In: Wu CY, Li HW (Eds) Flora Reipublicae Popularis Sinicae, vol. 65(2). Science Press, Beijing, 545–572.

Yan KJ, Fang D (2009) A supplement to the *Paraphlomis* (Lamiaceae) from Guangxi, China. Redai Yaredai Zhiwu Xuebao 17(7): 91–92. http://jtsb.ijournals.cn/jtsb_cn/article/issue/2009_17_1

Zhang RB, Deng T, Dou QL, Wei RX, He L, Ma CB, Zhao S, Hu S (2020) *Paraphlomis kuankuoshuiensis* (Lamiaceae), a new species from the limestone areas of northern Guizhou, China. PhytoKeys 139: 13–20. https://doi.org/10.3897/phytokeys.139.47055

Zhao F, Chen P, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, Min DZ, Peng H, Olmstead RG, Li B, Xiang CL (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biology 19(1): e2. https://doi.org/10.1186/s12915-020-00931-z

PhytoKeys 204: 9–21 (2022)
doi: 10.3897/phytokeys.204.82508
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Reinstatement of species rank for *Grimmia limprichtii* (Bryophyta, Grimmiaceae) based on molecular and morphological data

Chao Feng[1], Jin Kou[2], Ting-Ting Wu[2], Guo-Li Zhang[2]

1 *College of Grassland, Resources and Environment, Key Laboratory of Grassland Resources, Ministry of Education, China, Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization of Ministry of Agriculture, Inner Mongolia Agricultural University, Hohhot 010011, China* **2** *School of Life Sciences, Key Laboratory of Molecular Epigenetics of Ministry of Education, Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University, Changchun 130024, China*

Corresponding author: Jin Kou (kouj398@nenu.edu.cn)

Academic editor: Matt von Konrat | Received 19 February 2022 | Accepted 20 July 2022 | Published 2 August 2022

Citation: Feng C, Kou J, Wu T-T, Zhang G-L (2022) Reinstatement of species rank for *Grimmia limprichtii* (Bryophyta, Grimmiaceae) based on molecular and morphological data. PhytoKeys 204: 9–21. https://doi.org/10.3897/phytokeys.204.82508

Abstract

The genus *Grimmia* Hedw. has been considered taxonomically difficult because of its great morphological variability, and its treatments by different specialists have led to incongruent results. One of the debates in the genus is the species status of *Grimmia limprichtii* Kern, an Asian-European disjunct moss species that has been considered identical to *Grimmia anodon* Bruch & Schimp. or *Grimmia tergestina* Tomm ex Bruch & Schimp. It has also been regarded as the muticous-leaved male plants of *G. tergestina*. Based on a detailed analysis of the type and many non-type specimens combining the molecular and morphological data, the reinstatement of species rank for *G. limprichtii* is proposed. The diagnostic characteristics of *G. limprichtii* and its distinction from some closely related species, with which it may be confused, are discussed. *Grimmia obtusifolia* C. Gao & T. Cao is considered a synonym of *G. limprichtii* based on molecular and morphological data.

Keywords

Asia-Europe disjunction, *Grimmia obtusifolia*, phylogenetic taxonomy

Introduction

The genus *Grimmia* is one of the largest genera of the moss family Grimmiaceae (Feng et al. 2013). Its species are found on all continents, and most of them prefer dry and temperate or cold environments, and all of them are saxicolous with a marked preference for acidic bedrock (Hastings and Greven 2007). Its taxonomy is reputedly difficult because of great morphological variability in most of its species and the difficulty of properly assessing some crucial characteristics (Feng et al. 2014). Therefore, its treatment by different specialists has led to incongruent results (Muñoz 1999; Ignatova and Muñoz 2004). One example is the number of species accepted in the genus, ranging from 51, according to Maier (2010), who synonymized many names of morphologically diverging taxa, to 71, as reported by Muñoz and Pando (2000), to 95, following Hastings and Greven (2007). Some of the controversial species have recently been resolved based on molecular and morphological data (Hugonnot et al. 2018; Kou et al. 2019; Feng et al. 2021).

Grimmia limprichtii Kern was described in 1897. However, since it was discovered, this species has been considered identical to *Grimmia anodon* Bruch & Schimp. (Loeske 1930) and this treatment was accepted by following authors (such as Wijk et al. 1962; Muñoz and Pando 2000). In recent years, it was synonymized with *Grimmia tergestina* Tomm. ex Bruch & Schimp. by emphasizing the cell pattern, structural characteristics of the costa, and characteristics of the perigonial leaves, as well as the occasional presence of both muticous and hair-pointed leaves in the same plant of the latter species (Maier 2002). Soon afterwards, *G. limprichtii* was regarded as the muticous-leaved male plant of *G. tergestina*, as its male plants were associated with sporulating *G. tergestina* in Tibet (Greven 2009).

Grimmia obtusifolia C. Gao & T. Cao was first described in Tibet, China, and later, it was discovered in many other provinces, such as Qinghai, Xinjiang, Sichuan, Tibet of China, and three locations in Mongolia (Tsegmed and Ignatova 2007; Jia and He 2013). In addition, this species may appear in Pakistan (Gruber and Peer 2010). Although *G. obtusifolia* was accepted by some authors (Redfearn et al. 1996; Tan and Jia 1997; Muñoz and Pando 2000; Liu et al. 2011; Jia and He 2013), soon after it was described, *G. obtusifolia* was synonymized by other authors with *G. limprichtii* (Greven and Sotiaux 1995) and *G. tergestina* (Maier 2002, 2010; Greven 2009). Maier (2010) synonymized *G. obtusifolia* with *G. limprichtii* due to similarities in leaf shape, laminal basal cells, and costal architecture, while Greven (2009) believed that *G. obtusifolia* and *G. limprichtii* were muticous-leaved male plants of *G. tergestina*. Plants with muticous leaf apices are not rare in *G. tergestina* and *G. anodon*, and the similar leaf shape, areolation of the leaf base, and costal architecture explain the synonymization with *G. tergestina*, and the nearly unistratose upper laminal cells may explain that with *G. anodon* (Maier 2002, 2010). *Grimmia limprichtii* and *G. obtusifolia* have a similar habit, concave leaves, cucullate and rounded-obtuse leaf apex, architecture of the costa, and areolation of the leaf base. The only difference between the two species is that *G. obtusifolia* has nearly bistratose upper laminal cells, while *G. limprichtii* has unistratose cells with bistratose ridges (Maier 2002; Greven 2014).

Throughout our continuing investigation of xerophilic mosses, which are particularly prevalent in Tibet, many *Grimmia* specimens were collected. Some of them belong to either *G. obtusifolia* or *G. limprichtii*. Detailed observations revealed that these samples bear archegonia, which is contradictory compared to the point of view that *G. obtusifolia* and *G. limprichtii* are muticous-leaved male plants of *G. tergestina*. This discovery prompted us to conduct further morphological and molecular studies to confirm their systematic position.

Materials and methods

Morphological observations

Over 2000 specimens of the genus *Grimmia* including types were examined during our revision of Grimmiaceae in China and these specimens were mainly from herbaria investigations (mainly IFP, KUN) and more than 50 field surveys in recent years. All specimens were studied with the typical anatomical and morphological methods applied for the Grimmiaceae (Muñoz 1999; Maier 2010). The collected specimen was deposited at NMAC. Microscopic examinations and measurements were taken with a ZEISS Primo Star light microscope, and microphotographs were obtained with a Canon EOS 70D camera mounted on the microscope. Three plants were dissected from each collection, and for each shoot every possible structure from the gametophyte and sporophyte was examined and a record kept of what was found for each individual species. Specific morphological and anatomical features of taxonomic importance were assessed mainly following Maier (2010) and Muñoz (1999). Leaves were always taken from the upper middle of the stem, and cross-sections were made in the middle part of the stem. Measurements of leaf width were taken at the base, mid- and upper leaf. Cross-sections were made mid-leaf. For comparison the morphological characters of the types of *G. limprichtii*, *G. obtusifolia*, and the sequenced Chinese *G. limprichtii*, the key characters including habit, leaf, laminal basal cells and the cross-sections at mid-leaf of the three specimens were shown in Fig. 1.

Taxon sampling, DNA amplification, and sequencing

The only recent collection record from Europe is the material collected in 1993 (Greven and Sotiaux 1995). However, the collection was nearly thirty years ago, which could not be sequenced. To investigate the phylogenetic position of *G. tergestina*, *G. obtusifolia* and *G. limprichtii*, three specimens collected from Tibet were sequenced. Table 1 lists the accessions of the new sequences generated in this study, and Table 2 lists the accessions of the sequences downloaded from GenBank that were used in this study. We employed the nuclear (ITS) marker, which allowed the re-use of earlier results (Streiff 2006; Hernández-Maqueda et al. 2008). DNA extraction, amplification and sequencing of the target regions followed the protocols described in Feng et al. (2021). The PCR

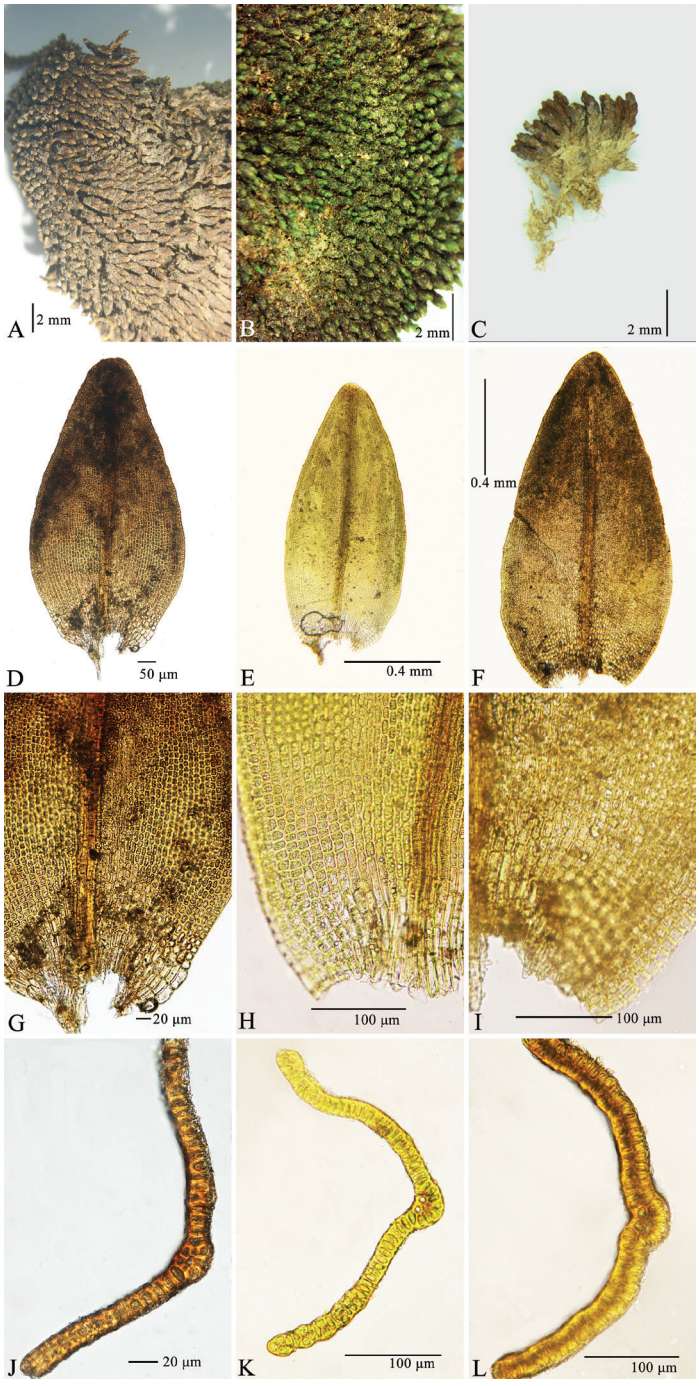


Figure 1. *Grimmia limprichtii* **A–C** habit **D–F** leaves **G–I** laminal basal cells **J–L** cross-sections at mid-leaf. [**A, D, G, J** lectotype of *Grimmia limprichtii*, *Kern* **B, E, H, K** Tibet, *Zi Wang 20180808022* **C, F, I, L** holotype of *Grimmia obtusifolia*, *Lang 1347*] Photos **A, D, G** and **J** courtesy of the Farlow Herbarium of Harvard University and others by Chao Feng.

Table 1. New sequences used in this study, including taxa vouchers information and GenBank accession numbers.

Species	Voucher information	ITS	*rps4*	*trnL-trnF*
*Grimmia tergestina*_F	China, Tibet, Zi Wang 20180809024	OL514232	OL450501	OL450510
*Grimmia limprichtii*_G	China, Tibet, Zi Wang 20180903002	OL514233	OL450502	OL450511
*Grimmia obtusifolia*_H	China, Tibet, Zi Wang 20180808022	OL514234	OL450503	OL450512

Table 2. Sequences from GenBank used in this study, including taxa and GenBank accession numbers.

Species	ITS	*rps4*	*trnL–trnF*
Coscinodon cribrosus	–	AJ845205	AJ847855
Dicranum muehlenbeckii	–	AF231276	AF231245
Ditrichum flexicaule	–	AJ845204	AJ847854
Drummondia obtusifolia	–	AF223038	AF229895
Dryptodon anomalus	EU343751	–	–
Dryptodon austrofunalis	EU343752	–	–
Dryptodon decipiens	EU343753	–	–
Dryptodon leibergii	EU343755	–	–
Dryptodon patens	EU343756	–	–
Dryptodon torquatus	EU343757	–	–
Funaria hygrometrica	–	AJ845203	AJ847853
Grimmia alpestris	–	AJ845237	AJ847887
Grimmia anodon	EU343758	AJ845209	AJ847859
Grimmia anomala	–	AJ845210	AJ847860
Grimmia austrofunalis	–	AJ845211	AJ847861
Grimmia bicolor	EU343759	–	–
Grimmia caespiticia	EU343760	AJ845212	AJ847862
Grimmia caespiticia	EU343761	–	–
Grimmia capillata	EU343762	–	–
Grimmia cribrosa	EU343763	–	–
Grimmia crinita	EU343764	AJ845213	AJ847863
Grimmia decipiens	–	AJ845215	AJ847865
Grimmia dissimulata	–	AJ845216	AJ847866
Grimmia donniana	EU343765	AJ845217	AJ847867
Grimmia elatior	EU343754	AJ845218	AJ847868
Grimmia elongata	EU343766	AJ845219	AJ847869
Grimmia funalis	EU343767	AJ845220	AJ847870
Grimmia funalis	EU343768	–	–
Grimmia funalis	EU343769	–	–
Grimmia funalis	EU343770	–	–
Grimmia fuscolutea	–	AJ845221	AJ847871
Grimmia hamulosa	EU343771	–	–
Grimmia hartmanii	–	AJ845222	AJ847872
Grimmia incrassicapsulis	EU343772	–	–
Grimmia incurva	EU343773	AJ845223	AJ847873
Grimmia involucrata	EU343774	–	–
Grimmia involucrata	EU343775	–	–
Grimmia khasiana	–	AJ845224	AJ847874
Grimmia laevigata	EU343776	AJ845225	AJ847875
Grimmia lisae	–	AJ845226	AJ847876
Grimmia longirostris	EU343777	AJ845227	AJ847877
Grimmia macroperichaetialis	EU343778	–	–
Grimmia meridionalis	–	AJ845228	AJ847878
Grimmia mollis	EU343779	–	–
Grimmia montana	EU343780	AJ845229	AJ847879
Grimmia montana	EU343781	–	–
Grimmia muehlenbeckii	–	AJ845230	AJ847880
Grimmia nevadensis	EU343782	–	–
Grimmia orbicularis	EU343783	AJ845231	AJ847881

Species	ITS	*rps*4	*trn*L–*trn*F
Grimmia orbicularis	EU343784	–	–
Grimmia ovalis	EU343785	AJ845232	AJ847882
Grimmia pilifera	EU343786	AJ845233	AJ847883
Grimmia plagiopodia	EU343787	AJ845234	AJ847884
Grimmia poecilostoma	EU343788	–	–
Grimmia pulvinata	EU343789	AJ845235	AJ847885
Grimmia pulvinata	EU343790	–	–
Grimmia ramondii	–	AJ845214	AJ847864
Grimmia reflexidens	EU343791	–	–
Grimmia serrana	EU343792	–	–
Grimmia sessitana	–	AJ845236	AJ847886
Grimmia tergestina	EU343793	AJ845238	AJ847888
Grimmia torquata	–	AJ845239	AJ847889
Grimmia trichophylla	–	AJ845240	AJ847890
Grimmia trinervis	EU343794	–	–
Grimmia ungeri	EU343795	–	–
Grimmia unicolor	EU343796	AJ845241	AJ847891
Grimmia wilsonii	EU343797	–	–
Hydrogrimmia mollis	–	AJ845206	AJ847856
Ptychomitrium gardneri	–	AF231290	AF231258
Racomitrium aciculare	EU343798	AJ845207	AJ847857
Racomitrium didymum	EU343799	–	–
Racomitrium elongatum	EU343800	–	–
Racomitrium heterostichum	EU343801	–	–
Schistidium apocarpum	–	AJ845208	AJ847858
Schistidium crassipilum	EU343802	–	–
Schistidium sp. *'lingulatum'*	EU343750	–	–
Scouleria aquatica	–	AF306984	AF231179

products were purified and directly sequenced by the Invitrogen Corporation Shanghai Representative Office. Double-stranded sequencing was performed, and all sequence fragments were edited and assembled using Vector NTI (Suite 11.5) to ensure accuracy.

Phylogenetic analyses

The sequences were aligned using MAFFT 7.222 (Kazutaka and Daron 2013) and then edited in BioEdit 7.0.1 (Hall 1999). The concatenation of each individual *rps*4 and *trn*L-*trn*F fragments was performed using our custom Perl script. Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML). MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) was used for BI analyses under the GTR substitute model. Two Markov Chain Monte Carlo (MCMC) searches were run for 1 million generations each, with a sampling frequency of 1000. The first 25% of the trees were discarded as burn-in. A posterior probability (PP) of 0.95–1.00 was considered strong support. The convergence between runs in all cases dropped below 0.01. ML analyses were executed in IQ-TREE 1.6.3 (Nguyen et al. 2014) under the TPM2u+F+G4 (for cpDNA) and TIM+F+I+G4 (for ITS) substitute models, respectively, selected by the ModelFinder program (Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion (BIC), and 1000 fast bootstrapping replicates were used. Nodes with bootstrap (BS) values of 70–89% were treated as moderate and 90–100% as well supported. The final tree obtained was visualized and edited in FigTree v.1.4.0 (Rambaut 2014).

Results

Molecular data

The chloroplast (cp) and ITS alignments comprised 1149 and 1509 nucleotide sites, respectively. The BI and ML phylogenetic trees had a consistent topology, although there were different levels of support depending on the method. Hence, only the topology with branch lengths from the BI tree is presented, with added support from the ML method on the respective trees (Figs 2, 3). The inference from ITS (Fig. 2)

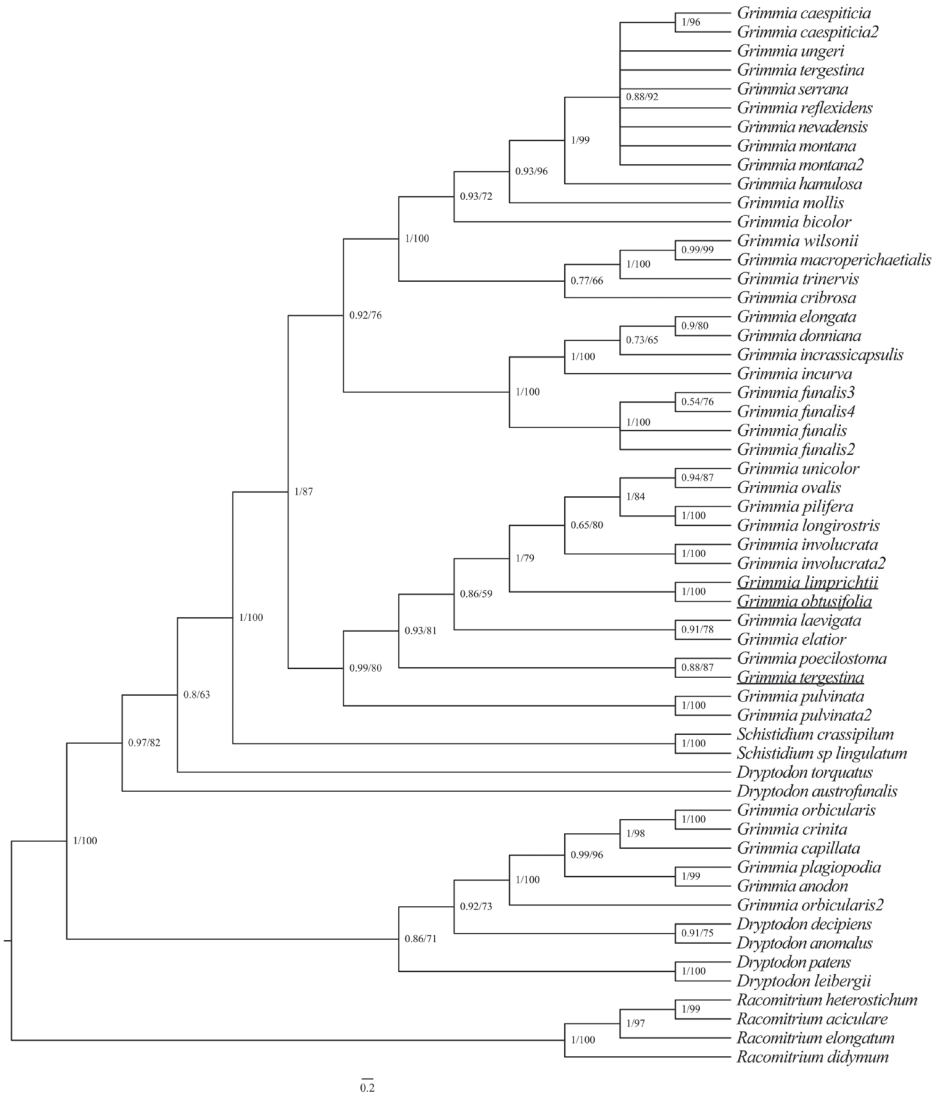


Figure 2. Phylogenetic relationships (50% majority consensus tree) from the Bayesian inference on the ITS dataset. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values for the ML analysis. The species investigated in this study were marked in underscore.

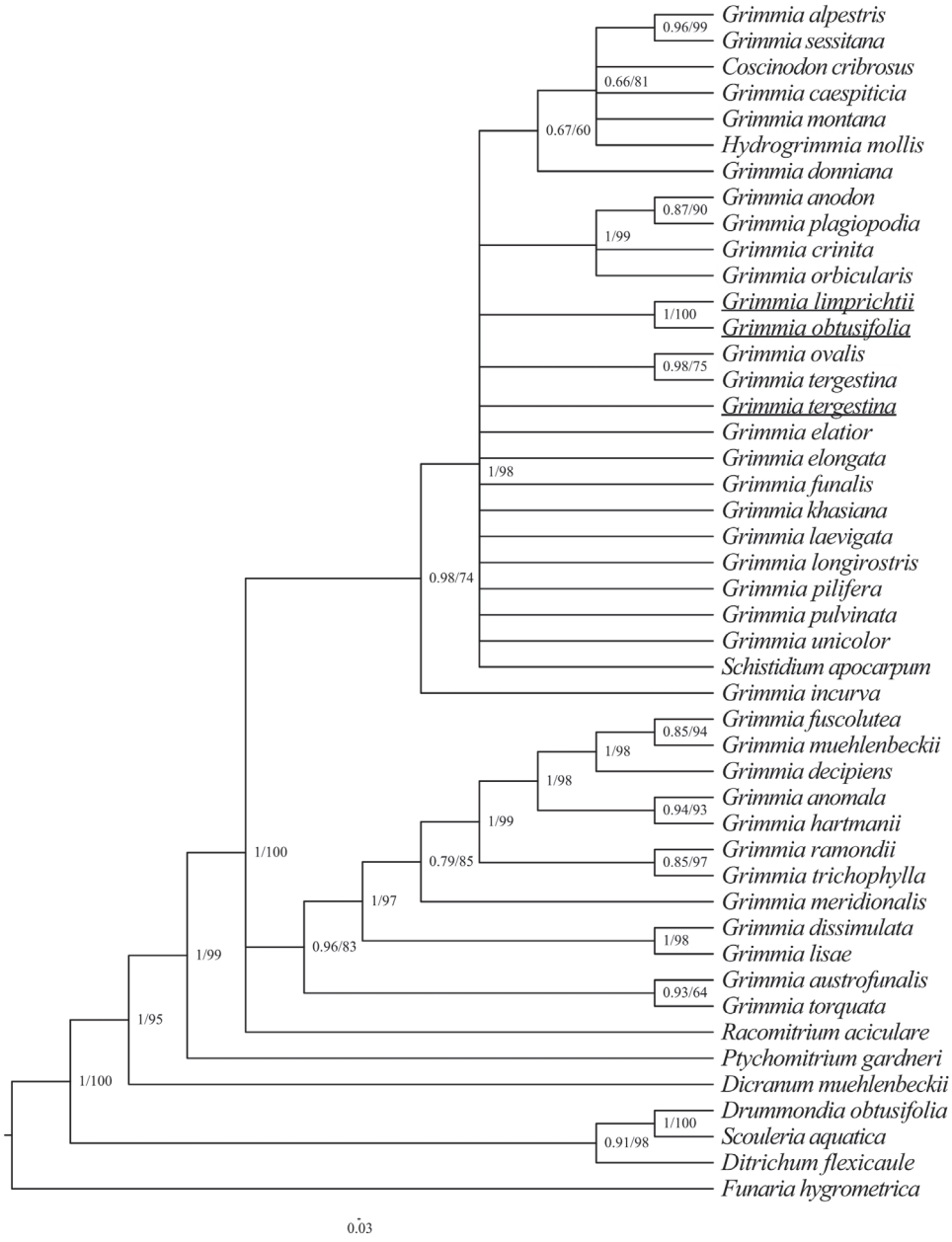


Figure 3. Phylogenetic relationships (50% majority consensus tree) from the Bayesian inference of the concatenated *rps*4 and *trn*M-*trn*V datasets. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values for the ML analysis. The species investigated in this study were marked in underscore.

and the chloroplast regions (Fig. 3) agree in most aspects. The topology of both ITS data and chloroplast data resolved *G. limprichtii* and *G. obtusifolia* as sister taxa in a strongly supported clade (BS = 100, PP = 1). *Grimmia limprichtii* and *G. obtusifolia* are not closely related to *G. tergestina*.

Taxonomic treatment

***Grimmia limprichtii* Kern, Revue Bryologique 24: 56. 1897.**
Figs 1, 4
Chinese name: 林氏紫萼藓

Grimmia obtusifolia C. Gao & T. Cao, Acta Botanica Yunnanica 3: 394. f. 4: 10–16. 1981.
Type: Tibet, Shuanghu Xian, Lang 1347 (holotype: IFP!; paratypes: IFP!, MO).

Type. Dolomiten, Palagrouppe: Felsgallerien am limone, bei 2100m. 29.7.96 Kern (lectotype: FH!; isolectotypes: Goet!, JE, PC).

For full description and illustration, see Cao and Vitt (1986), Greven and Sotiaux (1995), and Feng (2014).

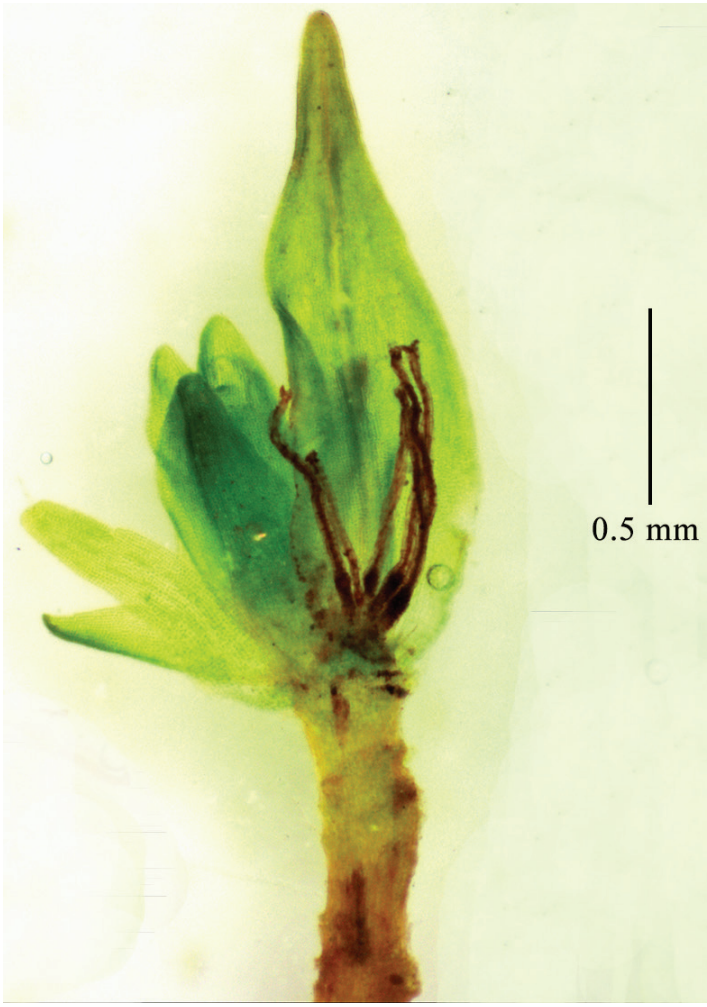


Figure 4. *Grimmia limprichtii* archegonia. Photos: Chao Feng (*Zi Wang 20180808022*).

Discussion

Grimmia limprichtii is a remarkable species characterized by small and slender plants, muticous, concave to somewhat keeled and oblong-ovate leaves, somewhat cucullate and rounded-obtuse leaf apex, plane leaf margins, and a costa ending below the apex. In addition, its sexual condition is dioicous. Although the androecia of *G. limprichtii* were discovered in Europe and Asia (Greven and Sotiaux 1995), its archegonia were usually found in our collections from Inner Mongolia (Feng 2014) and Tibet (Fig. 4), but androecia were not found. Our findings showed that the presumption that *G. limprichtii* is the muticous-leaved male plant of *G. tergestina* (Greven 2009) is unreliable. The generation of a single generative organ in a specific area may explain why the sporophytes are not generated. The characteristic bistratose, partially bistratose or unistratose with bistratose ridges in the upper part of laminal cells is an intraspecific variation influenced by ecological factors, based on our molecular and morphological results.

Morphologically, *G. limprichtii* is most similar to *G. tergestina*, a widely distributed species (Muñoz 1999; Ignatova and Muñoz 2004). Both species share similar leaf shapes, plane leaf margins, and indistinct costa. Additionally, some specimens of the latter species are found in leaves both with and without hair-points (Maier 2002). However, *G. limprichtii* can be readily distinguished from *G. tergestina* by its small and slender plants, costa ending below the apex, and costal guide cells in laminal parts that are distinct from laminal cells. While *G. tergestina* has rather stiff plants, costa percurrent and guide cells of the laminal part of the costa are hardly distinct or even indistinct from lamina cells, due to their similarity.

Grimmia crassiuscula H.C.Greven & C.Feng, a species that was recently described from the Helan mountains, China (Greven and Feng 2014), resembles *G. limprichtii* in the oblong-ovate and muticous leaves, cucullate leaf apex, plane leaf margins, and costa ending below the apex. Nevertheless, *G. crassiuscula* differs from *G. limprichtii* in having plants in loose and succulent mats, absence of a central strand of the stem, and costa without stereids.

Grimmia limprichtii was previously synonymized with *Grimmia anodon* Bruch & Schimp., a widely distributed species (Muñoz 1999; Hastings and Greven 2007). Although hair-point presence and length and the number of cell layers in leaf cross sections are variable in the latter species (Muñoz 1999), *G. anodon* can be separated readily from *G. limprichtii* by its keeled and broadly oblong-lanceolate leaves, elongate-rectangular laminal basal cells, and autoicous sexuality. *G. limprichtii*, by contrast, has concave and oblong-ovate leaves, quadrate to rectangular laminal basal cells, and dioicous sexuality.

Acknowledgements

We are very grateful to Dr Jesús Muñoz, Real Jardín Botánico, for his valuable advice while the authors were studying the genus *Grimmia* and for sending important literature to us. We really appreciate Genevieve E. Tocci of Harvard University Herbaria

(FH) for providing many fine photos of the type of *G. limprichtii* and correcting the draft. We thank Dr Wei Li and the curator of IFP for the loan of the type and non-type specimens of *G. obtusifolia* to us, and to the curator of FH and Dr Marc Appelhans of GOET for the loan of the type of *G. limprichtii*. We are very grateful to Dr Matt von Konrat of Field Museum and an anonymous reviewer for instructive advice, comments, and corrections to draft of this paper. This work was supported by the Natural Science Foundation of China (grant no. 32060051, 42001045, 31660051), Shenzhen Key Laboratory of Southern Subtropical Plant Diversity (grant no. 99203030), the Natural Science Foundation of Inner Mongolia (grant no. 2022MS03066), and the Innovative team of China's Ministry of Education-Research on the sustainable use of grassland resources (IRT_17R59).

References

Cao T, Vitt DH (1986) A taxonomic revision and phylogenetic analysis of *Grimmia* and *Schistidium* (Bryopsida; Grimmiaceae) in China. The Journal of the Hattori Botanical Laboratory 61: 123–247. https://doi.org/10.18968/jhbl.61.0_123

Feng C (2014) *Grimmia* Hedwig. In: Bai XL (Ed.) Illustrations of bryophyte of Helan Mountain. Ningxia People's Publishing House, Yinchuan, 252–265.

Feng C, Muñoz J, Kou J, Bai X-L (2013) *Grimmia ulaandamana* (Grimmiaceae), a new moss species from China. Annales Botanici Fennici 50(4): 233–238. https://doi.org/10.5735/086.050.0405

Feng C, Bai X-L, Kou J (2014) *Grimmia grevenii* (Grimmiaceae), a new species from the Wudalianchi volcanoes in northeast China and its comparison with *G. maido* and *G. longirostris*. The Bryologist 117(1): 43–49. https://doi.org/10.1639/0007-2745-117.1.043

Feng C, Kou J, Zhang G-L, Wang Z (2021) Range extension of two xerophytic mosses in China based on molecular and morphological data. Herzogia 33(2): 300–308. https://doi.org/10.13158/heia.33.2.2020.300

Greven H (2009) The end of *Grimmia limprichtii*. Field Bryology 99: 23.

Greven H (2014): Grimmias of the World. https://www.grimmiasoftheworld.com [accessed 16.02.2022]

Greven H, Feng C (2014) *Grimmia crassiuscula* sp. nov. (Grimmiaceae) from China, and its separation from *Grimmia tergestina* and *Grimmia unicolor*. Herzogia 27(1): 137–140. https://doi.org/10.13158/heia.27.1.2014.137

Greven H, Sotiaux A (1995) *Grimmia limprichtii*, a bryophyte with a disjunct distribution in the Alps and Himalayas. The Bryologist 98(2): 239–241. https://doi.org/10.2307/3243309

Gruber JP, Peer T (2010) A contribution to the knowledge of the bryophyte flora of the mountains of North Pakistan (Autonomous Region of Gilgit-Baltistan). Herzogia 25(2): 271–285. https://doi.org/10.13158/heia.25.2.2010.271

Hall T (1999) BioEdit: A user-friendly biological sequence alignment program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hastings RI, Greven H (2007) *Grimmia* Hedwig. In: Flora of North America Editorial Committee (Eds) Flora of North America north of Mexico (Vol 27). Oxford University Press, New York, 204–305.

Hernández-Maqueda R, Quandt D, Muñoz J (2008) Testing reticulation and adaptive convergence in the Grimmiaceae (Bryophyta). Taxon 57: 500–510. https://doi.org/10.2307/25066018

Hugonnot V, Porley RD, Ignatov MS (2018) A taxonomic conundrum resolved: The transfer of *Grimmia horrida* to *Coscinodon* based on sporophyte discovery in France, with support from molecular data. The Bryologist 121(4): 520–528. https://doi.org/10.1639/0007-2745-121.4.520

Ignatova EA, Muñoz J (2004) The genus *Grimmia* (Grimmiaceae, Musci) in Russia. Arctoa 13(1): 101–182. https://doi.org/10.15298/arctoa.13.13

Jia Y, He S (2013) Species catalogue of China. Volume 1 Plants, Bryophytes. Science Press, Beijing. [In Chinese]

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Kazutaka K, Daron MS (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kou J, Feng C, Li W (2019) The taxonomic status and range extension of *Grimmia exquisita* J.Muñoz (Grimmiaceae, Bryophyta). Journal of Bryology 41(1): 42–47. https://doi.org/10.1080/03736687.2018.1553017

Liu Y, Cao T, Ge X-J (2011) A case study of DNA barcoding in Chinese Grimmiaceae and a moss recorded in China for the first time. Taxon 60(1): 185–193. https://doi.org/10.1002/tax.601016

Loeske L (1930) Monographie der Europäischen Grimmiaceen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Maier E (2002) The genus *Grimmia* (Musci, Grimmiaceae) in the Himalaya. Candollea 57: 143–238.

Maier E (2010) The genus *Grimmia* Hedw. (Grimmiaceae, Bryophyta): A morphological-anatomical study. Boissiera 63: 1–377.

Muñoz J (1999) A revision of *Grimmia* (Musci, Grimmiaceae) in the Americas. 1: Latin America. Annals of the Missouri Botanical Garden 86(1): 118–191. https://doi.org/10.2307/2666219

Muñoz J, Pando F (2000) A world synopsis of the genus *Grimmia* (Musci, Grimmiaceae). Monographs in Systematic Botany from the Missouri Botanical Garden 83: 1–133. https://doi.org/10.2307/2666219

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Rambaut A (2014) FigTree, v1.4.2: Tree figure drawing tool. Molecular evolution, phylogenetics and epidemiology. https://github.com/rambaut/figtree/releases

Redfearn PL, Tan BC, He S (1996) A newly updated and annotated checklist of Chinese mosses. The Journal of the Hattori Botanical Laboratory 79: 163–357. https://doi.org/10.18968/jhbl.79.0_163

Ronquist F, Huelsenbeck J (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Streiff A (2006) Phylogenetic Study of *Grimmia* (Grimmiaceae) Based on Plastid DNA Sequences (*trn*L-*trn*F and *rps*4) and on Morphological Characters. The Bryologist 109(2): 224–235. https://doi.org/10.1639/0007-2745(2006)109[224:PSOGGB]2.0.CO;2

Tan BC, Jia Y (1997) Mosses of Qinghai-Tibetan Plateau, China. The Journal of the Hattori Botanical Laboratory 82: 305–320. https://doi.org/10.18968/jhbl.82.0_305

Tsegmed TS, Ignatova EA (2007) Distribution and ecology of *Grimmia* (Grimmiaceae, Bryophyta) in Mongolia. Arctoa 16(1): 157–162. https://doi.org/10.15298/arctoa.16.16

Wijk van der R, Margadant WG, Florschutz PA (1962) Index Muscorum. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.

PhytoKeys 204: 23–34 (2022)
doi: 10.3897/phytokeys.204.86045
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Hymenophyllum chamaecyparicola (Hymenophyllaceae), a new filmy fern species from Taiwan

Zhi-Xiang Chang[1*], Tian-Chuan Hsu[2,3*], Li-Yaung Kuo[4]

1 *Fushan Research Center, Taiwan Forestry Research Institute, No. 1, Fushan, Shuangpi Rd., Yilan 264013, Taiwan* **2** *Botanical Garden Division, Taiwan Forestry Research Institute, No. 53, Nanhai Rd., Taipei 10066, Taiwan* **3** *Department of Life Sciences, National Chung Hsing University, Taichung 402202, Taiwan* **4** *Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu 30013, Taiwan*

Corresponding author: Li-Yaung Kuo (lykuo@life.nthu.edu.tw)

Academic editor: Joel Nitta | Received 1 May 2022 | Accepted 15 July 2022 | Published 4 August 2022

Citation: Chang Z-X, Hsu T-C, Kuo L-Y (2022) *Hymenophyllum chamaecyparicola* (Hymenophyllaceae), a new filmy fern species from Taiwan. PhytoKeys 204: 23–34. https://doi.org/10.3897/phytokeys.204.86045

Abstract

Hymenophyllum chamaecyparicola T.C.Hsu & Z.X.Chang, a new filmy fern species (Hymenophyllaceae) has been described from Taiwan and illustrated based on morphological and phylogenetic evidence. Although the new species resembles members in the subgenus *Mecodium*, namely *H. wrightii*, our plastid phylogeny has revealed that it is genetically distant from *H. wrightii* and forms a clade nested within subg. *Hymenophyllum*. The most notable characteristic to differentiate *H. chamaecyparicola* from related species is the presence of minute spathulate hairs on the surface of the rachis and veins. *Hymenophyllum chamaecyparicola* is currently only known from a small area in northern Taiwan, and endemic to that country.

Keywords

Filmy fern, *Hymenophyllum*, new species, Taiwan

Introduction

Hymenophyllum is the largest subgenus among the ten subgenera in genus *Hymenophyllum* Sm., and includes at least 100 species (Ebihara et al. 2006; PPG I 2016). Generally, the members of this subgenus are distinguished by their long-creeping

* These authors contributed equally to this work.

rhizomes, pinnate to tripinnate laminae, denticulation on the segment margins, bivalvate involucres, and included receptacles (Ebihara et al. 2006; Hennequin et al. 2010). However, subg. *Hymenophyllum* as a whole varies greatly in many aspects, including cytology and morphology (Hennequin et al. 2010). With a considerably large number of morphologically disparate species, members of subg. *Hymenophyllum* have been scattered among different groups or genera in Hymenophyllaceae (Pryer et al. 2001; Ebihara et al. 2002; Hennequin et al. 2003, 2006, 2010; Hennequin 2004) leaving many systematic issues in this subgenus unsettled. In Taiwan, 26 species are currently known to belong to the genus *Hymenophyllum*. Among them, eight were recognized in subg. *Hymenophyllum* (Hsu et al. 2019; TPG 2019, 2021), including *H. barbatum* (Bosch) Baker, *H. blandum* Racib., *H. denticulatum* Sw., *H. devolii* M.J.Lai, *H. holochilum* (Bosch) C.Chr., *H. okadae* Masam., *H. oligosorum* Makino and *H. simonsianum* Hook. Subgenus *Hymenophyllum* can be distinguished from its sister subgenus *Mecodium* by the presence of indumentum along the stipe, rachis, costae and veins vs. these surfaces glabrous in subgen. *Mecodium*.

In 2019, the first author discovered a *Hymenophyllum* species with an uncertain assignment in a subtropical montane cloud forest of northern Taiwan. After observing its dwarf habit, superficially glabrous laminae, entire segments, and bivalvate, subentire involucres, we initially considered it to be a member belonging to subg. *Mecodium*, and tentatively identified it as *H. wrightii* Bosch, a small species distributed across East Asia and North America (Liu et al. 2013; Lee et al. 2014; Duffy et al. 2015). However, morphological distinctions between the specimen and *H. wrightii* were observed after careful investigation. This species produces apically distributed sori and minute clavate hairs along the rachis and costae (Figs 1, 2), which are absent in the subg. *Mecodium*, especially *H. wrightii*. In this study, we provided not only morphological but also phylogenetic evidence to circumscribe this uncertain species. With no recording of a similar species from previous literature, we determined it as a new species and have described it here as *Hymenophyllum chamaecyparicola* T.C.Hsu & Z.X.Chang, currently known to be endemic to Taiwan.

Materials and methods

Taxon sampling and molecular work

It total, we sampled 19 species, including most members of the East Asian subg. *Hymenophyllum*, all subg. *Mecodium* species in Taiwan, and *H. imbricatum* Blume from subg. *Globosa* as an outgroup (Hsu et al. 2019). This sampling also included three species from sect. *Pseudomecodium*, *H. exsertum* Wall. ex Hook., *H. oligosorum* and *H. pachydermicum* Ces., which was demonstrated to have frond characters similar to subg. *Mecodium* (Iwatsuki 1984, 1985). Their DNA was extracted using a modified CTAB protocol by Kuo (2015). Two chloroplast DNA (cpDNA) regions from these samples were sequenced: *rbcL* and *rps4-trnS* (*rps4* gene + *rps4-trnS* intergenic spacer).

PCR reactions were each prepared in a 15 μL volume containing 20 ng genomic DNA, 1 × SuperRed PCR Master Mix RED (TOOLS, New Taipei City, Taiwan), and 0.5 μM of each primer. PCR products were then cleaned using ExoSAP-IT (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and sequenced with ABI 3730XL (Thermo Fisher Scientific, Waltham, Massachusetts, USA) by the Genomics BioSci. & Tech. company (New Taipei City, Taiwan). Primers, voucher information and GenBank accession numbers are provided in the Appendices (Appendix 1 and Appendix 2).

Phylogenetic analyses

In total, 49 sequences were used for analyses, including 23 newly generated ones from 13 samples and those used in Hsu et al. (2019) and Chen et al. (2021). These sequences were first aligned using MUSCLE (Edgar 2004) implemented in AliView (Larsson 2014), and the resulting alignments of the two cpDNA regions were then concatenated. Seven partitions were initially identified in the concatenated alignment, including each of three codon positions in *rbcL*, each of three codon positions in *rps4*, and *rps4-trnS* intergenic spacer. The best partition scheme and nucleotide substitution models were inferred by ModelFinder (Kalyaanamoorthy et al. 2017) based on AICc criteria, and applied for maximum likelihood (ML) and Bayesian phylogenetic analysis. IQ-TREE v. 1.6.10 (Nguyen et al. 2015) was used to reconstruct ML phylogeny in CIPRES (Miller et al. 2011) with 1000 standard bootstrap replicates. The Bayesian phylogenetic analysis was performed using MrBayes v.3.2.6 (Ronquist et al. 2012) in CIPRES (Miller et al. 2011) with two simultaneous runs and four chains. In each chain, 20 million generations were run, and sampled every 1000 generations. Tracer v. 1.7.1 (Rambaut and Drummond 2013) was used to determine the convergence through generations among chains. The first 25% of the generations was discarded as burn-in, and the posterior probabilities (PP) as branch supports of a Bayesian tree were then summarized.

Results

The concatenated cpDNA dataset of *rbcL* (1365 bp) and *rps4-trnS* (1125 bp) contained a total of 2490 aligned sites. In our cpDNA phylogeny (Fig. 3), no conflicting relationship was found between ML and Bayesian trees. Our two samples of the uncertain *Hymenophyllum* (*H. chamaecyparicola* sp. nov.) possessed identical cpDNA sequences, and this species was found to be phylogenetically unrelated to *H. wrightii* in subg. *Mecodium*. Instead, it formed a strongly supported clade embedded in subg. *Hymenophyllum* and was well separated from all other species (Fig. 3). Interestingly, subg. *Hymenophyllum* as defined by Ebihara et al. (2006) was revealed to be non-monophyletic in our phylogenies because *H. simosianum* was found sister to subg. *Mecodium* with weak supporting values (BS/PP=73/0.6).

Taxonomic treatment

Hymenophyllum chamaecyparicola **T.C.Hsu & Z.X.Chang, sp. nov.**
urn:lsid:ipni.org:names:77302867-1
Figs 1, 2

Type. Taiwan. Yilan County: Datong Township, Mingchih, 1200–1300 m, 31 January 2019, *Z.X. Chang ZXC01438* (holotype: TAIF; isotype: TAI).

Diagnosis. Morphologically, *Hymenophyllum chamaecyparicola* is most similar to *H. wrightii* in sharing pinnate to bipinnatifid fronds, entire segment margins, and bivalvate, entire or subentire involucres. However, the new species could be clearly distinguished from *H. wrightii* by the presence of minute spathulate hairs on both surfaces of laminae (vs. glabrous laminae in *H. wrightii*) (Fig. 1H, I), the lack of two veinlets at the base of sori (vs. two veinlets at the base of sori in *H. wrightii*), and sori confined to apex or upper margins of laminae (vs. on short acroscopic segments close to rachis in *H. wrightii*) (Fig. 1J). This new species is phylogenetically related to *H. barbatum*, *H. devolii*, *H. exsertum*, *H. okadae* and *H. oligosorum*, while it could readily be distinguished from *H. barbatum*, *H. devolii* and *H. okadae* in having entire (vs. serrate) segment margins (Fig. 1D–G) and from *H. exsertum* and *H. oligosorum* in having pinnatifid to bipinnatifid (vs. bipinnatifid to tripinnatifid) laminae (Fig. 1D–G) sparsely covered with short (< 0.2 mm) clavate hairs (vs. densely covered with > 1 mm long acicular hairs) on abaxial surface of rachis and costae (Figs 1H, I, 2E).

Description. Plants epiphytic. Rhizomes long creeping, blackish brown, 0.2–0.3 mm in diam, covered with caducous golden brown multicellular hairs, turning glabrescent when aged. Fronds (1)3–7(10) mm apart, (0.7)1–2.5(4.5) cm long, usually pendent. Stipes dark brownish, (1)2–12(25) mm long, ca. 0.15 mm in diam., wingless, with very sparse caducous hairs similar to those on the rhizomes, turning glabrescent when aged. Laminae pinnatifid to bipinnatifid, flabellate-orbicular, ovate or elliptic, (0.8)1–2.2(3.5) × (0.4)0.6–1.1(1.5) cm, membranous, base obtuse, apex rounded, with minute pale brownish clavate hairs along both surfaces of rachis, costae and veins, otherwise glabrous; clavate hairs up to 0.15 mm long, very sparse adaxially, sparse to scattered abaxially; rachises brown, slightly zigzag, winged throughout or sometimes wingless at base, wings up to ca. 0.2 mm wide, flat, entire; pinnae 2–4(5) pairs, alternate, forming acute angles with rachis, lower pinnae usually forked, rarely more dissected, upper pinnae usually simple, (2)3–8(11) mm long; ultimate segments oblong, (1)2–7(10) × 1.2–1.5 mm, apex rounded, entire, flat or slightly involute; veins simple, greenish brown, ending slightly below the apical margin. Sori 1–3(6) per lamina, confined to apex of lamina or sometimes scattered along upper margins, solitary and terminal on ultimate segments, segment lamina usually slightly constricted below sori; involucres bivalvate, orbicular, ovate-orbicular or elliptic, 1.2–2 × 1–1.5 mm, with a few minute clavate hairs at base, margins entire or minutely erose; receptacles inserted. Spores chlorophyllous, 64 per sporangium.

Additional specimen examined. Taiwan. Yilan County: Datong Township, Mingchih, 1200–1300 m, 11 February 2019, *Chang ZXC01440* (TAIF); same loc., 11 July 2019, *Chang ZXC01670* (TAIF); same loc. and date, *Hsu 11888* (TAIF).

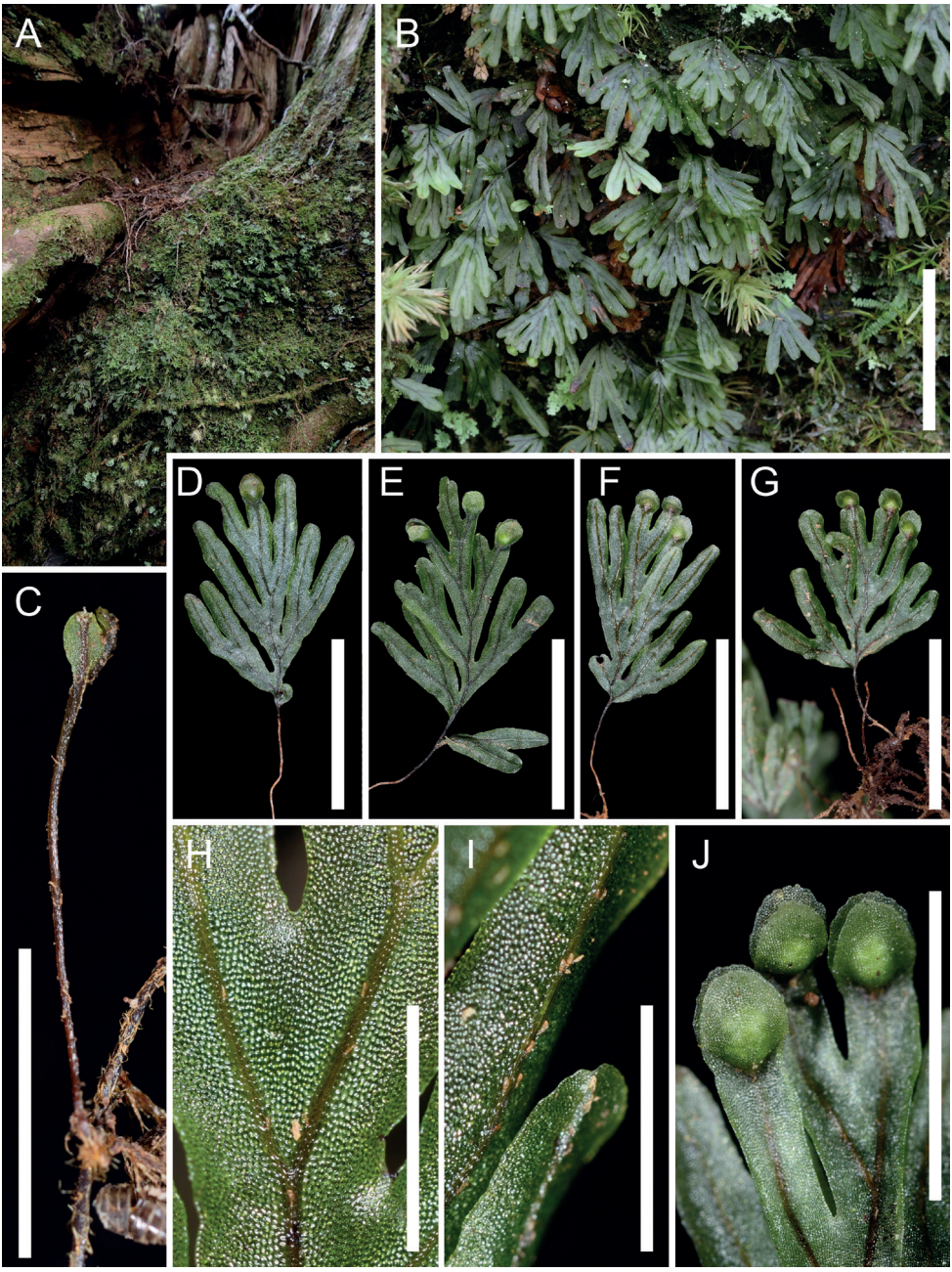


Figure 1. Habitat and morphology of *Hymenophyllum chamaecypericola*, from *Hsu 11888* (TAIF) **A, B** wild population growing on moss-covered basal trunk of a giant *Chamaecyparis obtusa* var. *formosana* **C** rhizome and young frond, showing the wingless and scarcely hairy stipe **D–G** fronds, adaxial views (**D, E**) and abaxial views (**F–G**) **H, I** laminae, adaxial view (**F**) and abaxial view (**G**), showing the minute yellow-brown clavate hairs on rachis and veins. J. Sori. Scale bars: 2 cm (**B**); 5 mm (**C, J**); 1 cm (**D–G**); 2 mm (**H, I**).

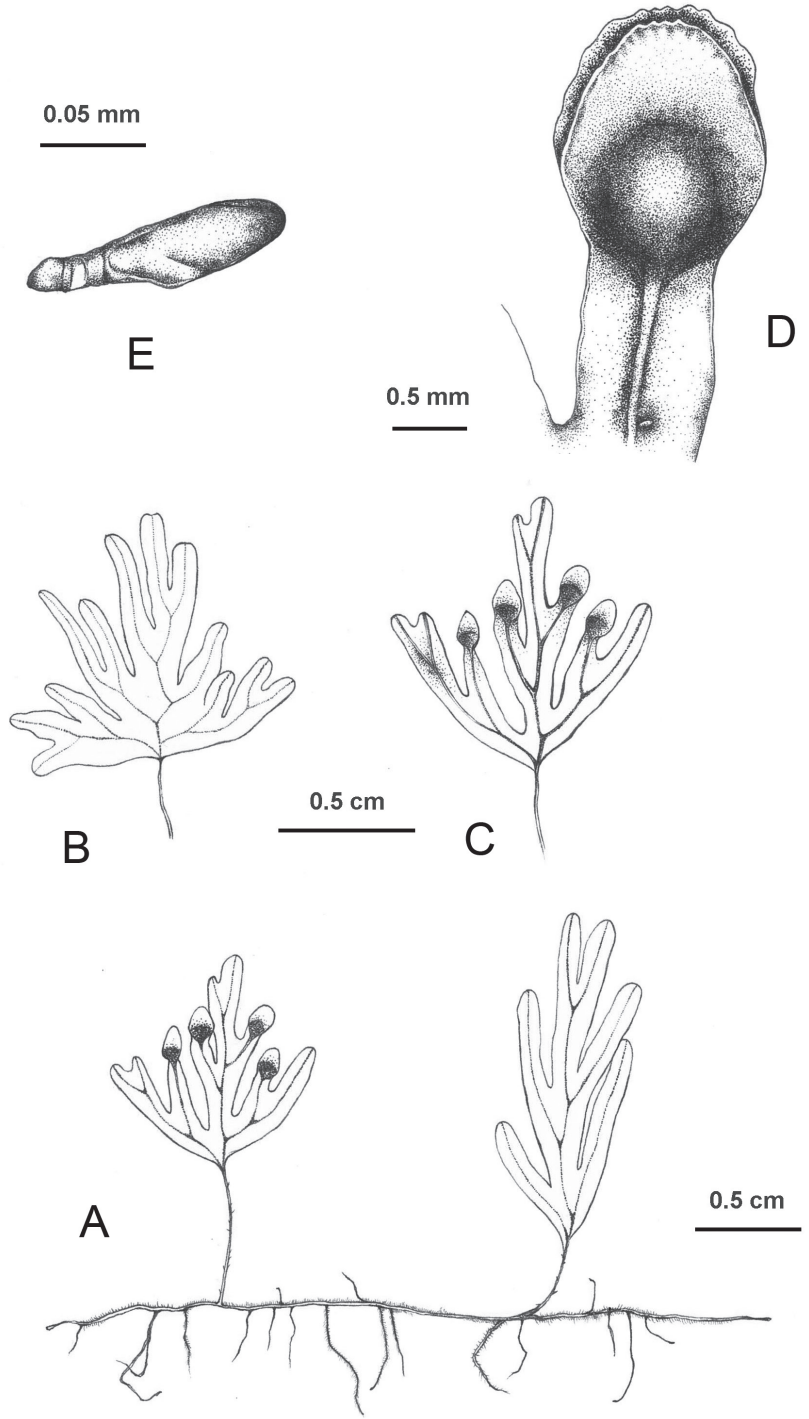


Figure 2. Line drawing of *Hymenophyllum chamaecypericola* T.C.Hsu & Z.X.Chang, sp. nov., based on the holotype *Z.X. Chang ZXC01438* (TAIF) **A** rhizome and fronds **B** sterile frond **C** fertile frond **D** sori **E** a clavate hair.

Distribution and habitat. *Hymenophyllum chamaecyparicola* is endemic to Taiwan and currently known from scattered populations on a single ca. 2000 m^2 mountain slope in *Chamaecyparis* montane mixed cloud forest (Li et al. 2013) around Mingchih (24.65361°N, 121.46950°E). It is epiphytic on bases of tree trunks and exposed roots of *Chamaecyparis obtusa* var. *formosana* (Hayata) Hayata.

Etymology. The specific epithet, a noun in apposition, is derived from *Chamaecyparis*, a Gymnosperm genus, and *–cola*, dweller, alluding to unusual habitat of the new species occurring on the lower trunk of the giant *C. obtusa* var. *formosana*.

Discussion

Our phylogeny generally agrees with the "modern" circumscriptions of *Hymenophyllum* subg. *Hymenophyllum* and subg. *Mecodium* (Ebihara et al. 2006; Hennequin et al. 2010; Hsu et al. 2019; Vasques et al. 2019) with only one exception – *H. simonsianum*, which was placed within subg. *Hymenophyllum* based on morphology (Ebihara et al. 2006) but resolved here as an isolated lineage sister to subg. *Mecodium* (Fig. 3). Given that this unexpected position of *H. simonsianum* was weakly supported in our tree based on only two cpDNA regions, we consider that more sequence data is required to ascertain its systematic placement.

The phylogenetic position of *Hymenophyllum chamaecyparicola*, nested within subg. *Hymenophyllum*, was somewhat surprising in the beginning due to its superficial resemblance to *H. wrightii* in subg. *Mecodium*. However, after a detailed examination of the specimens, we concluded that its placement in subg. *Hymenophyllum* is also morphologically evident. Though hardly visible to the naked eye, *H. chamaecyparicola* bears clavate hairs on stipes and rachis, and such laminar trichomes are common in subg. *Hymenophyllum* but absent in subg. *Mecodium* (Ebihara et al. 2006; Hennequin et al. 2010; Hsu et al. 2019). Moreover, from our examination, two veinlets can be found at the bases of sori in members of subg. *Mecodium* sori but not in *H. chamaecyparicola* and other species of subg. *Hymenophyllum* as implied previously (Dubuisson et al. 2018).

Obviously, our sampling of *Hymenophyllum* subg. *Hymenophyllum* (11 species), with an estimate of more than 100 species (Ebihara et al. 2006), remains insufficient. Even so, this study still provides some insights about interspecific relationships and systematics within this subgenus. We revealed for the first time that sect. *Pseudomecodium*, mainly defined by the combination of abaxially hairy veins and entire segments (Iwatsuki 1984, 1985), is non-monophyletic. In our tree, the four sampled species with entire segments, including *H. chamaecyparicola*, *H. exsertum*, *H. oligosorum* and *H. pachydermicum*, were placed in three different lineages (Fig. 3). In addition, our data strongly supported that *H. okadae*, recently reinstated from a synonym of *H. barbatum* based on a few subtle morphological characters (Knapp and Hsu 2017), is also phylogenetically distinct.

In addition to *H. chamaecyparicola*, *H. devolii* is another subg. *Hymenophyllum* species endemic to Taiwan. Our study then revealed that *H. devolii* is affiliated, not only morphologically but also phylogenetically, with its sympatric relatives, *H. okadae* and

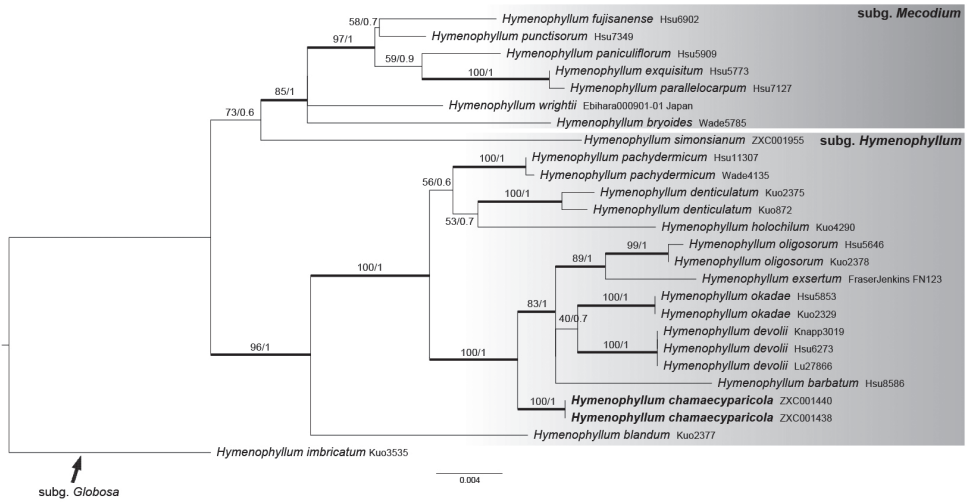


Figure 3. Maximum likelihood (ML) phylogeny of *Hymenophyllum* subg. *Hymenophyllum* and *Hymenophyllum* subg. *Mecodium* based on the chloroplast DNA dataset (*rbcL* + *rps4-trnS*). Branch support is indicated in ML bootstrap/ BI posterior probabilities.

H. barbatum, which are also distributed in other East Asian regions. It will be very worthy to further study the speciation pathways behind these endemic ferns in Taiwan. A comprehensive sampling in the subgenus, especially from Southeast Asia, and a dated phylogeny are ultimately necessary to clarify the evolutionary history of these Taiwan endemic ferns.

Key to subg. *Hymenophyllum* and *Mecodium* species in Taiwan

1 Laminae glabrous, indumentum absent along the stipes, rachises, and veins...**2**
– Indumentum present along the stipes, rachises, and veins **6**
2 Stipes wingless or only with decurrent wings at apexes **3**
– Stipes narrowly winged to base or at least to middle..... **4**
3 Stipes reddish brown, wingless; involucres orbicular, distinctly wider than joint segments..... ***H. punctisorum***
– Stipes dark brownish, only with decurrent wings at apexes; involucres ovate-orbicular or ovate, roughly as wide as joint segments .... ***H. parallelocarpum***
4 Laminae shorter than 6 cm; sori densely aggregated at lamina apexes..... ..... ***H. paniculiflorum***
– Laminae variable; sori never densely aggregated at lamina apexes **5**
5 Rachis and costa wings weakly crispate or flat; ultimate segments nearly flat; involucres ovate to ovate-triangular..... ***H. fujisanense***
– Rachis and costa wings strongly crispate; ultimate segments contorted; involucres oval to suborbicular..... ***H. exquisitum***
6 Segment margins entire..... **7**
– Segment margins serrate **8**

7 Laminae pinnatifid to bipinnatifid; minute pale brownish clavate hairs (ca. < 0.2 mm) present on both surfaces of rachises and veins ..... ***H. chamaecyparicola***
– Laminae bipinnate to tripinnatifid; brownish setae (ca. > 1 mm) present on both surfaces of rachises and veins ..... ***H. oligosorum***
8 Involucres obconic-tubular; receptacles exserted..... **9**
– Involucres cleft to base, not obconic-tubular; receptacles included in involucres **11**
9 Stipes and rachises wingless; involucres serrate at apexes ..... ***H. blandum***
– Stipes and rachises winged; involucres entire or toothed at apexes..... **10**
10 Laminae crispate; involucres toothed; spine-like protrusions present on base of involucres ..... ***H. denticulatum***
– Laminae flat; involucres entire; spine-like protrusions absent on base of involucres ..... ***H. holochilum***
11 Involucres orbicular to ovate **12**
– Involucres oblong to oval..... **13**
12 Rachis wings involute; involucres orbicular to oblate, dentate at apexes .... ***H. okadae***
– Rachis wings recurved to revolute; involucres orbicular to ovate, entire or sometimes slightly crenate at apexes..... ***H. devolii***
13 Laminae ovate; segments 2 mm broad; costae of sterile pinna with more than 2 pairs of costules..... ***H. barbatum***
– Laminae linear-oblong to linear-lanceolate; segments 2–4 mm broad; costae of sterile pinna only with 1 or 2 pair of costules..... ***H. simonsianum***

Acknowledgements

We are grateful to Ralf Knapp for his useful comments and enthusiastic participation in field trips. We thank Chih-Yun Sun for preparing line drawings; Alexandria Quinlan for English edits; the curators of K, L, MICH, NY, P, TAI, TAIF and US herbaria for access to their collections; Taiwan Pteridophyte Group (TPG) for maintaining DNA collections used in this study; Cheng-Wei Chen and Pei-Jung Xie for generating DNA sequences; Diego Tavares Vasques, Atsushi Ebihara, Joel Nitta, and one anonymous reviewer for their comments on the manuscript. This project was supported by MOST project (109-2621-B-007-001-MY3, 111-2628-B-007-006-MY3) in Taiwan, the Bioresource Conservation Research Center in College of Life Science from the Higher Education Sprout Project by MOE, Biodiversity Information Fund for Asia project (BIFA6_010).

References

Chen C-W, Ebihara A, Cheng K-Y, Hsu T-C, Huang Y-M, Dang VD, Luu HT, Le VS, Li C-W (2021) Studies of Vietnamese pteridophyte flora 1. Systematic Botany 46(3): 573–581. https://doi.org/10.1600/036364421X16312067913507

Dubuisson J-Y, Pechon TL, Bauret L, Rouhan G, Reeb C, Boucheron-Dubuisson E, Selosse M-A, Chaussidon C, Dajoz I, Pynee K, Grangaud E, Robert Y, Tamon J-M, Hennequin S (2018) Disentangling the diversity and taxonomy of Hymenophyllaceae (Hymenophyllales, Polypodiiadae) in the Mascarene archipelago, with ecological implications. Phytotaxa 375(1): 1–58. https://doi.org/10.11646/phytotaxa.375.1.1

Duffy AM, Stensvold MC, Farrar DR (2015) Independent gametophytes of *Hymenophyllum wrightii* in North America: Not as rare as we thought. American Fern Journal 105(1): 45–55. https://doi.org/10.1640/0002-8444-105.1.45

Ebihara A, Iwatsuki K, Kurita S, Ito M (2002) Systematic position of *Hymenophyllum rolandi-principis* Rosenst. or a monotypic genus *Rosenstockia* Copel. (Hymenophyllaceae) endemic to New Caledonia. Acta Phytotaxonomica et Geobotanica 53(1): 35–49. https://doi.org/10.18942/apg.KJ00003256664

Ebihara A, Dubuisson JY, Iwatsuki K, Hennequin S, Ito M (2006) A taxonomic revision of Hymenophyllaceae. Blumea 51(2): 221–280. https://doi.org/10.3767/000651906X622210

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Hasebe M, Omori T, Nakazawa M, Sano T, Kato M, Iwatsuki K (1994) *RbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proceedings of the National Academy of Sciences of the United States of America 91(12): 5730–5734. https://doi.org/10.1073/pnas.91.12.5730

Hennequin S (2004) Le genre *Hymenophyllum* Sm. (Hymenophyllaceae, Filicopsida): systématique phylogénétique, évolution morphologique et histoire biogéographique. Ph. D. Thesis, Pierre and Marie Curie University Paris, France.

Hennequin S, Ebihara A, Ito M, Iwatsuki K, Dubuisson J-Y (2003) Molecular systematics of the fern genus *Hymenophyllum* s.l. (Hymenophyllaceae) based on chloroplastic coding and noncoding regions. Molecular Phylogenetics and Evolution 27(2): 283–301. https://doi.org/10.1016/S1055-7903(02)00404-9

Hennequin S, Ebihara A, Ito M, Iwatsuki K, Dubuisson J-Y (2006) New insights into the phylogeny of the genus *Hymenophyllum* s.l. (Hymenophyllaceae): Revealing the polyphyly of *Mecodium.* Systematic Botany 31(2): 271–284. https://doi.org/10.1600/036364406777585775

Hennequin S, Ebihara A, Dubuisson J-Y, Schneider H (2010) Chromosome number evolution in *Hymenophyllum* (Hymenophyllaceae), with special reference to the subgenus *Hymenophyllum.* Molecular Phylogenetics and Evolution 55(1): 47–59. https://doi.org/10.1016/j.ympev.2010.01.001

Hsu T-C, Huang Y-F, Chao Y-S (2019) Systematics of *Hymenophyllum* subgenus *Mecodium* (Hymenophyllaceae) in Taiwan. Systematic Botany 44(4): 753–767. https://doi.org/10.1600/036364419X15710776741413

Iwatsuki K (1984) Studies in the systematics of filmy ferns: VII. A scheme of classification based chiefly on the Asiatic species. Acta Phytotaxonomica et Geobotanica 35(4–6): 165–179. https://doi.org/10.18942/bunruichiri.KJ00001078509

Iwatsuki K (1985) The Hymenophyllaceae of Asia, excluding Malesia. Journal of the Faculty of Science, University of Tokyo, section III. Botany 13: 501–551.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Knapp R, Hsu T-C (2017) Ferns and Fern Allies of Taiwan. Second Supplement. KBCC Press, Pingtung, Taiwan.

Kuo L-Y (2015) Polyploidy and biogeography in genus *Deparia* and phylogeography in *Deparia lancea*. Ph. D. Thesis, National Taiwan University, Taiwan.

Larsson A (2014) AliView: A fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30(22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lee CS, Lee K, Lee SG, Ebihara A (2014) A new taxon of *Hymenophyllum* (Hymenophyllaceae): *H. wrightii* f. *serratum.* Korean Journal of Plant Taxonomy 44(4): 233–237. https://doi.org/10.11110/kjpt.2014.44.4.233

Li C-F, Chytrý M, Zelený D, Chen M-Y, Chen T-Y, Chiou C-R, Hsia Y-J, Liu H-Y, Yang S-Z, Yeh C-L, Wang J-C, Yu C-F, Lai Y-J, Chao W-C, Hsieh C-F (2013) Classification of Taiwan forest vegetation. Applied Vegetation Science 16(4): 698–719. https://doi.org/10.1111/avsc.12025

Liu J, Zhang Q, Ebihara A, Iwatsuki K (2013) Hymenophyllaceae. In: Wu Z, Raven PH, Hong D (Eds) Flora of China, vol. 2–3. Missouri Botanical Garden Press, Beijing, China, St. Louis, USA, 93–109.

Miller MA, Pfeiffer W, Schwartz T (2011) The CIPRES science gateway: A community resource for phylogenetic analyses. In: Towns JW (Ed.) Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery ACM. New Orleans, LA, 1–8.

Nadot S, Bajon R, Lejeune B (1994) The chloroplast gene *rps4* as a tool for the study of Poaceae phylogeny. Plant Systematics and Evolution 191(1–2): 27–38. https://doi.org/10.1007/BF00985340

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

PPG I (2016) A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54(6): 563–603. https://doi.org/10.1111/jse.12229

Pryer KM, Smith AR, Hunt JS, Dubuisson J-Y (2001) *RbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). American Journal of Botany 88(6): 1118–1130. https://doi.org/10.2307/2657095

Rambaut A, Drummond AJ (2013) Tracer v1.6. http://tree.bio.ed.ac.uk/software/tracer/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Souza-Chies TT, Bittar G, Nadot S, Carter L, Besin E, Lejeune B (1997) Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene *rps4*. Plant Systematics and Evolution 204(1–2): 109–123. https://doi.org/10.1007/BF00982535

TPG (2019) Updating Taiwanese pteridophyte checklist: A new phylogenetic classification. Taiwania 64: 367–395. https://doi.org/10.6165/tai.2019.64.367

TPG (2021) Taiwan Pteridophyte Checklist v2021.2. https://groups.google.com/forum/#!forum/taiwan-pteridophyte-group [accessed 02.07.2021]

Vasques DT, Ebihara A, Hirai RY, Prado J, Motomi I (2019) Phylogeny of *Hymenophyllum* subg. *Mecodium* (Hymenophyllaceae), with special focus on the diversity of the *Hymenophyllum polyanthos* species complex. Plant Systematics and Evolution 305(9): 811–825. https://doi.org/10.1007/s00606-019-01609-y

Appendix 1

Table A1. Voucher and sequence information of *Hymenophyllum* species for the phylogenetic analyses. GenBank accessions (*rps4- trnS* and *rbcL*) are under their columns, respectively. The symbol "–" means not available; the symbol "* " means newly generated sequences in this study.

Taxon	Voucher specimen number	Collection locality	Herbarium	*rps4-trnS*	*rbcL*
H. barbatum	*Hsu 8586*	Taiwan (Taoyuan County)	TAIF	ON773153*	ON652817*
H. blandum	*Kuo 2377*	Taiwan (Yilan County)	TAIF	ON773147*	ON773829*
H. bryoides	*Wade 5785*	Vietnam	TAIF	MW478759	MW478758
H. chamaecyparicola	*ZXC001440*	Taiwan (Yilan County)	TAIF	ON773148*	ON773830*
H. chamaecyparicola	*ZXC001438*	Taiwan (Yilan County)	TAIF	ON773149*	ON773831*
H. denticulatum	*Kuo 872*	Taiwan (Pingtung County)	TAIF	ON773146*	ON773828*
H. denticulatum	*Kuo 2375*	Taiwan (Yilan County)	TAIF	–	ON773827*
H. devolii	*Knapp 3019*	Taiwan (Taitung County)	P	MF144616	MF144660
H. devolii	*Lu 27866*	Taiwan (Taitung County)	TAIF	–	ON773833*
H. devolii	*Hsu 6273*	Taiwan (Taitung County)	TAIF	MN266569	MN266660
H. exquistum	*Hsu 5773*	Taiwan (Hsinchu County)	TAIF	MH211098	MH211069
H. exsertum	*Fraser-Jenkins-FN123*	India	TAIF	ON773154 *	ON773836*
H. fujisanense	*Hsu 6902*	Taiwan (Taitung County)	TAIF	MH211087	MH211058
H. holochilum	*Kuo 4290*	Taiwan (Pingtung County)	TAIF	MH265124	MH265124
H. imbricatum	*Kuo 3535*	Philippines	TAIF	MH211105	MH211076
H. okadae	*Hsu 5853*	Taiwan (Taoyuan County)	TAIF	MH211103	MH211074
H. okadae	*Kuo 2329*	Taiwan (Yilan County)	TAIF	ON773145*	ON773826*
H. oligosorum	*Kuo 2378*	Taiwan (Yilan County)	TAIF	–	ON773825*
H. oligosorum	*Hsu 5646*	Taiwan (Hualien County)	TAIF	MH211102	MH211073
H. pachydermicum	*Hsu 11307*	Vietnam	TAIF	ON773151*	ON773834*
H. pachydermicum	*Wade 4135*	Vietnam	TAIF	ON773152*	ON773835*
H. paniculiflorum	*Hsu 5909*	Taiwan (Taichung County)	TAIF	MH211097	MH211068
H. parallelocarpum	*Hsu 7127*	Taiwan (Pingtung County)	TAIF	MH211101	MH211072
H. punctisorum	*Hsu 7349*	Taiwan (Nantou County)	TAIF	MH211083	MH211054
H. simonsianum	*ZXC001955*	Taiwan (Nantou County)	TAIF	ON773150*	ON773832*
H. wrightii	*Ebihara000901-01*	Japan	TI	AY775430	AB083277

Appendix 2

Table A2. Primers used in this study.

Name	Region	Sequence (5'-3')	Reference
aF	*rbcL*	ATGTCACCACAAACAGAGACTAAAGC	Hasebe et al. 1994
1379R	*rbcL*	TCACAAGCAGCAGCTAGTTCAGGACTC	Pryer et al. 2001
Fern rbcL fVGF	*rbcL*	GAGACTAAAGCAGGTGTTGGATTCA	This study
Fern rbcL rVVG	*rbcL*	GTTCCCCYTCTAGTTTRCCTACTAC	This study
rps5	*rps4-trnS*	ATGTCCCGTTATCGAGGACCT	Nadot et al. 1994
trnS	*rps4-trnS*	TACCGAGGGTTCGAATC	Souza-Chies et al. 1997

PhytoKeys 204: 35–41 (2022)
doi: 10.3897/phytokeys.204.86647
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Ardisia whitmorei (Primulaceae-Myrsinoideae), a new species from north east of Peninsular Malaysia

Avelinah Julius[1], Timothy M.A. Utteridge[2]

1 *Forest Research Institute Malaysia, Kepong, Selangor, 52109, Malaysia* **2** *Royal Botanic Gardens, Kew, Richmond, TW9 3AE, UK*

Corresponding author: Avelinah Julius (plagiovel80@yahoo.com)

Academic editor: Wendy Achmmad Mustaqim | Received 17 May 2022 | Accepted 20 July 2022 | Published 4 August 2022

Citation: Julius A, Utteridge TMA (2022) *Ardisia whitmorei* (Primulaceae-Myrsinoideae), a new species from north east of Peninsular Malaysia. PhytoKeys 204: 35–41. https://doi.org/10.3897/phytokeys.204.86647

Abstract

Ardisia whitmorei Julius & Utteridge, **sp. nov.** (Primulaceae-Myrsinoideae), a member of *Ardisia* subgenus *Stylardisia* on account of the style protruding from the closed petals prior to anthesis, is herein described and illustrated as a new species. This new species is easily distinguished by the combination of the inflorescences with a slender rachis branched to two orders, the corolla lobes are abaxially glabrous with usually up to only two gland-dots near the apex and the brochidrodromous secondary veins with double loops near the margin.

Keywords

Endemic, Ericales, Gunung Padang, IUCN status, Malesia, *Stylardisia*, taxonomy, Terengganu

Introduction

The genus *Ardisia* Sw. is one of the largest tropical genera in the Primulaceae subfamily Myrsinoideae (containing the woodier, tropical members), having a pantropical distribution with approximately 725 species (POWO 2022). In Peninsular Malaysia, the last comprehensive account of the genus was that of Stone (1989) who treated 74 species in an annotated key in the Tree Flora of Malaya (because most *Ardisia* species do not reach the arborescent limit to merit full descriptions in the Tree Flora). An additional five species were added by Hu (2002; two species), Julius and Utteridge (2012, 2021; two species) and Julius et al. (2017; one species), bringing the total number of species in Peninsular Malaysia to 79.

Ardisia is classified into 16 subgenera (indicated here with the silcrow: §) using characters of habit, leaf morphology, inflorescence position and floral morphology (Mez 1902; Stone 1993; Larsen and Hu 1995), with ten subgenera present in Malesia (see Stone 1982; Larsen and Hu 1995). In Peninsular Malaysia, all these subgenera are present with most speciose groups being §*Tinus* and §*Stylardisia*, with 16 and 15 species, respectively.

A new species from southern Peninsular Malaysia, *Ardisia gasingoides* Julius & Utteridge, was initially assigned to §*Stylardisia*, based on collections of fruiting material (Julius et al. 2017). However, recent molecular results suggest it is better placed in §*Acrardisia* (Julius 2019; Julius et al. 2021). This example shows the importance of having flowering specimens, or sequenced material, available for a definitive subgeneric placement in *Ardisia*. Stone (1989) annotated specimens of §*Stylardisia* in the Herbarium of the Forest Research Institute Malaysia at Kepong (KEP) during his work for the Tree Flora account, but several could not be identified due to incomplete material. A single fruiting collection from Gunung (G.) Padang collected by Timothy Whitmore in 1969 was annotated by Stone (6 Oct 1980) as '*Ardisia* sp. "Y" near *A. sessilis* Scheff. but distinct', but Stone (1989) did not list this taxon in his annotated key to the genus in his Tree Flora account. Recently, flowering material of the species was collected during an expedition to G. Padang in 2010 (Ummul-Nazrah et al. 2011), allowing us to critically scrutinise the floral and fruit morphology against the known species in the subgenus from Peninsular Malaysia. After careful examination of the specimens and the relevant literature of species known from §*Stylardisia*, we confirm that this is an undescribed taxon and it is formally described and illustrated as new to science here. The new taxon described here brings the number of §*Stylardisia* species native to Peninsular Malaysia to 16.

Materials and methods

This study was based on examination of herbarium specimens at K, KEP and the relevant taxonomic literature (e.g. Stone 1982, 1992; Larsen and Hu 1991; Chen and Pipoly 1996; Hu 2002); in addition, specimen images from Global Plants JSTOR (http://plants.jstor.org/), Kew Herbarium Catalogue (http://apps.kew.org/herbcat/gotoHomePage.do) and Plants of the World Online (**POWO**: http://www.plantsoftheworldonline.org/) were consulted. All measurements were taken from herbarium specimens and rehydrated material for the floral description; shape terminology follows Systematics Association Committee (1962). Flowering and fruiting materials are indicated by 'fl.'and 'fr.', respectively. The conservation assessment of the species was undertaken using IUCN categories of threat (see IUCN 2012 and IUCN Standards and Petitions Committee 2022) following the guidelines and procedures developed at FRIM for the Malaysia Plant Red List (Chua and Saw 2006).

Taxonomy

***Ardisia whitmorei* Julius & Utteridge, sp. nov. (*§Stylardisia*)**
urn:lsid:ipni.org:names:77302868-1
Fig. 1

Diagnosis. Amongst the Peninsular Malaysian members of subgenus (§) *Stylardisia*, the new species is easily recognised by the following combination characters: lateral veins brochidrodromous with double loops towards the margin and prominent on both surfaces, the relatively large leaves (15–23 cm long), the inflorescences with a slender rachis and branched to two orders and the glabrous corolla lobes with usually up to only two gland-dots near the apex abaxially (Fig. 1).

Type. Malaysia. Peninsular Malaysia: Terengganu, Hulu Terengganu, G. Padang, trail to summit of G. Padang, 4°51.06'N, 102°53.22'E, 1236 m alt., 20 March 2010 (fl.), *Mohd. Hairul et al.*, *FRI70884* (holotype KEP!).

Description. A woody shrub with about 2 m high. *Indumentum* of scale or short, brown, simple or branched trichomes, with or without glands on vegetative and reproductive part. *Leaves* alternate; petiole stout, 1–2 cm long, covered with dense scale; lamina subcoriaceous, elliptic-oblong, 15–23 × 5.5–7.5 cm, base cuneate-attenuate, margin entire, apex acuminate, acumen 1–1.5 cm long, glabrous on both surfaces, except the dense, brown scale beneath; mid-rib flat above, raised below; lateral veins 21–28 pairs, closely spaced, brochidrodromous with double looping in the margin, distinct on upper surface, prominent beneath, intersecondary veins present within each pair; intercostal veins reticulate, distinct on both surfaces. *Inflorescences* axillary in the uppermost axils on lateral branches (see Notes), paniculate, ca. 12 cm long, 2 times branched, with flowers umbelliform at the ends of alternate branches, laxly to closely arranged on branchlets; peduncle and rachis 10 cm long, flexuous and winged, densely hairy; bracts lanceolate, ca. 1 mm long, glabrous on both surfaces, margin ciliate, deciduous. *Flower* 5-merous; pedicels 4–10 mm long, slender and obconically flared towards calyx base, covered with simple brown hairs, sparsely to glabrescent; calyx lobes not overlapping, spreading, covered with 2–4 brown gland-dots abaxially, glabrous on both surfaces, triangular, 1–1.2 × 1 mm, margin ciliate, with laxly spaced, pale brown hairs, apex obtuse; corolla contorted, lobes pinkish, with up to two gland-dots near apex abaxially, ovate-acuminate, ca. 3.5 × 1.5 mm, glabrous on both surfaces; stamens subsessile, anther lanceolate-mucronate, ca. 2 × 0.8 mm, glabrous, except densely covered with gland-dots near mid-rib abaxially, thecae not locellate, dehiscent by longitudinal slits; ovary globose, ca. 1 × 1 mm, glabrous, style and stigma slender, ca. 4 mm long, ovules ca. 12 in two series. *Fruits* with dense gland-dots, globose, ca. 4 × 4 mm, glabrous.

Distribution. Endemic in Peninsular Malaysia, Terengganu (G. Padang).

Ecology. Growing in primary lower montane forest.

Etymology. The species is named after the late Dr Timothy C. Whitmore (1935–2002), a tropical botanist whose interests pertained to all aspects of tropical rain forests and who first collected this species from G. Padang.

Conservation status. Least Concern (LC). This species is found only in one locality and G. Padang is under Taman Negara, which is a protected area. In addition, the habitat is an intact mossy forest where a healthy population was observed along the steep slopes ridge towards the summit plateau (Mohd. Hairul Mohd. Amin, pers. com.). Therefore, it is assessed as Least Concern (LC) according to the Malaysia Red List (Chua and Saw 2006) and IUCN Red List Categories and Criteria (IUCN 2012) and guideline version 15 (IUCN Standards and Petitions Committee 2022).

Additional specimen examined. Malaysia. Peninsular Malaysia: Terengganu, Gunong [Gunung] Padang Expedition, Summit plateau G. Padang, closed 40 ft. [14 m alt.] lower montane type forest on eastern side of plateau [4°51'N, 102°52'E], 4200 ft. [1280 m alt.], 20 Sept 1969 (fr.), *T. C. Whitmore*, *FRI 12727* (KEP!).

Notes. This species was initially flagged as distinct by Stone who assumed it to be similar to *Ardisia sessilis* Scheff., no doubt due to the leaf size and the venation, but to date, there is no valid name for this taxon. Although the new species shows some similarity to *A. sessilis* in the shape of the leaves (elliptic-oblong), which are in the same size range (15–25 cm long) and in the reticulation (intercostal veins ± reticulate), it differs from the latter in several morphological characteristics, such as the marginal veins absent (but double marginal veins present in *A. sessilis*), the inflorescence rachis is slender (vs. stout) and the pedicel is longer and slender (vs. short or almost sessile and thick).

There are several members of §*Stylardisia* that have large leaves and slender inflorescences rachis, but the new species most resembles *Ardisia nurii* Furtado in having elliptic-oblong leaves and a brochidrodromous venation. However, the inflorescence in *A. nurii* is usually branched to three and rarely two orders, whereas in *A. whitmorei*, it is branched to two orders. In addition, the brochidrodromous venation is double looped in *A. whitmorei*, but not in *A. nurii*. The new species is also similar to *A. pterocaulis* Miq. (*A. platyclada* King & Gamble sensu Stone (1989)), also with the inflorescence rachis being slender, but *A. whitmorei* has inflorescences branched to two orders (vs. to three orders in *A. pterocaulis*), has longer leaves, 15–23 cm long (compared to the shorter leaves, 9.5–13 cm long) with a flat lamina surface (vs. bullate), the brochidrodromous lateral veins (vs. meet in prominent looped intramarginal veins) and the corolla lobes are pinkish (vs. waxy white), that are abaxially covered with only two gland-dots near the apex (vs. over the entire surface).

The material of *A. whitmorei* currently available for study is rather poor and the inflorescences are found in axils of terminal leaves, but as these inflorescences are large, multi-flowered and paniculate, as well as the flowers having the style projecting from the bud, we are confident this species is best placed within §*Stylardisia*. The material appears to be of plants that have had the terminal bud removed (they appear damaged at the apex) and we assume the inflorescences have had to appear from lower axils. Other subgenera with axillary inflorescences include §*Pimelandra* and §*Akosmos*, but the new species has none of the characters for those taxa, i.e. short axillary inflorescences or axillary and terminal inflorescences and both with no style extension prior to anthesis.

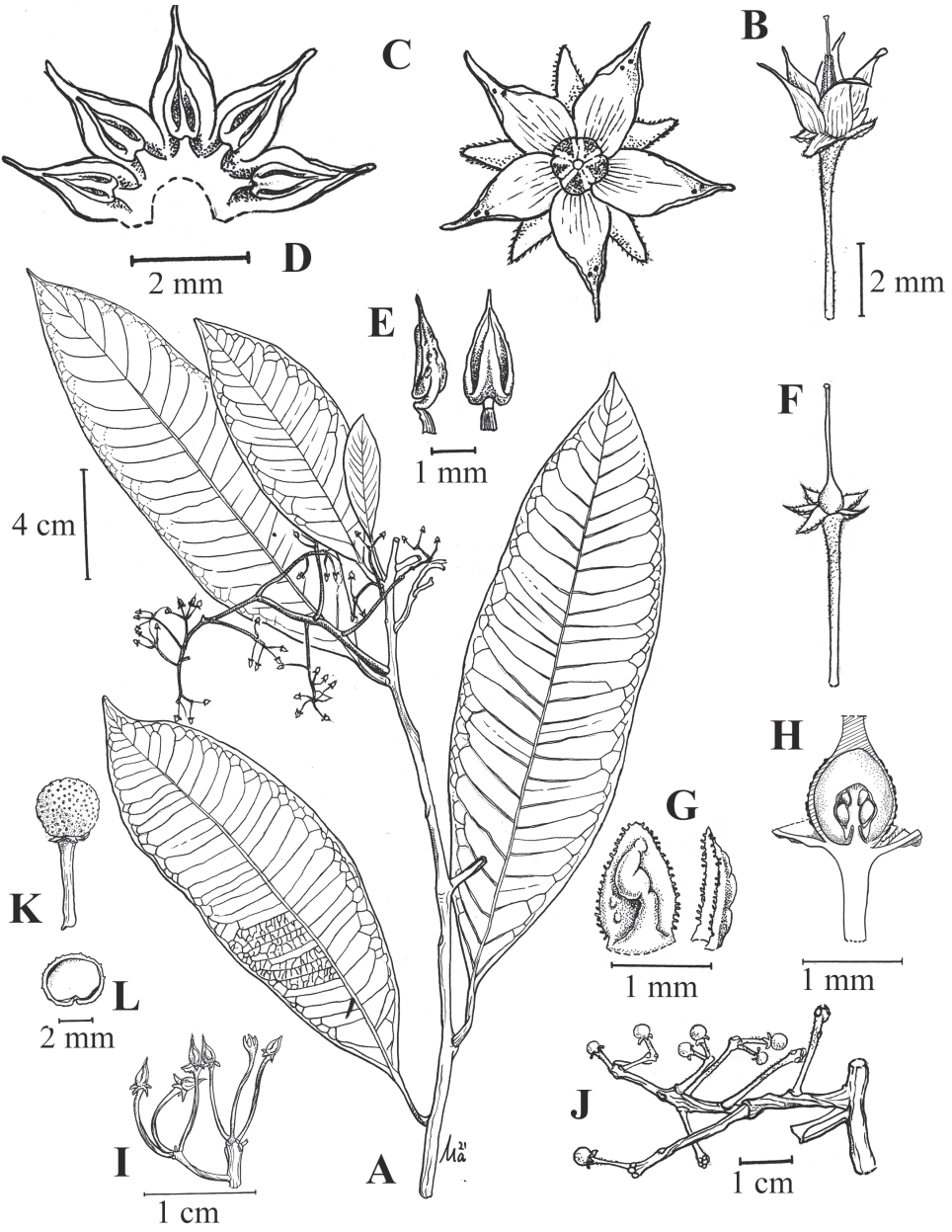


Figure 1. *Ardisia whitmorei* Julius & Utteridge, sp. nov. **A** flowering leafy twig **B** mature flower **C** aerial view of opened flower **D** flower dissected to show the stamen arrangement **E** anther, lateral (left) and front view (right) **F** petals removed to show calyx and pistil **G** calyx, abaxial (left) and lateral view (right) **H** ovary dissected to show the ovules **I** flower prior to anthesis, showing one flower with exerted style **J** infructescence **K** fruit **L** fruit, cross-section. (Illustration by Mohd Aidil Nordin **A–I** from *Mohd. Hairul et al.*, *FRI70884* **J–L** from *T.C. Whitmore*, *FRI12727*: scale bar for **C** similar to **D, F** similar to **B** and **K** similar to **L**).

Excluding *Conamomum utriculosum* Ridl. (synonym: *Amomum utriculosum* (Ridl.) Holttum), about ten taxa are listed as endemic to G. Padang (Ummul-Nazrah et al. 2011; with more not yet named due to incomplete material, but known to be distinct from known species). The addition of the new species described here brings the total number of endemic species for G. Padang to 11, suggesting that there are very likely more taxa that may be endemic and waiting to be described.

Acknowledgements

This research was carried out as part of the Flora of Peninsular Malaysia Project at the Forest Research Institute Malaysia (FRIM). Financial support for the Flora of Malaysia projects, including the fieldwork undertaken for this paper, was supported by the Ministry of Energy and Natural Resources (KeTSA) of Malaysia under the 11th Malaysian Plans (SPPII No: P23085100018003). The encouragement and useful comments from an anonymous reviewer for reviewing and proofreading our manuscript are greatly acknowledged. We thank Wendy YSY for valuable assistance with the conservation assessments; Mohamad Aidil Noordin for the botanical illustration; and the dedicated staff of KEP Herbarium at FRIM for their support in the field.

References

Chen J, Pipoly III JJ (1996) Myrsinaceae. In: Wu ZY, Raven PH (Eds) Flora of China 15. Science Press, Beijing, and Missouri Botanical Garden Press, St Louis, 1–38.

Chua LSL, Saw LG (2006) Malaysia Plant Red List: Guide for Contributors. Forest Research Institute Malaysia, 28 pp.

Hu CM (2002) New species of *Ardisia* (Myrsinaceae) from Malesia. Blumea 47: 493–512.

IUCN (2012) IUCN Red List Categories and Criteria.Version 3.1. 2nd edn. IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK.

IUCN Standards and Petitions Committee (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15. Prepared by the Standards and Petitions Committee. [accessed 28 February 2022]

Julius A (2019) Evolution of leaf nodulation in Myrsinoideae (Primulaceae). PhD dissertation, Chiba University, Japan, 116 pp.

Julius A, Utteridge TMA (2012) Revision of *Ardisia* subgenus *Bladhia* in Peninsular Malaysia; studies in Malaysian Myrsinaceae I. Kew Bulletin 67(3): 379–388. https://doi.org/10.1007/s12225-012-9374-4

Julius A, Utteridge TMA (2021) *Ardisia recurvisepala* (Primulaceae-Myrsinoideae), a new species from Peninsular Malaysia. Phytotaxa 480(1): 79–84. https://doi.org/10.11646/phytotaxa.480.1.7

Julius A, Kajita T, Utteridge TMA (2017) *Ardisia gasingoides* (Primulaceae-Myrsinoideae), a new species from southern Peninsular Malaysia. Phytotaxa 291(4): 281–286. https://doi.org/10.11646/phytotaxa.291.4.5

Julius A, Gutiérrez-Ortega JS, Sabran S, Tagane S, Naiki A, Darnaedi D, Aung M, Dang S, Nguyen N, Binh H, Watano Y, Utteridge TMA, Kajita T (2021) Phylogenetic relationship of tropical Asian *Ardisia* and relatives (Primulaceae) shows non-monophyly of recognized genera and subgenera. Shokubutsu Kenkyu Zasshi 93: 149–165.

Larsen K, Hu CM (1991) New taxa of Myrsinaceae from Thailand. Nordic Journal of Botany 11(1): 61–78. https://doi.org/10.1111/j.1756-1051.1991.tb01795.x

Larsen K, Hu CM (1995) Reduction of *Tetrardisia* to *Ardisia*. Nordic Journal of Botany 15(2): 161–162. https://doi.org/10.1111/j.1756-1051.1995.tb00134.x

Mez C (1902) Myrsinaceae. In: Engler A (Ed.) Das Pflanzenreich, Heft 9, IV. Fam. 236. Verlag von Wilhelm Engelmann, Leipzig, 1–473.

POWO (2022) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. http://www.plantsoftheworldonline.org/

Stone BC (1982) New and noteworthy Malaysian Myrsinaceae, I. Malaysian Forester 45: 101–121.

Stone BC (1989) New and noteworthy Malesian Myrsinaceae, III. On the genus *Ardisia* Sw. in Borneo. Proceedings. Academy of Natural Sciences of Philadelphia 141: 263–306.

Stone BC (1992) A revision of the genus *Tetrardisia* Mez (Myrsinaceae). Malayan Nature Journal 46: 1–11.

Stone BC (1993) New and noteworthy Malesian Myrsinaceae, VI. *Scherantha*, a new subgenus of *Ardisia*. Pacific Science 47(3): 276–294.

Systematics Association Committee (1962) IIa. Terminology of simple symmetrical plane shapes (Chart 1a)*. Taxon 11(8): 245–247. https://doi.org/10.2307/1217034 [Addendum]

Ummul-Nazrah AR, Rafidah AR, Kiew R, Imin K (2011) The botany of Gunung Padang, Terengganu. Malayan Nature Journal 63(4): 625–641.

PhytoKeys 204: 43–56 (2022)
doi: 10.3897/phytokeys.204.85746
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Hymenasplenium tholiformis (Aspleniaceae), a new fern species from southeastern Xizang, China based on morphological and molecular evidence

Yong-Lin Qiu[1*], Ke-Wang Xu[2*], Wen-Bin Ju[3], Wang-Lin Zhao[4,5], Liang Zhang[6]

1 *School of Ecology and Environmental Science, Yunnan University, Kunming, 650091, China* **2** *Co-Innovation Center for Sustainable Forestry in Southern China & Key Laboratory of National Forestry and Grassland Administration on Subtropical Forest Biodiversity Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing, 210037, China* **3** *Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, 610041, China* **4** *College of Science, Tibet University, Lhasa, 850000, China* **5** *Motuo Observation and Research Center for Earth Landscape and Earth System, Chinese Academy of Sciences, Linzhi 860712, China* **6** *CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences(CAS), Kunming, 650201, China*

Corresponding author: Liang Zhang (zhangliang@mail.kib.ac.cn)

Academic editor: Blanca León | Received 24 April 2022 | Accepted 3 July 2022 | Published 5 August 2022

Citation: Qiu Y-L, Xu K-W, Ju W-B, Zhao W-L, Zhang L (2022) *Hymenasplenium tholiformis* (Aspleniaceae), a new fern species from southeastern Xizang, China based on morphological and molecular evidence. PhytoKeys 204: 43–56. https://doi.org/10.3897/phytokeys.204.85746

Abstract

A new species of Aspleniaceae, *Hymenasplenium tholiformis* sp. nov., is described from Medog County in southeastern Xizang, China. The new species is morphologically similar to *H. apogamum* and *H. szechuanense*, but the former has ascending pinnae, pinna apex obtuse to rounded, pinna-marginal teeth entire, and veins terminating just below marginal teeth. Phylogenetic analysis based on five plastid markers confirmed that this new species represents a diverging lineage in the *H. excisum* subclade of *Hymenasplenium*.

Keywords

Hymenasplenium, *H. excisum* subclade, Medog, pinna morphology

* These authors contributed equally.

Introduction

Hymenasplenium Hayata is one of two genera in the species-rich fern family Aspleniaceae, comprising more than 60 species worldwide (Murakami and Moran 1993; Murakami 1995; PPG I 2016; Schneider et al. 2017; Xu et al. 2018b). The genus is characterized by the stelar structure of the long-creeping rhizome, dorsiventrally symmetrical steles, swollen petiole bases, and unique rachis-costae structure (Murakami 1995; Xu et al. 2018b). Extensive cryptic speciation in *Hymenasplenium* has been demonstrated based on recent studies (Chang et al. 2018; Xu et al. 2018b), which resulted in the discovery of a large number of new species (Chang et al. 2018; Xu et al. 2018a, 2019a, 2019b).

Located in southeastern Xizang, Medog County is one of the biodiversity hotspots in China, which has rich plant diversity in the Eastern Himalaya (Sun and Zhou 1996). Recently, new taxa of seed plants have continuously added to the flora of this region (Li et al. 2018; Li et al. 2021; Tian et al. 2021; Ya et al. 2021; Zhou et al. 2021). Previous taxonomic studies on the ferns in Xizang have partially uncovered the fern diversity in Medog (Wu 1983; Ching and Ling 1984a, 1984b; Zhang et al. 2004); recent work has added more new species and new records to the fern flora of this region (Wei et al. 2018; Fan et al. 2021; Qiu et al. 2022). As a continuous effort to clarify the species diversity of pteridophytes in Medog, we conducted a 12-day fieldwork in Medog in October 2021. During that trip, we collected some specimens of a species of *Hymenasplenium* that were obviously different from all other species of the genus. Our later morphological study and phylogenetic analysis confirmed that these specimens represent a distinctive species.

Materials and methods

Morphological studies

Morphological characters of the new species were observed in the field. Herbarium specimens of *Hymenasplenium* at KUN and PYU were studied. Digital specimens of other related species of *Hymenasplenium* were examined from the online database CVH (https://www.cvh.ac.cn/) and JSTOR Global Plants (https://plants.jstor.org/). Spore samples were taken from the type specimens and coated with gold particles using the BAL-TEC SCD 005 Cool Sputter Coater (BAL-TECAG., Liechtenstein) and imaged via a QUANTA 200 Scanning Electron Microscope (SEM; FEI Co., USA) at Yunnan University, Kunming, China.

Phylogenetic analyses

To clarify the phylogenetic position of the new species, we sampled representatives of all the three major clades of the genus and six subclades in the Old World clade

(Xu et al. 2018b). DNA sequences from five plastid markers (*atpB*, *psbA*, *rbcL*, *rps4* & *rps4-trnS*, and *trnL* & *trnL-F*) of 102 accessions representing 40 species of *Hymenasplenium* were sampled. Sixteen species of *Asplenium* were used as outgroups. Voucher information and GenBank accession numbers for each sampled taxon are provided in Appendix I.

Total genomic DNA was extracted from silica-gel-dried leaves using the TIANGEN plant genomic DNA extraction kit (TIANGEN Biotech., Beijing, China) following the manufacturers' protocols. Five plastid markers (*atpB*, *psbA*, *rbcL*, *rps4* & *rps4-trnS*, and *trnL* & *trnL-F*) were selected for amplification and sequencing. The PCR system uses the ready-to-use rapid PCR master mix gold Mix (green) 25 μl amplification system developed by Beijing Qingke Xinye Biotechnology Co., Ltd. The new sequences were viewed and edited using Sequencher v.4.14 (Gene Codes Corporation, Ann Arbor, Michigan). The total sequences were automatically aligned in MAFFT ver. 7 (Katoh and Standley 2013) and manually adjusted in BioEdit (Hall 1999). jModeltest2 (Darriba et al. 2012) was used to select the best-fitting likelihood model for maximum likelihood (ML; Felsenstein 1973) and Bayesian analyses. The Akaike information criterion (Akaike 1974) was used to select among models instead of the hierarchical likelihood ratio test, following Pol (2004) and Posada and Buckley (2004). Maximum likelihood (ML) bootstrapping was conducted with 1000 rapid bootstrap (BS) analyses followed by a search for the best-scoring tree in a single run in RAxML v. 8 (Stamatakis et al. 2008). Bayesian inference (BI) was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with two runs of four Markov chain Monte Carlo chains, each beginning with a random tree and sampling one tree every 1000 generations for 10,000,000 generations. Both ML and BI analyses were conducted on Cipres (Miller et al. 2010).

Results and discussion

Morphological comparison

Like most species in *Hymenasplenium*, the new species have long-creeping rhizome, once-pinnate laminae, asymmetrical pinnae, reddish-brown rachis, and elliptic to reniform spores, but can be distinguished from other species in the genus by the combined characters of pinna apex obtuse to rounded, ascending pinnae, relatively fewer pairs of pinnae, and pinna-marginal teeth entire, veins terminating just below marginal teeth. The pinna shape of *H. tholiformis* is most distinct in the genus, with acroscopic margins curved and irregularly toothed, basiscopic margins truncate or slightly curved and entire, and pinna apex obtuse to rounded. A few species in the genus sometimes also have pinna apex obtuse, for example, *H. apogamum* (N.Murak. & Hatan.) Nakaike, *H. szechuanense* (Ching) Viane & S.Y. Dong (Table 1), but none of them have obviously curved margins on the acroscopic side of pinnae.

Table 1. Comparison of morphological characters to differentiate *Hymenasplenium tholiformis*, *H. szechuanense*, *H. apogamum,* and *H. pseudobscurum*.

Characters	*H. tholiformis*	*H. szechuanense*	*H. apogamum*	*H. pseudobscurum*
Size of lamina	13–16 × 3–5 cm	15–25 × 3–5 cm	10–20 × 3–5 cm	20–25 × 5–10 cm
Number of lateral pinnae	15–21 pairs	20–25 pairs	15–25 pairs	15–30 pairs
Pinna shape	trapeziform to trapeziform-lunate	trapeziform	quadrangular-trapeziform	trapeziform-falcate
Size of middle pinnae	2.5–3 × 0.6–1 cm	1.5–2.3 × 0.7–1 cm	2–3.5 × 0.6–1 cm	2.5–4 × 0.8–1.8 cm
Shape of pinna apex	obtuse to rounded	truncate to obtuse	obtuse to subacute	obtuse to subacute
Stipe color	shiny, black purple	shiny, dark purple	shiny, purple	not shiny or purple
Rachis color	purple	purple	purple	grayish green
Teeth	entire	retuse	entire	entire
Sori position	medial	inframedial	medial	supramedial
Indusium	single	single	single	double
Number of basalveins lacking	3–4	3–4	1–2	3–5

Molecular phylogenetic analyses

The alignment of five plastid markers was 5,309 bp, of which 3,848 sites were identical, 938 characters were parsimony informative, and 523 variable characters were parsimony-uninformative. A total of 12 sequences are newly generated for this study (Appendix I). The monophyly of *Hymenasplenium* was confirmed by our reconstructed phylogeny. *Hymenasplenium tholiformis* was strongly supported as a member of the *H. excisum* subclade in the Old World clade (Xu et al. 2018b). Its affinity with *H. pseudobscurum* Viane was weakly supported (Fig. 2). To date, only three species have been recognized in the *H. excisum* subclade (Xu et al. 2018b); our discovery adds one more species to this subclade. Within the subclade, *H. tholiformis* is different from *H. obscurum* (Blume) Tagawa and *H. pseudobscurum* by having black-purple to dark purple petioles and pinna apex obtuse to rounded, and different from *H. excisum* (C. Presl) S. Lindsay by having smaller habit and truncate or slightly curved margins of the basiscopic side of pinnae.

Taxonomic treatment

Hymenasplenium tholiformis **Liang Zhang, W.B. Ju & K.W. Xu, sp. nov.**
urn:lsid:ipni.org:names:77302869-1

Type. China. Xizang: Medog County, Beibeng Xiang, Xirang, ca. 600 m from the Yarlung Zangbo River, 29°11'17.63"N, 95°03'42.27"E, 720 m elev., 28 Oct 2021, *Liang Zhang & Wen-Bin Ju 4781* (holotype: KUN1543824!; isotypes: CDBI!, KUN!).

Diagnosis. *Hymenasplenium tholiformis* is morphologically most similar to *H. szechuanense*, but different by having larger pinnae (middle pinnae 2.5–3 cm vs. 1.5–2.3 cm), extremely ascending upper pinnae (vs. spreading or slightly ascending), curved margins of acroscopic side of pinnae (vs. truncate), and pinna-marginal teeth

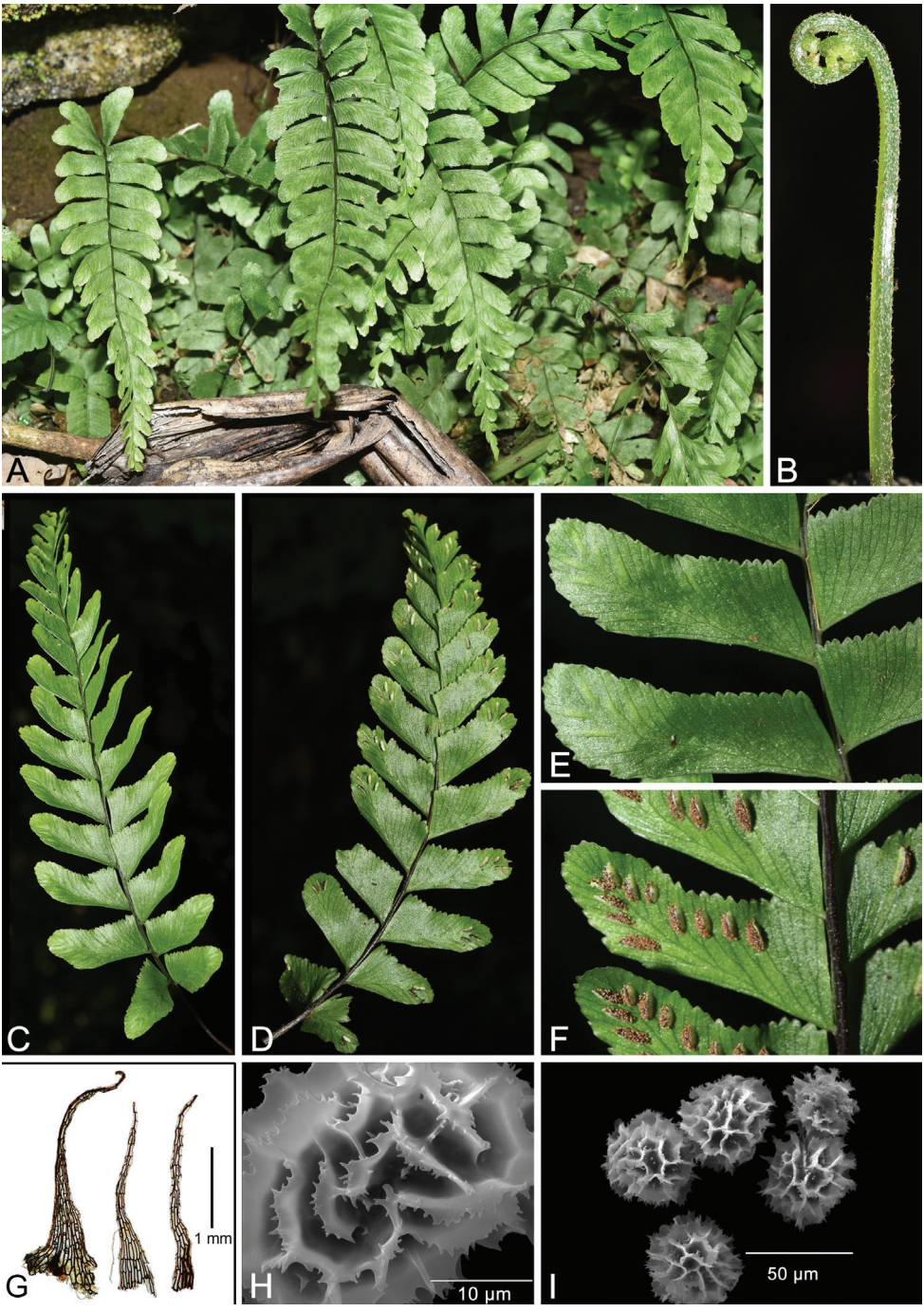


Figure 1. *Hymenasplenium tholiformis* sp. nov. **A** habit **B** crozier **C** adaxial lamina **D** abaxial lamina **E** adaxial pinnae **F** abaxial pinnae, showing sori **G** rhizome scales **H** spore surface **I** spores. Photos credit: **A–F** by L. Zhang; **G–I** by W.-Z. Ma & Y.-L. Qiu.

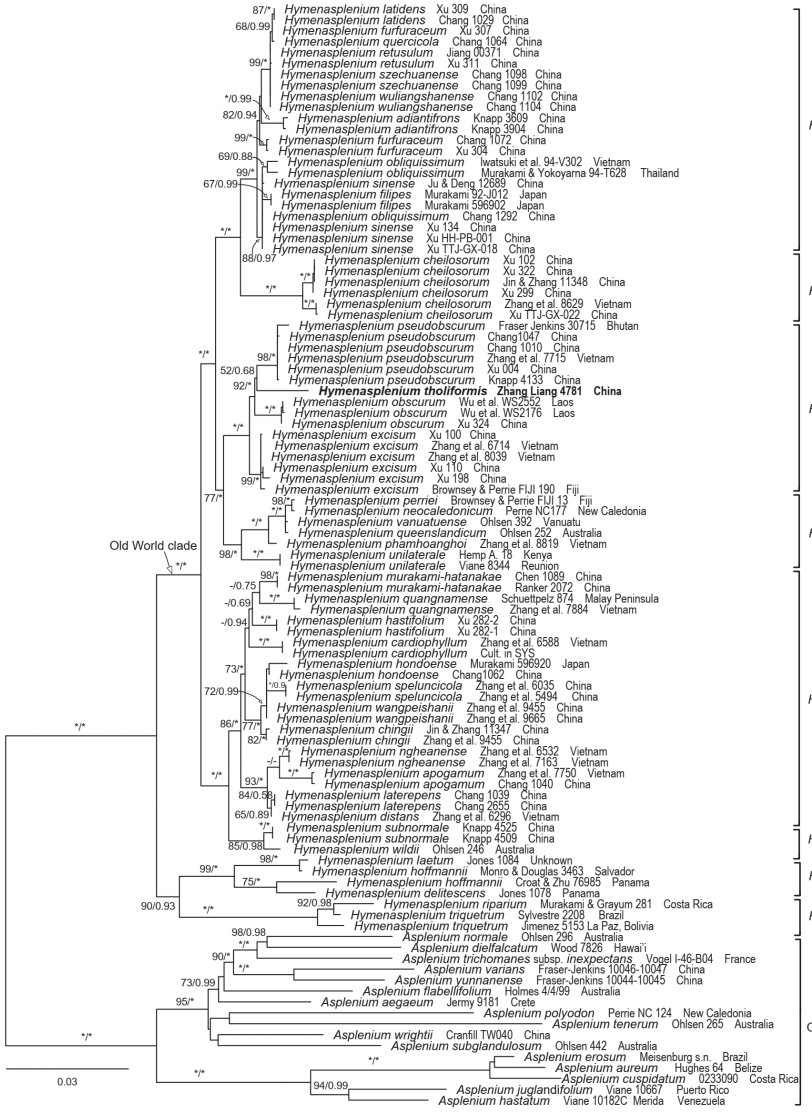


Figure 2. Maximum likelihood phylogeny of *Hymenasplenium* based on five plastid markers (*atpB*, *psbA*, *rbcL*, *rps4* & *rps4-trnS*, and *trnL* & *trnL-F*). The numbers associated with branches are maximum likelihood bootstrap support (MLBS) and Bayesian posterior probability (BIPP). The asterisk indicates MLBS = 100, BIPP = 1.00. The subclades are indicated following Xu et al. (2018b).

entire and veins terminating just below marginal teeth (vs. pinna-marginal teeth retuse to emarginate and veins terminating just below these notches).

Description. Plants perennial, 20–36 cm. Rhizome long-creeping, ca. 2 mm in diam., apex scaly; scales dark brown, lanceolate or narrowly triangular, 0.5–1 × 0.2–0.3 mm, margins entire; roots yellowish brown when dried. Fronds remote, 10–12 mm apart, subglabrous; stipe shiny, black purple, 8–13 cm long, base ca. 2–3 mm in diam.,

with scales similar to those on rhizome; lamina herbaceous, once pinnate, narrowly oblong to lanceolate, 13–16 × 3–5 cm, base truncate and slightly reduced, apex acuminate to caudate; rachis 0.5–1 mm in diam., wingless, narrowly grooved adaxially, shiny, glabrous, black purple to dark purple; pinnae 15–21 pairs, trapeziform to trapeziform-lunate, basal pinnae nearly opposite, spreading or slightly ascending, upper pinnae alternate, extremely ascending, middle pinnae alternate, ascending, 2.5–3 × 0.6–1 cm, dimidiate, pinna asymmetrical, base largest, upper part of pinna enlarged, similar width as, or slightly wider than, the middle part of pinna, apex obtuse to rounded, acroscopic margins curved and irregularly toothed, teeth entire, basiscopic margins truncate or slightly curved and entire (Fig. 1). Veins visible on both sides of pinnae, free, forking and terminating in marginal teeth, 3–4 basal basiscopic veins lacking. Sori medial, linear or semi-elliptic, 5–8 pairs per pinna (Fig. 1), 2–3 mm long; indusia persistent, brown, linear or semi-elliptic, membranous, entire, opening toward costa. Spores elliptic to reniform, perispore fimbriate-alate, 43–47 μm in diam. (Fig. 1); 32 spores per sporangium.

Distribution and conservation assessment. *Hymenasplenium tholiformis* is endemic to Medog County. Currently, only one large population with ca. 35 individuals was found. According to IUCN Red List criteria B2a or D (IUCN 2022), this species should be listed as critically endangered (CR). More extensive fieldwork at low elevations in nearby mountains will be needed to accurately assess its conservation status.

Ecology. *Hymenasplenium tholiformis* was observed in a shady place at the bottom of a large rock in the disturbed forest, at an elevation of 720 m, ca. 600 m from the Yarlung Zangbo River. High humidity and cool conditions are important for the growth of the new species.

Etymology. The specific epithet alludes to dome shape of pinna apex.

Vernacular name. yuan ding mo ye tie jiao jue (圆顶膜叶铁角蕨; Chinese name).

Comments. In Medog County, ferns are highly diverse along the Yarlung Zangbo River and its tributaries. In this region, at elevations between 650 m and 4500 m, we have discovered four species in three subclades of *Hymenasplenium*, including *H. cheilosorum* (Kunze ex Mett.) Tagawa in the *H. cheilosorum* subclade, *H. obliquissimum* (Hayata) Sugim. in the *H. obliquissimum* subclade, and *H. excisum* and *H. tholiformis* in the *H. excisum* subclade. Of the four species, *H. tholiformis* is distributed at the lowest elevation, while *H. obliquissimum* is at the highest elevation between 2100 m to 2250 m.

Acknowledgements

The research was supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0502) and the National Natural Science Foundation of China (#32100167). We thank Dr. Bo Xu and Dr. Meng Li for their assistance in the field.

References

Akaike H (1974) A new look at the statistical model identification. Automatic Control, IEEE Transactions 19: 716–723. https://doi.org/10.1109/TAC.1974.1100705

Chang Y-F, Hori K, Murakami N, Cao L-M, Lu SG, Schneider H (2018) Validation of *Hymenasplenium laterepens* (Aspleniaceae): Evidence from morphology and molecular analyses. Phytotaxa 374(4): 277–290. https://doi.org/10.11646/phytotaxa.374.4.1

Ching R-C, Ling Y-X (1984a) A supplement to the Flora of Xizang (Tibet) (1). Acta Pytotaxonomica Snica 22: 193–204.

Ching R-C, Ling Y-X (1984b) A supplement to the Flora of Xizang (Tibet) (2). Acta Pytotaxonomica Snica 22: 397–408.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): e772. https://doi.org/10.1038/nmeth.2109

Fan X-P, Zhang L, Zhang L-B, Zhang L (2021) *Selliguea wusugongii* (Polypodiaceae), a new fern species from southeastern Xizang, China based on morphological and molecular evidence. Phytotaxa 480(1): 57–68. https://doi.org/10.11646/phytotaxa.480.1.5

Felsenstein J (1973) Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. Systematic Biology 22(3): 240–249. https://doi.org/10.1093/sysbio/22.3.240

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

IUCN (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Li J-W, Tan Y-H, Wang X-L, Wang C-W, Jin X-H (2018) *Begonia medogensis*, a new species of Begoniaceae from Western China and Northern Myanmar. PhytoKeys 103: 13–18. https://doi.org/10.3897/phytokeys.103.25392

Li J-W, Ya J-D, Ye D-P, Liu C, Liu Q, Pan R, He Z-X, Pan B, Cai J, Lin D-L, Jin X-H (2021) Taxonomy notes on *Vandeae* (Orchidaceae) from China: Five new species and two new records. Plant Diversity 43(5): 379–389. https://doi.org/10.1016/j.pld.2021.01.009

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, 1–8. https://doi.org/10.1109/GCE.2010.5676129

Murakami N (1995) Systematics and evolutionary biology of the fern genus *Hymenasplenium* (Aspleniaceae). Journal of Plant Research 108(2): 257–268. https://doi.org/10.1007/BF02344351

Murakami N, Moran RC (1993) Monograph of the neotropical species of *Asplenium* sect. *Hymenasplenium* (Aspleniaceae). Annals of the Missouri Botanical Garden 80(1): 1–38. https://doi.org/10.2307/2399820

Pol D (2004) Empirical problems of the hierarchical likelihood ratio test for model selection. Systematic Biology 53(6): 949–962. https://doi.org/10.1080/10635150490888868

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53(5): 793–808. https://doi.org/10.1080/10635150490522304

PPG I (2016) A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54(6): 563–603. https://doi.org/10.1111/jse.12229

Qiu Y-L, Zhang L, Zhang L-B, Zhou X-M, Zhang L (2022) *Athyrium aberrans* (Athyriaceae), a new species of the lady ferns from southeastern Xizang, China, based on morphological and molecular evidence. Phytotaxa 533(3): 165–172. https://doi.org/10.11646/phytotaxa.533.3.2

Schneider H, Liu H-M, Chang YF, Ohlsen D, Perrie LR, Shepherd L, Kessler M, Karger DN, Hennequin S, Marquardt J, Russell S, Ansell S, Lu NT, Kamau P, Lóriga J, Regalado L, Heinrichs J, Ebihara A, Smith AR, Gibby M (2017) Neo-and Paleopolyploidy contribute to the species diversity of *Asplenium* – The most species-rich genus of ferns. Journal of Systematics and Evolution 55(4): 353–364. https://doi.org/10.1111/jse.12271

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57(5): 758–771. https://doi.org/10.1080/10635150802429642

Sun H, Zhou Z (1996) The characters and origin of the flora from the big bend gorge of Yalutsangpu (Brahmabutra) river, eastern Himalyas. Yunnan Zhi Wu Yan Jiu 18: 185–204.

Thiers B (2016) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. 68: 324–333. https://doi.org/10.1007/s12228-016-9423-7

Tian D-K, Wang W-G, Dong L-N, Xiao Y, Zheng M-M, Ge B-J (2021) A new species (*Begonia giganticaulis*) of Begoniaceae from southern Xizang (Tibet) of China. PhytoKeys 187: 189–205. https://doi.org/10.3897/phytokeys.187.75854

Wei H-J, Zhou X-L, Jin D-M, Yan Y-H (2018) New records of pteridophytes in Xizang. Guihaia 38: 397–407.

Wu Z-Y (1983) Flora Xizangica Vol.1. Science Press, Beijing.

Xu K-W, Zhang L, Lu NT, Zhou X-M, He H, Luong TT, Knapp R, Liao W-B, Zhang L-B (2018a) Nine new species of *Hymenasplenium* (Aspleniaceae) from Asia. Phytotaxa 358(1): 1–25. https://doi.org/10.11646/phytotaxa.358.1.1

Xu K-W, Zhou X-M, Yin Q-Y, Zhang L, Lu NT, Knapp R, Luong TT, He H, Fan Q, Zhao W-Y, Gao X-F, Liao W-B, Zhang L-B (2018b) A global plastid phylogeny uncovers extensive cryptic speciation in the fern genus *Hymenasplenium* (Aspleniaceae). Molecular Phylogenetics and Evolution 127: 203–216. https://doi.org/10.1016/j.ympev.2018.05.021

Xu K-W, Chen C-W, Kamau P, Liao W-B, Zhang L-B (2019a) Four new species of the fern genus *Hymenasplenium* (Aspleniaceae) from Africa and Asia. Phytotaxa 416(1): 34–42. https://doi.org/10.11646/phytotaxa.416.1.4

Xu K-W, Lorence D, Wood KR, Liao W-B, Zhang L-B (2019b) A revision of the *Hymenasplenium unilaterale* subclade (Aspleniaceae; Pteridophyta) with the description of nine new species. Phytotaxa 419(1): 1–27. https://doi.org/10.11646/phytotaxa.419.1.1

Ya J-D, Zhang T, Pandey TR, Liu C, Han Z-D, Ye D-P, He D-M, Liu Q, Yang L, Huang L, Zhang R-Z, Jiang H, Cai J (2021) New contributions to Goodyerinae and Dendrobiinae

(Orchidaceae) in the flora of China. Plant Diversity 43(5): 362–378. https://doi.org/10.1016/j.pld.2021.05.006

Zhang X-C, Shen Z-H, Dong S-Y (2004) Additions to the pteridophyte flora of Xizang (II). Bulletin of Botanical Research 24: 274–277.

Zhou Z, Guo Y-J, Yue J-P, Chen H-L, Tan Y-H, Sun H (2021) *Amblyanthus chenii* (Primulaceae), a new species and generic record from Xizang, China. Systematic Botany 46(1): 152–161. https://doi.org/10.1600/036364421X16128061711250

Appendix I

Voucher specimens and GenBank accession numbers for DNA sequences used in this study. Information is presented in the following order: species, voucher, locality, GenBank numbers for *rbcL*, *trnL* intron & *trnL-F* spacer, *rps4* & *rps4-trnS*, *atpB*, *psbA*. "*" represents the newly published sequences in this study; "–" no data. Herbaria acronyms follow Index Herbariorum (Thiers 2016).

Asplenium aegaeum Lovis, Reichst. & Greuter in Reichst.; Jermy 9181 (BM); Crete; AY300103 (Schneider et al. 2005); AY300050 (Schneider et al. 2005); AY549774 (Schneider et al. 2005); –; –. ***A. aureum*** Cav.; Hughes 64 (BM); Belize; AF240651 (Pinter et al. 2002); AF240667 (Pinter et al. 2002); AY549759 (Pinter et al. 2002); –; –. ***A. cuspidatum*** Lam.; Grantham & Parsons 0233090 (UC); Costa Rica; AY300111 (Schneider et al. 2004); AY300058 (Schneider et al. 2004); AY549760 (Schneider et al. 2004); –; –. ***A. dielfalcatum*** Viane; Wood 7826 (PTBG); Hawaii; AY549738 (Schneider et al. 2005); AY549841 (Schneider et al. 2005); AY549787 (Schneider et al. 2005); –; –. ***A. erosum*** Maxon; Unknow; –; KX397706 (Germain-Aubrey,C. C et al., Unpublished); –; –; –; –. ***A. flabellifolium*** Cav.; Holmes 4/4/99 (BM); Australia; AY300115 (Schneider et al. 2005); AY300062 (Schneider et al. 2005); AY549779 (Schneider et al. 2005); –; –. ***A. hastatum*** Klotzsch ex Kunze; Viane 10182C; Merida, Venezuela; GU929869 (Leroux et al. 2011); –; –; –; –. ***A. juglandifolium*** Lam.; Viane 10667; Puerto Rico; GU929870 (Leroux et al. 2011); –; –; –; –. ***A. normale*** D. Don; Ohlsen 296 (MELU); Queensland, Australia; KP774926 (Ohlsen et al. 2015); KP851904 (Ohlsen et al. 2015); KP835419 (Ohlsen et al. 2015); –; –. ***A. polyodon*** G. Forst; Perrie NC 124 (WELT); New Caledonia; KP774900 (Ohlsen et al. 2015); KP835397 (Ohlsen et al. 2015); KP835433 (Ohlsen et al. 2015); –; –. ***A. subglandulosum*** subsp. ***papaverifolium*** (Kunze) Salvo, Prada & Consuelo Díaz; Prada & Consuelo Díaz Ohlsen 442 (MELU); Victoria, Australia; KP774929 (Ohlsen et al. 2015); KP851909 (Ohlsen et al. 2015); KP835456 (Ohlsen et al. 2015); –; –. ***A. tenerum*** G. Forst; Ohlsen 265 (MELU); Queensland, Australia; KP774858 (Ohlsen et al. 2015); KP835346 (Ohlsen et al. 2015); KP835437 (Ohlsen et al. 2015); –; –. ***A. trichomanes*** subsp. ***inexpectans*** Lovis; Vogel I-46-B04; France; AY549743 (Schneider et al. 2005); AY549846 (Schneider et al. 2005); AY549792 (Schneider et al. 2005); –; –. ***A. varians*** Wall. ex Hook. & Grev.; Fraser-Jenkins 10046–10047 (BM); China; AY300147 (Schneider et al. 2005); AY300094 (Schneider et al. 2005); –; –; –. ***A. wrightii*** D. C. Eaton ex Hook.; Cranfill TW040 (UC); Taiwan, China; AY549730 (Schneider et al. 2005);

AY549833 (Schneider et al. 2005); AY549766 (Schneider et al. 2005); –; –. ***A. yunnanense*** Franch.; Fraser-Jenkins 10044–10045 (BM); China; AY300149 (Schneider et al. 2005); AY300096 (Schneider et al. 2005); AY549803 (Schneider et al. 2005); –; –. ***Hymenasplenium adiantifrons*** (Hayata) Viane & S. Y. Dong; Knapp 3609 (P); Taiwan, China; –; MH065559 (Xu et al. 2018); MH065322 (Xu et al. 2018); –; –. ***H. adiantifrons***; Knapp 3904 (P); Taiwan, China; –; MH065560 (Xu et al. 2018); MH065323 (Xu et al. 2018); –; –. ***H. apogamum*** N. Murak. & Hatan.; Zhang et al. 7750 (CDBI, MO, VNMN); Thua Thien-Hue, Vietnam; MH065437 (Xu et al. 2018); MH065604 (Xu et al. 2018); MH065376 (Xu et al. 2018); MH065518 (Xu et al. 2018); –. ***H. apogamum***; Chang 1040 (HITBC); Yunnan, China; MH884808 (Chang et al. 2018); –; MH884830 (Chang et al. 2018); –; MH884838 (Chang et al. 2018). ***H. cardiophyllum*** (Hance) Nakaike; Sysu 2; Cult. in Sun-yat Sen University; MH065387 (Xu et al. 2018); MH065534 (Xu et al. 2018); MH065306 (Xu et al. 2018); MH065454 (Xu et al. 2018); –. ***H. cardiophyllum***; Zhang et al. 6588 (CDBI, MO, VNMN); Lang Son, Vietnam; –; MH065584 (Xu et al. 2018); –; MH065501 (Xu et al. 2018); –. ***H. cheilosorum*** (Kunze ex Mett.) Tagawa; Xu GX022 (SYS); Guangxi, China; MH065381 (Xu et al. 2018); –; MH065343 (Xu et al. 2018); MH065448 (Xu et al. 2018); –. ***H. cheilosorum***; Xu 102 (SYS); Hainan, China; MH065385 (Xu et al. 2018); –; MH065346 (Xu et al. 2018); MH065452 (Xu et al. 2018); –. ***H. cheilosorum***; Xu 299 (SYS); Guangxi, China; MH065402 (Xu et al. 2018); –; MH065350 (Xu et al. 2018); MH065473 (Xu et al. 2018); –. ***H. cheilosorum***; Xu 322 (SYS); Yunnan, China; MH065405 (Xu et al. 2018); –; MH065352 (Xu et al. 2018); MH065476 (Xu et al. 2018); –. ***H. cheilosorum***; Zhang et al. 8629 (CDBI, MO, PHH); Lam Dong, Vietnam; MH065415 (Xu et al. 2018); –; MH065357 (Xu et al. 2018); MH065487 (Xu et al. 2018); –. ***H. cheilosorum***; Jin & Zhang 11348 (CDBI); Yunnan, China; –; MH065571 (Xu et al. 2018); MH065359 (Xu et al. 2018); MH065489 (Xu et al. 2018); –. ***H. chingii*** K. W. Xu, Li Bing Zhang & W. B. Liao; Jin & Zhang 11347 (CDBI); Yunnan, China; MH065428 (Xu et al. 2018); MH065586 (Xu et al. 2018); MH065364 (Xu et al. 2018); MH065502 (Xu et al. 2018); –. ***H. chingii***; Zhang et al. 9455 (CDBI); Guizhou, China; MH065430 (Xu et al. 2018); MH065588 (Xu et al. 2018); MH065333 (Xu et al. 2018); MH065504 (Xu et al. 2018); –. ***H. delitescens*** (Maxon) L. Regalado & Prada; Jones 1078 (MO); Panama; MH065443 (Xu et al. 2018); MH065609 (Xu et al. 2018); MH065338 (Xu et al. 2018); MH065522 (Xu et al. 2018); –. ***H. distans*** Li Bing Zhang, K. W. Xu & Liang Zhang; Zhang et al. 6296 (CDBI, MO, VNMN); Hoa Binh, Vietnam; –; MH065591 (Xu et al. 2018); MH065335 (Xu et al. 2018); MH065507 (Xu et al. 2018); –. ***H. excisum*** (C. Presl) S. Linds.; Brownsey & Perrie FIJI 190 (WELT); Fiji; KP774884 (Ohlsen et al. 2015); KP851914 (Ohlsen et al. 2015); KP851882 (Ohlsen et al. 2015); –; –. ***H. excisum***; Zhang et al. 8039 (CDBI, MO, VNMN); Quang Nam, Vietnam; MH065419 (Xu et al. 2018); MH065573 (Xu et al. 2018); MH065361 (Xu et al. 2018); –; –. ***H. excisum***; Xu 100 (SYS); Hainan, China; MH065383 (Xu et al. 2018); MH065531 (Xu et al. 2018); MH065344 (Xu et al. 2018); MH065450 (Xu et al. 2018); –. ***H. excisum***; Xu 110 (SYS); Hainan, China; MH065384 (Xu et al. 2018); MH065532 (Xu et al. 2018); MH065345 (Xu et al. 2018); MH065451 (Xu et al.

2018); –. ***H. excisum***; Xu 198 (SYS); Hainan, China; MH065394 (Xu et al. 2018); MH065541 (Xu et al. 2018); MH065341 (Xu et al. 2018); MH065462 (Xu et al. 2018); –. ***H. excisum***; Zhang et al. 6714 (CDBI, MO, VNMN); Bac Kan, Vietnam; MH065436 (Xu et al. 2018); MH065603 (Xu et al. 2018); MH065375 (Xu et al. 2018); MH065517 (Xu et al. 2018); –. ***H. filipes*** (Copel.) Sugim., Murakami 596902 (TI); Kagoshima, Japan; U30605 (Hasebe et al. 1995); –; –; –; –. ***H. filipes***; Murakami 92-J012; Kagoshima, Japan; AB016176 (Murakami et al. 1998); –; –; –; –. ***H. furfuraceum*** (Ching) Viane & S. Y. Dong; Xu 304 (SYS); Yunnan, China; MH065406 (Xu et al. 2018); MH065553 (Xu et al. 2018); MH065353 (Xu et al. 2018); MH065477 (Xu et al. 2018); –. ***H. furfuraceum***; Xu 307 (SYS); Yunnan, China; MH065409 (Xu et al. 2018); MH065557 (Xu et al. 2018); MH065320 (Xu et al. 2018); MH065481 (Xu et al. 2018); –. ***H. furfuraceum***; Chang1072 (HITBC); Yunnan, China; MW194198 (Zhang et al. 2021); –; MW194221 (Zhang et al. 2021); –; MW194182 (Zhang et al. 2021). ***H. hastifolium*** Ke Wang Xu, Li Bing Zhang &W. B. Liao; Xu 282-2 (SYS); Guangxi, China; MH065397 (Xu et al. 2018); MH065546 (Xu et al. 2018); MH065312 (Xu et al. 2018); MH065468 (Xu et al. 2018); –. ***H. hastifolium***; Xu 282-1 (SYS); Guangxi, China; MH065398 (Xu et al. 2018); MH065547 (Xu et al. 2018); MH065313 (Xu et al. 2018); MH065469 (Xu et al. 2018); –. ***H. hoffmannii*** (Hieron.) L. Regalado & Prada; Croat & Zhu 76985 (MO); Canal, Panama; MH065441 (Xu et al. 2018); MH065607 (Xu et al. 2018); MH065378 (Xu et al. 2018); –; –. ***H. hoffmannii***; Monro & Douglas 3463 (MO); Santa Ana, El Salvador; MH065442 (Xu et al. 2018); MH065608 (Xu et al. 2018); MH065337 (Xu et al. 2018); MH065521 (Xu et al. 2018); –. ***H. hondoense*** (N.Murak. & Hatan.) Nakaike; Murakami 596920 (KYO); Kouchi, Japan; AB014705 (Murakami et al. 1999); –; –; –; –. ***H. hondoense***; Chang1062 (HITBC); Yunnan, China; MH884814 (Chang et al. 2018); –; MH884833 (Chang et al. 2018); –; MH884840 (Chang et al. 2018). ***H. laetum*** (Sw.) L. Regalado & Prada; Jones 1155 (TUR); –; –; –; –; KM114105 (Lehtonen,S et al., Unpublished); –. ***H. laterepens*** N. Murak. & X. Cheng ex Y. Fen Chang & K. Hori; Chang 2655 (HITBC); Yunnan, China; MH884806 (Ohlsen et al. 2015); –; –; –; –. ***H. laterepens***; Chang 1039 (HITBC); Yunnan, China; MH884807 (Chang et al. 2018); –; MH884829 (Chang et al. 2018); –; MH884837 (Chang et al. 2018). ***H. latidens*** (Ching) Viane & S. Y. Dong; Knapp 3672 (P); Taiwan, China; –; MH065566 (Xu et al. 2018); –; –; –. ***H. latidens***; Xu 309 (SYS); Yunnan, China; MH065407 (Xu et al. 2018); MH065554 (Xu et al. 2018); MH065318 (Xu et al. 2018); MH065478 (Xu et al. 2018); –. ***H. latidens***; Y.-F. Chang1029 (HITBC); Yunnan, China; MW194204 (Zhang et al. 2021); –; MW194219 (Zhang et al. 2021); –; MW194180 (Zhang et al. 2021). ***H. murakami***-hatanakae Nakaike; Ranker 2072 (COLO); Taiwan, China; EF452140 (Schuettpelz et al. 2007); – (Schuettpelz et al. 2007); – (Schuettpelz et al. 2007); EF452020 (Schuettpelz et al. 2007); –. ***H. murakami-hatanakae*** Nakaike; C.-C. Chen 1089 (HITBC); Taiwan, China; MH884823 (Chang et al. 2018); –; –; –; –. ***H. neocaledonicum*** Li Bing Zhang & K. W. Xu; PerrieNC177 (WELT); New Caledonia; KP774896 (Ohlsen et al. 2015); KP851915, (Ohlsen et al. 2015); KP851878 (Ohlsen et al. 2015); –; –. ***H. ngheanense*** Li Bing Zhang, K. W. Xu & N. T. Lu; Zhang et al. 6532 (CDBI, MO, VNMN); Phu Tho,

Vietnam; MH065426 (Xu et al. 2018); MH065583 (Xu et al. 2018); MH065331 (Xu et al. 2018); MH065500 (Xu et al. 2018); –. ***H. ngheanense***; Zhang et al. 7163 (CDBI, MO, VNMN); Nghe An, Vietnam; MH065439 (Xu et al. 2018); MH065605 (Xu et al. 2018); MH065336 (Xu et al. 2018); MH065519 (Xu et al. 2018); –. ***H. obliquissimum*** (Hayata) Sugim.; Murakami & J. Yokoyarna 94-T628; Chiang Mai, Thailand; AB016178 (Murakami et al. 1998); –; –; –; –. ***H. obliquissimum***; lwatsuki et al. 94-V302; Hoang Lien Son, Vietnam; AB016187 (Murakami et al. 1998); –; –; –; –. ***H. obliquissimum***; Ju & Deng 12689 (CDBI); Sichuan, China; –; MH065597 (Xu et al. 2018); MH065370 (Xu et al. 2018); MH065511 (Xu et al. 2018); –. ***H. obliquissiumum***; Chang1292 (HITBC); Tibet, China; MW194212 (Zhang et al. 2021); –; MW194232 (Zhang et al. 2021); –; MW194192 (Zhang et al. 2021). ***H. obscurum*** (Blume) Tagawa; Xu 324 (SYS); Yunnan, China; MH065404 (Xu et al. 2018); MH065552 (Xu et al. 2018); MH065351 (Xu et al. 2018); MH065475 (Xu et al. 2018); –. ***H. obscurum***; Zhang et al. 7715 (CDBI, MO, VNMN); Thanh Hoa, Vietnam; MH065411 (Xu et al. 2018); MH065567 (Xu et al. 2018); MH065354 (Xu et al. 2018); MH065483 (Xu et al. 2018); –. ***H. obscurum***; Wu et al. WS2176 (MO); Xiangkhoang, Laos; ON859869 *; ON859878 *; ON859875;ON859872 *; –. ***H. obscurum***; Wu et al. WS2552 (MO); Louangphrabang, Laos; ON859870 *; ON859879 *; ON859876*;ON859873 *; –. ***H. perriei*** Li Bing Zhang & K. W. Xu; Brownsey & Perrie FIJI 13 (WELT); Fiji; KP774885 (Ohlsen et al. 2015); KP851918 (Ohlsen et al. 2015); KP851880 (Ohlsen et al. 2015); –; –. ***H. phamhoanghoi*** Li Bing Zhang, K. W. Xu & T. T. Luong; Zhang et al. 8819 (CDBI, MO, PHH); Khanh Hoa, Vietnam; MH065432 (Xu et al. 2018); MH065590 (Xu et al. 2018); MH065334 (Xu et al. 2018); MH065506 (Xu et al. 2018); –. ***H. pseudobscurum*** Viane; Knapp 4133 (P); Taiwan, China; –; MH065565 (Xu et al. 2018); –; –; –. ***H. pseudobscurum***; Xu 004 (SYS); Hong Kong, China; MH065380 (Xu et al. 2018); MH065530 (Xu et al. 2018); MH065342 (Xu et al. 2018); MH065447 (Xu et al. 2018); –. ***H. pseudobscurum***; Fraser-Jenkins30715; Bhutan; MH884826 (Chang et al. 2018); –; MH884834 (Chang et al. 2018); –; –. ***H. pseudobscurum***; Chang 1010 (HITBC); Yunnan, China; MH884827 (Chang et al. 2018); –; MH884835 (Chang et al. 2018); –; MH884841 (Chang et al. 2018). ***H. pseudobscurum***; Chang 1047 (HITBC); Yunnan, China; MH884828 (Chang et al. 2018); –; MH884836 (Chang et al. 2018); –; MH884842 (Chang et al. 2018). ***H. quangnamense*** Li Bing Zhang, K. W. Xu & Liang Zhang; Zhang et al. 7884 (CDBI, MO, VNMN); Quang Nam, Vietnam; MH065412 (Xu et al. 2018); MH065568 (Xu et al. 2018); MH065355 (Xu et al. 2018); MH065484 (Xu et al. 2018); –. ***H. quangnamense***; Schuettpelz 874; Malay Peninsula; MH884824 (Chang et al. 2018); –; –; –; –. ***H. queenslandicum*** Li Bing Zhang & K. W. Xu; Ohlsen 252 (MELU); Queensland, Australia; KP774849 (Ohlsenet al. 2015); KP851916 (Ohlsenet al. 2015); KP851879 (Ohlsen et al. 2015); –; –. ***H. quercicola*** (Ching) Viane & S. Y. Dong; Chang1064 (HITBC); Yunnan, China; MW194205 (Zhang et al. 2021); –; MW194220 (Zhang et al. 2021); –; MW194181 (Zhang et al. 2021). ***H. retusulum*** (Ching) Viane & S. Y. Dong; Jiang 00371 (SYS); Yunnan, China; MH065379 (Xu et al. 2018); MH065529 (Xu et al. 2018); MH065304 (Xu et al. 2018); MH065446 (Xu et al. 2018); –. ***H. retusulum***; Xu 311 (SYS); Yunnan, China;

MH065408 (Xu et al. 2018); MH065556 (Xu et al. 2018); MH065319 (Xu et al. 2018); MH065480 (Xu et al. 2018); –. ***H. riparium*** (Liebm.) L. Regalado & Prada; Murakami & Grayum 281 (KYO); Virgen del Socorro, Costa Rica; AB014708 (Murakami et al. 1999); –; –; –; –. ***H. sinense*** K. W. Xu, Li Bing Zhang & W. B. Liao; Xu PB001 (SYS); Yunnan, China; MH065386 (Xu et al. 2018); MH065533 (Xu et al. 2018); rps4 & MH065347 (Xu et al. 2018); MH065453. (Xu et al. 2018); –. ***H. sinense***; Xu 134 (SYS); Jiangxi, China; MH065388 (Xu et al. 2018); MH065535 (Xu et al. 2018); MH065348 (Xu et al. 2018); MH065455 (Xu et al. 2018); –. ***H. sinense***; Xu GX018 (SYS); Guangxi, China; –; –; MH065349 (Xu et al. 2018); MH065465 (Xu et al. 2018); –. ***H. speluncicola*** Li Bing Zhang, K. W. Xu & H. He; Zhang et al. 5494 (CDBI, MO); Guangxi, China; –; MH065592 (Xu et al. 2018); MH065366 (Xu et al. 2018); MH065508 (Xu et al. 2018); –. ***H. speluncicola***; Zhang et al. 6035 (CDBI); Guizhou, China; –; MH065594 (Xu et al. 2018); MH065368 (Xu et al. 2018); MH065509 (Xu et al. 2018); –. ***H. subnormale*** (Copel.) Nakaike; Knapp 4525 (P); Taiwan, China; –; MH065612 (Xu et al. 2018); –; MH065526 (Xu et al. 2018); –. ***H. subnormale***; Knapp 4509 (P); Taiwan, China; –; MH065613 (Xu et al. 2018); –; MH065527 (Xu et al. 2018); –. ***H. szechuanense*** (Ching) Viane & S. Y. Dong; Chang1099 (HITBC); Sichuan, China; MW194207 (Zhang et al. 2021); –; MW194223 (Zhang et al. 2021); –; MW194183 (Zhang et al. 2021). ***H. szechuanense***; Chang1098 (HITBC); Sichuan, China; MW194206 (Zhang et al. 2021); –; MW194222 (Zhang et al. 2021); –; MW194193 (Zhang et al. 2021). ***H. tholiformis*** Liang Zhang, W.B. Ju & K.W. Xu; Zhang Liang 4781 (KUN); Tibet, China; ON859868 *; ON859877 *; –; ON859871 *; ON859874 *. ***H. triquetrum*** (N. Murak. & R. C. Moran) L. Regalado & Prada; Sylvestre 2208 (RB); Brazil; KT329398 (Mynssen et al. 2016); –; –; –; –. ***H. triquetrum***; Jimenez 5153 (MO); La Paz, Bolivia; MH065444 (Xu et al. 2018); MH065610 (Xu et al. 2018); MH065339 (Xu et al. 2018); MH065523 (Xu et al. 2018); –. ***H. unilaterale*** (Lam.) Hayata; Viane 8344; Reunion; GU929873 (Leroux et al. 2011); –; –; –; –. ***H. unilaterale***; Hemp A. 18 (BM); Kenya; AF240652 (Pinter et al. 2002); AF525232 (Pinter et al. 2002); –; –; –. ***H. vanuatuense*** Li Bing Zhang & K. W. Xu; Ohlsen 392 (MELU); Tanna, Vanuatu; KP774898 (Ohlsen et al. 2015); KP851917 (Ohlsen et al. 2015); KP851881 (Ohlsen et al. 2015); –; –. ***H. wangpeishanii*** Li Bing Zhang & K. W. Xu; Zhang et al. 9665 (CDBI); Guizhou, China; MH065423 (Xu et al. 2018); MH065577 (Xu et al. 2018); –; MH065494 (Xu et al. 2018); –. ***H. wangpeishanii***; Zhang et al. 9455 (CDBI); Guizhou, China; MH065430 (Xu et al. 2018); MH065588 (Xu et al. 2018); MH065333 (Xu et al. 2018); MH065504 (Xu et al. 2018); –. ***H. wildii*** (Bail.) D.J.Ohlsen; Ohlsen 246 (MELU); Queensland, Australia; KP774927 (Ohlsen et al. 2015); KP851919 (Ohlsen et al. 2015); KP851877 (Ohlsen et al. 2015); –; –. ***H. wuliangshanense*** (Ching) Viane & S. Y. Dong; Y.-F. Chang1102 (HITBC); Yunnan, China; MW194208 (Zhang et al. 2021); –; MW194224 (Zhang et al. 2021); –; MW194184 (Zhang et al. 2021). ***H. wuliangshanense***; Chang1104 (HITBC); Yunnan, China; MW194209 (Zhang et al. 2021); –; MW194225 (Zhang et al. 2021); –; MW194185 (Zhang et al. 2021).

PhytoKeys 204: 57–72 (2022)
doi: 10.3897/phytokeys.204.85256
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

A new phylogeny of *Rumex* (Polygonaceae) adds evolutionary context to the diversity of reproductive systems present in the genus

Kirstie D. Grant[1], Daniel Koenemann[2], Janet Mansaray[3], Aisha Ahmed[4], Hamid Khamar[5,6], Jalal El Oualidi[5], Janelle M. Burke[2]

1 *Department of Biological Sciences, Florida Agricultural & Mechanical University, Tallahassee, FL, USA* **2** *Department of Biology, Howard University, Washington, DC, USA* **3** *Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA* **4** *College of Medicine, Howard University, Washington, DC, USA* **5** *Département de Botanique et Ecologie Végétale, Institut Scientifique, Université Mohammed V de Rabat, Rabat, Morocco* **6** *Université Ibn Tofail, Faculté des Sciences, Laboratoire de Botanique et de Protection des plantes, B.P. 133, Kénitra, Morocco*

Corresponding authors: Kirstie D. Grant (kirstie.grant@famu.edu), Janelle M. Burke (Janelle.burke@howard.edu)

Academic editor: Bo Li | Received 22 April 2022 | Accepted 12 July 2022 | Published 5 August 2022

Citation: Grant KD, Koenemann D, Mansaray J, Ahmed A, Khamar H, El Oualidi J, Burke JM (2022) A new phylogeny of *Rumex* (Polygonaceae) adds evolutionary context to the diversity of reproductive systems present in the genus. PhytoKeys 204: 57–72. https://doi.org/10.3897/phytokeys.204.85256

Abstract

Rumex is one of about 50 genera in the knotweed family, Polygonaceae. The genus comprises about 200 species with bisexual, or more commonly, unisexual flowers, with the species displaying monoecious, dioecious, synoecious (hermaphroditic) or polygamous reproductive systems. Some of the dioecious species have heteromorphic sex chromosomes, which is rare amongst angiosperms. We here present a plastid phylogeny of 67 species, representing all four subgenera. For this study, we used three chloroplast markers, *rbcL*, *trnH-psbA*, *trnL-F* and dense taxon sampling to reconstruct the most comprehensive molecular phylogeny of *Rumex* to date. The reconstructed phylogeny for this work resolves six major clades and one large grade in *Rumex* subg. *Rumex*. In addition, the species with known dioecious reproductive systems are resolved within a broader clade we term "the dioecious clade". These results suggest that the species with divergent reproductive systems are more closely related to each other than to other species comprising the rest of the *Rumex* genus.

Keywords

Dioecious, *Emex*, heteromorphic sex chromosome systems, monoecious, synoecious

Introduction

Commonly known as docks and sorrels, *Rumex* L. (Polygonaceae) is a relatively large genus. *Rumex* encompasses four circumscribed subgenera, approximately 200 species and hundreds of described subspecies or varieties. Many species in *Rumex* are cosmopolitan in nature, spanning six continents of the world. However, many individual species are either regionally endemic, native or introduced on particular continents (Rechinger 1937).

The cosmopolitan distribution of *Rumex* species is indicative of their ability to thrive in a wide variety of environmental conditions. Described species are just as recurrent in dry and sandy soils as they are in marshes and cultivated fields, spanning the arctic, subarctic, boreal, temperate, tropical and subtropical localities (Löve and Kapoor 1967). Although several biological species demonstrate little to no niche preference (e.g. *Rumex crispus* L., *Rumex obtusifolius* L.), there are others that exhibit exceedingly precise ecological requirements (e.g. *Rumex bipinnatus* L.f., *Rumex pictus* Forrsk.). The large variation in the distribution of *Rumex* species might also account for the large deviation observed in the morphology of these species (Fig. 1), whereby some reach almost seven metres in height and others rarely exceed a few centimetres (Rechinger 1949; Löve and Kapoor 1967; Rechinger 1990).

In the 20th Century, progress in the taxonomic and cytological study of *Rumex* was largely accomplished by two researchers: Áskell Löve and Karl Heinz Rechinger (Rechinger 1937; Rechinger 1954a; Löve and Kapoor 1967). Löve extensively documented the cytological diversity of *Rumex* and he proposed a generic status for *Acetosa* and *Acetosella* (the groups with species bearing heteromorphic sex chromosomes) and subgeneric status for *Axillares* and *Platypodium*. Löve also considered *Rumex* to be composed of several smaller genera corresponding to a number of cytotypes (Löve 1957; Löve and Kapoor 1967; Mariotti et al. 2006, 2009).

Over the course of his career, Rechinger effectively monographed *Rumex*, using plant morphology and geographic distribution in his taxonomic treatments (Rechinger 1933, 1937, 1939, 1949, 1954a, b, 1984, 1990; Brandbyge and Rechinger 1989). By the mid-1900s, Rechinger had proposed a subgeneric status for *Platypodium* and maintained *Acetosa*, *Acetosella* and *Lapathum* as comparable subgenera (Rechinger 1954a; Table 1). Rechinger chose to retain *Rumex* as a single genus.

The reproductive systems of *Rumex* species vary extensively. Species of *Rumex* exhibit synoecious (hermaphroditic), monoecious, dioecious and polygamous

Table 1. Summary of the recognised subgenera in Rumex, with species diversity and reproductive systems present.

Subgenus	No. of species	Sexual system	Sex chromosomes
Acetosa	41	Dioecious, Gynodioecious, Polygamous	Yes (in part)- XX/XY_1Y_2
Acetosella	5	Dioecious (rarely polygamous)	Yes- XX/XY
Rumex (= *Lapathum*)	126	Synoecious, Monoecious	No
Platypodium	1	Synoecious	No

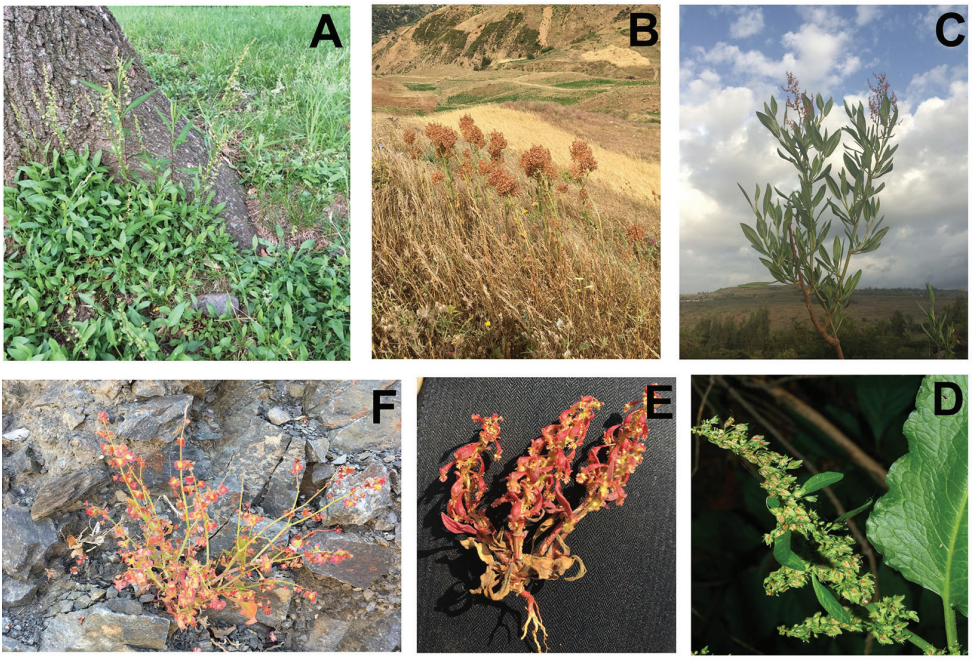


Figure 1. Depiction of morphological variation amongst the different subgenera of *Rumex*. **A** *Rumex acetosella* growing in Virginia, USA (subg. *Acetosella*) **B** *Rumex thyrsoides* growing in Morocco (subg. *Acetosa*) **C** *Rumex nervosus* growing in Ethiopia (subg. *Acetosa*) **D** *Rumex obtusifolius* growing in New York, USA (subg. *Rumex*) **E** *Rumex bucephalophorus* collected on the Moroccan coast (subg. *Platypodium*) **F** *Rumex papilio* growing in Morocco (subg. *Acetosa*). All photo credits J.M. Burke.

reproductive systems (Rechinger 1949; Rechinger 1954a; Löve and Kapoor 1967; Mosyakin 2005; Navajas-Pérez et al. 2005). Most of the reproductive system diversity has been described in subgenera *Acetosa* or *Acetosella*. In particular, most species in these subgenera are dioecious (Rechinger 1937, 1949, 1954a, 1984). A few species in subgenus *Rumex* have variable systems, especially between synoecy and monoecy (e.g. *Rumex crispus*, J. Burke, pers. obs.). There are also three species of *Rumex* endemic to the Hawaiian Islands (*Rumex albescens* Hillebr., *R. giganteus* Aiton and *R. skottsbergii* O. Deg. & I. Deg.), which are all monoecious (Wagner et al. 1999).

Rumex has two different sex chromosome systems exhibited in many of the dioecious species, classified in *Rumex* subg. *Acetosa* and *Rumex* subg. *Acetosella*. In *Rumex*, the documented sex chromosomes are heteromorphic. Two sex-determining chromosomal mechanisms are known: XX/XY and XX/XY_1Y_2 (Löve 1940, 1942,1943, 1944; Löve and Löve 1948; Shibata et al. 1999, 2000; Navajas-Pérez et al. 2005; Cunado et al. 2007; Ming et al. 2011). The XX/XY_1Y_2 system is dosage-dependent and plant sex is based on the autosome to sex-chromosome ratio. In this system, female individuals have 14 chromosomes and male individuals have 15 chromosomes (Löve 1940, 1944; Löve and Kapoor1967; Navajas-Pérez et al. 2005).

Recent molecular phylogenetic work has sought to resolve the placement of *Rumex* in the Polygonaceae more broadly (Sanchez and Kron 2008; Sanchez et al. 2009; Burke et al. 2010; Burke and Sanchez 2011; Sanchez et al. 2011; Schuster et al. 2011, 2013, 2015). These studies have placed *Rumex* alongside the other Rumices of Campderá (*Emex* and *Oxyria*), with the addition of *Rheum* as either sister to *Oxyria* (Burke et al. 2010; Schuster et al. 2011) or to *Rumex* + *Emex* (Schuster et al. 2013, 2015). One area that lacks clarity has been the placement of *Emex*, which sometimes appears to be nested within *Rumex* (e.g. Sanchez et al. 2011) and is sometimes placed as sister to *Rumex* (e.g. Burke et al. 2010). Moreover, the relationships of species within *Rumex*, including the relationship between *Rumex* and *Emex*, continue to be poorly understood due to insufficient sampling and paucity of data. To date, the relationships amongst species placed within Rechinger's subgenus *Rumex* are particularly obscure.

Here we present a new phylogeny of *Rumex*, constructed using three plastid gene regions (*trnH-psbA*, *rbcL* and *trnL-F*) and 67 *Rumex* species. We have used this phylogeny to test the placement and monophyly of its circumscribed subgenera, as well as discuss the broad patterns in the evolution of reproductive systems within *Rumex*.

Materials and methods

Taxon sampling and DNA Isolation

DNA was isolated from 109 accessions, representing 67 *Rumex* species. Of the 109 included accessions, a total of 99 *Rumex* accessions, six *Rheum* L. species, three *Emex* L. accessions and one species of *Persicaria* L. (Mill.) are represented. *Persicaria virginiana* (L.) Gaertn., *Rheum alexandrae* Batalin, *Rheum emodii* Wall., *Rheum nobile* Hook. f. & Thomson, *Rheum officinale* Baill., *Rheum palmatum* L. and *Rheum rhabarbarum* L. were included as outgroup species. Additional plant samples were obtained through the GenBank sequence database (Appendix A1). Samples were taken from a combination of herbarium specimens (K, NY, OSC, RAB, US), field collections and cultivated samples from collaborators. Herbarium acronyms follow the Index Herbariorum (Thiers 2019).

All fresh leaf samples were dried using silica gel. Plant tissue was homogenised using the FastPrep-24 5G Sample Preparation System (M. P. Biomedicals, LLC Santa Ana CA, USA). Total genomic DNA was extracted from herbarium specimen-sampled and silica-dried leaf tissues using a BIOLINE ISOLATE II Plant DNA Kit (Cat No. BIO-52070). Modification for herbarium material proceeded as follows: Cell lysis was carried out using 300 µl of buffer (PA1 or PA2) and 30 µl of proteinase K (20 µg/ml) and incubated for 18 hours at 65 °C on an orbital shaker.

Marker selection

For this first comprehensive phylogeny of the genus, we focused on plastid marker selection. Previous authors utilised nrITS as a nuclear marker (Schuster et al. 2011; Schuster et al. 2015). However, we did not utilise nrITS for this phylogeny due to

a number of issues that would interfere with accurate reconstruction of evolutionary relationships: 1) nrITS is extremely variable and difficult to align (66% of nrITS sequence data was excluded in Schuster et al. [2015] publication) and 2) Due to widespread polyploidy documented in multiple *Rumex* species, sequences of nrITS would not necessarily be low copy and there would be substantial issues with paralogy and orthology across multiple polyploidy events.

For plastid marker selection, we screened multiple markers that had previously been used in Polygonaceae reconstruction (Burke et al. 2010; Burke and Sanchez 2011; Koenemann and Burke 2020). We selected markers that both showed sufficient variation across the genus and were easily amplified for most taxa.

PCR amplification and sequencing

Amplification of DNA markers was completed for three plastid regions: *rbcL*, *trnH-psbA* and *trnL-F* (Table 2). *rbcL* was amplified using the following PCR conditions: 94 °C for 1 min, followed by 34 cycles of 94 °C/15 s, 54 °C/15 s and 72 °C/30 s and a final extension period of 5 min at 72 °C. *trnH-psbA* was amplified using the following PCR conditions: 94 °C for 2 min, followed by 34 cycles of 94 °C/30 s, 55 °C/30 s and 72 °C/30 s and a final extension period of 7 min at 72 °C. *trnL-F* was amplified using the following PCR conditions: 80 °C for 5 min, followed by 34 cycles of 94 °C/1 min, 55 °C/1 min and 72 °C/2 min and a final extension period of 5 min at 72 °C. PCR and gel electrophoresis were performed following standard protocols with no special conditions. PCR experiments were performed separately with only fresh or only herbarium material to help prevent cross-contamination.

Table 2. Gene regions used: name of primers, total length of region, % parsimony informative characters.

Gene region	Reference	Primer names	Total aligned length	PIC (%)
rbcL	Fazekas et al 2008	rbcLF, rbcLR	539	24 (4.5)
trnH-psbA	Shaw 2007	psbA, trnH	596	132 (22.1)
3trnL-F	Shaw 2005	3'trnL^{UAA}F, trnF ^{GAA}	442	65 (14.7)
Combined			1577	221 (14.0)

PCR amplicons were sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing. Sequences were edited using Geneious v. 10 (Biomatters Ltd.). Reviewed sequences were aligned with MUSCLE (Edgar 2004) and concatenated using MESQUITE (Maddison 2005).

Phylogeny reconstruction

All phylogenetic analyses were completed using the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). Prior to the phylogenetic reconstructions, we performed ModelTest-NG (Darriba et al. 2020) for the concatenated matrix to determine the suggested model of evolution. ModelTest-NG indicated that the best fit was the General Time Reversible (GTR) model.

We performed Maximum Likelihood (ML) phylogeny reconstruction using GARLI v. 2.01.1067 (Zwickl 2006). We used the default GARLI parameters with the following exceptions: we performed 1000 search replications (10 iterations of 100 search replicates). In order to better search tree space, we increased the attachments per taxon setting to 150 and extended the generations without improvement parameter to 50000. To evaluate support for phylogenetic relationships, statistical bootstrapping was performed, specifying only one search replicate per bootstrap iteration for 100 iterations. All bootstrap trees were downloaded and used to generate a majority rule consensus tree in MESQUITE (Maddison 2005). The consensus tree was visualised in FigTree version 1.4.3 (Rambaut 2014).

We performed Bayesian Inference phylogeny reconstruction in MrBayes 3.2.7a (Ronquist et al. 2012). The priors were set to the defaults (Dirichlet). We set the seed number at 123. We conducted two independent Markov Chain Monte Carlo (MCMC) runs, each with four chains employing BEAGLE library acceleration (as recommended by CIPRES). Each MCMC run was set to complete 5 million generations, with trees sampled every 1,000 generations. The first 25% of trees in each run were discarded as burn-in. MrBayes then synthesised the two independent runs and extracted the majority rule consensus tree with posterior probabilities.

Posterior probability and bootstrap values were visualised using FigTree version 1.4.3 (Rambaut 2014) and MESQUITE (Maddison 2005). Posterior probabilities above 90% and bootstrap support values above 70% were considered significant and annotated in the final phylogeny.

Results

The most likely tree was generated using 109 specimen accessions. This included seven outgroup species, three accessions of *Emex* and 99 accessions of *Rumex*. The present phylogeny represents 67 *Rumex* species, more than twice the number of species of *Rumex* sampled in previous phylogenies (31 species in Navajas-Pérez et al. 2005; 13 species in Schuster et al. 2015). A total of 47 sequences were missing from the final matrix, yielding 14.4% missing data in the final analysis (Grant 2022). Table 2 summarises the variability of each of the gene regions. The most variable region was *trnH-psbA*, which consisted of 22.1% parsimony informative characters. The least variable region was *rbcL* which consisted of 4.5% parsimony informative characters.

The most likely tree recovered by GARLI received a likelihood score of Ln = -5767.548440.

The genus *Rumex* was recovered as monophyletic with strong support (100 Bayesian Posterior Probability/98 Maximum Likelihood Bootstrap) (Fig. 2). The analysis did not recover *Rumex* subgenus *Rumex*, the subgenus with the most species diversity, as monophyletic. In our phylogeny, species of subgenus *Rumex* form a grade at the base of the tree ("Basal Grade" – Fig. 2). *Emex* was recovered as monophyletic, just above the Basal Grade and sister to the dioecious clade. While the results indicate

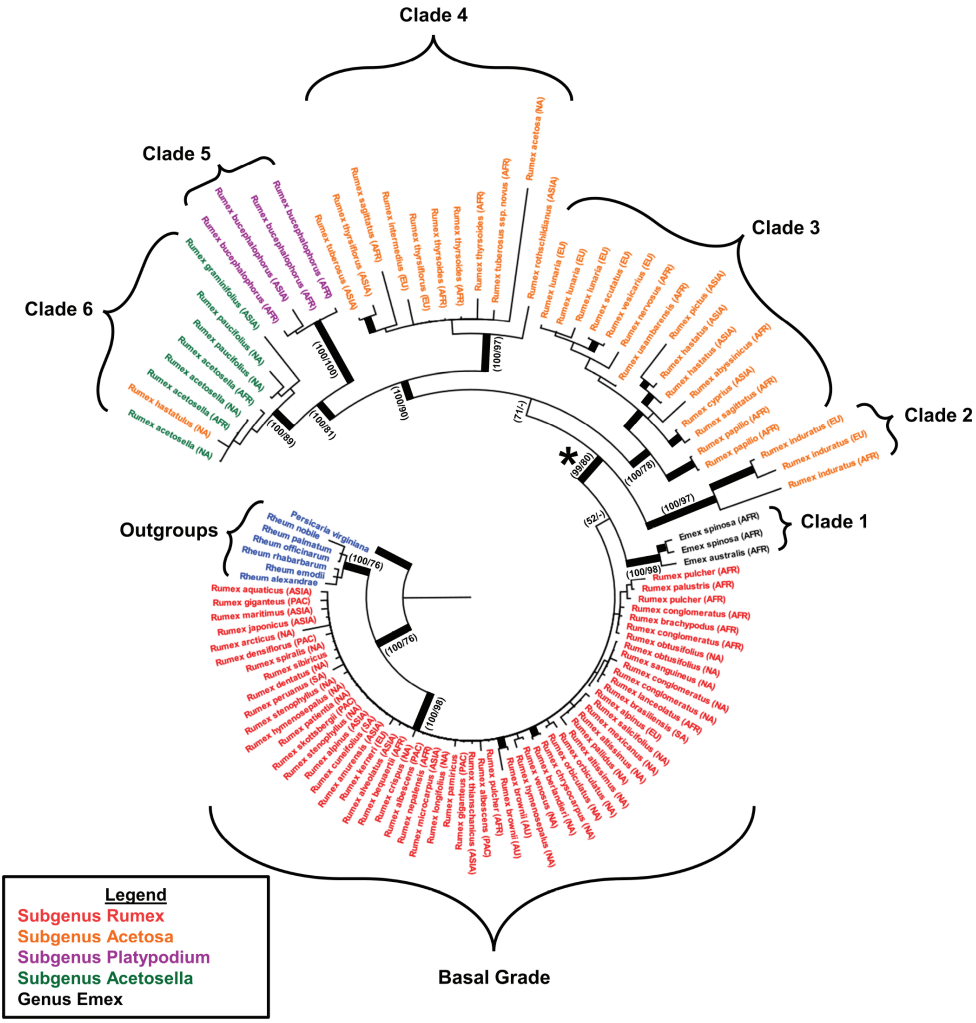


Figure 2. Bayesian phylogenetic reconstruction for *Rumex* species using three chloroplast sequences (*rbcL*, *trnH-psbA* and *trnL-F*). Thickened branch indicates simultaneous posterior probability above 90% and bootstrap support above 70%. Exact support values are indicated at important nodes (Bayesian Posterior Probability/Maximum Likelihood Bootstrap). Outgroup species (*Rheum* and *Persicaria*) are shown in blue. *Rumex* species, traditionally placed in subgenus *Rumex*, are shown in red. Species, traditionally placed in the sister genus *Emex*, are shown in black. *Rumex* species, traditionally placed in subgenus *Acetosa*, are shown in gold. *Rumex* species, traditionally placed in subgenus *Platypodium*, are shown in purple. *Rumex* species, traditionally placed in subgenus *Acetosella*, are shown in green. The asterisk denotes the transition to dioecy "Dioecious Clade" referenced in the text.

strong support for the relationship between the known *Emex* species, *E. australis* and *E. spinosa* (100/98), they are conflicting and show poor support for the placement of *Emex* within *Rumex*. Posterior probability support for the placement of *Emex* is only 52% and the most likely GARLI tree placed *Emex* within the Basal Grade of subgenus

Rumex. Furthermore, different gene regions reconstructed conflicting topologies for the placement of *Emex*. The *rbcL* phlyogeny placed *Emex* within *Rumex* subgenus *Rumex* (50% bootstrap support). Both *trnh-psbA* and *trnL-F* placed *Emex* as sister to the *Rumex* genus (*trnh-psbA* < 50% bootstrap support and *trnL-F* 91% bootstrap support) (results not shown).

The remaining taxa, comprising the subgenera *Acetosa*, *Acetosella* and *Platypodium* form a highly supported (99/80) monophyletic group (Fig. 2). This group is denoted as "the dioecious clade" because it is here that we see the initial transition to dioecy of the known dioecious *Rumex* species resolved in this group. The relationships of the clades within this group are also well-supported. Our recovered phylogenetic tree did not recover subgenus *Acetosa* as monophyletic. Within the dioecious clade, subgenus *Acetosa* is comprised of three well-supported, monophyletic groups, Clade 2 (100/97), Clade 3 (100/78) and Clade 4 (100/97) and is nested below a pair of clades, represented by subgenus *Platypodium* (Clade 5) and subgenus *Acetosella* (Clade 6). The pair (*Platypodium* + *Acetosella*) is also well supported (100/81). Subgenus *Platypoidium* was recovered as monophyletic with strong support (100/100) and consists of four accessions of its only circumscribed species: *Rumex bucephalophorus*. Species in subgenus *Acetosella* were recovered together with strong support (100/89), but the inclusion of *Rumex hastatulus* means the subgenus was not recovered as monophyletic.

In addition to corresponding largely to the established subgeneric system, the topology also largely corresponds to the diversity of the reproductive and sex chromosome systems present in *Rumex*. Species in subgenus *Rumex* (Basal Grade) are mostly hermaphroditic with no documented heteromorphic sex chromosomes. With no documented heteromorphic sex chromosomes, *Emex* is also represented as a clade and consists of purely monoecious species. Subgenus *Acetosa* consists entirely of dioecious species, with some members exhibiting the sex chromosome system XX/XY_1Y_2. Subgenus *Platypodium*, another hermaphroditic group with no reported sex chromosomes, is nested between subgenera *Acetosa* and *Acetosella*. Subgenus *Acetosella* consists of species that are both dioecious and have the sex chromosome system XX/XY.

Discussion

Our results produced a phylogeny of *Rumex*, with six major clades and one grade, largely congruent with Rechinger's subgeneric classification. The placement of *Emex* conflicted, based on the molecular markers used. In our phylogeny, it is sister to the dioecious clade, but without strong support.

Within the phylogeny, the basal grade is mostly made up of species from *Rumex* subgenus *Rumex*. That subgenus *Rumex* was recovered as a grade rather than a clade is not surprising given the known extensive hybridisation amongst species of this subgenus. This phenomenon most certainly contributed to the lack of resolution in species-level relationships within subgenus *Rumex*. Additionally, although hybridisation between species in subgenus *Rumex* and species in the other subgenera are not

well documented, it is possible that such hybrids exist and serve to hinder our ability to distinguish subgenus *Rumex* as a clade. We suspect that increased taxon sampling and genetic data, especially from the nuclear genome, will help to resolve relationships amongst species in subgenus *Rumex*.

Although dioecious, the species included in Clade 2 and Clade 3 have no reported heteromorphic sex chromosome systems. The species included in Clade 4 exhibit a complex sex chromosome system (XX/XY_1Y_2). This placement suggests that this heteromorphic sex chromosome system was derived from dioecious ancestors. The genetic origin of heteromorphic sex chromosomes in *Rumex* is beyond the scope of this manuscript, but this result provides a framework to investigate potentially intermediary taxa that may contain homomorphic or transitionary sex chromosome systems.

Subgenus *Platypodium* (Clade 5) was resolved as monophyletic and nested within "the dioecious clade". Based on its plant and chromosome morphology, earlier studies concerning *Rumex bucephalophorus* have referred to it as the link between subgenus *Rumex*, which is predominantly synoecious and subgenus *Acetosella*, which is predominantly dioecious (Löve 1944). Although morphologically variable, *R. bucephalophorus* consistently exhibits a synoecious reproductive system. Its derivation from amongst the dioecious species in this phylogeny suggests a reversal from a dioecious condition.

Subgenus *Acetosella* (Clade 6) was not recovered as monophyletic. Known dioecious species, *R. hastatulus*, of subgenus *Acetosa* is nested within subgenus *Acetosella*. *Rumex hastatulus* is documented to exhibit two distinct karyotypes: a complex sex chromosome system (XX/XY_1Y_2, North Carolina karyotype) which is characteristic of subgenus *Acetosa* and the simple sex chromosome system (XX/XY, Texas karyotype) which is characteristic of subgenus *Acetosella* (Navajas-Pérez et al. 2005; Mariotti et al. 2009; Hough et al. 2014). In addition, Rechinger's 1937 treatment indicates a polygamous reproductive system for *R. hastatulus* (Rechinger 1937). Given the variability found within this species, *R. hastatulus* could have been placed in either subgenus (*Acetosa* or *Acetosella*), where species appear to have diversified according to the type of sex chromosome system they exhibit. This finding suggests the plasticity of reproductive and sex chromosome systems within *Rumex*, as a single species can exhibit two different karyotypes.

One of the striking features of the phylogeny recovered in this study is its congruence with the taxonomic system established by Rechinger (Rechinger 1933, 1937, 1939, 1949, 1954a, 1954b, 1984, 1990). Rechinger retained the diversity of species as a single genus, but divided them into four subgenera: *Rumex* (*Lapathum*), *Platypodium*, *Acetosa* and *Acetosella*. Each subgenus is prominently present in the topology. Subgenus *Platypodium* is monophyletic. Subgenus *Acetosella* is monophyletic even with the inclusion of *Rumex hastatulus*, whose placement has been ambiguous. The two largest subgenera, *Acetosa* and *Rumex*, were recovered as grades. The grade of subgenus *Acetosa* is well-resolved and well-supported. The grade of subgenus *Rumex* is both less well-resolved and less well-supported. The recovered topology, nevertheless, serves to confirm the major relationships amongst species in the genus, relationships for which Rechinger had proposed using only morphology.

In all, this work has provided a reconstructed phylogeny that differs from those currently published (Navajas-Pérez et al. 2005; Schuster et al. 2015) and has tested the placement and monophyly of its circumscribed subgenera. This work builds on those previous studies by providing an increased taxon sampling density, which has resulted in a more comprehensive reconstruction of the evolutionary history of *Rumex* and a more thorough examination of the stability of the subgeneric system. This work has provided an early outline of the evolution of reproductive systems in *Rumex*, suggesting an ordered plasticity and transitions from synoecy to dioecy to dioecy with heteromorphic sex chromosomes. Additionally, this work suggests a possible reversal from a dioecious condition. Future directions in *Rumex* research include the identification and application of nuclear markers that will allow for a more robust phylogeny, particularly with respect to the placement of *Emex*. Additionally, future genomic studies will serve to elucidate the evolution of the sex chromosomes and sex determining regions in *Rumex*.

Acknowledgements

This study was largely funded by award NSF-HRD #1601031 to J.M. Burke. The authors would like to thank staff at the following herbaria for facilitating access to specimens for sampling: K, NY, OSC, RAB and US. Special thanks to Spencer Barrett and Joanna Rifkin for access to cultivated *Rumex* material. We also thank Daniel Atha for providing us with a record of his *Rumex* collections and facilitating access to silica-dried material. Special thanks to Mr. Ibrahim El Hafid for his assistance with transportation during the Morocco expedition. We also thank Ms. Maria Ramos for her assistance with plant cultivation at the Howard University greenhouse.

References

Brandbyge J, Rechinger KH (1989) A new *Rumex* from Ecuador. Nordic Journal of Botany 9(2): 203–204. https://doi.org/10.1111/j.1756-1051.1989.tb02115.x

Burke JM, Sanchez A (2011) Revised subfamily classification for Polygonaceae, with a tribal classification for Eriogonoideae. Brittonia 63(4): 510–520. https://doi.org/10.1007/s12228-011-9197-x

Burke JM, Sanchez A, Kron KA, Luckow MA (2010) Placing the woody tropical genera of Polygonaceae: A hypothesis of character evolution and phylogeny. American Journal of Botany 97(8): 1377–1390. https://doi.org/10.3732/ajb.1000022

Cunado N, Navajas-Pérez R, de la Herrán R, Rejón CR, Rejón MR, Santos JL, Garrido-Ramos MA (2007) The evolution of sex chromosomes in the genus *Rumex* (Polygonaceae): Identification of a new species with heteromorphic sex chromosomes. Chromosome Research 15(7): 825–833. https://doi.org/10.1007/s10577-007-1166-6

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new scalable tool for the selection of DNA and protein evolutionary models. Molecular Biology and Evolution 37(1): 291–294. https://doi.org/10.1093/molbev/msz189

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy DM, Hajibabaei M, Barrett SC (2008) Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS ONE 3(7): e2802. https://doi.org/10.1371/journal.pone.0002802

Grant KD (2022) A new phylogeny of Rumex (Polygonaceae) adds evolutionary context to the diversity of reproductive systems present in the genus. Dryad, Dataset.

Hough J, Hollister JD, Wang W, Barrett SC, Wright SI (2014) Genetic degeneration of old and young Y chromosomes in the flowering plant *Rumex hastatulus.* Proceedings of the National Academy of Sciences of the United States of America 111(21): 7713–7718. https://doi.org/10.1073/pnas.1319227111

Koenemann DM, Burke JM (2020) A Molecular Phylogeny for the Genus *Coccoloba* (Polygonaceae) with an Assessment of Biogeographic Patterns. Systematic Botany 45(3): 567–575. https://doi.org/10.1600/036364420X15935294613536

Löve Á (1940) Polyploidy in *Rumex acetosella* L. Nature 145(3670): 351. https://doi.org/10.1038/145351a0

Löve Á (1942) Cytogenetic studies in *Rumex*: III. Some notes on the Scandinavian species of the genus. Hereditas 28(3-4): 289–296. https://doi.org/10.1111/j.1601-5223.1942.tb03281.x

Löve Á (1943) A Y-linked inheritance of asynapsis in *Rumex acetosa.* Nature 152(3856): 358–359. https://doi.org/10.1038/152358b0

Löve Á (1944) Cytogenetic studies on *Rumex* subgenus *Acetosella.* Hereditas 30(1–2): 1–136. https://doi.org/10.1111/j.1601-5223.1944.tb03303.x

Löve Á (1957) Sex determination in *Rumex.* Proceedings of the Genetic Society of Canada 2: 31–36.

Löve Á, Kapoor BM (1967) A chromosome atlas of the collective genus *Rumex.* Cytologia 32(3–4): 328–342. https://doi.org/10.1508/cytologia.32.328

Löve Á, Löve D (1948) Chromosome numbers of northern plant species. Department of Agriculture, Reykjavik, University Institute of Applied Sciences.

Maddison WP (2005) Mesquite: a modular system for evolutionary analysis. Version 1.06. http://mesquiteproject.org [accessed 30.11.2019]

Mariotti B, Navajas-Pérez R, Lozano R, Parker JS, De La Herrán R, Rejón CR, Rejon MR, Garrido-Ramos M, Jamilena M (2006) Cloning and characterization of dispersed repetitive DNA derived from microdissected sex chromosomes of *Rumex acetosa.* Genome 49(2): 114–121. https://doi.org/10.1139/g05-089

Mariotti B, Manzano S, Kejnovský E, Vyskot B, Jamilena M (2009) Accumulation of Y-specific satellite DNAs during the evolution of *Rumex acetosa* sex chromosomes. Molecular Genetics and Genomics 281(3): 249. https://doi.org/10.1007/s00438-008-0405-7

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), Institute of Electrical and Electronics Engineers, New Orleans, 1–8. http://doi.org/10.1109/GCE.2010.5676129

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annual Review of Plant Biology 62(1): 485–514. https://doi.org/10.1146/annurev-arplant-042110-103914

Mosyakin A (2005) Rumex. In: Flora of North America Editorial Committee (Eds) Flora of North America North of Mexico, Volume 5. Oxford University Press, New York, 1–690.

Navajas-Pérez R, de la Herrán R, González GL, Jamilena M, Lozano R, Rejón CR, Rejón MR, Garrido-Ramos MA (2005) The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) inferred from nuclear and chloroplastidial sequence data. Molecular Biology and Evolution 22(9): 1929–1939. https://doi.org/10.1093/molbev/msi186

Rambaut A (2014) FigTree version 1.4.3. http://www.ac.uk/software/figtree [accessed 30.11.2019]

Rechinger KH (1933) Vorarbeiten zu einer Monographie der Gattung Rumex, III. Die Süd- und zentralamerikanischen Arten der Gattung *Rumex*. Arkiv för botanik 26A(3): 1–58.

Rechinger KH (1937) The North American species of *Rumex.* Field Museum of Natural History, 1–151.

Rechinger KH (1939) Polygonaceae. In: Hegi G, Hanser C (Eds) Illustrierte flora von Mittel – Europa. Verlag, Munich, 352–436.

Rechinger KH (1949) Rumices asiatici: Vorarbeiten zu einer Monographie der Gattung Rumex VII. Candollea 12: 9–162. https://www.jstor.org/stable/43336109

Rechinger KH (1954a) Monograph of the genus *Rumex* in Africa: vorarbeiten zu einer Monographie der Gattung Rumex VIII. Gleerup, 1–114.

Rechinger KH (1954b) Beiträge zur kenntnis von *Rumex* XII: Some new American species of Rumex. Leaflets of Western Botany 7: 133–135.

Rechinger KH (1984) *Rumex* (Polygonaceae) in Australia: A reconsideration. Nuytsia 5(1): 75–122. https://florabase.dpaw.wa.gov.au/nuytsia/article/98

Rechinger KH (1990) *Rumex* subgen. *Rumex* sect. *Axillares* (Polygonaceae) in South America. Plant Systematics and Evolution 172: 151–192. https://doi.org/10.1007/BF00937805

Ronquist FM, Teslenko P, Van Der Mark DL, Ayres A, Darling S, Hohna B, Larget L, Liu MA, Suchard J, Huelsenbeck P (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sanchez A, Kron KA (2008) Phylogenetics of Polygonaceae with an Emphasis on the Evolution of Eriogonoideae. Systematic Botany 33(1): 87–96. https://doi.org/10.1600/036364408783887456

Sanchez A, Schuster TM, Kron KA (2009) A Large-Scale Phylogeny of Polygonaceae Based on Molecular Data. International Journal of Plant Sciences 170(8): 1044–1055. https://doi.org/10.1086/605121

Sanchez A, Schuster TM, Burke JM, Kron KA (2011) Taxonomy of Polygonoideae (Polygonaceae): A new tribal classification. Taxon 60(1): 151–160. https://doi.org/10.1002/tax.601013

Schuster TM, Reveal JL, Kron KA (2011) Phylogeny of Polygoneae (Polygonaceae: Polygonoideae). Taxon 60(6): 1653–1666. https://doi.org/10.1002/tax.606010

Schuster TM, Setaro SD, Kron KA (2013) Age estimates for the buckwheat family Polygonaceae based on sequence data calibrated by fossils and with a focus on the Amphi-Pacific Muehlenbeckia. PLoS ONE 8(4): e61261. https://doi.org/10.1371/journal.pone.0061261

Schuster TM, Reveal JL, Bayly MJ, Kron KA (2015) An updated molecular phylogeny of Polygonoideae (Polygonaceae): Relationships of Oxygonum, Pteroxygonum, and Rumex, and a new circumscription of Koenigia. Taxon 64(6): 1188–1208. https://doi.org/10.12705/646.5

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller, J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal Of Botany 92(1): 142–166. https://doi.org/10.3732/ajb.92.1.142

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal Of Botany 94(3): 275–288. https://doi.org/10.3732/ajb.94.3.275

Shibata F, Hizume M, Kuroki Y (1999) Chromosome painting of Y chromosomes and isolation of a Y chromosome-specific repetitive sequence in the dioecious plant Rumex acetosa. Chromosoma 108(4): 266–270. https://doi.org/10.1007/s004120050377

Shibata F, Hizume M, Kuroki Y (2000) Differentiation and the polymorphic nature of the Y chromosomes revealed by repetitive sequences in the dioecious plant, *Rumex acetosa.* Chromosome Research 8(3): 229–236. https://doi.org/10.1023/A:1009252913344

Thiers B (2019) Index Herbariorum: The William & Lynda Steere Herbarium, The New York Botanical Garden (updated continuously). http://sweetgum.nybg.org/science/ih/ [accessed 30.11.2019]

Wagner WL, Sohmer SH, Herbst DR (1999) Manual of the flowering plants of Hawai'i. University of Hawaii Press, 1–1952.

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Thesis, The University of Texas at Austin, Austin, Texas.

Appendix A1. List of taxa sampled and vouchers specimens

Abbreviations of herbaria where the voucher is housed are listed after the collection number. Sequences can be found on the Dryad database: https://doi.org/10.5061/dryad.69p8cz8zs

For sequences that we did not generate, accession information is given as found on GenBank.

GenBank sequences used for this study:

rbcL: *Rumex pamiricus* Rech. f. – JF944139.1, *Rumex sibiricus* Hulten- KC483892.1
trnH-psbA: *Rumex pamiricus*- JN047053.1

Table A1. DNA Sequences Generated for this Study.

Scientific name	Voucher
Emex australis Steinh.	P.C. Zietsma 4053, NY
Emex spinosa (L.) Campd.	Schuhwerk 90/328, NY
Emex spinosa (L.) Campd.	J.M. Burke 302, HUDC
Persicaria virginiana (L.) Gaertn.	J.M. Burke s.n., BH
Rheum alexandrae Batalin	Cultivated Material, HUDC
Rheum emodii Wall.	Cultivated Material, HUDC
Rheum officinale Baill.	Cultivated Material, HUDC
Rheum palmatum var. *taguticaum* L.	Cultivated Material, HUDC
Rheum rhabarbarum L.	Cultivated Material, HUDC
Rheum nobile Hook. f. & Thomson	Pradham 820581, BH
Rumex abyssinicus Jacq.	J.M. Burke 251, HUDC
Rumex acetosa L.	K.D. Grant s.n., HUDC
Rumex acetosella L.	R. Brand 1336, NY
Rumex acetosella L.	D.E. Atha 10521, NY
Rumex acetosella L.	K.D. Grant s.n., HUDC
Rumex acetosella L.	J.M. Burke 309, HUDC
Rumex albescens Hillebr.	Lorence 5224, K
Rumex albescens Hillebr.	Wood 14959, US
Rumex alpinus L.	Larsen 20708, US
Rumex alpinus L.	D.E. Atha 5114, NY
Rumex altissimus Alph, Wood	Shultz 8717, US
Rumex altissimus Alph. Wood	D.E. Atha 10857, NY
Rumex alveolatus Los.-Losinsk.	Rechinger 48318, US
Rumex amurensis F. Schmidt ex Maxim.	Barrett Lilan22p
Rumex aquaticus L.	Elias 7251, US
Rumex arcticus Trautv.	Shetler 4560, US
Rumex arifolius All.	K. Deguchi 4023, NY
Rumex bequaertii De Wild.	Germishuizen 3447, US
Rumex berlandieri Meisn.	Thieret 17178, US
Rumex brachypodus Rech. f.	J.M. Burke 312, HUDC
Rumex brasiliensis Link	R. Wasum 1655, NY
Rumex brownii Campd.	Wilson 10250, NY
Rumex brownii Campd.	Wilson 10250, US
Rumex bucephalophorus L.	Barrett 17RBTA5
Rumex bucephalophorus L.	J.M. Burke 293, HUDC
Rumex bucephalophorus L.	J.M. Burke 301, HUDC
Rumex bucephalophorus L.	J.M. Burke 304, HUDC
Rumex chrysocarpus Moris	D.E. Atha 13012, NY
Rumex conglomeratus Murray	D.E. Atha 10045, NY
Rumex conglomeratus Murray	J.M. Burke 271, HUDC
Rumex conglomeratus Murray	J.M. Burke 298, HUDC

Scientific name	Voucher
Rumex conglomeratus Murray	J.M. Burke 299, HUDC
Rumex crispus L.	J.M. Burke 268, HUDC
Rumex cuneifolius Campd.	J.C. Solomon 13044, US
Rumex cyprius Murb.	Kocher B-273, US
Rumex densiflorus Osterh.	Pinkava P12626, US
Rumex dentatus L.	D.G. Kelch 07.328, OSC
Rumex giganteus Aiton	K. Thorne 6736, NY
Rumex giganteus Aiton	Canfield 1304, US
Rumex graminifolius Gerogi ex Lamb.	Petrosky 1811, US
Rumex hastatulus Baldwin	D.E. Atha 10503, NY
Rumex hastatus D. Don	MacArthur 1291, US
Rumex hastatus D. Don	Barrett s.n.
Rumex hymenosepalus Torr.	Cultivated material, HUDC
Rumex hymenosepalus Torr.	A. Tiehm 15727, OSC
Rumex induratus Bioss. et Reut.	M.W. Chase 925, K
Rumex induratus Bioss. et Reut.	Barrett s.n.
Rumex induratus Bioss. et Reut.	J.M. Burke 310, HUDC
Rumex intermedius DC.	Rainha 5270, US
Rumex japonicus Houtt.	Bai-Zhang 4049, US
Rumex kerneri Borbás	Barta 2004-390, US
Rumex lanceolatus Thunb.	H.J. Venter 10295, NY
Rumex longifolius DC.	D. E. Atha 8858, NY
Rumex lunaria L.	NR. 8879, NY
Rumex lunaria L.	Barrett 17RLLM1
Rumex lunaria L.	Barrett 17RLTF1
Rumex maritimus L.	Shiu Ying Hu 13127, US
Rumex mexicanus Meisn.	D.E. Breedlove 13305, US
Rumex microcarpus Campd.	Barrett MJ-P40 (Seed)
Rumex nepalensis Spreng.	J.M. Burke 248, HUDC
Rumex nervosus Vahl	J.M. Burke 252, HUDC
Rumex obtusifolius L.	J.M. Burke s.n., BH
Rumex obtusifolius L.	J.M. Burke 270, HUDC
Rumex orbiculatus A. Gray	Ruee 43716, US
Rumex orbiculatus A. Gray	D.E. Atha et al. 8883/2010, NY
Rumex pallidus Bigelow	D.E. Atha 13922, NY
Rumex palustris Sm.	J.M. Burke 306, HUDC
Rumex papilio Coss. & Balansa,	S.L. Jury 13659, K
Rumex papilio Coss. & Balansa	J.M. Burke 303, HUDC
Rumex patientia L.	D.E. Atha 10674, NY
Rumex paucifolius Nutt.	Barrett 17RpCOT3.2
Rumex paucifolius Nutt.	Barrett 17RpCMC15.2
Rumex peruanus Rech. f.	V. Quipuscoa 1349, NY
Rumex pictus Forssk.	Barrett 17Rp.AR1
Rumex pulcher L.	J.M. Burke 294, HUDC
Rumex pulcher L.	J.M. Burke 295, HUDC
Rumex pulcher L.	J.M. Burke 296, HUDC
Rumex rothschildianus Aarons. ex Evenari	Barrett 17Rrs3.2
Rumex sagittatus Thunb.	Strobach B55575, US
Rumex sagittatus Thunb.	H.J. Venter 9995, NY
Rumex salicifolius Weinm.	W. Wood s.n., OSC

Scientific name	Voucher
Rumex sanguineus L.	J.M. Burke 316., HUDC
Rumex scutatus L.	Barrett s.n.
Rumex skottsbergii O.Deg. & I.Deg.	Degener 35050, US
Rumex spiralis Small.	D.E. Atha 9727, NY
Rumex stenophyllus Ledeb.	D.E. Atha 11389, NY
Rumex stenophyllus Ledeb.	R.L. McGregor 40643, OSC
Rumex tianschanicus Losinsk.	Barrett SH1-A-2007454
Rumex thyrsiflorus Fingerh.	Ollegard 261, US
Rumex thyrsiflorus Fingerh.	Elias 7282, US
Rumex thyrsoides Desf.	J.M. Burke 305, HUDC
Rumex thyrsoides Desf.	J.M. Burke 313, HUDC
Rumex thyrsoides Desf.	J.M. Burke 307, HUDC
Rumex tuberosus L.	S. Omar et al. 52591, K
Rumex tuberosus subsp. nov	J.M. Burke 308, HUDC
Rumex usambarensis (Dammer) Dammer	Ellemann 889, NY
Rumex venosus Pursh.	R.E. Brainerd 428, OSC
Rumex vesicarius L.	Brummit 15271, US

Supplementary material 1

Aligned Data Matrix

Authors: Kirstie D. Grant

Data type: FASTA

Explanation note: Aligned sequence file.

Link: https://doi.org/10.3897/phytokeys.204.85256.suppl1

PhytoKeys 204: 73–81 (2022)
doi: 10.3897/phytokeys.204.84397
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Spiradiclis liboensis (Rubiaceae), a new species from limestone mountain areas in Guizhou, China

Xiao-Fei Song[1], Wen-Jian Liu[2], Ao-Xue Chen[1], Zheng-Ming Yao[3], Hong-Bo Lan[3], Lei Wu[1]

1 *College of Forestry, Central South University of Forestry and Technology, Changsha 410004, Hunan, China* **2** *Hunan Qingyanghu State-owned Forest Farm, Changsha 410627, Hunan, China* **3** *Maolan National Nature Reserve, Qiannan 558400, Guizhou, China*

Corresponding author: Lei Wu (wuleiibk@163.com)

Academic editor: Andre Simões | Received 29 March 2022 | Accepted 19 July 2022 | Published 8 August 2022

Citation: Song X-F, Liu W-J, Chen A-X, Yao Z-M, Lan H-B, Wu L (2022) *Spiradiclis liboensis* (Rubiaceae), a new species from limestone mountain areas in Guizhou, China. PhytoKeys 204: 73–81. https://doi.org/10.3897/phytokeys.204.84397

Abstract

Spiradiclis liboensis L. Wu & W. J. Liu, a new species in tribe Ophiorrhizeae of Rubiaceae from limestone mountain areas of Guizhou, south-western China, is described and illustrated. It is similar to *S. guangdongensis* and *S. jingxiensis*, but differs from the latter two by the following traits: stipule triangular, inflorescence sessile or with peduncle up to 0.5 mm long, pedicel 0.8–2.2 mm long, corolla white, salverform, corolla tube 1.6–2.2 cm long, corolla tube of long-styled morph inside with a villous ring and stigmas positioned at the throat of the corolla tube. The conservation status is assessed as "Vulnerable" (VU) according to the IUCN Red List Categories and Criteria.

Keywords

China, limestone, Rubiaceae, *Spiradiclis*, taxonomy

Introduction

Spiradiclis Blume, a member of the tribe Ophiorrhizeae (Rubiaceae), is a poorly known and taxonomically complicated genus (Razafimandimbison and Rydin 2019; Tong et al. 2020; Li et al. 2021). There are approximately 59 species worldwide, of which 52 known species are distributed in southern and south-western China (Deb and Rout 1989; Chen and Taylor 2011; Tong et al. 2020; Wen et al. 2021) and these are one of

the most representative herbs in limestone areas in the country (Wang 2002; Chen and Taylor 2011; Wen et al. 2019; Wu et al. 2019a; Li et al. 2021).

Spiradiclis most closely resembles *Ophiorrhiza* L. and the two genera are in the same tribe Ophiorrhizeae, based on morphological characters (Verdcourt 1958; Darwin 1976; Lo 1999; Chen and Taylor 2011; Wu et al. 2019b) and molecular evidence (Bremer and Manen 2000; Bremer 2009; Rydin et al. 2009; Wikström et al. 2013). Even so, the monophyly of the two genera is questioned (Razafimandimbison and Rydin 2019). However, *Spiradiclis* is morphologically different from *Ophiorrhiza* by its linear-oblong or subglobose capsules with four valves (vs. obcordate and compressed capsules with two valves) when mature. Since the delimitation and relationship of the two genera still need further research, we prefer to accept the traditional concept of *Spiradiclis* here due to its unique capsule form.

During fieldwork in Libo County, Guizhou Province, Chen Yaping (Kunming Institute of Botany, Chinese Academy of Sciences) came across a peculiar population of *Spiradiclis* in flower on a limestone hill and consulted us for identification. This population was initially considered to be *S. guangdongensis* H. S. Lo or *S. jingxiensis* R. J. Wang by its creeping habit, small leaves, 1–2-flowered inflorescences and subglobose capsules. However, after revisiting relevant literature (Lo et al. 1983; Chen and Taylor 2011; Wang et al. 2015; Wen et al. 2015; Wu et al. 2015a, b, 2016, 2019a, b; Pan et al. 2016, 2019; Wang 2016; Liu et al. 2017; Zhang et al. 2018; Wen et al. 2019; Tong et al. 2020; Li et al. 2021), as well as specimens, this population could be distinguished from these two species. Hence, this population is assumed to represent an undescribed new taxon, which is here described.

Materials and methods

Materials were deposited at the Herbarium of forest plants in the Central South University of Forestry and Technology (CSFI), Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and the Chinese Academy of Sciences (IBK), South China Botanical Garden, Chinese Academy of Sciences (IBSC) (acronyms according to Thiers 2018). Morphological observations and measurements of the new species were based on living material and specimens. Morphological terms follow Harris and Harris (2001).

Taxonomic treatment

Spiradiclis liboensis **L. Wu & W. J. Liu, sp. nov.**
urn:lsid:ipni.org:names:77303030-1
Figs 1, 2

Type. China. Guizhou Province: Libo County, Maolan National Nature Reserve, 107°56'E, 22°5'N, alt. 900 m, 9 May 2018 (flower), *L. Wu & F. L. Chen 6410* (holotype: CSFI barcode 069626, isotypes: CSFI, IBK).

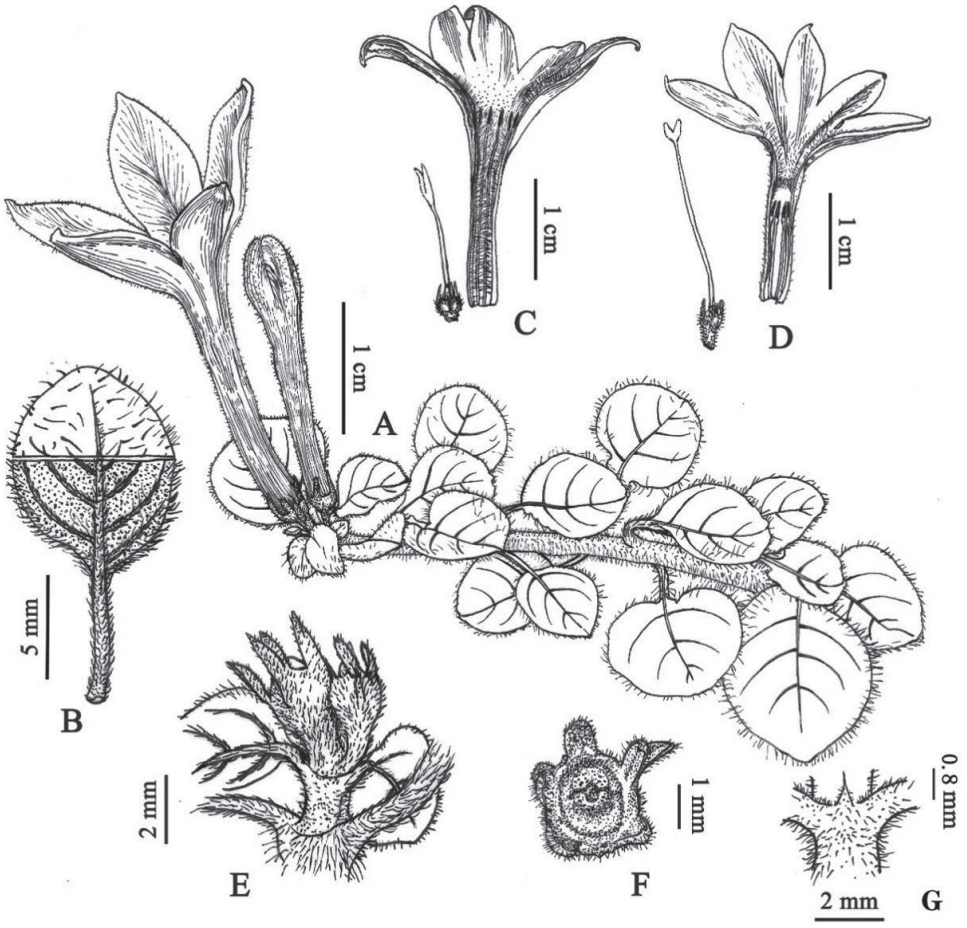


Figure 1. *Spiradiclis liboensis* L. Wu & W. J. Liu. **A** habit **B** leaf blade, adaxial and abaxial views **C** short-styled flower **D** long-styled flower **E** infructescence **F** calyx and disc in face view **G** stipules. Drawn by X.Y. Zeng.

Diagnosis. The new species is similar to *S. guangdongensis* and *S. jingxiensis*, but differs from the former by the triangular (vs. linear), ca. 0.8 (vs. 1–3.5) mm long stipule, 1.6–2.2 (vs. 0.8–1) cm long corolla tube and stigma and anthers positioned at (vs. exserted 5 mm above) the throat of the corolla tube in the long-styled form and short-styled form, respectively, and from the latter by its green (vs. purple) stem, 0.8–2.2 mm (vs. 3–5 mm) long pedicels, white (vs. pink) corolla with tube ca. 1.2 mm wide at the lower part, enlarged at the upper part and ca. 4.8 mm wide at the throat (vs. ca. 2 mm).

Description. Perennial herbs, creeping, rooting at most nodes; stems terete, slender, densely pubescent, internodes 1–5 mm long. Petiole 2–9 mm long, sparsely pubescent; leaf blades papery when dry, adaxially green to dark green, abaxially lightly green, ovate to orbicular, 0.3–1.4 × 0.3–1.2 cm, base truncate to rounded, apex subacute to obtuse, adaxially sparsely hispidulous, abaxially pubescent along veins; secondary veins 3–4 on each side; stipules triangular, ca. 0.8 mm long, pubescent, usually deciduous.

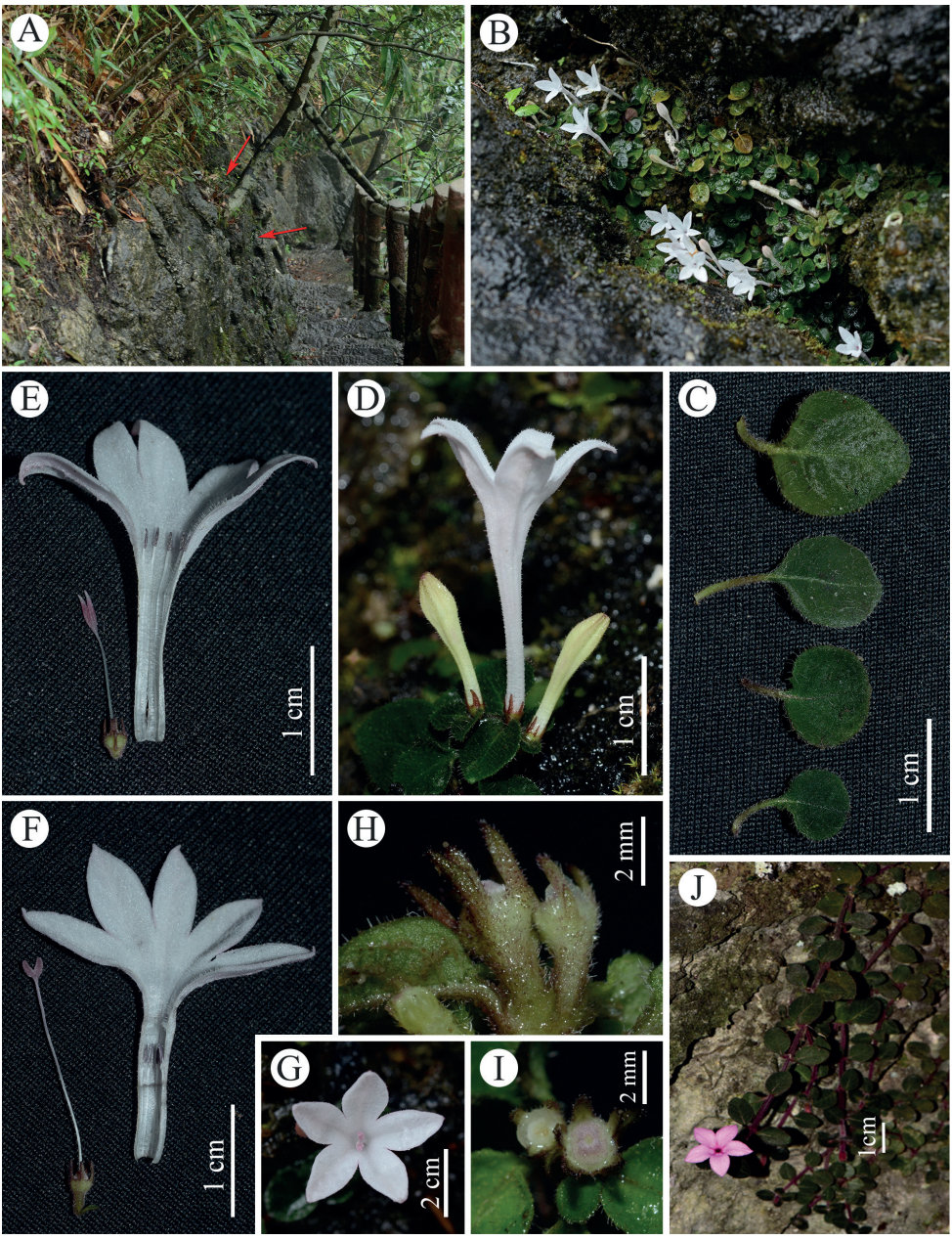


Figure 2. *Spiradiclis liboensis* L. Wu & W. J. Liu. **A** habitat (the arrow shows the place of growth) **B** habit **C** leaves showing variation range **D** inflorescence, side view **E–F** dissected short-styled flower and long-styled flower showing floral parts **G** long-styled flower, frontal view **H** young capsules **I** discs in face view. *S. jingxiensis* **J** habit. (Designed by Lei Wu and Xiao-Fei Song).

Inflorescence terminal, cymose, with 1–2 flowers; peduncle 0–0.5 mm long; bracteoles linear or narrowly triangular, 1–2.3 mm long, puberulent. Flowers distylous, 5-merous; pedicels pubescent, 0.8–2.2 mm long. Calyx pubescent; hypanthium obconical, 1.3–1.6 mm long; lobes linear, 1.3–1.8 mm long, outside puberulent. Corolla white, salverform, puberulent outside; tube 1.6–2.2 cm long, ca. 1.2 mm wide at the lower part, enlarged at the upper part, ca. 4.8 mm wide at the throat; lobes subovate, 6–9 × 3–6 mm, puberulent inside; stamens 5; anthers linear-oblong, 1.4–1.6 mm long; stigma bilobed; ovary 2-celled. Long-styled flowers: corolla tube inside with a villous ring below throat and densely pubescent from the throat to the base of the corolla lobes; stamens inserted at the middle of the corolla tube; filaments ca. 0.4 mm long; stigmas positioned at the throat of the corolla tube; lobes elliptic, ca. 1.7 mm long; styles 1.5–1.8 cm long, glabrous. Short-styled flowers: corolla tube pubescent inside; stamens positioned at the throat of the corolla tube; filaments ca. 1.5 mm long; stigmas inserted slightly above the middle of the corolla tube; stigma lobes lanceolate, ca. 3 mm long; styles 7–9 mm long, glabrous. Capsules subglobose, ca. 2.5 mm in diam., pubescent, untwisted valves when mature.

Distribution and habitat. *Spiradiclis liboensis* is currently only known from limestone hills in the Maolan National Nature Reserve, Libo County, Guizhou Province, south-western China. It grows on humid slopes or within crevices under the evergreen broad-leaved forest, at an altitude of 850–950 m. The forest here is dominated by trees of Fagaceae (e.g. *Cyclobalanopsis glauca* (Thunb.) Oerst.), Lauraceae (e.g. *Phoebe calcarea* S. K. Lee et F. N. Wei and *Lindera megaphylla* Hemsl.) and Sapindaceae (e.g. *Handeliodendron bodinieri* (H. Lév.) Rehder).

Phenology. Flowering from May to June, fruiting from June to October.

Etymology. The specific epithet is derived from the type locality, Libo County, southern China. The Chinese name is given as "荔波螺序草" (lì bō luó xù cǎo).

Additional specimens examined (*paratypes*). China. Guizhou Province: Libo County, the type locality, 9 May 2019 (fruit), *F. C. Chen & Z. B. Xiong* CFL5029 (IBSC!), 14 October 2019, *F. L. Chen* 19101401 (CSFI!, IBSC!).

Provisional conservation status. After a series of investigations into limestone areas of Guizhou Province, five populations of the new species with approximately 60 individuals at each site have been observed. All the individuals are distributed in Maolan National Nature Reserve and the habitats are mostly in good condition, except for the population adjacent to a tourism road. Hence, according to the IUCN Standards and Petitions Subcommittee (2019) Guidelines, this species is currently evaluated as 'Vulnerable' [VU].

Discussion. *Spiradiclis liboensis* is morphologically very similar to *S. guangdongensis* and *S. jingxiensis*. According to the classification of Lo (1999), the three species belong to the subgenus *Sinospiradiclis*. However, the new species differs from *S. guangdongensis* H. S. Lo mainly by its triangular, shorter stipule, much longer corolla tube and stigma and anthers positioned at the throat of the corolla tube in the long-styled form and short-styled form, respectively, and from *S. jingxiensis* R. J. Wang mainly by its green

Table 1. Morphological comparison of *Spiradiclis guangdongensis*, *S. jingxiensis* and *S. liboensis*.

Characters	*Spiradiclis guangdongensis*	*S. jingxiensis*	*S. liboensis*
Petioles length	2–6 mm long	0.5–3 mm long	2–9 mm long
Leaf blades	orbicular; base rounded, apex obtuse, mucronulate or acute	oval to ovate; base broadly cuneate to rounded; apex obtuse to acute	ovate to orbicular, base truncate to rounded
Stipules	linear-subulate, 2–3 mm long	simple or 2-lobed, lobes linear, 1.5–3.0 mm long	triangular, ca. 0.8 mm long
Inflorescence	1–3-flowered, usually one flower	1–2-flowered	1–2-flowered
Peduncle length	Sessile	0–5 mm	sessile or 0.5 mm
Pedicel length	3–7.5 mm	3–5 mm	0.8–2.2 mm
Corolla	white, slender funnelform	pink, salverform	white, salverform
Corolla tubes	0.8–1 cm long, 0.8–2 mm wide, with almost the same width from base to top	1.3–1.9 cm long, ca. 2 mm wide, with almost the same width from base to top	1.6–2.2 cm long, ca. 1.2 mm wide at lower part, enlarged at upper part, ca. 4.8 mm wide at the throat
Corolla lobes	lobes 4–7 × ca. 3 mm	oval, ca. 5.5–6.5 × 2.5–3.0 mm	subovate, 6–9 × 3–6 mm
Position of the stigma and anthers	exserted 5 mm above the throat of corolla tube in long-styled form and short-styled form, respectively	positioned at the throat of corolla tube in both forms	positioned at the throat of corolla tube in both forms
Styles in short-styled form	6–10 mm long	ca. 4.5 mm long	7–9 mm long
Flowering	March to April	May to June	May to June

stem, shorter pedicels, white corolla with tube ca. 1.2 mm wide at the lower part, enlarged at the upper part, ca. 4.8 mm wide at the throat. The detailed morphological comparisons amongst them are listed in Table 1.

Currently, there are nine other species of *Spiradiclis* with creeping or decumbent habits and small leaf blades shorter than 3 cm, viz., *S. danxiashanensis* R. J. Wang, *S. glandulosa* L. Wu & Q. R. Liu, *S. hainanensis*, *S. lui* Liu Yan & L. Wu, *S. karstana* L. Wu, X. Li & Q. R. Liu, *S. pauciflora* L. Wu & Q.R. Liu, *S. tubiflora* L. Wu, B. M. Wang & B. Pan, *S. pengshuiensis* B. Pan & R. J. Wang and *S. umbelliformis* H. S. Lo (Chen and Taylor 2011; Wang et al. 2015; Wu et al. 2015a, b, 2019b; Zhang et al. 2018). To better differentiate these species, a key is provided.

Key to the species of *Spiradiclis* with stems creeping or decumbent and leaf blades shorter than 3 cm

1 Calyx lobes ca. 4–6 mm long, oblong-lanceolate ..... ***S. glandulosa***
– Calyx lobes shorter than 3.5 mm, linear, triangular or subulate **2**
2 Corollas tubular-funnelform with tubes distinctly enlarged ..... ***S. tubiflora***
– Corolla funnelform or salverform, usually with a slender tube **3**
3 Leaf blades usually shorter than 1.5 cm; inflorescences 1–3-flowered **4**
– Leaf blades usually longer than 1.5 cm (except *S. pauciflora*); inflorescence many-flowered (more than 3 flowers) **7**
4 Stigmas and anthers distinctly excluded in long-styled and short-styled forms... **5**
– Stigmas and anthers included in long-styled and short-styled forms **6**
5 Inflorescence 2–3-flowered; corolla tube 1.1–1.5 cm long.... ***S. danxiashanensis***
– Inflorescence usually 1-flowered; corolla tube 0.8–1 cm long .... ***S. guangdongensis***

6 Peduncles longer than 5 mm; long-styled corolla inside without a ring of long hairs **7**
– Inflorescences sessile or peduncles shorter than 5 mm; long-styled corolla inside with a ring of long hair **8**
7 Leaf blade usually cordiform-orbicular, base cordulate to truncate .... ***S. hainanensis***
– Leaf blade ovate, base cuneate or broadly cuneate, decurrent **9**
8 Stem green; pedicels 0.8–2.2 mm long; corolla white; corolla tube ca. 1.2 mm wide at lower part, enlarged at upper part, and ca. 4.8 mm wide at the throat; styles 7–9 mm long in short-styled form ..... ***S. liboensis***
– Stem purple; pedicels 3–5 mm long; corolla pink; corolla tube ca. 2 mm wide, cylindrical, with almost the same width from base to top; styles 4.5 mm long in short-styled form ..... ***S. jingxiensis***
9 Stipules shorter than 2 mm, usually deciduous **10**
– Stipules 5–10 mm long, persistent **12**
10 Corollas funnelform, tubes 7–9 mm long ..... ***S. pauciflora***
– Corollas salverform, tubes longer than 9 mm **11**
11 Secondary veins 5–12 pairs; corolla tube 15–25 mm long with a ring of long hairs inside in long-styled form ..... ***S. karstana***
– Secondary veins 4–6 pairs; corolla tube 9–15 cm long without a ring of long hairs inside in long-styled form ..... ***S. pengshuiensis***
12 Stems densely multicellular villosulous; bracts shorter than 1 mm ..... ***S. umbelliformis***
– Stems glabrous; bracts 5.5–8.5 mm long ..... ***S. lui***

Acknowledgements

The authors are grateful to the staff of the Herbaria BNU, GXMI, IBK, IBSC, KUN, PE and SYS for permission to examine specimens. We also thank Miss Ya-Ping Chen (KUN) for helping in the field investigation and Xin-Yi Zeng for her excellent drawing. This study was supported by the Natural Science Foundation of Hunan (grant. no. 2021JJ31152) and the Scientific Research Project of Education Department of Hunan Province (grant. no. 18B178).

References

Bremer B (2009) A review of molecular phylogenetic studies of Rubiaceae. Annals of the Missouri Botanical Garden 96(1): 4–26. https://doi.org/10.3417/2006197

Bremer B, Manen J (2000) Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). Plant Systematics and Evolution 225(1–4): 43–72. https://doi.org/10.1007/BF00985458

Chen T, Taylor CM (2011) *Spiradiclis*. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China. Vol 19. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 330–339.

Darwin SP (1976) The Pacific species of *Ophiorrhiza* L. (Rubiaceae). Lyonia 1: 48–101.

Deb DB, Rout RC (1989) Two new species of the genus *Spiradiclis* (Rubiaceae) from India. Candollea 44: 225–229.

Harris JG, Harris MW (2001). Plant Identification Terminology: An Illustrated Glossary. Spring Lake Publishing, 1–188.

IUCN Standards and Petitions Subcommittee (2019) Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf [accessed 4 October 2020]

Li JL, Yuan Q, Liu Y, Song XF, Pan B, Qu CH, Wu L (2021) Two new species of *Spiradiclis* (Rubiaceae) from limestone areas in southwestern China. Nordic Journal of Botany 39: e02979. https://doi.org/10.1111/njb.02979

Liu J, Pan B, Li SW, Xu WB (2017) *Spiradiclis quanzhouensis* (Rubiaceae): A new species from limestone area in Guangxi, China. Nordic Journal of Botany 36(3): e01595. https://doi.org/10.1111/njb.01595

Lo HS (1999) Spiradiclis Blume. In: Lo HS (Ed.) Flora Reipublicae Popularis Sinicae. Vol. 71 (1). Science Press, Beijing, 86–110.

Lo HS, Sha WL, Chen XX (1983) A revision of the genus *Spiradiclis* Bl. Acta Botanica Austro Sinica 1: 27–36.

Pan B, Ma HS, Wang RJ (2016) *Spiradiclis pengshuiensis* (Ophiorrhizeae, Rubioideae), a new species from Chongqing, China. PhytoKeys 63: 41–45. https://doi.org/10.3897/phytokeys.63.8016

Pan B, Tu RH, Hareesh VS, Wu L (2019) *Spiradiclis cavicola* (Rubiaceae), a new species from limestone caves in south-western China. Annales Botanici Fennici 56(1–3): 1–4. https://doi.org/10.5735/085.056.0101

Razafimandimbison SG, Rydin C (2019) Molecular-based assessments of tribal and generic limits and relationships in Rubiaceae (Gentianales): Polyphyly of Pomazoteae and paraphyly of Ophiorrhizeae and *Ophiorrhiza*. Taxon 68(1): 72–79. https://doi.org/10.1002/tax.12023

Rydin C, Kainulainen K, Razafimandimbison SG, Smedmark JEE, Bremer B (2009) Deep divergences in the coffee family and the systematic position of Acranthera. Plant Systematics and Evolution 278(1–2): 101–123. https://doi.org/10.1007/s00606-008-0138-4

Thiers B (2018) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [accessed 26 June 2021]

Tong YH, Xia NH, Wu L, Vu TC (2020) Critical notes on *spiradiclis purpureocaerulea* H.S. Lo (Rubiaceae) from vietnam. Adansonia 42(19): 291–296. https://doi.org/10.5252/adansonia2020v42a19

Wang RJ (2002) Two new species of *Spiradiclis* (Rubiaceae) from China. Novon 12(3): 420–423. https://doi.org/10.2307/3393092

Wang RJ (2016) *Spiradiclis jingxiensis* sp. nov. (Rubiaceae) from Guangxi, China. Nordic Journal of Botany 34(5): 550–552. https://doi.org/10.1111/njb.01134

Wang RJ, Wen HZ, Deng SJ, Zhou LX (2015) *Spiradiclis danxiashanensis* (Rubiaceae), a new species from south China. Phytotaxa 206(1): 30–36. https://doi.org/10.11646/phytotaxa.206.1.5

Wen HZ, Wang RJ, Deng SJ (2015) *Spiradiclis longanensis*, a new species of Rubiaceae from China. PhytoKeys 55: 113–117. https://doi.org/10.3897/phytokeys.55.4975

Wen ZJ, Yang JC, Xu YF, Wu L (2019) *Spiradiclis densa* sp. nov. (Rubiaceae) from limestone areas in Guangxi, China. Nordic Journal of Botany 37(6): e02190. https://doi.org/10.1111/njb.02190

Wen ZJ, Huang YF, Hu YH, Nguyen KS, Wu L (2021) *Spiradiclis detianensis* (Rubiaceae, Ophiorrhizeae), a new species from south-western Guangxi, China. PhytoKeys 184: 103–110. https://doi.org/10.3897/phytokeys.184.69886

Wikström N, Neupane S, Kårehed J, Motley TJ, Bremer B (2013) Phylogeny of *Hedyotis* L. (Rubiaceae: Spermacoceae): redefining a complex Asian-Pacific assemblage. Taxon 62(2): 357–374. https://doi.org/10.12705/622.2

Wu L, Wang JL, Mo SS, Liu QR (2015a) *Spiradiclis glandulosa* sp. nov. (Rubiaceae) from limestone areas in southern China. Nordic Journal of Botany 33(1): 79–82. https://doi.org/10.1111/njb.00577

Wu L, Wang JL, Liu QR (2015b) *Spiradiclis pauciflora* (Rubiaceae), a new species from limestone areas in Guangxi, China. Annales Botanici Fennici 52(3–4): 257–261. https://doi.org/10.5735/085.052.0318

Wu L, Tong Y, Pan B, Liu QR (2016) *Spiradiclis glabra* sp. nov. (Rubiaceae) from limestone areas in Guangdong, China. Nordic Journal of Botany 34(6): 718–721. https://doi.org/10.1111/njb.01156

Wu L, Li X, Liu WJ, Liu QR (2019a) *Spiradiclis karstana* (Rubiaceae), a new species from Yunnan, China. PhytoKeys 117: 1–8. https://doi.org/10.3897/phytokeys.117.28281

Wu L, Wang BM, Pan B, Yu XL (2019b) *Spiradiclis tubiflora* (Rubiaceae), a new cave-dwelling species from southern China. PhytoKeys 130: 217–224. https://doi.org/10.3897/phytokeys.130.34625

Verdcourt B (1958) Remarks on the calassification of the Rubiaceae. Bulletin van den Rijksplantentuin, Brussel 28: 209–281.

Zhang F, Liu Y, Wen ZJ, Wu L (2018) *Spiradiclis lui*, a new species of Rubiaceae from Guangxi, China. Nordic Journal of Botany 36(6): e01786. https://doi.org/10.1111/njb.01786

PhytoKeys 204: 83–95 (2022)
doi: 10.3897/phytokeys.204.84886
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Taxonomic study of a novel terrestrial alga, *Spongiosarcinopsis qinghaiensis* sp. nov. (Protosiphonaceae, Chlorophyta), from the Qinghai-Tibet Plateau

Qiufeng Yan[1,2], Huan Zhu[1], Jiao Fang[1,2], Benwen Liu[1], Guoxiang Liu[1]

1 *Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China* **2** *University of Chinese Academy of Sciences, Beijing 100049, China*

Corresponding author: Guoxiang Liu (liugx@ihb.ac.cn)

Academic editor: Wolf-Henning Kusber | Received 4 April 2022 | Accepted 29 July 2022 | Published 11 August 2022

Citation: Yan Q, Zhu H, Fang J, Liu B, Liu G (2022) Taxonomic study of a novel terrestrial alga, *Spongiosarcinopsis qinghaiensis* sp. nov. (Protosiphonaceae, Chlorophyta), from the Qinghai-Tibet Plateau. PhytoKeys 204: 83–95. https://doi.org/10.3897/phytokeys.204.84886

Abstract

There is only one species of *Spongiosarcinopsis* in the literature currently. It was found in gray soil in Russia for the first time. According to molecular data analysis results, the isolated algal strain is most closely related to *Spongiosarcinopsis terrestris*. Unlike *Spongiosarcinopsis terrestris*, the isolated strain was found on soil surfaces at high altitudes, the young vegetative cell is spherical, vegetative cells are relatively large, and pyrenoids are generally fewer. In view of such morphological differences, phylogenetic analysis results, and comparison of ITS2 secondary structure and ultrastructure, the strain isolated in the present study was proposed to be a novel species.

Keywords

ITS2 secondary structure, new species, phylogenetic analysis, *Spongiosarcinopsis*

Introduction

Algae exist in almost all terrestrial environments on earth, and always appear on and below the soil surface (Metting 1981). Since the end of the 19th century, algae have been recognized as an integral part of the soil microbial community (Starks et al. 1981). Algae that grow on or under soil play important roles in agro-ecosystems and are indicators of soil quality (Trofim et al. 2013). Soil algae have a positive effect on soil

formation and stabilization, increasing soil nitrogen content through nitrogen fixation (Metting 1981; Goyal 1997; Kuzyakhmetov 1998). The soil environment is highly heterogeneous, which provides diverse habitats for exploitation by microorganisms, including algae (Trevors and Wellington 1997). Many algae are found in soil habitats, including *Spongiosarcinopsis* (Temraleeva et al. 2018; Temraleeva 2019), *Chlorococcum* (Nostoch 1842), *Hormidium* (Kützing 1843), *Tetracystis* (Brown and Bold 1964), *Chlorosarcinopsis* (Herndon 1958), *Protococcus* (Agardh 1824), *Neochloris* (Starr 1955), and *Sphaerocystis* (Chodat 1897). In view of the diversity and importance of soil green algae, it is necessary to classify and identify them accurately.

Spongiosarcinopsis was proposed in 2018, due to its unique morphological characteristics (chloroplasts are spongy, which distinguish it from other genera in the family) and molecular sequence, it is classified within Protosiphonaceae (Temraleeva et al. 2018; Temraleeva 2019). Protosiphonales are divided into four groups, out of which three groups include Stephanosphaeraceae, Chlorococcaceae, and Protoiphonaceae. Protoiphonaceae (Chlorophyta, Chlorophyceae) are characterized by naked zoospores, one to several pyrenoids and a starch envelope. In addition, most algae in the family can produce excessive secondary carotenoids, and most of the algae turn orange (Temraleeva et al. 2017). Based on a literature review, recent studies rarely rely exclusively on morphology when classifying species. This is because many algae exhibit large intraspecific morphological variability, genetically controlled polymorphisms, or environment-induced plasticity (Lurling 2003; Logares et al. 2007). Consequently, more comprehensive taxonomic evidence is required to support the conclusions of taxonomists, including 18S ribosomal DNA (rDNA) and internal transcribed spacer (ITS) rRNA molecular markers, which are commonly used in green algae, in addition to compensatory base pair changes (CBCS), for species differentiation (Leliaert et al. 2014).

The aim of the present study was to combine morphological and molecular phylogenetic analyses of 18S rDNA and ITS rRNA sequences, and compare ITS2 secondary structure and ultrastructure between the two species in the genus *Spongiosarcinopsis*. A novel *Spongiosarcinopsis* sp. was proposed.

Materials and methods

Isolation and culture of algal strains

Collection of algal strains from the surface of loose sandy soil near Qinghai Lake in September 2020 (37°02'N, 100°44'E, altitude: 3201.8 m). Take an appropriate amount of soil sample into a 2 ml centrifuge tube, add an appropriate amount of distilled water, mix well with a vortex shaker, and inoculate an appropriate amount of suspension with a pipette tip on BG-11 solid medium containing 1.3% agar, and then use a triangular glass rod coated solid slab (Allen 1968), and then incubated at a constant temperature of 25 °C under a 12/12-h light/dark cycle until visible colonies

appeared. Then, the algal colonies were transferred to fresh medium until a pure single algal culture was obtained. Transfer the single algal culture to a 96-well plate containing BG-11 liquid medium. The algal strains were stored in the freshwater algae culture bank (Freshwater Algae Culture Collection at the Institute of Hydrobiology) of Institute of aquatic biology, Chinese Academy of Sciences, China(No. 7, Donghu South Road, Wuhan, Hubei), under the Accession No. FACHB-(3451). Morphological observations of native and cultured algal strains were performed using a Leica DM5000B microscope, and photomicrographs were obtained using a Leica DFC320 digital camera.

DNA extraction, PCR amplification, sequencing

The pure unialgal cells were disrupted using a beader (3110BX, Biospec Products, Buttersville, USA) with mini beads. The total DNA was extracted using HP Plant DNA Kit (Omega Bio-Tek, GA, USA). PCR amplification was performed using 6 μL of template DNA, 1 μL of each primer, and 42 μL of Master Mix in a 50-μL reaction volume. The 18S rDNA sequences were amplified using the primers 18SR and 18SF (Medlin et al. 1988), and the amplification conditions were as follows: 94 °C for 5 min, followed by 94 °C for 50 s, 55 °C for 50 s, 72 °C for 90 s, and a final extension at 72 °C for 10 min. The primers used to amplify the ITS sequence were NS7m and LR1850 (Bhattacharya et al. 1996), and the amplification conditions were as follows: 32 cycles of 94 °C for 5 min, 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °Cor 10 min. The PCR products were sequenced by TSINGKE Biotechnologies (China), and the sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/) with login numbers OM333733 and OM333734.

Molecular phylogenetic analyses

Based on BLAST search selection as well as possible phylogenetic relationships and broader green algae, 45 18S rDNA and 19 ITS sequences were downloaded from GenBank, and preliminarily aligned using MAFFT 7.3 (Standley 2013). Manual optimization via Seaview (v.5.0) (Gouy et al. 2021). Pairwise distances were plotted against model-corrected distances using MEGA (v.11.0)(Tamura et al. 2021) to evaluate mutational saturation of the alignments saturation in the variable positions, and neither transversion nor transformation reached saturation. Phylogenies were estimated using maximum likelihood (ML) in PhyloSuite (v.1.2.1) (Zhang et al. 2020) and Bayesian inference in MrBayes (v.3.2.2) (Mishra and Thines 2014). The best-fit evolutionary model was selected using hiearchial likelihood ratio tests and Akaike information criterion through Modeltest-NG (Darriba et al. 2020). K2+G+I and K2+G were found to be the best-fit models for 18S rDNA and ITS, respectively. For ML analysis, tree search was realized using a heuristic search option with random addition of sequences (10 replicates) and tree bisection and reconnection branch-swapping algorithm. Statistical reliability was estimated by Bootstrap analysis with

1000 replicates of the dataset for ML. Four Markov chains (three heated chains, one cold chain) were run for Bayesian Markov Chain Monte Carlo analysis for 20 million generations with tree sampling performed for every 10,000 generations. Stationary distribution was assumed when the average standard deviation of split frequencies between the two runs was lower than 0.01. The first 25% of the trees was discarded, the consensus tree with the remaining samples was constructed, and a posteriori probability was inferred. The phylogenetic trees were edited using Figtree1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Comparison of ITS2 secondary structure

Implemented the prediction of ITS2 secondary structure in the ITS2 database (http://its2.bioapps.biozentrum.uni-wuerzburg.de/). By comparing the secondary structures of ITS2 of the two algae, the differences in the two structures were analyzed. Cluster W (Larkin et al. 2007) multiple alignment sequence in 4SALE version 1.7 software was used to align ITS2 sequence and secondary structure (Seibel et al. 2008). Secondary structure images edited with Varna v3.1 (Darty et al. 2009).

Results

***Spongiosarcinopsis qinghaiensis* Q. YAN & G. LIU, sp. nov.**

Description. Found on the soil surface around brackish lakes. Unicellular, spherical (Figs 1, 2), and cell diameter of 6–15 μm. Mature cells often divide into two cells by means of diads (Fig. 3). Cells contain one net-like chloroplast (Fig. 6), lateral, almost filling the entire cell at maturity (Figs 2, 8), one nucleus, and one pyrenoid covered by starch envelope, although not obvious (Figs 7, 8).

The vegetative cell wall is rough and there is an unidentified gelatinous layer outside the cell wall (Fig. 9). The pyrenoid is covered with a segmented starch envelope and penetrated by straight thylakoids (Fig. 10).

Asexual reproduction is achieved through diads or release of aplanospores (Figs 3–5). Aplanosporangia contain eight to 16 spherical aplanospores, and the release process can be observed (Figs 4, 5). No sexual reproduction was observed.

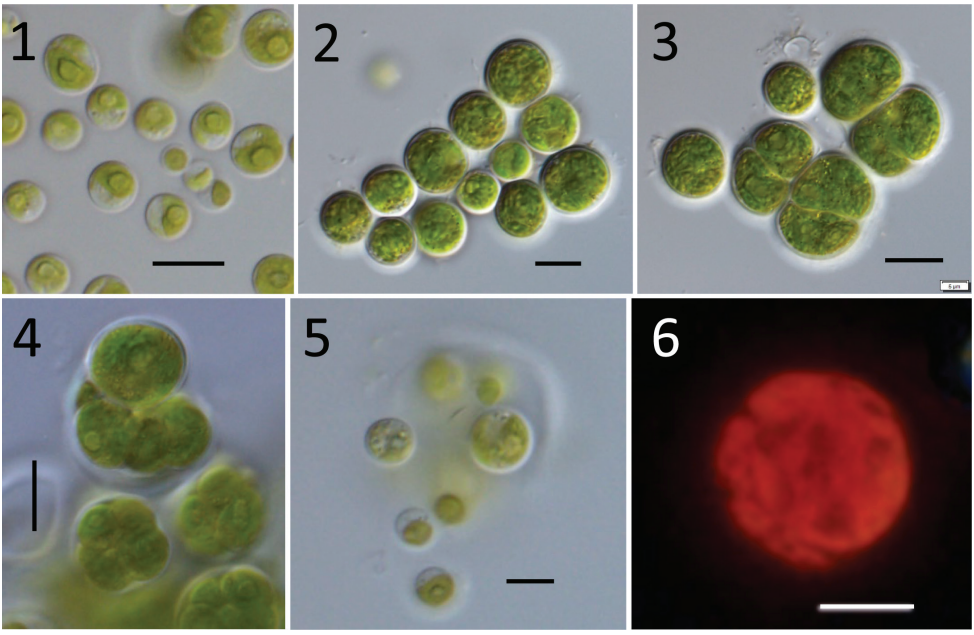
Etymology. The species epithet refers to the Holotype locality (Qinghai Province).

Type locality. Qinghai Lake National Nature Reserve (37°02'N, 100°44'E), Qinghai Province, China; on soil surface.

Iconotype. Fig. 2.

Holotype. QH2015 (HBI), collected by Qiu–Feng Yan and Huan Zhu, 22 September 2020; deposited in the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China.

Distribution. At present, the algal isolate is only found in China. It grows on loose and moist soil surfaces around brackish lakes.



Figures 1–6. Culture sample morphology of *Spongiosarcinopsis qinghaiensis* sp. nov.: **1** the normal growth state of the algal strain, young cells have a larger protein nucleus **2** mature vegetative cells **3** vegetative cells reproduce through diads **4** aplanosporangia containing aplanospores covered by thin cell walls **5** aplanospores released from aplanosporangia **6** net-like chloroplast under fluorescence. Scale bar: 10 μm (**1–4**); 5 μm (**5–6**).

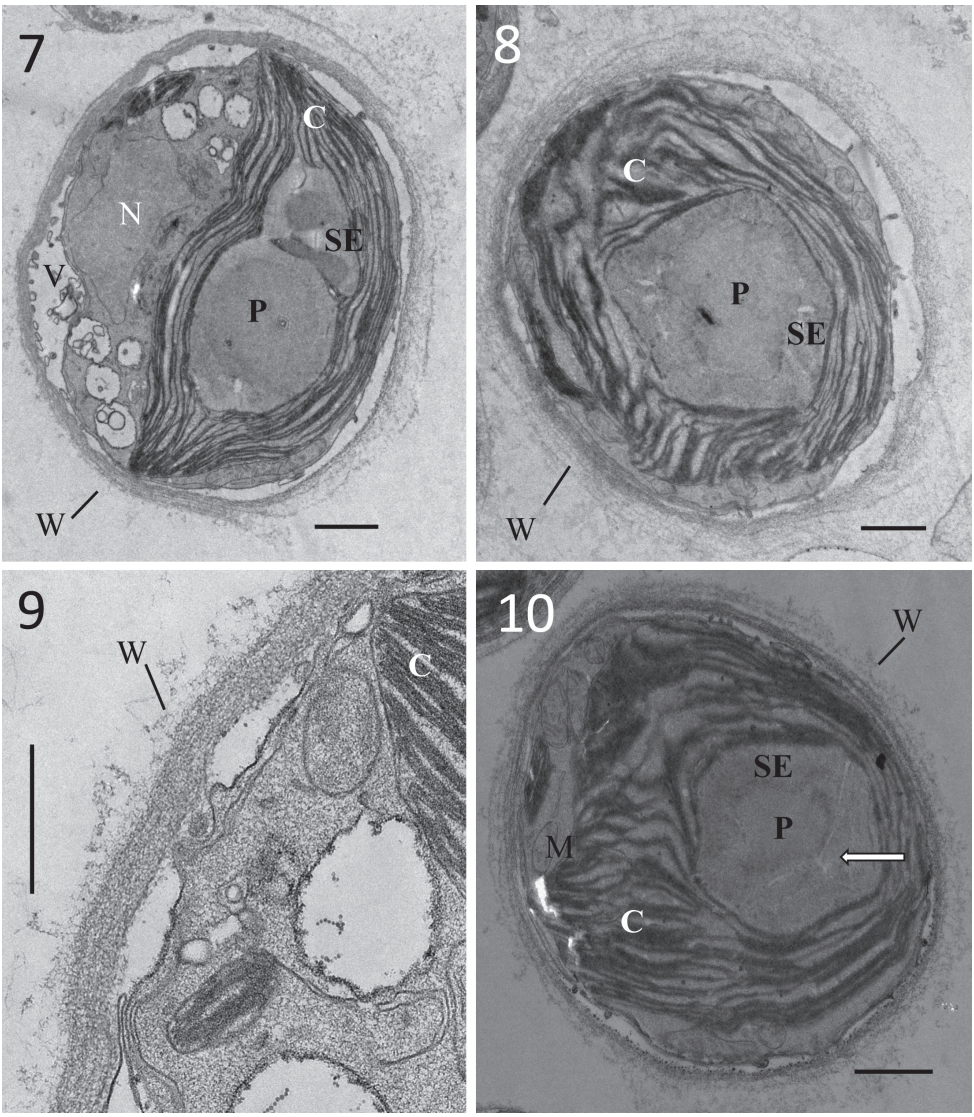
Authentic culture. Culture strain FACHB-3451 is deposited in the Freshwater Algae Specimen Station, Institute of Hydrobiology, Chinese Academy of Sciences (http://algae.ihb.ac.cn/).

Therefore, *Spongiosarcinopsis qinghaiensis* was found to be different from *Spongiosarcinopsis terrestris*, with respect to young cell shape, habitat, size of vegetative cells, and number of pyrenoids. Registration. http://phycobank.org/103211.

Phylogenetic analyses

The 18S rDNA alignment applied 45 sequences (including 15 Protosiphonaceae sequences), which consisted of 1686 sites, out of which 300 (17.8%) and 208 (12.3%) were variable and parsimony-informative sites, respectively. Nineteen ITS sequences were used for alignment and 546 sites, out of which 327 (59.9%) and 232 (42.5%) were variable and parsimony-informative sites, respectively. Table 1 presents detailed information about the alignment and nucleotide substitution in 18S rDNA and ITS concatenated phylogenies for molecular phylogenetic analysis.

The phylogenetic tree was constructed using the Bayesian approach based on 18S rDNA and ITS alignments (Figs 11, 12), with the Bayesian posterior probabilities and



Figures 7–10. TEM images of *Spongiosarcinopsis qinghaiensis* sp. nov.: **7** cells contain one nucleus, one chloroplast, one pyrenoid covered by starch envelope **8** the starch envelope covering the pyrenoid is not obvious **9** unidentified gelatinous layer outside the cell wall **10** the pyrenoid is covered by segmented starch envelope and penetrated by straight thylakoids. C = chloroplast, N = nucleus, P = pyrenoid, SE = starch envelope of the pyrenoid, W = cell wall. Arrows indicate thylakoids penetrating into the pyrenoid matrix. Scale bar: 1 μm (**7, 8, 10**); 500 nm (**9**).

ML bootstrap support reported. The topology of our 18S rDNA phylogeny (Fig. 11) is essentially consistent with that reported in previous studies (Temraleeva et al. 2017). The tree based on 18S rDNA showed a strong *Spongiosarcinopsis* clade, comprising the algal strains isolated in the present study; hence, the algal isolates were classified into

Table 1. Detailed information of alignment and nucleotide substitution in 18S and *tuf*A concatenated phylogenetic for ML analysis.

Dataset	18S	ITS
Alignment length	1686	546
Number of sequences	45	19
Parsimony-informative sites	208	232
Invariant sites	1386	219
Best-fit model	K2+G+I	K2+G
Base frequency (A/C/G/T)	0.25/0.21/0.28/0.26	0.25/0.26/0.24/0.25
Saturation test (*Iss*/*Iss*.c)	0.056<0.836	0.397<0.721

the genus *Spongiosarcinopsis* (Fig. 11). The tree based on ITS sequences prsesented the interspecific relationships within the genus *Spongiosarcinopsis*, and the algal strain isolated in the present study formed a distinct branch separate from *Spongiosarcinopsis terrestris* (Fig. 12).

ITS2 secondary structure

The ITS2 secondary structure was annotated, and two *Spongiosarcinopsis* strains were detected (Fig. 13), namely, *Spongiosarcinopsis terrestris* (MF687232) and *Spongiosarcinopsis qinghaiensis* (OM333733). The differences between the two strains of algae were compared. Three CBCs and four hemi-compensatory base changes (h-CBCs) were detected between the two strains.

Discussion

Protosiphonaceae used to include *Chlorochytrium* (Cohn 1872) and *Kentrosphaera* (Borzì 1883; Kostikov 2001); however, according to algaebase records, there are only three genera left in Protosiphonaceae, namely: *Spongiosarcinopsis* (Temraleeva et al. 2018; Temraleeva 2019), *Protosiphon* (Klebs 1896), and *Urnella* (Playfair 1918). Nakada et al. (2008) includes Protoiphonaceae in Stephanosphaerinia clade, but does not take into account morphological features. Recently, Protoiphonaceae (Chlorophyta, Chlorophyceae) have been reported to be characterized by naked zoospores, one to several pyrenoids, and a starch envelope; furthermore, most algae in the family can produce excessive secondary carotenoids, so that the algae turn orange (Temraleeva et al. 2017).

In the present study, a phylogenetic tree constructed using the Bayesian approach based on 18S rDNA revealed that the algal isolates formed a robust branch and were closely related to *Spongiosarcinopsis terrestris* (MF687231) (Fig. 11). Furthermore, intraspecific, interspecific, and adjacent genera (Protosiphonaceae) showed high ML support and Bayesian posterior probability. As a boundary, *Spongiosarcinopsis* is clearly separated from the adjacent genus to form two distinct clades. Such molecular

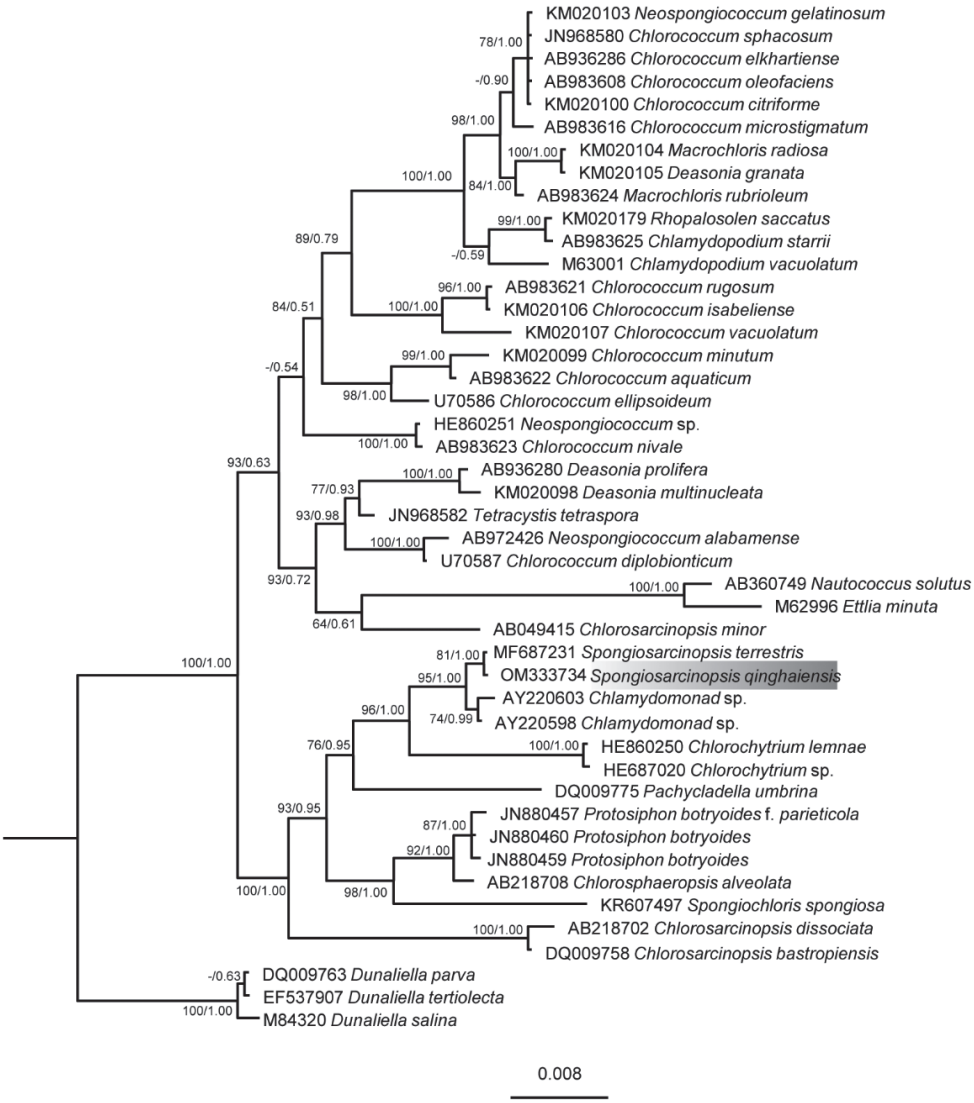


Figure 11. Phylogenetic tree constructed by Bayesian approach based on 18S rDNA sequences. Maximum likelihood bootstrap values and Bayesian posterior probabilities are given on nodes. Values above 0.50 for BI and 50 for ML are given. The gray part shows the new species of this study.

phylogenetic data support the classification of our novel algal isolate into the genus *Spongiosarcinopsis*. The phylogenetic tree constructed using the Bayesian approach based on ITS sequence showed that *S. terrestris* (MF687232) and *S. qinghaiensis* (OM333733) formed a clade but are clearly separated, and had high ML support and Bayesian posterior probability (Fig. 12). The finding was confirmed by the evolutionary tree developed based on ITS2 secondary structure (Fig. 13).

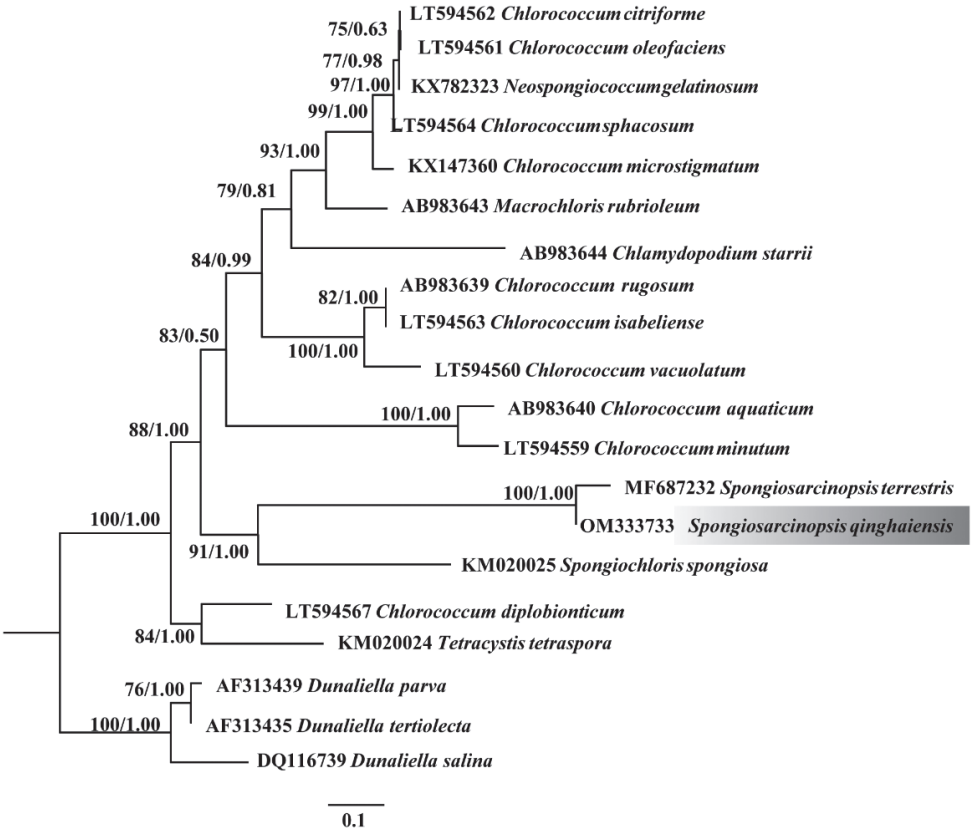


Figure 12. Phylogenetic tree constructed by Bayesian approach based on ITS sequences. Maximum likelihood bootstrap values and Bayesian posterior probabilities are given on nodes. Values above 0.50 for BI and 50 for ML are given. The gray part shows the new species of this study.

Three CBCs and four h-CBCs were detected between *S. terrestris* (MF687232) and *S. qinghaiensis* (OM333733) based on ITS2 secondary structure analysis. Analysis of the four main spiral branches revealed that the two strains had varying characteristics to different degrees. The presence of CBCs is often used as an indicator of isolated species or genera (Vanormelingen et al. 2007; Bock et al. 2011a, b). The difference suggests that *S. qinghaiensis* (OM333733) is unique.

Morphologically, unlike *S. terrestris*, the isolated strain was characterized based on habitat, young vegetative cell shape, size of vegetative cells, and number of pyrenoids. The thylakoid bundle shape is straight; however, this may not be its true state, and is not used as a distinguishing feature here. Both algal strains were found on the soil surface; however, the soil types were different. *S. qinghaiensis* was found in loose soil around a brackish lake, whereas *S. terrestris* was found in a gray forest soil. Such gaps in geography and habitat may be some of the reasons

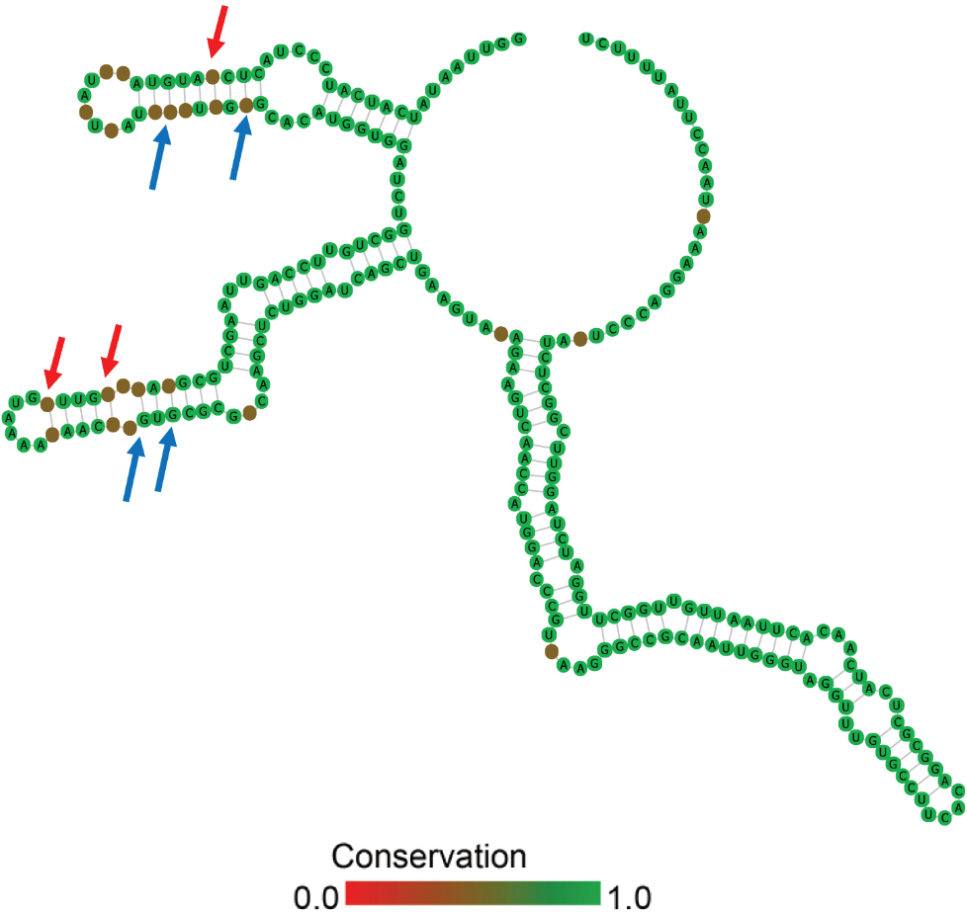


Figure 13. The ITS2 secondary structure between the two strains in *Spongiosarcinopsis*. The darker the color, the more significant the variation. Differences between the two species have been indicated by arrows in the figure, red for CBCs and blue for h-CBCs.

for the unique *S. qinghaiensis* morphology and phylogeny. To better distinguish *S. qinghaiensis* and *S. terrestris*, their morphological characteristics were compared. The algal strain isolated in the present study is spherical at the young stage, whereas *S. terrestris* is ellipsoidal. Furthermore, *S. qinghaiensis* has one pyrenoid and a cell diameter of 6–15 μm. In contrast, *S. terrestris* has 1–5 pyrenoids and a cell diameter of 5–10 μm. The *Spongiosarcinopsis* strain isolated in the present study was derived from the soil surface next to a brackish lake. The extreme environments with a high altitude (3201.8 m), high salinity, and low temperature suggest that there may still be numerous undiscovered algal species growing under similar environments. The molecular data and morphological characteristics support *Spongiosarcinopsis qinghaiensis* as a novel species.

Conclusions

Based on phylogenetic analysis, ITS2 secondary structure comparison, morphological characteristics, and ultrastructure, *Spongiosarcinopsis qinghaiensis* is proposed as a novel *Spongiosarcinopsis* sp. Algal strains in highland areas are often not readily observed, which may lead to algal diversity being partially overlooked, so that more research should be undertaken.

References

Agardh CA (1824) Systema algarum. Literis Berlingianis. https://doi.org/10.5962/bhl.title.1829

Allen MM (1968) Simple conditions for growth of unicellular blue-green algae on plates 1, 2. Journal of Phycology 4(1): 1–4. https://doi.org/10.1111/j.1529-8817.1968.tb04667.x

Bhattacharya D, Friedl T, Damberger S (1996) Nuclear-encoded rDNA group I introns: Origin and phylogenetic relationships of insertion site lineages in the green algae. Molecular Biology and Evolution 13(7): 978–989. https://doi.org/10.1093/oxfordjournals.molbev.a025666

Bock C, Paoutová M, Krienitz L (2011a) Phylogenetic position of *Coronastrum ellipsoideum* and description of *Parachlorella hussii* sp. nov. Biologia 66(4): 585–594. https://doi.org/10.2478/s11756-011-0056-8

Bock C, Pröschold T, Krienitz L (2011b) Updating the genus *Dictyosphaerium* and description of *Mucidosphaerium* gen. nov. (Trebouxiophyceae) based on morphological and molecular data. Journal of Phycology 47(3): 638–652. https://doi.org/10.1111/j.1529-8817.2011.00989.x

Borzi A (1883) Studi algologici: saggio di richerche sulla biologia delle alghe / Antonino Borzi. https://doi.org/10.5962/bhl.title.1607

Brown Jr RM, Bold HC (1964) Comparative studies of the algal genera *Tetracystis* and *Chlorococcum*. University of Texas Publication 6417: 1–213. https://doi.org/10.1111/j.1529-8817.1965.tb04558.x

Chodat R (1897) Algues pélagiques nouvelles. Bulletin de l'Herbier Boissier 5: 119–120.

Cohn F (1872) Über parasitische Algen. Beitr. Biol. Pflanzen 1(1): 87–106.

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. Molecular Biology and Evolution 37(1): 291–294. https://doi.org/10.1093/molbev/msz189

Darty K, Denise A, Ponty Y (2009) VARNA: Interactive drawing and editing of the RNA secondary structure. Bioinformatics 25(15): 1974–1975. https://doi.org/10.1093/bioinformatics/btp250

Gouy M, Tannier E, Comte N, Parsons DP (2021) Seaview version 5: a multiplatform software for multiple sequence alignment, molecular phylogenetic analyses, and tree reconciliation. In Multiple Sequence Alignment. Humana, New York, 241–260. https://doi.org/10.1007/978-1-0716-1036-7_15

Goyal SK (1997) Algae and the soil environment. Phykos (Algiers) 36: 1–13.

Herndon W (1958) Studies on chlorosphaeracean algae from soil. American Journal of Botany 45(4): 298–308. https://doi.org/10.1002/j.1537-2197.1958.tb13130.x

Klebs GA (1896) Ueber Die Fortpflanzungs-Physiologie der niederen Organismen, der Protobionten. Specieller Theil.

Kostikov I (2001) Soil algae of Ukraine (history and methods of study, system, flora conspect). Fitosociocentre Press, Kiev.

Kützing FT (1843) Phycologia generalis: oder Anatomie, Physiologie und Systemkunde der Tange. Brockhaus. https://doi.org/10.5962/bhl.title.4746

Kuzyakhmetov GG (1998) Productivity of algocenoses in zonal arable soils of steppe and forest-steppe. Eurasian Soil Science 31(4): 406–410.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics (Oxford, England) 23(21): 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Leliaert F, Verbruggen H, Vanormelingen P, Steen F, López-Bautista JM, Zuccarello GC, Clerck O (2014) DNA-based species delimitation in algae. European Journal of Phycology 49(2): 179–196. https://doi.org/10.1080/09670262.2014.904524

Logares R, Rengefors K, Kremp A, Shalchian-tabrizi K, Boltovskoy A, Tengs T, Shurtleff A, Klaveness D (2007) Phenotypically different microalgal morphospecies with identical ribosomal DNA: A case of rapid adaptive evolution? Microbial Ecology 53(4): 549–561. https://doi.org/10.1007/s00248-006-9088-y

Lurling M (2003) Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology. Annales de Limnologie 39(2): 85–101. https://doi.org/10.1051/limn/2003014

Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71(2): 491–499. https://doi.org/10.1016/0378-1119(88)90066-2

Metting B (1981) The systematics and ecology of soil algae. Botanical Review 47(2): 195–312. https://doi.org/10.1007/BF02868854

Mishra B, Thines M (2014) siMBa—A simple graphical user interface for the Bayesian phylogenetic inference program MrBayes. Mycological Progress 13(4): 1255–1258. https://doi.org/10.1007/s11557-014-1010-2

Nakada T, Misawa K, Nozaki H (2008) Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18S rRNA phylogenetic analyses. Molecular Phylogenetics and Evolution 48(1): 281–291. https://doi.org/10.1016/j.ympev.2008.03.016

Nostoch M (1842) [1843] Monographia Nostochinearum Italicarum addito specimine de Rivulariis / auctore J[osepho] Meneghini, Prof. Augustae Taurinorum.

Playfair GI (1918) New and rare freshwater algae. Proceedings of the Linnean Society of New South Wales 43: 497–543. [pls LIV–LVII.]

Seibel PN, Müller T, Dandekar T, Wolf M (2008) Synchronous visual analysis and editing of RNA sequence and secondary structure alignments using 4SALE. BMC Research Notes 1(1): 1–7. https://doi.org/10.1186/1756-0500-1-91

Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Starks TL, Shubert LE, Trainor FR (1981) Ecology of soil algae: A review. Phycologia 20(1): 65–80. https://doi.org/10.2216/i0031-8884-20-1-65.1

Starr RC (1955) A comparative study of *Chlorococcum* meneghini and other spherical, zoospore-producing genera of the Chlorococcales. quarterly review of biology. Science Series 20: [i–viii] 1–111. [239 figs.]

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Temraleeva A (2019) Validation of *Spongiosarcinopsis terrestris* gen. et sp. nov. (Protosiphonaceae, Chlorophyta). Notulae Algarum 98: 1–2.

Temraleeva AD, Moskalenko SV, Bachura YM (2017) Morphology, ecology, and 18S rDNA phylogeny of the green microalgal order Protosiphonales (Chlorophyceae, Chlorophyta). Microbiology 86(2): 159–169. https://doi.org/10.1134/S0026261717020205

Temraleeva A, Moskalenko S, Mincheva E, Bukin Y, Sinetova M (2018) *Spongiosarcinopsis terrestris* gen. et sp. nov. (chlorophyta, chlorophyceae): A new genus of green algae from gray forest soil, russia. Phytotaxa 376(6): 291. https://doi.org/10.11646/phytotaxa.376.6.4

Trevors JT, Wellington E (1997) Modern soil microbiology. Marcel Dekker.

Trofim A, Donţu N, Şalaru V, Gheorhiţa C (2013) Taxonomic and ecological structure of the edaphic algae flora from certain agrocenoses of the Republic of Moldova. http://studiamsu.eu/nr-1-61-2013/

Vanormelingen P, Hegewald E, Braband A, Kitschke M, Friedl T, Sabbe K, Vyverman W (2007) The systematics of a small spineless *Desmodesmus* species, d. costato-granulatus (Sphaeropleales, Chlorophyceae), based on its2 rdna sequence analyses and cell wall morphology 1. Journal of Phycology 43(2): 378–396. https://doi.org/10.1111/j.1529-8817.2007.00325.x

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

PhytoKeys 204: 97–108 (2022)
doi: 10.3897/phytokeys.204.89690
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

A new freshwater species *Achnanthidium kangdingnese* (Bacillariophyta, Achnanthidiaceae) from Sichuan Province, China

Pan Yu[1,2], Qingmin You[1], Yonghong Bi[2], Quanxi Wang[1]

1 *College of Life Sciences, Shanghai Normal University, Shanghai 200234, China* **2** *Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China*

Corresponding author: Quanxi Wang (wangqx@shnu.edu.cn)

Academic editor: Kalina Manoylov | Received 30 June 2022 | Accepted 9 August 2022 | Published 12 August 2022

Citation: Yu P, You Q, Bi Y, Wang Q (2022) A new freshwater species *Achnanthidium kangdingnese* (Bacillariophyta, Achnanthidiaceae) from Sichuan Province, China. PhytoKeys 204: 97–108. https://doi.org/10.3897/phytokeys.204.89690

Abstract

A new freshwater diatom species, *Achnanthidium kangdingnese* Yu, You & Wang, **sp. nov.** from Sichuan Province, China, is described. The morphology of this species was analyzed with scanning electron microscopy (SEM) and light microscopy (LM). *A. kangdingnese* belongs to the *A. initium*-like subgroup, which has external distal raphe ends curved in opposite directions of the valve. The main characteristics of *A. kangdingnese* are its linear shape, rounded apices and transpically-elongated areolae on the both valves. The central area is well defined with one or two spaced striae of the raphe valve. And on the internal valve, areolae are occluded by hymens perforated by delicate slits, and each hymen is closely joined with the adjacent hymen. We compared the new species with other similar species of *Achnanthidium*, *A. kangdingnese* is considered to be sufficiently different from other similar species based on valve outline, shape of the axial and center areas, and striae density. The new species is known only from its type locality, a mountain lake in Kangding County.

Keywords

Diatom, morphology, Mugecuo Lake, new species, taxonomy

Introduction

The genus *Achnanthidium* Kützing was initially described by Kützing (1844) as a subgenus of *Achnanthes* Bory de Saint-Vincent (1822), with *A. microcephalum* Kützing (Kützing 1844) as the type of species (Pérès et al. 2014). *Achnanthidium* was

re-established by Round et al. (1990) and redefined by Round and Bukhtiyarova (1996). The number of species in *Achnanthidium* now exceeds 200 (Kociolek et al. 2018; You et al. 2021; Yu et al. 2022). Based on the characteristics of distal raphe ends, and valve and areolar shapes, the species of this genus have been divided into three major subgroups. The species of the *A. minutissimum* complex have straight external distal raphe ends and linear to linear-lanceolate valve shapes. Species in the *A. pyrenaicum* complex have external distal raphe ends that are deflected to one side and slit-like areolar openings. Members of the *A. exiguum* complex have external distal raphe ends curved in opposite directions (Yu et al. 2018, 2019a; Miao et al. 2020; Tseplik et al. 2021; Yu et al. 2022). *A. exiguum* and its relatives have since been segregated into the genus of *Gogorevia* Kulikovskiy, Glushchenko, Maltsev and Kociolek (Kulikovskiy et al. 2020). Karthick et al. (2017) also proposed the *A. initium*-like subgroup based on the external distal raphe ends which curve in opposite directions. At present, only four species belong to this latter group, including *A. contrarea* (Lange-Bertalot and Steindorf) H. Lange-Bertalot (Moser et al. 1998), *A. peridotiticum* (Moser, Lange-Bertalot and Metzeltin) H. Lange-Bertalot (Moser et al. 1998), *A. indicatrix* (Lange-Bertalot and Steindorf) H. Lange-Bertalot (Moser et al. 1998), and *A. initium* Karthick, J.C. Taylor and P.B. Hamilton (Karthick et al. 2017).

Members of *Achnanthidium* have long been considered to belong to the family Achnanthaceae. In China, 48 species of *Achnanthidium* have been reported compared to 155 taxa of *Achnanthes* (Liu et al. 2021), including 11 new *Achnanthes* species (Hustedt 1922; Jao 1964; Jao et al. 1974; Qi and Xie 1984; Zhu and Chen 1994, 1996; Kociolek et al. 2020; Liu et al. 2021). It is possible these species could belong to *Achnanthidium*, but the lack of the type material makes it difficult to confirm their taxonomic position. It is therefore necessary to collect samples from the type locality for taxonomic clarification. From 2001 to 2022, 17 new *Achnanthidium* species were described from China (Liu et al. 2016; Yu et al. 2018, 2019a, b, 2022; You et al. 2019, 2021; Liu et al. 2021; Ge et al. 2022). In the present study, we described a new freshwater diatom species, *Achnanthidium kangdingnese* from Mugecuo Lake in Kangding County, Sichuan Province, China. We documented its valve morphology with a light microscope (LM) and scanning electron microscopy (SEM), and compared its morphological characters with similar species.

Materials and methods

Four diatom samples were collected from Mugecuo Lake in August, 2015. The new species was only found in one sample (MGC201508036) (30°08'43"N, 101°51'35"E). Mugecuo Lake is located at an altitude of 3780 m in Kangding County, Sichuan Province, China in the northern Hengduan Mountains between the Sichuan Basin and the Qinghai-Tibet Plateau (Chen et al. 2013). Several water chemistry characteristics were also recorded, including: pH, temperature and conductivity. These were all measured using a YSIPro Plus multiparameter meter (YSI, Ohio, USA). Diatom samples were

collected from natural substrates by brushing them off with clean toothbrushes. Samples were placed in sample bottles and preserved with formalin (4% final concentration).

In the laboratory, diatom samples (10 mL) were cleaned with concentrated nitric acid (10 mL) using the Microwave Accelerated Reaction System (Model MARS, CEM Corporation, Charlotte, USA) (Parr et al. 2004), with a pre-programmed digestion scheme (temperature, 180 °C) (Yu et al. 2019a). Next, samples were alternately centrifuged for 8 min at 3000 rpm (TDZ5-WS, Luyi Corporation, Shanghai, China) and washed five times using distilled water. The resulting diatom samples were preserved with 95% ethanol. Permanent diatom slides were made with Naphrax (Brunel Microscopes Ltd, Chippenham Wiltshire, U.K) for light microscopy (LM), and the cleaned diatom samples were air-dried onto cover slips and mounted onto alloy stubs for observation with the scanning electron microscope (SEM). LM studies were made with a ZEISS AXIO Imager A2 microscope fitted with DIC optics and at 1000× magnification (1.4 numerical aperture). SEM examination was made using a SU8010 (Hitachi High-Technologies Corp., Tokyo, Japan) at 2 kV, and at a working distance of less than 6 mm. Images were compiled with Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, C.A., U.S.A.). Morphological terminology follows Round et al. (1990). All of the diatom samples and permanent slides are housed in the Biology Department Diatom Herbarium, Shanghai Normal University (SHTU).

Results

***Achnanthidium kangdingnese* P. Yu, Q.M. You & Q.X. Wang, sp. nov.**
Figs 1–5

Description. LM observations (Fig. 1A–AD), valves are linear in shape, with rounded apices. Some individuals were slightly constricted in the middle. Valve length 10.8–23.5 μm, breadth 3.8–4.0 μm (n = 200). On both valves striae are radiate throughout, and striae count cannot be performed with LM. Raphe valve is concave, with a narrow, linear axial area slightly expanded near the center. The central area is well defined with one or two spaced striae. Rapheless valve is convex, with a narrow linear axial area weakly expanded at the middle portion of the valve. The central area is a small oval or absent.

SEM observations (Figs 2–5), both valves have a narrow hyaline area at the valve face-mantle junction (Figs 2A, B, 4A, B). Raphe valve: Externally, the raphe is filiform and straight (Fig. 2A, B), distal raphe ends are deflected in opposite directions (Fig. 2 A–D), and proximal raphe ends are straight and teardrop-shaped (Fig. 2A, B, E). The number of striae is 34–36 in 10 μm at the middle portion, and 33–38 in 10 μm near the apices (Figs 2A, B; 3A). Areolae are round or oval. The uniseriate striae are composed of 4–7 areolae in the middle portion of the valve (Fig. 2A, B, E), and 1–7 areolae at the apex (Fig. 2 A–D). Valve mantle with a single row of linear areolae extend around the apices with a small interruption at the ends (Fig. 2A, C–E). Internally, the thickening widens at the end (Fig. 3A, C), and the raphe terminates in raised helic-

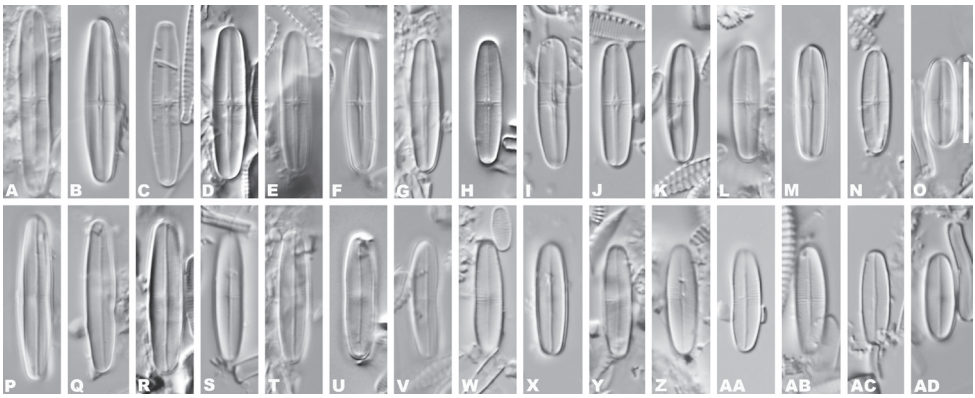


Figure 1. A–AD LM valve views of *Achnanthidium kangdingnese* sp. nov. Scale bar: 10 μm.

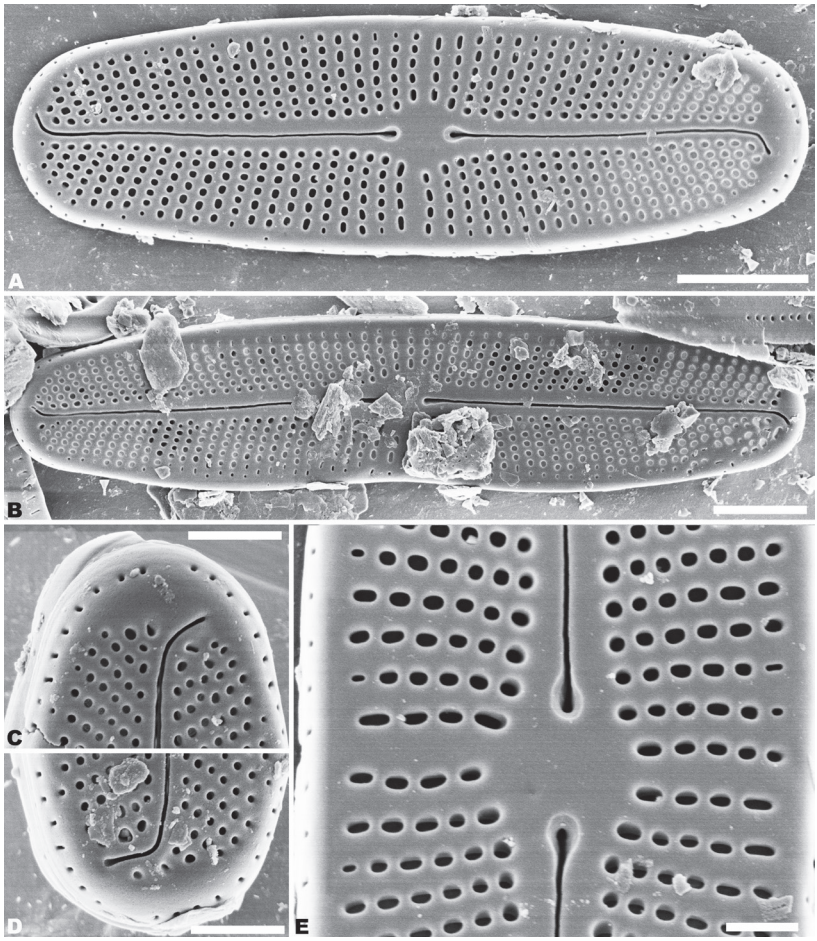


Figure 2. A–E *Achnanthidium kangdingnese* sp. nov. SEM external views of raphe valve **A, B** entire raphe valve **C, D** valve apex, showing the distal raphe ends **E** central area of the valve, showing the proximal raphe ends. Scale bars: 2 μm (**A, B**); 1 μm (**C, D**); 0.5 μm (**E**).

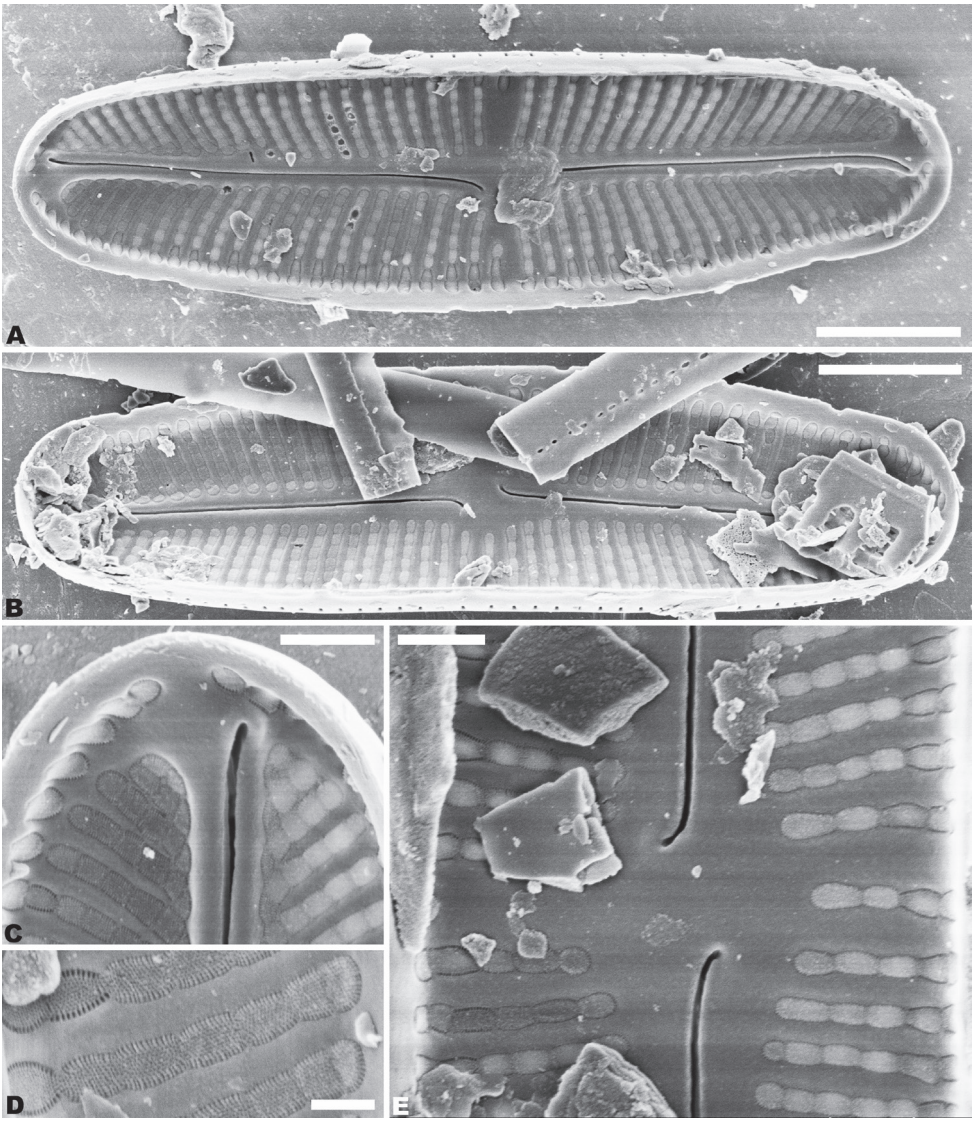


Figure 3. A–E *Achnanthidium kangdingnese* sp. nov. SEM internal views of raphe valve **A, B** entire raphe valve **C** valve apex, showing the distal raphe ends **E** central area of the valve, showing the proximal raphe ends **D** internal areola occluded with fine hymenate structures. Scale bars: 2 μm (**A, B**); 1 μm (**C**); 0.5 μm (**E**); 0.2 μm (**D**).

toglossae close to the apices (Fig. 3A–C). Proximal raphe ends are distinctly deflected in opposite directions (Fig. 3B, E). Areolae are transapically elongated in throughout valve (Fig. 3C, E). Areolae are occluded by hymene perforated by delicate slits, and each hymen is closely joined with the adjacent hymen (Fig. 3D).

Rapheless valve: the single row of pores on the mantle is continuous (Figs 4A–C, 5A, C). Externally, the axial area is linear and weakly expanded in the central area (Fig. 4A, B).

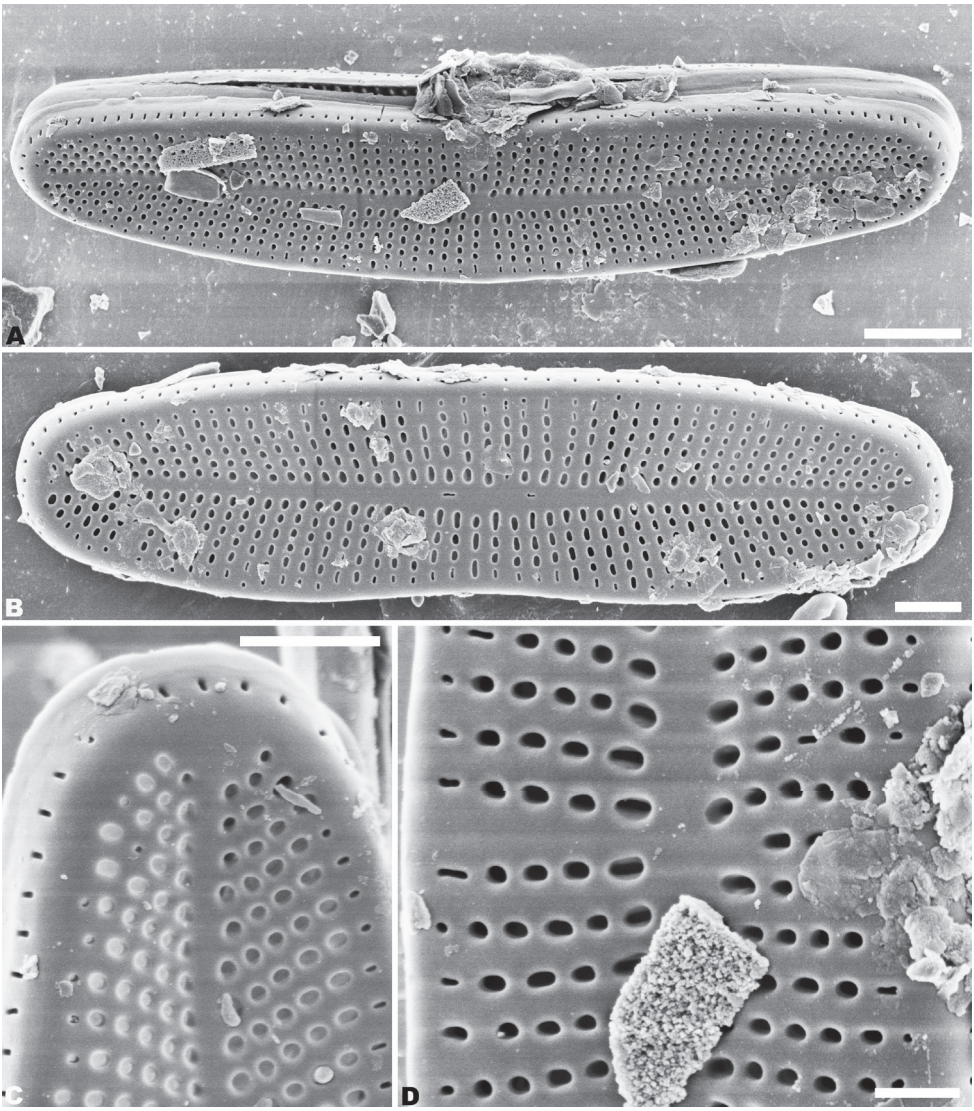


Figure 4. A–D *Achnanthidium kangdingnese* sp. nov. SEM external views of rapheless valve **A, B** entire raphe valve **C** valve apex **D** central area of the valve. Scale bars: 2 μm (**A, B**); 1 μm (**C**); 0.5 μm (**D**).

On some valves, there are two slit-like areolae oriented longitudinally in the middle region of the axial area (Fig. 4B). Striae are uniseriate, comprised of 3–6 round or transapically oriented areolae in the central area (Fig. 4A, B, D), and 1–5 round or oblong areolae at the apices (Fig. 4A–C). A row of slit-like areolae is present on the mantle (Fig. 4A, B). Internally, the axial area is slightly raised (Fig. 5A). Areolae are transapically oval in the valve (Fig. 5B, C). The number of striae is 34–38 in 10 μm in the center, and 38–40 in 10 μm near the apices (Figs 4A, B; 5A). Areolae are occluded by hymens perforated by delicate slits, and each hymen is closely joined with an adjacent hymen (Fig. 5B, C).

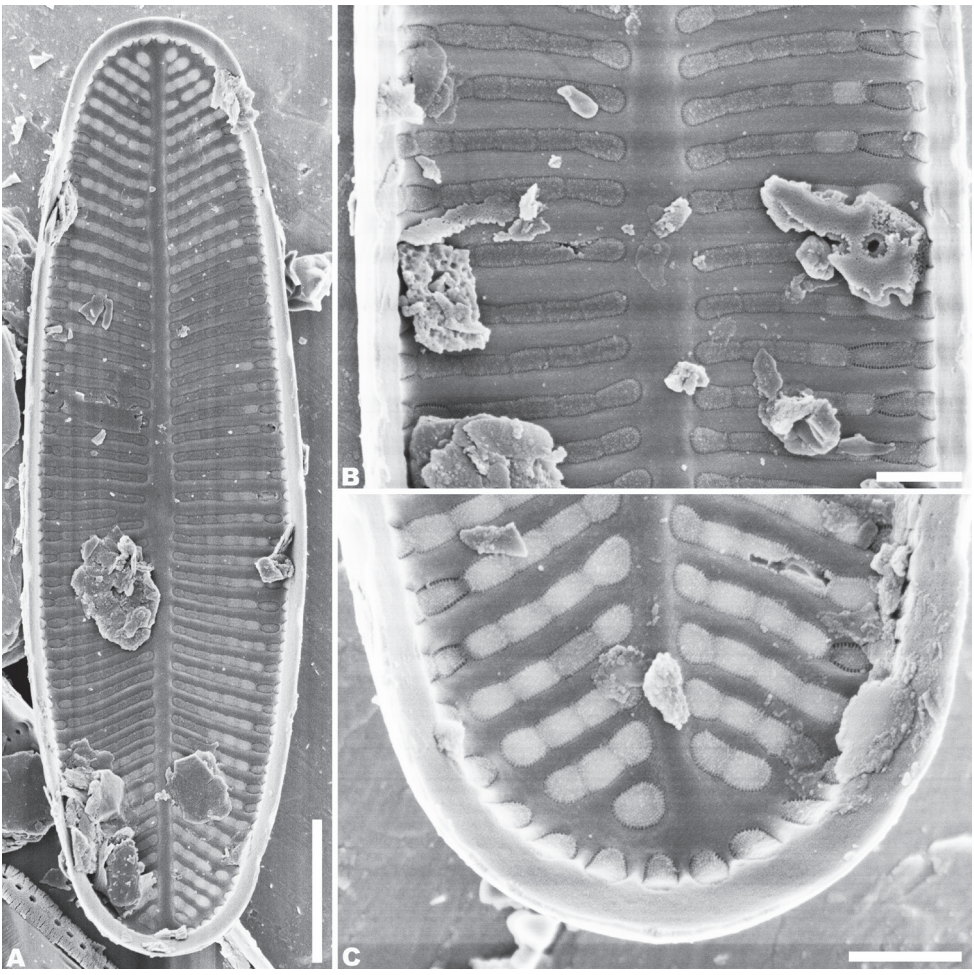


Figure 5. A–C *Achnanthidium kangdingnese* sp. nov., SEM internal views of rapheless valve **A** entire raphe valve **B** central area of the valve **C** valve apex. Scale bars: 2 μm (**A**); 0.5 μm (**B, C**).

Holotype (designated here). SHTU! Slide MGC201508036 in Lab of Algae and Environment, College of Life Sciences, Shanghai Normal University, Shanghai, China. Holotype illustrated in Fig 1D, R.

Type locality. China. Mugecuo lake, Sichuan Province, 30°08'43"N, 101°51'35"E, altitude: 3780 m, *leg. Quanxi Wang in August 2015.*

Etymology. The species so named refers to Kangding County where the holotype was collected.

Ecology. Periphytic diatom samples collected in Mugecuo Lake (MGC201508036), pH 7.8, water temperature 12.5 °C, Conductivity 35 μs.cm^{-1}). The sample of this new species occurred at less than 2% relative abundance (total counted 400 valves). There are 5 species that accounted for more than 5% of sample MGC201508036: *Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & E. Ács (Ács et al. 2016) (47.5%), *Brachysira blancheana*

H. Lange-Bertalot & G. Moser (Lange-Bertalot and Moser 1994) (9.6%), *Encyonema silesiacum* (Bleisch) D.G. Mann (Round et al. 1990) (7.3%), *Staurosira pseudoconstruens* (Marciniak) H. Lange-Bertalot (Krammer and Lange-Bertalot 2000) (7.1%), and *Nitzschia frustulum* (Kützing) A. Grunow (Cleve and Grunow 1880) (5.2%).

Distribution. The new species is known only from the type locality.

Discussion

Achnanthidium kangdingnese sp. nov. possesses features characteristic of the genus *Achnanthidium*. These characteristics include a linear shape, with rounded apices, uniseriate striae, transpically-elongated areolae on the both valves, fine raphe, and deflected external distal raphe fissures (Ponader and Potapova 2007). The deflected external distal raphe fissures support its inclusion in the *A. initium*-like group (Karthick et al. 2017).

Achnanthidium kangdingnese can be compared with several conspecific representatives within the genus based on the outline and structure of the valve. Similar species used for comparison include *A. contrarea*, *A. peridotiticum*, *A. indicatrix*, and *A. initium* (Table 1). In terms of features viewed in the LM, the outline of the valves of *A. kangdingnese* are linear with rounded apices, while those of *A. contrarea* were expanded linear to linear-elliptical with broad capitate apices, *A. peridotiticum* and *A. indicatrix* are linear to linear-elliptical with rounded capitate apices. The valves of *A. kangdingnese* are shorter (10.8–23.5 μm) than the valves of *A. contrarea* (15 to 37.0 μm) and *A. indicatrix* (20.0–35.0 μm). The valves of *A. kangdingnese* are wider (3.8–4.0 μm) than the valves of *A. initium* (3.1–3.6 μm), and narrower than *A. contrarea* (6.0–8.0 μm) and *A. indicatrix* (5.0–7.5 μm). *A. kangdingnese* also has a small oval or absent central area, but *A. peridotiticum* and *A. indicatrix* possess a rhombic-shaped central area, *A. contrarea* has a rhombic to rectangular central area, and *A. initium* has an asymmetrical transverse central area. Additionally, the density of striae of *A. kangdingnese* is higher on both valves than in *A. contrarea* (28–32 in 10 μm on both valves), *A. peridotiticum* (~30 in 10 μm on both valves), *A. indicatrix* (24–27 in 10 μm on the raphe valve, 25–30 in 10 μm on the rapheless valve), and *A. initium* (29–34 in 10 μm on the raphe valve, 32–35 in 10 μm on the rapheless valve).

Achnanthidium kangdingnese is easily separated from *A. minutissimum* complex and *A. pyrenaicum* complex species in this genus by having external distal raphe ends curved in opposite directions of the valve. In contrast to other *Achnanthidium* species, in an internal view, the areolae of *A. kangdingnese* are occluded by hymens, and each hymen closely joins with the adjacent hymen on the both valves (Figs 3C–E, 5A–C).

Achnanthidium kangdingnese has only been found on stones in Mugecuo Lake. This lake has a slightly alkaline pH (7.8) and low conductivity (35 μs.cm^{-1}). Among the four samples taken from Mugecuo Lake, *A. kangdingnese* was found, in low numbers, only in one sample. At the type locality, other monoraphid species co-occur with these new species. The co-occurring monoraphid taxa include *A. pyrenaicum* (Hustedt) P. Kobayasi (Kobayashi 1997), *A. rivulare* M.G. Potapova and K.C. Ponader (Potapova and Ponader

Table 1. Comparison of morphological characteristics of *Achnanthidium kangdingnese* sp. nov. and closely related taxa.

Species/Feature	***A.kangdingnese* sp. nov.**	***A. contrarea* (Lange-Bertalot & Steindorf) Lange-Bertalot**	***A. peridotiticum* (Moser, Lange-Bertalot & Metzeltin) Lange-Bertalot**	***A. indicatrix* (Lange-Bertalot & Steindorf) Lange-Bertalot**	***A. initium Karthick, Taylor* & Hamilton**
Valve length (μm)	10.8–23.5	15.0–37.0	15.0–27.0	20.0–35.0	11.0–25.5
Valve width (μm)	3.8–4.0	6.0–8.0	3.5–4.8	5.0–7.5	3.1–3.6
Valve outline	Linear	Expanded linear to linear- elliptical	Linear to linear-elliptical	Expanded linear to linear- elliptical	Linear-lanceolate to lanceolate
Valve apices	Rounded	Broad capitate	Rounded capitate	Rounded capitate	Rounded to weakly rostrate rounded
Raphe valve					
Axial area	Linear	Linear, linear-lanceolate	Linear-lanceolate	Linear	Narrow linear
Central area	Small oval or absent	Rhombic to rectangular	Rhombic	Small, rhombic	Asymmetrical transverse
Raphe	Distal fissures deflected to opposite directions	Distal fissures are hooked towards the opposite side	Distal fissures are strongly hooked towards the opposite side	Distal fissures are strongly hooked towards the opposite side	Distal fissures are strongly hooked towards the opposite side
Density of striae (10 μm)	34–36 (middle), 33–38 (apices)	28–32	~30	24–27	29–34
Number of areolae per stria	4–7 (middle), 1–7 (apices)	1–5 (middle), 1–4 (apices)	No data	5–6 (middle), 1–5 (apices)	2–5 (middle), 1–4 (apices)
Rapheless valve					
Axial area	Narrow, linear	Lanceolate	Linear	Linear	Narrow linear
Central area	Absent	Absent	Absent	Absent	Absent or weakly elliptical
Density of striae (10 μm)	34–38 (middle), 38–40 (apices)	28–32	~30	25–30	32–35
Number of areolae per stria	3–6 (middle), 1–5 (apices)	1–2 (middle), 1–3 (apices)	No data	No data	4–5 (middle), 1–3 (apices)
References	Current study	Moser et al. (1998)	Moser et al. (1998)	Moser et al. (1998)	Karthick et al (2017)

2004), *A. minutissimum* (Kützing) D.B. Czarnecki (Czarnecki 1994), and *Eucocconeis laevis* (Østrup) H. Lange-Bertalot (Lange-Bertalot and Genkal 1999). Further studies are needed to clarify the relationship between diatom diversity and ecology in this region.

Acknowledgements

This research was funded and supported by National Natural Science Foundation of China (No. 32100165, 32170205, 31770222). We would like to thank Yue Cao, Hao Liu, and Fen Luo for help in the field and in the preparation of samples for microscopy. We also thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

References

Ács E, Ari E, Duleba M, Dressler M, Genkal SI, Jakó E, Rimet F, Ector L, Kiss KT (2016) *Pantocsekiella*, a new centric diatom genus based on morphological and genetic studies. Fottea, Olomouc 16(1): 56–78. https://doi.org/10.5507/fot.2015.028

Bory de Saint-Vincent JBGN (1822) Achnanthe. *Achnanthes*. Dictionnaire Classique d'Histoire Naturelle 1: 79–80.

Chen Y, Lei B, Luo CD, Wang HY, Ma D, Liu L (2013) Study on soil organic matter and predict its storage of abies faxoniana forest in Mugecuo, Kangding. Journal of Soil and Water Conservation 27(4): 252–257.

Cleve PT, Grunow A (1880) Beiträge zur Kenntniss der arctischen Diatomeen. Kongliga Svenska Vetenskaps-Akademiens Handlingar 17(2): 121.

Czarnecki DB (1994) The freshwater diatoms culture collection at Loras College, Dubuque, Iowa. In: Kockiolek JP (Ed.) Proceedings of the 11th International Diatom Symposium. Memoirs of the California Academy of Sciences 17: 155–174.

Ge DY, Zhang SM, Liu HY, Liu Y, Kociolek PJ, Zhu H, Liu GX, Fan YW (2022) One new species of *Achnanthidium* Kützing (Bacillariophyta, Achnanthidiaceae) from Tibet, China. Phytotaxa 538(4): 268–280. https://doi.org/10.11646/phytotaxa.538.4.1

Hustedt F (1922) Bacillariales aus Innerasien. Gesammelt von Dr. Sven Hedin. In: Hedin S (Ed.) Southern Tibet, discoveries in former times compared with my own researches in 1906–1908. Lithographic Institute of the General Staff of the Swedish Army, Stockholm 6(3): 107–152. https://doi.org/10.5962/bhl.title.64226

Jao C (1964) Some fresh-water algae from southern Tibet. Oceanologia et Limnologia Sinica 6(2): 169–192. https://doi.org/CNKI:SUN:HYFZ.0.1964-02-003

Jao C, Zhu H, Lee Y (1974) The fresh-water algae from Mount Qomolangma district (in Tibet). Report of the Scientific Survey of Mount Qomolangma district 1966–1968: 92–126.

Karthick B, Taylor JC, Hamilton PB (2017) Two new species of *Achnanthidium* Kützing (Bacillariophyceae) from Kolli Hills, Eastern Ghats, India. Fottea 17(1): 65–77. https://doi.org/10.5507/fot.2016.020

Kobayashi H (1997) Comparative studies among four linear-lanceolate *Achnanthidium* species (Bacillariophyceae) with curved terminal raphe endings. Nova Hedwigia 65(1–4): 147–164. https://doi.org/10.1127/nova.hedwigia/65/1997/147

Kociolek JP, Balasubramanian K, Blanco S, Coste M, Ector L, Liu Y, Kulikovskiy M, Lundholm N, Ludwig T, Potapova M, Rimet F, Sabbe K, Sala S, Sar E, Taylor J, Van de Vijver B, Wetzel CE, Williams DM, Witkowski A, Witkowski J (2018) In DiatomBase. http://www.diatombase.org [Accessed: 2018–03–15]

Kociolek JP, You Q, Liu Q, Liu Y, Wang Q (2020) Continental diatom biodiversity discovery and description in China: 1848 through 2019. PhytoKeys 160: 45–97. https://doi.org/10.3897/phytokeys.160.54193

Krammer K, Lange-Bertalot H (2000) Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. Spektrum Akademischer Verlag, Heidelberg, 578 pp.

Kulikovskiy MS, Maltsev YI, Glushchenko AM, Kuznetsova IV, Kapustin DA, Lange-Bertalot H, Genkal SI, Kociolek JP (2020) *Gogorevia*, a new monoraphid diatom genus for

Achnanthes exigua and allied taxa (Achnanthidiaceae) described on the basis of an integrated molecular and morphological approach. Journal of Phycology 56(6): 1601–1613. https://doi.org/10.1111/jpy.13064

Kützing FT (1844) Die Kieselschaligen Bacillarien oder Diatomeen. W. Köhne, Nordhausen, 152 pp. https://doi.org/10.5962/bhl.title.64360

Lange-Bertalot H, Genkal SI (1999) Diatoms from Siberia I - Islands in the Arctic Ocean (Yugorsky-Shar Strait) Diatomeen aus Siberien. I. Insel im Arktischen Ozean (Yugorsky-Shar Strait). Iconographia Diatomologica 6: 271.

Lange-Bertalot H, Moser G (1994) *Brachysira*. Monographie der Gattung und *Naviculadicta* nov. gen. Biblioteca Diatomologica 29: 212.

Liu B, Blanco S, Long H, Jingjing XU, Jiang X (2016) *Achnanthidium sinense* sp. nov. (Bacillariophyta) from the Wuling Mountains Area, China. Phytotaxa 284(3): 194–202. https://doi.org/10.11646/phytotaxa.284.3.4

Liu Y, Tan X, Kociolek JP, Kulikovskiy M, Lu XX, Fan YW (2021) One new species of *Achnanthidium* Kützing (Bacillariophyta, Achnanthidiaceae) from the upper Han River, China. Phytotaxa 516(2): 187–194. https://doi.org/10.11646/phytotaxa.516.2.6

Miao M, Li Z, Hwang EA, Kim HK, Lee H, Kim BH (2020) Two New Benthic Diatoms of the Genus *Achnanthidium* (Bacillariophyceae) from the Hangang River, Korea. Diversity (Basel) 12(7): 285. https://doi.org/10.3390/d12070285

Moser G, Lange-Bertalot H, Metzeltin D (1998) Insel der Endemiten Geobotanisches Phänomen Ne-ukaledonien (Island of endemics New Caledonia -a geobotanical phenomenon). Bibliotheca Diatomologica 38: 464.

Parr JF, Taffs KH, Lane CM (2004) A microwave digestion technique for the extraction of fossil diatoms from coastal lake and swamp sediments. Journal of Paleolimnology 31(3): 383–390. https://doi.org/10.1023/B:JOPL.0000021857.32734.c6

Pérès F, Cohu RL, Delmont D (2014) *Achnanthidium barbei* sp. nov. and *Achnanthidium costei* sp. nov., two new diatom species from French rivers. Diatom Research 29(4): 387–397. https://doi.org/10.1080/0269249X.2014.890956

Ponader KC, Potapova MG (2007) Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): Ecology, distribution and taxonomic notes. Limnologica 37(3): 227–241. https://doi.org/10.1016/j.limno.2007.01.004

Potapova MG, Ponader KC (2004) Two common North American diatoms, *Achnanthidium rivulare* sp. nov. and *A. deflexum* (Reimer) Kingston: Morphology, ecology and comparison with related species. Diatom Research 19(1): 33–57. https://doi.org/10.1080/0269249X.2004.9705606

Qi YZ, Xie SQ (1984) The diatom in moss swamp from Hubei Shennongjia. Jinan Yili Xuebao 1984(3): 86–92.

Round FE, Bukhtiyarova L (1996) Four new genera based on *Achnanthes* (*Achnanthidium*) together with re-definition of *Achnanthidium*. Diatom Research 11(2): 345–361. https://doi.org/10.1080/0269249X.1996.9705389

Round FE, Crawford RM, Mann DG (1990) The Diatoms. Biology and morphology of the genera. Cambridge University Press, Cambridge, 747 pp.

Tseplik ND, Maltsev YI, Glushchenko AM, Kuznetsova IV, Genkal SI, Kociolek JP, Kulikovskiy MS (2021) *Achnanthidium tinea* sp. nov.-a new monoraphid diatom (Bacillariophyceae)

species, described on the basis of molecular and morphological approaches. PhytoKeys 174: 147–163. https://doi.org/10.3897/phytokeys.174.60337

You QM, Cao Y, Yu P, Kociolek JP, Zhang LX, Wu B, Lowe R, Wang QX (2019) Three new subaerial *Achnanthidium* (Bacillariophyta) species from a karst landform in the Guizhou Province, China. Fottea 19(2): 138–150. https://doi.org/10.5507/fot.2019.005

You QM, Zhao K, Wang YL, Yu P, Kociolek JP, Pang WT, Wang QX (2021) Four new species of monoraphid diatoms from Western Sichuan Plateau in China. Phytotaxa 479(3): 257–274. https://doi.org/10.11646/phytotaxa.479.3.3

Yu P, Kociolek JP, You QM, Wang QX (2018) *Achnanthidium longissima* sp. nov. (Bacillariophyta), a new diatom species from Jiuzhai Valley, Southwestern China. Diatom Research 33(3): 339–348. https://doi.org/10.1080/0269249X.2018.1545704

Yu P, You Q, Kociolek JP, Wang Q (2019a) Three new freshwater species of the genus *Achnanthidium* (Bacillariophyta, Achnanthidiaceae) from Taiping Lake, China. Fottea 19(1): 33–49. https://doi.org/10.5507/fot.2018.015

Yu P, You QM, Pang WT, Cao Y, Wang QX (2019b) Five new Achnanthidiaceae species (Bacillariophyta) from Jiuzhai Valley, Sichuan Province, Southwestern China. Phytotaxa 405(3): 147–170. https://doi.org/10.11646/phytotaxa.405.3.5

Yu P, You Q, Pang W, Wang Q (2022) Two new freshwater species of the genus *Achnanthidium* (Bacillariophyta, Achnanthidiaceae) from Qingxi River, China. PhytoKeys 191: 11–28. https://doi.org/10.3897/phytokeys.191.78489

Zhu HZ, Chen JY (1994) Study on the diatoms of the Wuling Mountain Region. Compilation of reports on the survey of algal resources, 405 pp.

Zhu HZ, Chen JY (1996) New taxa of diatom (Bacillariophyta) from Xizang (Tibet). (II). Zhiwu Fenlei Xuebao 34(1): 102–104. https://doi.org/CNKI:SUN:ZWFX.0.1996-01-011

PhytoKeys 204: 109–119 (2022)
doi: 10.3897/phytokeys.204.86857
https://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Pilea danxiaensis (Urticaceae), a new species in the Danxia landform from Guangdong, China including a description of the entire chloroplast genome

Long-Fei Fu[1], Chi Xiong[1], Alexandre K. Monro[2], Qiang Fan[3,4], Zai-Xiong Chen[5], Fang Wen[1], Zi-Bing Xin[1], Yi-Gang Wei[1], Wen-Bo Liao[3,4]

1 *Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, CN-541006 Guilin, China* **2** *The Royal Botanic Gardens, Kew, Identification & Naming Department, Richmond, Surrey TW9 3AE, UK* **3** *State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China* **4** *Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China* **5** *Administrative Commission of Danxiashan National Park, Shaoguan 512300,China*

Corresponding authors: Yi-Gang Wei (weiyigang@aliyun.com), Wen-Bo Liao (lsslwb@mail.sysu.edu.cn)

Academic editor: Clifford Morden | Received 23 May 2022 | Accepted 8 August 2022 | Published 19 August 2022

Citation: Fu L-F, Xiong C, Monro AK, Fan Q, Chen Z-X, Wen F, Xin Z-B, Wei Y-G, Liao W-B (2022) *Pilea danxiaensis* (Urticaceae), a new species in the Danxia landform from Guangdong, China including a description of the entire chloroplast genome. PhytoKeys 204: 109–119. https://doi.org/10.3897/phytokeys.204.86857

Abstract

Pilea danxiaensis L.F.Fu, A.K.Monro & Y.G.Wei, a new species of Urticaceae from Danxia landform, Guangdong, China, is described and photographed. Phylogenetic analyses based on three DNA regions (ITS, *trnL-F* and *rbcL*) suggest that the new species belongs to *P.* sect. *Pilea*. Within the section, the new species is morphologically most similar to *P. sinocrassifolia* and *P. peploides*. Plastid genome and ribosomal DNA (rDNA) sequences of the new species are assembled and annotated. The plastid genome is 151,857 bp in length and comprises two inverted repeats (IRs) of 25,307 bp separated by a large single-copy of 82,836 bp and a small single-copy of 18,407 bp. A total of 113 functional genes are recovered, comprising 79 protein-coding genes, 30 tRNA genes, and four rRNA genes. A global conservation assessment suggests that *P. danxiaensis* should be classified as of Least Concern (LC).

Keywords

Danxia landscape, new taxon, plastome, taxonomy

Introduction

Pilea Lindl. is the largest genus in the Urticaceae that comprises *ca* 715 species worldwide (Monro 2004; Fu et al. 2022). *Pilea* has a pantropical and subtropical distribution with the exception of Australia and New Zealand and is characterized by succulent herbs, shrubs and epiphytes and many point-endemic species. Outside the Neotropics, Indomalaya is the main center of diversification for *Pilea* (Monro 2006; Fu et al. 2022). Within Indomalaya, China contains more than 90 species (Chen and Monro 2003, Chen et al. 2007; Monro et al. 2012; Wang 2014, 2016; Fu et al. 2017; Yang et al. 2018).

A recent systematic study has demonstrated that *Pilea* is monophyletic after the exclusion of species of *Achudemia* and *Lecanthus* (Fu et al. 2022). The newly circumscribed genus has been classified into eight sections: *Pilea* sect. *Tetraphyllae* Y.G.Wei & A.K.Monro, sect. *Trimeris* Y.G.Wei & A.K.Monro, sect. *Lecanthoides* C.J.Chen, sect. *Angulatae* L.F.Fu & Y.G.Wei, sect. *Tetrameris* C.J.Chen, sect. *Verrucosae* L.F.Fu & Y.G.Wei, sect. *Plataniflorae* L.F.Fu & Y.G.Wei and sect. *Pilea* (Fu et al. 2022). These sections can be delimited by leaf margin morphology, stipule length, inflorescence architecture, flower sepal number and achene ornamentation (Fu et al. 2022).

While conducting field investigations into the Danxia flora of Guangdong, China, we encountered an unknown species of *Pilea* with 3-parted female flowers, 4-parted male flowers, short stipules (≤10 mm), entire leaf margins and ornamented or rarely smooth achenes that placed it within *Pilea* sect. *Plataniflorae* or sect. *Pilea* (Fu et al. 2022). After a thorough literature survey and review of herbarium specimens at IBK, IBSC, K, PE and SYS, along with molecular studies, we confirmed that this plant was a hitherto undescribed species of *Pilea* sect. *Pilea*.

Materials and methods

Morphological observations and conservation assessment

All morphological characters were observed from field and herbarium specimens using an Olympus SZX16 binocular microscope (Japan). For achene morphology, we also undertook scanning electron micrograph (SEM) observations. Achene materials were collected from specimens, dried, and mounted using double-sided adhesive tape and coated with gold using a sputter coater. The materials were then observed and photographed under a ZEISS EVO18 scanning electron microscope. At least ten achenes were used to determine their size and surface ornamentation.

A species conservation assessment was undertaken for the new species described here using IUCN criteria (IUCN 2019). Calculations of the extent of occurrence (EOO) and area of occupation (AOO) were undertaken using the online conservation assessment tool GeoCATAT (Bachman et al. 2011). The AOO was calculated using a cell width of 2 km as recommended by IUCN (2019).

Genomic DNA extraction and sequencing

Leaf material for DNA extraction was dried using silica gel (Chase and Hills 1991). Genomic DNA was extracted using a modified CTAB protocol (Chen et al. 2014). The total gDNA sample was sent to Majorbio (http://www.majorbio.com/, China) for library construction and next-generation sequencing. Short-insert (350 bp) paired-end read libraries preparation and 2 × 150 bp sequencing were performed on an Illumina (HiSeq4000) genome analyzer platform. Approximately 2 Gb of raw data for the new species was filtered using the FASTX-Toolkit to obtain high-quality clean data by removing adaptors and low-quality reads (http://hannonlab.cshl.edu/fastx_toolkit/download.html).

Plastid genome and ribosomal DNA (rDNA) assembly and annotation

Clean reads were paired and imported in Geneious Prime (Kearse et al. 2012). For plastid genome assembly, the clean reads were mapped to published plastid genome sequence as reference (Fu et al. 2019) using the Fine Tuning option in Geneious Prime (iterating set as 10 times) to exclude nuclear and mitochondrial reads. Then, de novo assembly was performed using Geneious Prime with a medium-low sensitivity setting to assemble plastid genome sequence. The generated contigs were mapped by the clean reads using the Fine Tuning option in Geneious Prime (iterating set as 10 times) to fill gaps. Contigs were able to be concatenated using the Repeat Finder option implemented in Geneious Prime until a ~130 kb contig (including SSC, IR and LSC) being built. The Inverted repeat (IR) region was determined by the Repeat Finder option in Geneious Prime and was reverse copied to obtain the complete plastid genome. The annotation approach of the assembled plastid genome was performed using CPGAVAS2 and PGA (Qu et al. 2019; Shi et al. 2019). The process of rDNA assembly is similar to the above with the exception of different reference (Fu et al. 2021) and iterating as none. The annotation approach of rDNA was performed using Annotate option in Geneious Prime.

Phylogenetic analyses

We generated a phylogeny using sequences data from previous phylogenies of *Pilea* s.l. (Fu et al. 2022). We extracted three DNA regions (ITS, *trnL-F* and *rbcL*) from assembled rDNA and complete plastid genome sequences, respectively, of the new species and downloaded all sequences data used in Fu et al. (2022) from GenBank (details see Suppl. material 1). This resulted in 145 accessions representing 131 taxa in total, 112 taxa of which belong to *Pilea* s.l. as in-group, and 21 species of which belong to *Elatostema* s.l., other tribes of Urticaceae, Moraceae and Cannabaceae, as out-group (Appendix 1). Three datasets (ITS, *trnL-F* and *rbcL*) were aligned independently using multiple alignment using fast Fourier transform (MAFFT) version 7.0 (Katoh and Standley 2013) with default settings, followed by manual adjustment. As there was no incongruence that affected the topology of the ingroup taxa as described in Fu et al.

(2022), phylogenies were reconstructed based on the combined dataset using Maximum Likelihood (ML) and Bayesian inference (BI). The BI and ML analyses were performed following Fu et al. (2022).

Results

Characteristics of the complete plastid genome and ribosomal DNA

The complete plastid genome and ribosomal DNA (rDNA) sequences of *Pilea danxiaensis* comprised 151,857 bp (Fig. 1) and 5,788 bp, respectively. The characteristics and statistics of plastid genome are summarized in Tables 1 and 2.

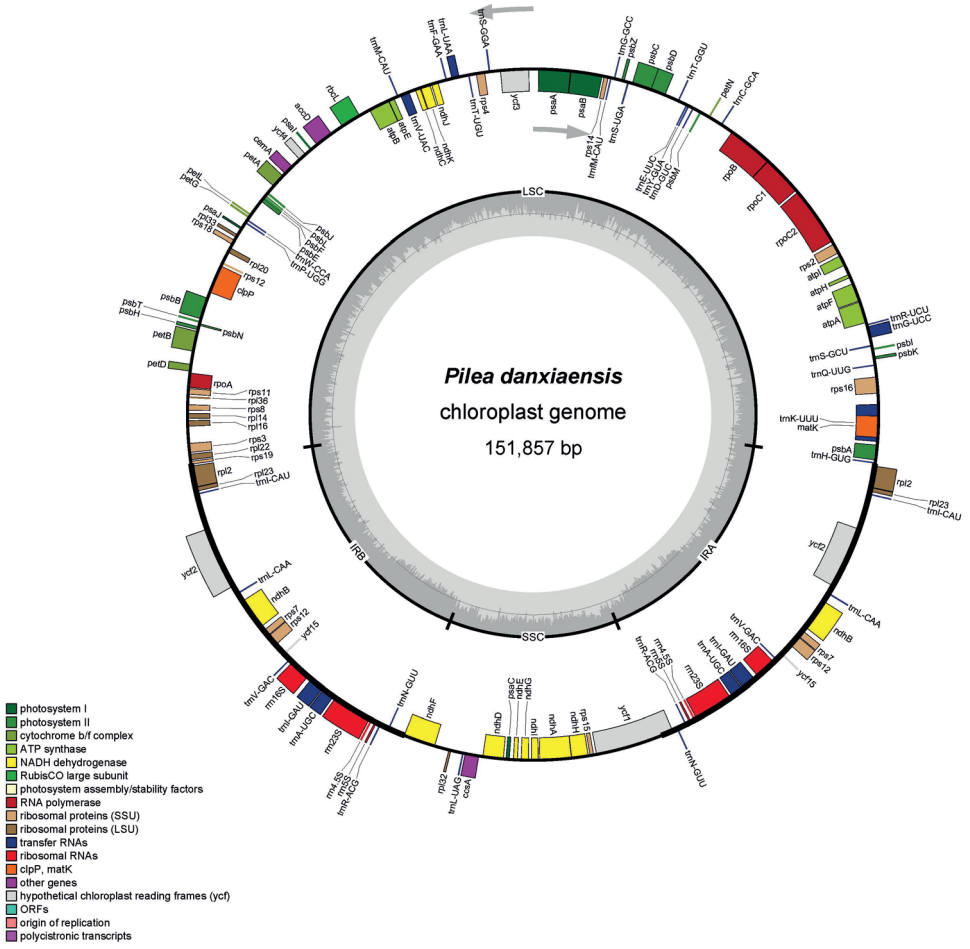


Figure 1. Plastid genome map of *Pilea danxiaensis*. The thick lines on the outer complete circle identify the inverted repeat regions (IRa and IRb). The innermost track of the plastome shows the GC content. Genes on the outside and inside of the map are transcribed in clockwise and counter directions, respectively.

Table 1. Summary of whole plastid genome of *Pilea danxiaensis*.

Characteristic	*Pilea danxiaensis*
Size (bp)	151,857
LSC length (bp)	82,836
SSC length (bp)	18,407
IR length (bp)	25,307
Number of genes	113
Protein-coding genes	79
rRNA genes	4
tRNA genes	30
LSC GC%	34.30%
SSC GC%	30.70%
IR GC%	42.80%

Table 2. Genes encoded in plastid genome of *Pilea danxiaensis*.

Group of genes	Gene name
tRNA genes	*trnA-UGC* (×2), *trnC-GCA*, *trnD-GUC*, *trnE-UUC*, *trnF-GAA*, *trnfM-CAU*, *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*
rRNA genes	*rrn16* (×2), *rrn23* (×2), *rrn4.5* (×2), *rrn5* (×2)
Ribosomal small subunit	*rps16**, *rps2*, *rps14*, *rps4*, *rps18*, *rps12*** (×2), *rps11*, *rps8*, *rps3*, *rps19*, *rps7* (×2), *rps15*
Ribosomal large subunit	*pl33*, *rpl20*, *rpl36*, *rpl14*, *rpl16**, *rpl22*, *rpl2** (×2), *rpl23* (×2), *rpl32*
DNA-dependent RNA poly merase	*poC2*, *rpoC1**, *rpoB*, *rpoA*
Photosystem I	*psaB*, *psaA*, *psaI*, *psaJ*, *psaC*
Photosystem II	*psbA*, *psbK*, *psbI*, *psbM*, *psbC*, *psbZ*, *psbG*, *psbJ*, *psbL*, *psbF*, *psbE*, *psbB*, *psbT*, *psbN*, *psbH*
Large subunit of rubisco	*rbcL*
NADH dehydrogenase	*ndhJ*, *ndhK*, *ndhC*, *ndhB** (×2), *ndhF*, *ndhD*, *ndhE*, *ndhG*, *ndhI*, *ndhA**, *ndhH*
Cytochrome b/f complex	*petN*, *petA*, *petL*, *petG*, *petB**, *petD**
ATP synthase	*atpA*, *atpF**, *atpH*, *atpI*, *atpE*, *atpB*
Maturase	*matK* (The *matK* is localized between the exons coding for the *trnK-UUU*)
Subunit of acetyl-CoA carboxylase	*accD*
Envelope membrane protein	*cemA*
Protease	*clpP***
Translational initiation factor	*infA*
C-type cytochrome synthesis	*ccsA*
Conserved open reading frames	*ycf3***, *ycf4*, *ycf2* (×2), *ycf1*, *ycf15* (×2)

Note: Genes with one or two introns are indicated by one (*) or two asterisks (**), respectively. Genes in the IR regions are followed by the (× 2) symbol.

Table 3. Statistics for the molecular datasets used in this study.

	Number of sequences	Aligned length (bp)	Variable characters (bp)	Parsimony information characters (bp)	Model used
ITS	142	528	419	339	-
trnL-F	139	677	667	38	-
rbcL	84	637	318	315	-
Combined	142	1,842	1,404	692	GTR+GAMMA

Phylogenetic reconstruction

The characteristics and statistics of the datasets used in this study are summarized in Table 3. ML and BI analyses of dataset of three DNA regions (ITS, *trnL-F* and *rbcL*) resulted in the same tree topologies that both indicate the new species recovering in the clade C8a of *Pilea* (PP = 1, BS = 100%) (Fig. 2).

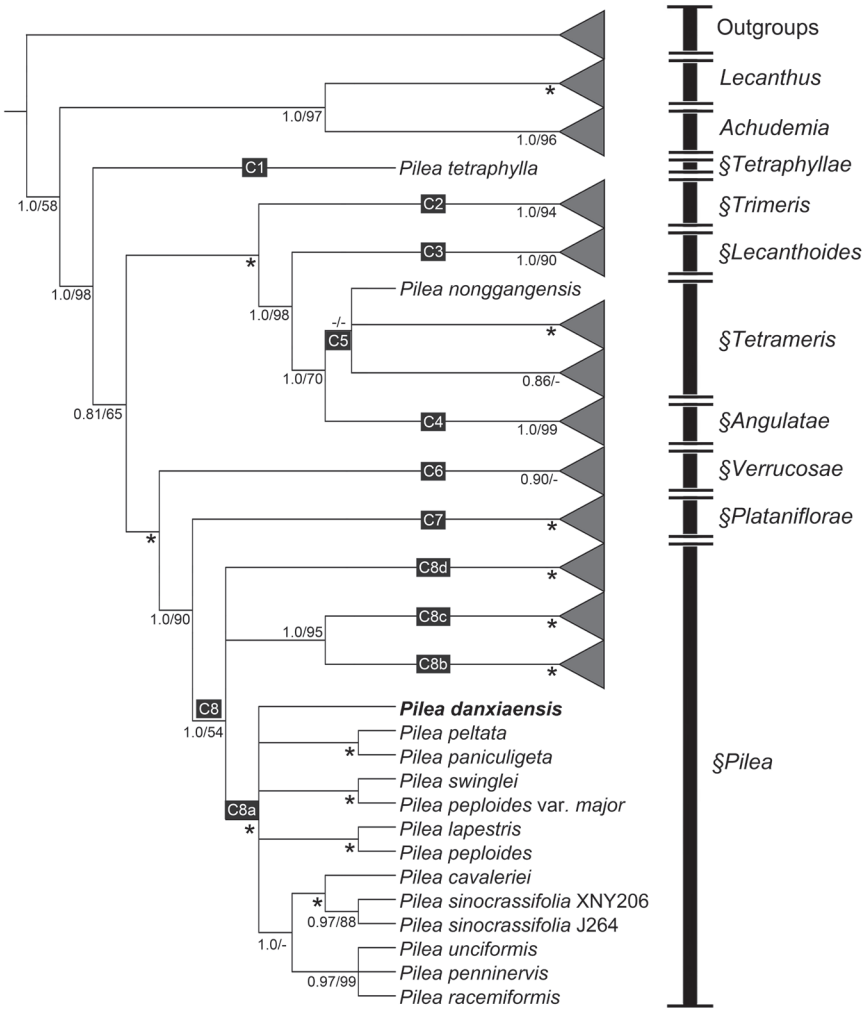


Figure 2. Phylogenetic tree of *Pilea* s.l. generated from Bayesian Inference (BI) of combined dataset (ITS, *trnL-trnF* and *rbcL*). Numbers below the branches indicate the posterior probability (≥0.5) of BI and bootstrap values (≥50%) of the ML analyses. '*' indicates supports of 1.0/100. The bold (***Pilea danxiaensis***) indicates the new species.

Taxonomic treatment

Pilea danxiaensis **L.F.Fu, A.K.Monro & Y.G.Wei, sp. nov.**
urn:lsid:ipni.org:names:77303547-1
Fig. 3

Type. China. Guangdong: Danxiashan National Park, Renhua County, Shaoguan City, 25.020°N, 113.752°E (WGS84), elev. 134 m, 2 April 2022, Liao Wen-Bo, Fan Qiang and Liao Li-Juan DNPC1728 (holotype IBK; isotypes IBK, SYS).

Diagnosis. Most similar to *Pilea sinocrassifolia* C.J.Chen from which it can be distinguished by the longer stipule (1.3–1.5 mm versus 1 mm), petiole (2–8 mm versus 0.2–0.6 mm) and staminate peduncle (8–25 mm versus 1.5–7 mm).

Description. Herbs prostrate, stem 30–200 × 1 mm, light green when fresh, drying yellowish-brown, glabrous, succulent, cystoliths fusiform, 0.2–0.4 mm long. Stipules, 1.3–1.5 × 1.7–2.1 mm, reniform, drying brown, papery, with dense cystoliths. Leaves petiolate, distichous, clustered towards the stem apex; petioles 2–8 mm long, glabrous, cystoliths densely scattered; laminae at each node equal or subequal, 3–15 × 5–20 mm, length: width ratio 0.7–0.9:1, suborbicular to broadly ovate, succulent, papery when dry; adaxial surface drying grey-green, dark green when fresh, glabrous, with cystoliths densely scattered, *ca* 0.3 mm, linear or fusiform; abaxial surface drying light green, green when fresh, glabrous, rugose when dry, 3-veined, secondary veins 3–6 pairs, borne at 45–60° to the midrib, with cystoliths sparsely scattered, *ca* 0.3 mm, linear or fusiform; apex obtuse or subretuse, base cuneate, rounded or subtruncate, margin entire and revolute. Inflorescences in upper nodes, appearing terminal, monoecious. Staminate inflorescences 10–30 mm, bearing 10–25 flowers in a capitulum or occasionally a glomerule; peduncle 8–25 × 0.5 mm, glabrous; pedicels *ca* 0.8 mm, glabrous. Staminate flowers 1 × 1 mm, green, drying light green, sepals 4, *ca* 1.8 mm; valvate, elliptic, glabrous, the subapical appendage *ca* 0.1 mm, corniculate, glabrous; stamens 4. Pistillate inflorescences 10–20 mm, bearing 20–50 flowers in a cyme or glomerule; peduncle 8–18 × *ca* 0.5 mm, glabrous; pedicels *ca* 0.5 mm. Pistillate flowers *ca* 0.5 mm, sepals 3, subequal, *ca* 0.3 mm, valvate, triangular-ovate, glabrous, the subapical appendage *ca* 0.1 mm. Infructescences 15–20 mm; peduncle 15–18 mm; achenes 0.68–0.72 × 0.40–0.46 mm, ovoid, spinulose-verrucose, rarely smooth.

Distribution and habitat. *Pilea danxiaensis* L.F.Fu, A.K.Monro & Y.G.Wei is known from a single locality in Renhua County, Shaoguan City, Guangdong, China, where it grows in a ravine on the Danxia landform, a petrographic geomorphology formed from Cretaceous sandstones and conglomerates.

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

Etymology. The species epithet is named after the Danxia landform with which the species is associated.

Vernacular name. dān xiá lěng shuǐ huā (Chinese pronunciation); 丹霞冷水花 (Chinese name).

Conservation status. At present, *Pilea danxiaensis* is known from a single locality, the Danxiashan National Park. The park covers 140 km^2 and the massif from which the type collection was made encompasses *ca* 114 km^2 (Google Earth Pro). Within that locality, the population of *P. danxiaensis* is estimated to number between 1,000 and 5,000 individuals distributed between 10 sub-populations, of which only one has been directly observed. A remote survey of the Danxiashan National Park using Google Earth Pro, suggests that the protected area itself is well protected and we observed no active threat or continuing decline in population size. *Pilea danxiaensis* is therefore classified as Least Concern (**LC**).

Additional specimen examined. China. Guangdong: Danxiashan National Geopark, Renhua County, Shaoguan City, 25.004°N, 113.655°E (WGS84), elev. 466 m, 20 April 2018, *Fan Qiang and Huang Yan-Shuang 16993* (IBK, SYS).

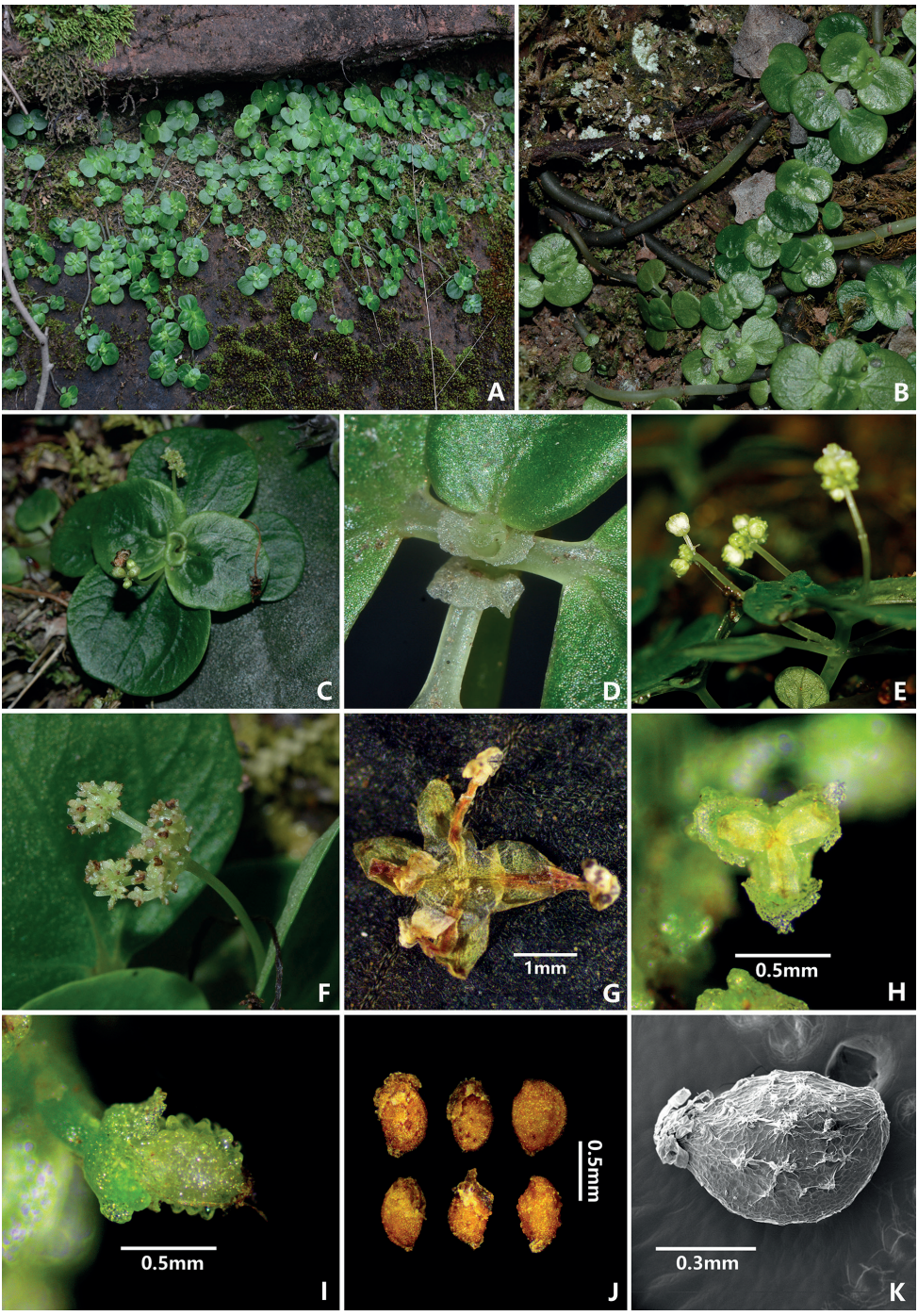


Figure 3. Plate of *Pilea danxiaensis* **A** habitat **B** habit **C** leaves and inflorescence **D** stipules **E** staminate inflorescence **F** pistillate inflorescence **G** staminate flower **H** pistillate flower **I** achene with pistillate sepals **J** LM of achene **K** SEM of achene.

Discussion

Fu et al. (2022) proposed a new infrageneric classification based on molecular and morphological evidence that suggested the leaf margin, stipule length, inflorescence architecture, flower sepal-number and achene ornamentation can be reliably used to place taxa into sections. Our research demonstrates that *Pilea danxiaensis* sits within clade C8a (Fig. 2) corresponding to *P.* sect. *Pilea*. Section *Pilea* is the most species-rich section in *Pilea* and has its center of species-richness in the neotropics from where several new species have been described in recent years (Monro 2006; Cabral et al. 2020; Beutelspacher and García-Martínez 2021). The morphology of *Pilea danxiaensis*, and specifically the 3-parted female flowers, 4-parted male flowers, short stipules (≤10 mm) and un-ornamented achenes, are congruent with it belonging to this section. SEM results indicate the achene length of *P. danxiaensis* to be 0.68–0.72 mm (≤ 0.8 mm), further supporting the inclusion of this species in *P.* sect. *Pilea*, and of a shift to smaller fruits as more lineages have formed (Fu et al. 2022).

Within *Pilea* sect. *Pilea*, the new species is most morphologically similar to *P. sinocrassifolia* and *P. peploides* from which it is distinguished in Table 4.

Table 4. Diagnostic comparison of *Pilea danxiaensis*, *P. sinocrassifolia* and *P. peploides*.

Characters	*P. danxiaensis*	*P. sinocrassifolia*	*P. peploides*
Stipule shape and length	reniform, 1.3–1.5 mm	triangular, *ca* 1 mm	triangular, *ca* 0.5 mm
Petiole length	2–8 mm	0.2–0.6 mm	3–20 mm
Staminate peduncle length	8–25 mm	1.5–7 mm	2–5 mm
Pistillate tepal number	3	NA	2

Conclusions

This study describes a new species of *Pilea* based on morphological and molecular evidence. Our results support the new infrageneric classification proposed by Fu et al. (2022). The reported plastid genome provides informative data to support further studies on the systematics, evolution, and conservation of the genus.

Acknowledgements

We would like to thank the staff at IBK, IBSC, K, PE and SYS who provided help for checking specimens. We are also grateful to Ms. Liao Li-Juan for participating in fieldwork. This work was supported by the Guangdong Provincial Special Research Grant for the Creation of National Parks (2021GJGY034), the National Natural Science Foundation of China (grant number 31860042), and the Light of West China Program of the Chinese Academic of Sciences ([2020]82).

Reference

Bachman S, Moat J, Hill AW, De la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCATAT: geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity science. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Beutelspacher CR, García-Martínez R (2021) Una nueva especie de *Pilea* (Urticaceae) de Chiapas, México.

Cabral FS, Gaglioti AL, Fiaschi P (2020) Two new species of *Pilea* Lindl. (Urticaceae) from southern Brazil. Systematic Botany 45(4): 822–825. https://doi.org/10.1600/036364420X16033962925268

Chase MW, Hills H (1991) Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon 40(2): 215–220. https://doi.org/10.2307/1222975

Chen CJ, Monro AK (2003) *Pilea*. In: Wu ZY, Raven PH (Eds) Flora of China, vol. 5. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 76–189.

Chen CJ, Monro AK, Chen L (2007) Name changes for Chinese *Pilea* (Urticaceae). Novon 17: 24–26. https://doi.org/10.3417/1055-3177(2007)17[24:NCFCPU]2.0.CO;2

Chen LY, Song MS, Zha HG, Li ZM (2014) A modified protocol for plant genome DNA extraction. Plant Diversity and Resources 36: 375–380. https://doi.org/10.7677/ynzwyj201413156

Fu LF, Huang SL, Monro AK, Liu Y, Wen F, Wei YG (2017) *Pilea nonggangensis* (Urticaceae), a new species from Guangxi, China. Phytotaxa 313(1): 130–136. https://doi.org/10.11646/phytotaxa.313.1.9

Fu LF, Xin ZB, Wen F, Li S, Wei YG (2019) Complete chloroplast genome sequence of *Elatostema dissectum* (Urticaceae). Mitochondrial DNA Part B, Resources 4(1): 838–839. https://10.1080/23802359.2019.1567292

Fu LF, Monro AK, Yang TG, Wen F, Pan B, Xin ZB, Zhang ZX, Wei YG (2021) *Elatostema qinzhouense* (Urticaceae), a new species from limestone karst in Guangxi, China. PeerJ 9: e11148. https://doi.org/10.7717/peerj.11148

Fu LF, Wen F, Maurin O, Rodda M, Gardner EM, Xin ZB, Wei YG, Monro AK (2022) A revised delimitation of the species-rich genus *Pilea* (Urticaceae) supports the resurrection of *Achudemia* and a new infrageneric classification. Taxon 71(4): 796–813. https://doi.org/10.1002/tax.12711

IUCN (2019) Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommitttee. [Available at] http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Monro AK (2004) Three new species, and three new names in *Pilea* (Urticaceae) from New Guinea. Kew Bulletin 59(4): 573–579. https://doi.org/10.2307/4110914

Monro AK (2006) Revision of species-rich genera: A phylogenetic framework for the strategic revision of *Pilea* (Urticaceae) based on cpDNA, nrDNA, and morphology. American Journal of Botany 93(3): 426–441. https://doi.org/10.3732/ajb.93.3.426

Monro AK, Wei YG, Chen CJ (2012) Three new species of *Pilea* (Urticaceae) from limestone karst in China. PhytoKeys 19(0): 51–66. https://doi.org/10.3897/phytokeys.19.3968

Qu X, Moore MJ, Li D, Yi T (2019) PGA: A software package for rapid, accurate, and flexible batch annotation of plastomes. Plant Methods 15(1): 50. https://doi.org/10.1186/s13007-019-0435-7

Shi L, Chen H, Jiang M, Wang L, Wu X, Huang L, Liu C (2019) CPGAVAS2, an integrated plastome sequence annotator and analyzer. Nucleic Acids Research 47(W1): W65–W73. https://doi.org/10.1093/nar/gkz345

Wang WT (2014) Two new species of Urticaceae from Tibet. Zhiwu Kexue Xuebao 32: 24–26.

Wang WT (2016) Notes on the genus *Pilea* (Urticaceae) of China. Bulletin of Botanical Research 36(4): 484–490.

Yang F, Wang YH, Qiao D, Wang HC (2018) *Pilea weimingii* (Urticaceae), a new species from Yunnan, southwest China. Annales Botanici Fennici 55(1–3): 99–103. https://doi.org/10.5735/085.055.0112

Supplementary material 1

Appendix S1

Authors: Long-Fei Fu, Chi Xiong, Alexandre Monro, Qiang Fan, Zai-Xiong Chen, Fang Wen, Zi-Bing Xin, Yi-Gang Wei, Wen-Bo Liao

Data type: GenBank accession numbers.

Explanation note: Taxa and GenBank accession numbers of DNA sequences used in this study.

Link: https://doi.org/10.3897/phytokeys.204.86857.suppl1