

Paracladopus chiangmaiensis (Podostemaceae), a new generic record for China and its complete plastid genome

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Abstract

The genus *Paracladopus* was established based on the type species *P. chiangmaiensis* in 2006. The two *Paracladopus* species are distributed in Thailand and Laos; however, neither of them has been documented in China to date. During our field work in 2020, we collected a river-weed in Wuzhi Mountain, Hainan Province of China. After checking the morphological characters, it was identified as *P. chiangmaiensis*. Then, we assembled and annotated its chloroplast genome based on the genome skimming data. The results showed that the complete chloroplast genome was 133,748 bp with 35% GC content, consisting of 76 protein-coding genes, 30 tRNA genes, and 4 rRNA genes. A maximum-likelihood tree constructed based on the *matK* genes showed that WuMS109 was clustered with *P. chiangmaiensis* (AB537420, AB698348) without base difference and together with the remains of *Paracladopus* formed a sister clade to *Cladopus*. This is the first report of *P. chiangmaiensis* that represents a new generic record for China. The discovery of this river-weed could lay the foundation for investigating their biogeographical patterns and species evolution in further studies.

Keywords

aquatic, chloroplast genome, *matK*, new generic record, *Paracladopus*, river-weeds, Wuzhi Mountain

Introduction

Podostemaceae, also known as river-weeds or podostems (Gustafsson et al. 2002; Koi and Kato 2012), which are the largest family of strictly aquatic angiosperms (De Mello et al. 2011), grow attached to waterworn rock surfaces, stones or even wood in rapids and waterfalls with pristine hydrology and high water quality (Rutishauser 1995; Koi and Kato 2020; Xue et al. 2020). They are found worldwide in rivers or streams with open and sunny habitats (Werukamkul et al. 2016), but distributed mainly in the tropical to warm-temperate regions with seasonal climates (Kato 2011). The species diversity center of river-weeds is located in the equatorial region of South America (De Mello et al. 2011). Its habitat and morphology have obviously undergone high specializations and extensive reductions (Gustafsson et al. 2002). These species lack double fertilization and the vascular tissue is reduced or lost (Johri et al. 1992; Kato 2016; Khanduri et al. 2016). The vegetative body of most river-weeds resembles algae, lichens, or mosses. So, they are easily overlooked and more field works are needed to investigate the species diversity. River-weeds are usually not obvious at conventional demarcation of stem, leaf and root (Willis 1902). The interpretation of the vegetative body also evokes much controversy (Mohan Ram and Sehgal 1992; Ota et al. 2001; Sehgal et al. 2002). In this text, we adopt the classical root-shoot model with its structural categories ‘roots’, ‘shoots’. The term ‘root’ refers to photosynthetic structures when endogenous shoot buds are developed but there are no exogenous leaves. Shoots are apparently absent or reduced, borne adventitiously on root. The Podostemaceae show an amazing diversity of root types. They vary from thread-like to ribbon-like and further to crustose (Mathew and Satheesh 1997; Jäger-Zürn 2000; Koi et al. 2019; Krishnan et al. 2019).

The life cycle of river-weeds is dictated by high and low water periods (Lalruatsanga 2020). During the rainy season, the plants submerge in violent currents exclusively in the vegetative phase (Kato 2016). When the water level lowers during the dry season, the plants emerge from the water and enter into the reproductive phase (Kelly et al. 2010; Koi and Kato 2012, 2015). In the early dry season, the plants form flower buds underwater, and subsequently come into flower and fruit when exposed to the air. The exposed plants wither and die while seeds are dispersed from their capsules. They are annual herbs, but perennial when submerged all year round (Kato et al. 2019). Exposure is necessary for reproduction, otherwise the plants will be unable to complete their life cycle (Kato 2016).

Podostemaceae comprises ca. 351 species (excluding infraspecies) assigned to ca. 50 genera in three subfamilies (POWO 2021). Podostemoideae, the largest subfamily (ca. 43 genera and ca. 322 species) of Podostemaceae, is distributed worldwide; subfamily Tristichoideae (6 genera and ca. 28 species) is mainly distributed in Asia, with the exception of *Tristicha trifaria* (Bory ex Willd.) Spreng., which is widely dispersed from Central and South America to Africa; while subfamily Weddellinoideae (a monotypic genus) is confined to South America (Kato 2006a). The phylogenetic position of Podostemaceae has been revealed by molecular data. They were included in the order Malpighiales, forming a sister group with Hypericaceae and related to Clusiaceae and Callophyllaceae based on plastid and nuclear markers or plastoms (APG IV

2016; Magallón et al. 2015; Li et al. 2021). However, Podostemaceae was a more basal isolated-clade in rosids and was recovered as Podestemales according to nuclear genes (Baker et al. 2021) or genomes (Xue et al. 2020).

The genus *Paracladopus* M.Kato, belonging to subfamily Podostemoideae, was established by Kato in 2006 based on the type species *P. chiangmaiensis* M.Kato, which was first discovered in northern Thailand (Kato 2006b). Two years later, another new species *P. chantaburiensis* Koi & M.Kato was discovered in southeastern Thailand (Koi et al. 2008). The two *Paracladopus* species are distributed in Thailand and Laos; however, neither of them has been documented in China to date. The ribbon-like roots and ellipsoidal or globose capsule of *Paracladopus* are similar to *Cladopus* H.Möller, but can be distinguished by its capsules with ribs or stripes, shoots borne on the sinuses of root branching and flanks of the root between successive root branches, and holdfasts presenting on ventral surface of root under the shoots (Koi and Kato 2012, 2015). Molecular phylogenetic analysis showed that *Paracladopus* was sister to *Cladopus*, and the two genera were in turn sister to the ‘*Hydrobryum*’ clade (including *Ctenobryum* Koi & M.Kato, *Hydrodiscus* Koi & M.Kato, *Hanseniella* C.Cusset, *Hydrobryum* Endl. and *Thawatchaia* M.Kato, Koi & Y.Kita) (Koi et al. 2012, 2019).

During a field trip to Wuzhi Mountain, Wuzhishan City, Hainan Province, China in December 2020, a river-weed (Fig. 1A-C) without flower and fruit attached to rock



Figure 1. The natural habitat of *Paracladopus chiangmaiensis* in Wuzhi Mountain **A, B** the river-weed submerging in water with open and sunny habitat **C** the river-weed attached to waterworn rock surfaces in rapids **D** several individuals emerging from water and enter into reproductive phase.

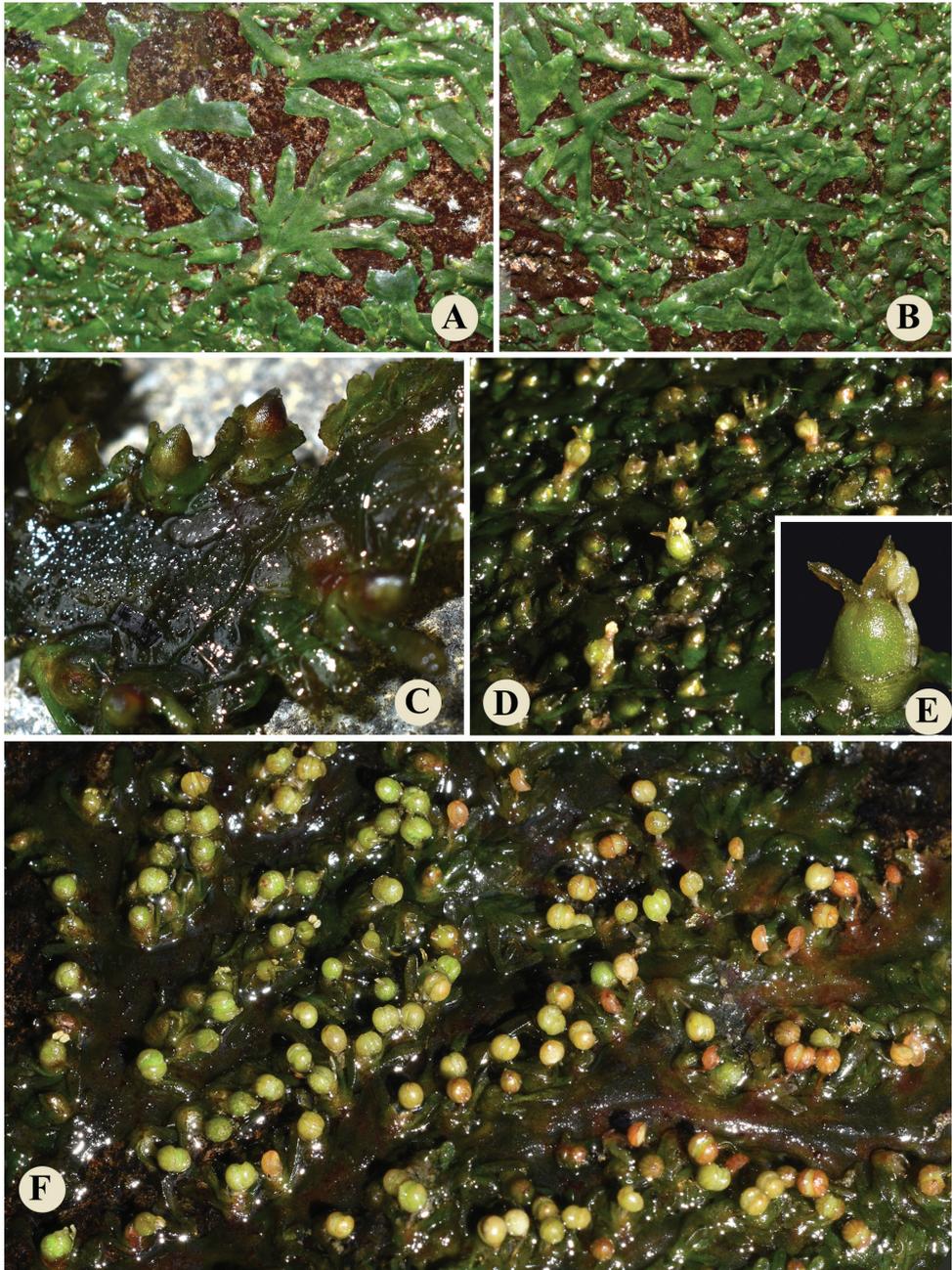


Figure 2. *Paracladopus chiangmaiensis* in natural habit **A** inconspicuous shoots are observed **B** ribbon-like roots with tufts of leaves on flanks **C** flowering shoots on flanks of root **D, E** flowers with indehiscent anthers **F** nearly ripe fruits with dehiscent capsule valves.

surfaces in rapids caught the first author's attention. At first sight, it resembled *Cladopus* species by its ribbon-like structure; however, ensiform leaves and shoots borne on both sides of the ribbon-like structure (Fig. 2A, B) confused us. In March 2021, we revisited Wuzhi Mountain and found a few residual flowers and a large number of mature fruits. The flower possessed a single stamen and a single pistil with 2 stigmas (Fig. 2D, E). The fruit was globose with some stripes. After a survey of literature, we confirmed the morphological characters of the plants matched that of the species *Paracladopus chiangmaiensis*, distributed in Thailand and Laos. This species and genus has not been previously reported to occur in China. So, its discovery in Hainan Province represented the first record of this genus for China.

Materials and methods

Material

Plant samples were collected from Wuzhishan Tropical Rainforest Scenic Area in Shuiman Village, Hainan Province (18°52'2.68"N, 109°40'51.41"E). The voucher specimen (Mingsong Wu, WuMS109) was deposited in the Traditional Chinese Medicine Herbarium of Hainan Province.

Total DNA extract, genome assembly and annotation

Total genomic DNA was extracted from the entire thallus dried immediately by silica gel using a modified CTAB method (Doyle and Doyle 1987). Genome skimming was performed using next-generation sequencing technologies on the Illumina Novaseq 6000 platform with 150 bp paired-end reads and 350 bp insert size by Novogene Bioinformatics Technology Co. Ltd. (Tianjin, China). A total of 3.85 Gb paired-end sequencing data was generated to assemble plastome using GetOrganelle pipeline (Jin et al. 2020b). The assembly graph viewer Bandage (Wick et al. 2015) was used to visualize the assemblies. The program Plastid Genome Annotator (Qu et al. 2019) was employed as the annotation tool with *Tristicha trifaria* (NC_049109) designated as the reference. Geneious Prime 2020.1.2 (Biomatters Ltd., Auckland, New Zealand) was used to adjust the start/stop codons, intron boundaries and tRNA genes for the preliminary annotation result. The *matK* gene was extracted from the chloroplast genome.

Phylogenetic analyses

To confirm our identification results based on morphological characters, and reveal the phylogenetic relationship of this species within *Paracladopus* and closely related genera, we downloaded the *matK* gene sequences from Genbank as shown in Fig. 6, which contains 24 species from 8 genera. Among them, three species of *Podostemum* were used as

outgroups. Based on the GTRGAMMA substitution model and 1000 bootstraps, we constructed a maximum likelihood tree using the RAxML-HPC2 (Stamatakis 2014) on XSEDE (8.2.6) in Cipres Science Gateway (Miller et al. 2012).

Results and discussion

New generic record for China

We discovered *Paracladopus chiangmaiensis* from Hainan province, representing a new generic record for China. Currently, only one population was discovered in Wuzhishan Tropical Rainforest Scenic Area. They live in turbulent rivers adhering to submerged rock surface with open and sunny habitat as shown in Fig. 1. When the water level dropped in the dry season, the plants were exposed to air to produce flowers and fruits shortly afterwards (Fig. 1D).

The roots, shoots, flowers and capsules of *P. chiangmaiensis* in the natural habitat were recorded in Fig. 2. Meanwhile, photographs under the stereoscope based on the fixed material were taken and shown in Figs 3, 4. The shoots were borne on the flanks of root and the sinuses of root branching (Figs 2B, 3A, E). The flowering shoots were very short with 4–6 ensiform bracts (Figs 2C, 3B, C). During the flower bud period, the pistil and stamen were covered by a spathe (Figs 2C, 3C). Flower was terminal in the short-shoot composed of one stamen with one linear tepal on each side (Fig. 4A) and one pistil with two stigma and a smooth ovary (Figs 2D, E, 4B). Capsule stalked was globose with 10–14 narrow stripes (Figs 2F, 4G, H), and dehisced by two equal valves (Fig. 4I, J). The flowering and fruiting period is from January to March. All morphological characteristics of this river-weed newly discovered in Wuzhi Mountain in China are consistent with the species described by Kato in Thailand and Laos (Kato 2006b; Koi and Kato 2012). The discovery of this river-weed represents a new generic record for China, and lays the foundation for investigating their biogeographical patterns and species evolution in further studies.

The complete plastid genome

The Podostemaceae possess one of the smallest known plastid genomes among the Malpighiales covering approximately 130 kb in length (Bedoya et al. 2019; Jin et al. 2020a). The complete chloroplast genome of *P. chiangmaiensis* was successfully assembled and annotated. The result showed that the length of the complete chloroplast genome was 133,748 bp with 35% GC content, consisting of a typical quadripartite plastid structure with two inverted repeats (20,854 bp) separated by a large single-copy region (79,537 bp) and a small single-copy region (12,503 bp) (Fig. 5). *Paracladopus chiangmaiensis* contained 110 unique genes, including 76 protein-coding genes, 30 tRNA genes, and 4 rRNA genes, and lost *rps16* gene, *rp123* genes, the intron of the *clpP* gene and *rps12* gene, and the second intron of the *ycf3* gene. An uncommon loss of *ycf1* and *ycf2* and a major

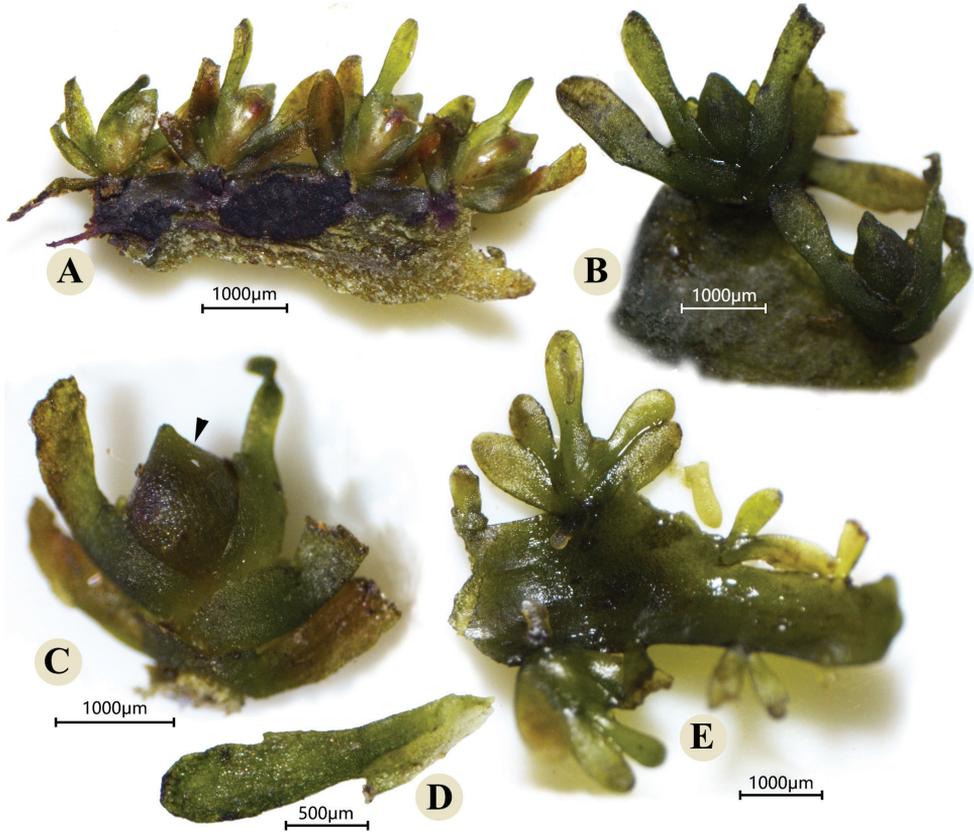


Figure 3. Morphology of *Paracladopus chiangmaiensis* shoots **A** four flowering shoots borne on the root **B** reproductive shoot with ensiform bracts and terminal floral bud enclosed by spathe **C** lateral view of ensiform bracts covering flower bud, arrow means spathe **D** ensiform leaves with sheaths on the inner edge **E** vegetative shoots borne on flanks of root.

inversion over 50 kb were also found. The inversion contained 46 genes spanning from *trnK-UUU* to *rbcL* in the large single-copy region. The *rps15* gene relocated from SSC to IR for the expansion of IRs. GC content in the IRs was higher than in other regions of the plastid. All plastid characters of *P. chiangmaiensis* were similar to other plastomes reported in Podostemaceae, excluding *Tristicha trifaria* whose *rps15* gene was located at the SSC/IRA boundary (Bedoya et al. 2019; Jin et al. 2020a). The annotated plastome was deposited in GenBank under the accession number MZ645928. Our results could provide essential data to investigate the phylogeny and evolution of river-weeds in the future.

The phylogenetic analysis

The *matK* gene sequence extracted from complete chloroplast genome of Wuzhishan river-weed (WuMS109) was 1,527 bp in length. The phylogenetic

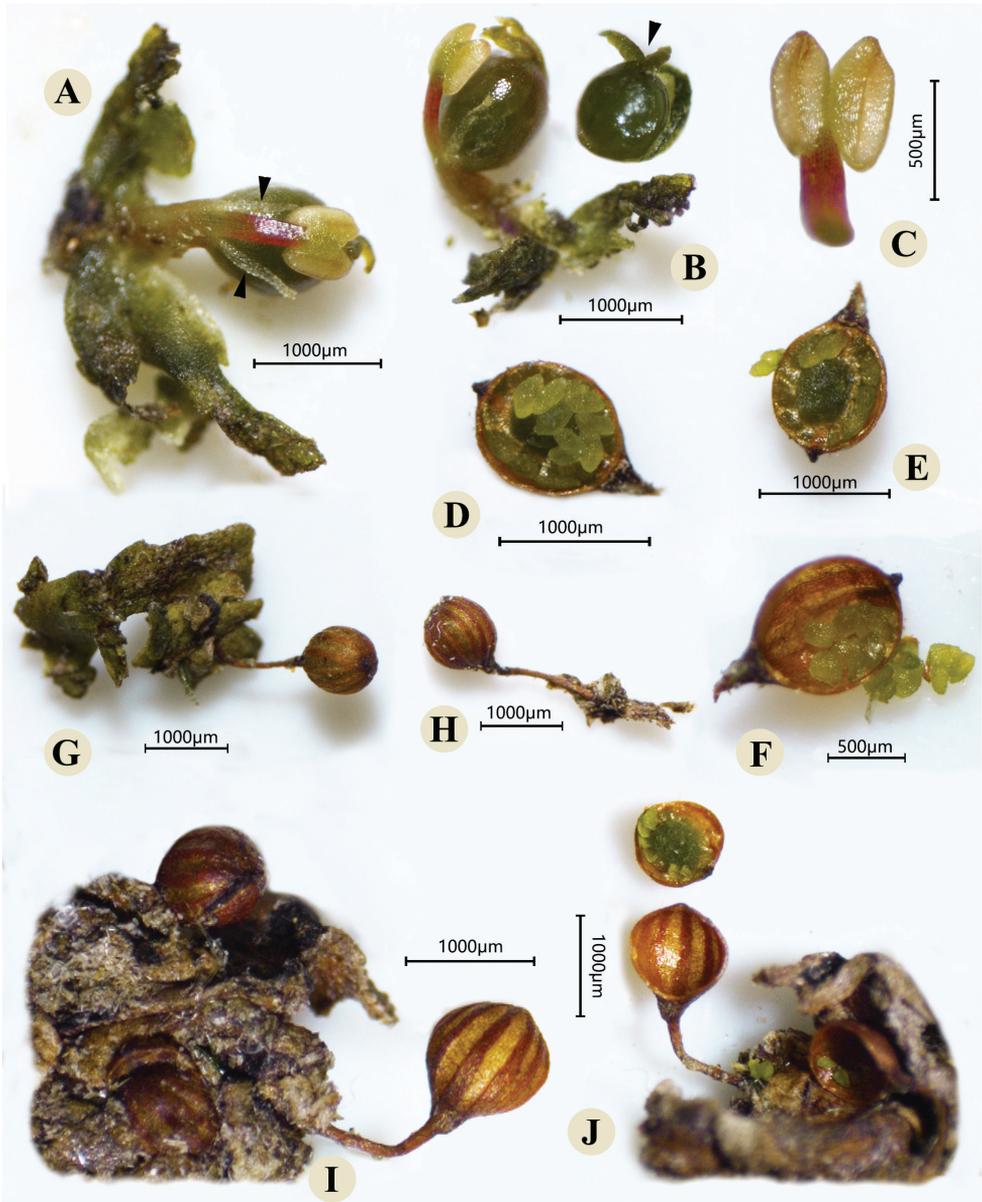


Figure 4. Flower and fruit of *Paracladopus chiangmaiensis* **A** a flower bud (spathella removed), arrows mean two tepals on sides of stamen **B** lateral view of a flower bud (spathella and bracts removed), showing single stamen and two stigmas (as shown by the arrow) **C** stamen with indehiscent anther **D, E, F** fruit and seeds **G, H** stalked capsules covered with stripes **I, J** two persistent capsule valves with some stripes.

relationship constructed based on the *matK* gene sequences showed that WuMS109 and *P. chiangmaiensis* (AB537420, AB698348) were clustered together without base difference (Fig. 6), which was consistent with the result of morphological

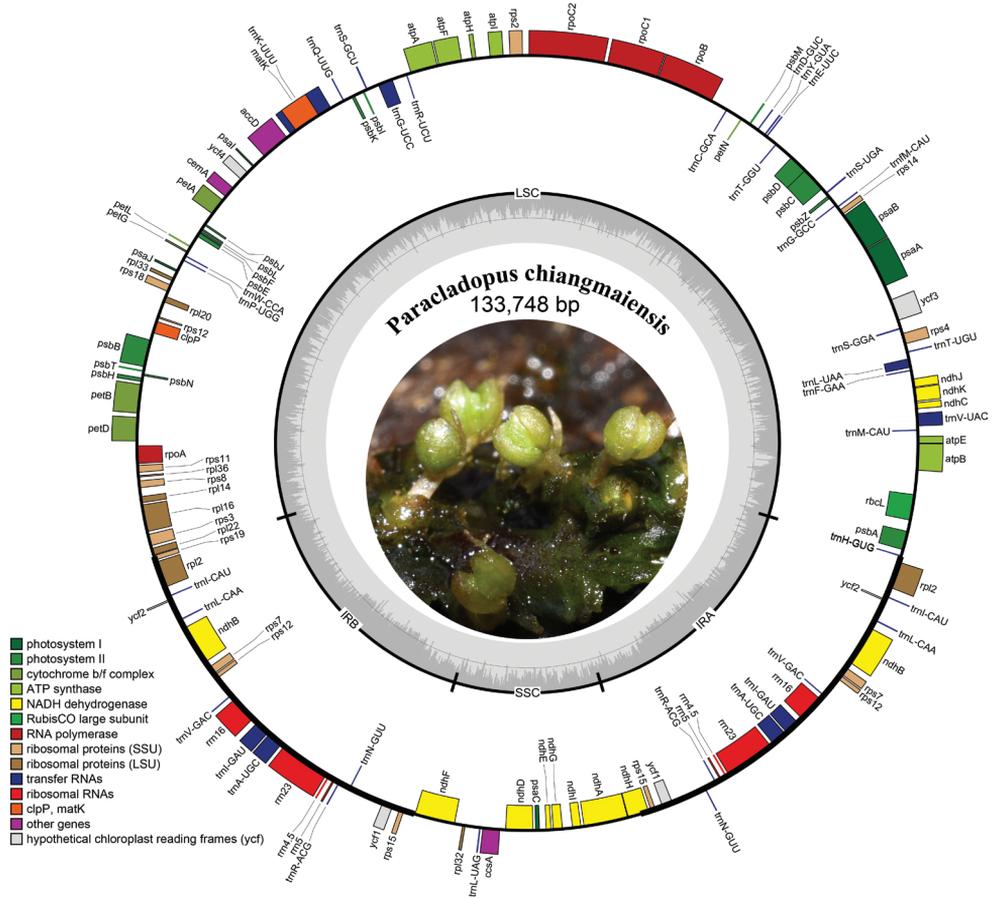


Figure 5. Gene map of *Paracladopus chiangmaiensis* complete chloroplast genomes. Genes inside the circle are transcribed clockwise, genes outside the circle counter clockwise. The circle inside the GC content graph marks the 50% threshold.

identification, so, the river-weed in Wuzhi Mountain was identified as *P. chiangmaiensis* with certainty. The genus *Paracladopus* is similar to *Cladopus* in ribbon-like roots and globose or ellipsoidal capsule, but can be distinguished by its shoots borne on the sinuses of root branching and flanks of the root, holdfasts presenting on ventral surface of root under the shoots and capsules with ribs or stripes. According to the key in ‘Flora of China’ (Qiu and Philbrick 2003), the pericarp of capsule with ribs denoted the genus *Hydrobryum* in Podostemaceae, but roots crustose and tufts of linear leaves scattered on its dorsal surface made the *Hydrobryum* markedly distinct from *Cladopus*. Molecular phylogenetic analysis showed that *Paracladopus* was a sister to *Cladopus*, but distantly related to *Hydrobryum*. The phylogenetic result was consistent with the previous research (Koi et al. 2012; Kato et al. 2019).

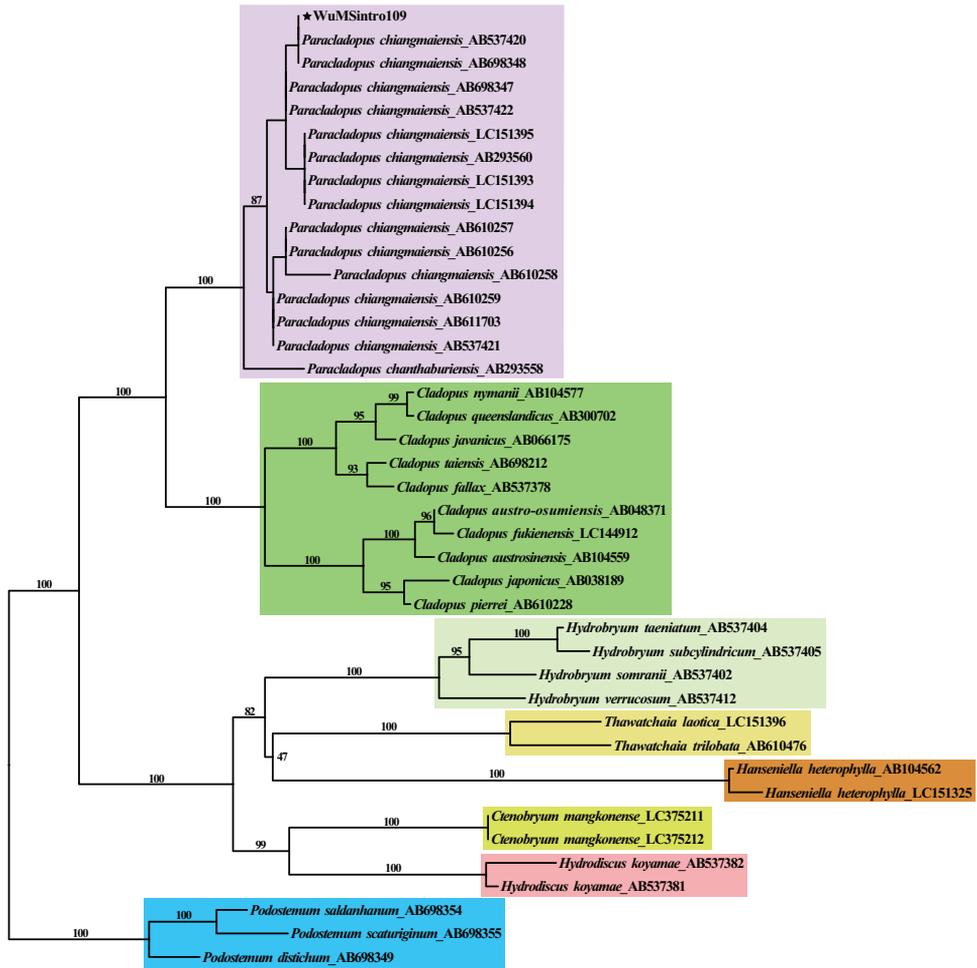


Figure 6. The plastid phylogeny of the *Paracladopus* and closely related genera. Maximum-likelihood (ML) tree inferred from *matK* gene. The number at each node indicates the ML bootstrap values. Species are color-coded according to genus. Three species of *Podostemum* (sky blue) are designed as outgroups. The sequence newly generated in this study marked by black pentagram.

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The Identity of *Rubus pekinensis* Focke and *R. crataegifolius* Bunge (Rosaceae)

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Abstract

A critical examination of specimens, with literature and a field survey have shown that *Rubus pekinensis* is conspecific with *R. crataegifolius*. Their morphological variations range can be defined as: leaves at the base may be ovate, suborbicular, narrowly ovate, entire, at the middle, ovate, narrowly ovate, oblong-lanceolate, palmately 3-lobed or 5-lobed and at the top, ovate, lanceolate, entire or 3-lobed; flowers solitary in the axillae or several flowers clustered at the terminal of branchlets or formed into short racemes. Therefore, we treat the former species as a synonym for the latter one.

Keywords

Identity, new synonym, *R. crataegifolius*, Rosaceae, *Rubus pekinensis*

Introduction

Rubus crataegifolius Bunge (1835: 98) was published, based on the collection from Pan-schan (Panshan), Hebei (now Tianjin), China A. Bunge s. n. (Syntypes LE [photo!], LE01015265, LE01015266, LE01015267, Fig. 1A–C). In the protologue, critical characteristics of the species were described as “Fruticosus erectus glabriusculus, ramis petiolis foliorum nervis pedicellisue aculeatis, foliis cordatis

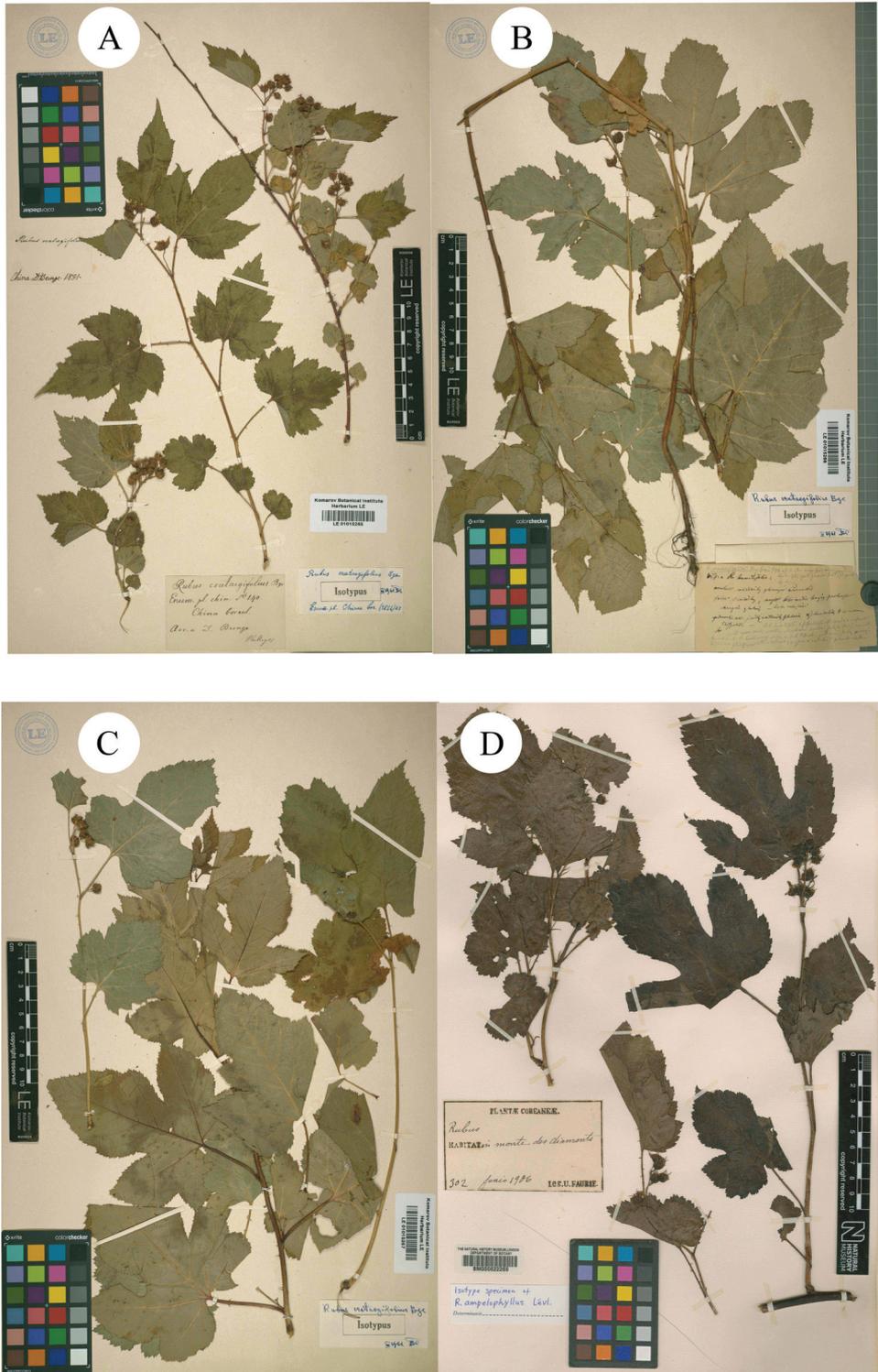


Figure 1. A–C syntypes of *R. crataegifolius* D syntype of *R. ampelophyllus*.



Figure 2. A–D syntypes of *R. wrightii*.

trifidis; lobis lateralibus acutis terminali acuminato acute inciso-dentatis, floribus axillaribus solitariis terminalibusque subracemosis; sepalis glabriusculis ovatis acuminatis erectis, petalis longe unguiculatis subspathulatis emarginatis, carpellis subexsuccis glabris”.

A. Gray (1858: 387) described a new species *R. wrightii* A. Gray on the basis of specimens collected from Hokkaido, Japan (Syntypes GH [photo!] GH00040766; K [photo!] K000737782, K000737783; NY [photo!] NY00391934; US [photo!] US00095501, Figs 2A–D, 3A). H. Léveillé and Vaniot (1905: 62) described *R. ouensanensis* H. Léév. & Vaniot in Bull. Soc. Agric. Sarthe, based on the collection from Ouen san, Corea U. J. Faurie 83 (Holotype E [photo!] E00010580; Isotypes A [photo!] A00040688 (Image of E00010580); G [photo!] G00437174; P [photo!] P00755199, P00755200, Figs 3B–D, 4A, B). H. Léveillé (1908: 279) described *R. ampelophyllus* H. Léév. in Repert. Spec. Nov. Regni Veg., based on the collection from Quelpaert, Corea U. J. Faurie 302 (Syntype BM [photo!] BM000622269, Fig. 1D). These three species were treated as synonyms of *R. crataegifolius* by Lu (1985: 117) and Lu & Boufford (2003: 236) in Flora of China.

H. Léveillé and Vaniot (1905: 62) published a new species *R. itoensis* H. Léév. & Vaniot, based on the specimens collected from Takeo, Kiushu, Japan U. J. Faurie 5365 (Syntypes BM [photo!] BM000622252; E [photo!] E00010657, Fig. 4C, D). According to its protologue, this species was described as similar to *R. grossularia* H. Léév. & Vaniot and *R. incisus* Thunb., but it differed by having peduncles, with large and numerous flowers. However, it was treated as a synonym of *R. crataegifolius* in Flora of Japan (Naruhashi 2001).

Focke (1917: 104) described a new species *R. pekinensis* Focke on the basis of the collection of O. Warburg 6549 from Miaofangshan (Miaofengshan), Beijing, China (Holotype B [photo!] B101154579, Fig. 5A). According to the holotype, features of the specimen can be described as “shrubs with slightly curved prickles; leaves simple, 3-lobed or undivided, margin irregularly incised-serrate; stipules linear, adnate; several flowers cluster in axillae”. In the protologue, the characters of this species can be defined as: “Praesto est ramus exsiccatu unicus florens non incolumis speciem vero memorabilem indicans. Ramus foliorum marginibus a petiolis decurrentibus subangulatus, cum petiolis glabriusculus et aculeis lanceolatis vel falcatis sparsis armatus. Folia longe petiolata, majuscula, circ. 16 cm. longa, 18 cm. inter angulorum lateralium apices lata, inaequaliter sat grosse serrata, utrinque parce pilosa; folia inferiora subquinqveloba, intermedia e fundo cordato-emarginata triloba, inaequalia, lobis oblongo-lanceolatis; suprema integra, lanceolata. Stipulae petiolorum basi insertae, lineares. – Flores in ramulis axillaribus complures, longe pedunculati, alii superiores terminales fasciculati; pedunculi 3–6 cm. longi, laxe pilosi, interdum aculeolo instructi; calyces parce pilosi, sepalis saepe mucronatis vel appendice subulata terminatis; petala, ut videtur, sepala vix superantia.”. However, the name *R. pekinensis* has never been used, except in its original publication, from the date of its publication. Thus, it is necessary to identify the species.

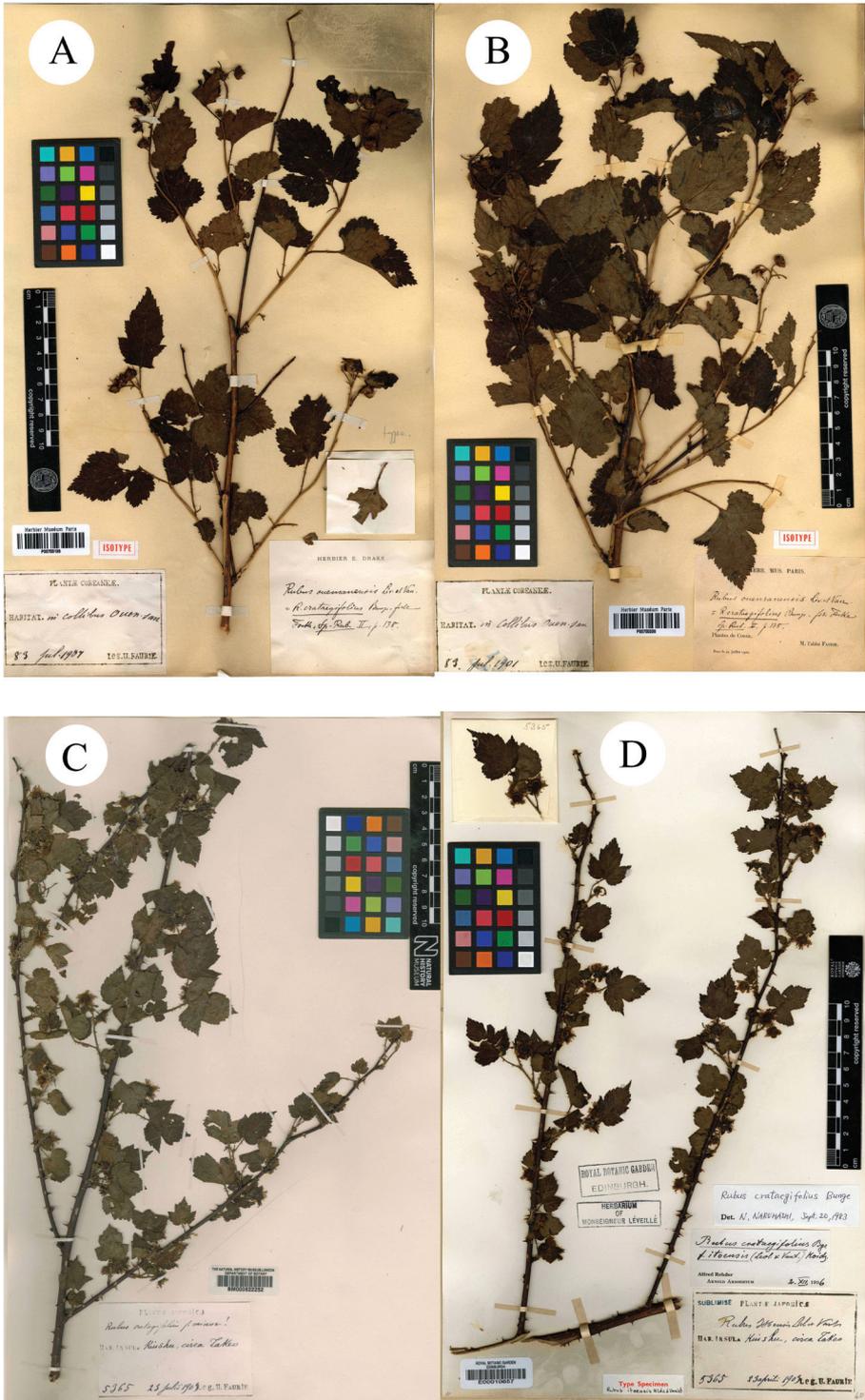


Figure 4. A, B isotypes of *R. ouensanensis* C, D syntypes of *R. itoensis*.

Materials and methods

Herbarium specimens, including all kinds of type specimens deposited in A, B, BM, E, G, GH, K, LE, NY, P and US were critically examined and a field survey in the type localities was conducted. Six populations from Miaofangshan and Pan-schan were surveyed and collected in 2021. High resolution pictures of leaves and fruits were taken by a SONY α 7II.

Results

Firstly, the examination of herbarium specimens, identified as *R. ampelophyllus*, *R. crataegifolius*, *R. itoensis*, *R. ouensanensis* and *R. wrightii*, indicated that they represented the same species. According to Art. 11.4 of the “International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code)” (Turland et al. 2018), *R. crataegifolius* is the correct name of this species. A comparison of type materials of *R. crataegifolius* and *R. pekinensis* showed that there are only a few differences in leaf morphology and inflorescence. So they also probably represented the same species.

Secondly, during the field survey to the type localities, three populations of the species *R. pekinensis* and *R. crataegifolius* were found from each location. All of the populations were at the fruiting stage and several observations regarding species identity were carried out in this *Rubus* group. The results can be described as: a) the morphological diversity of leaves is rich in different populations (Figs 1–6), even on the same plant (Fig. 8A–E). As Fig. 7 shows, leaves at the base may be ovate, suborbicular, narrowly ovate, entire, at the middle, ovate, narrowly ovate, oblong-lanceolate, palmately 3-lobed or 5-lobed and at the top, ovate, lanceolate, entire or 3-lobed. b) the diversity of inflorescence can be seen in *R. crataegifolius* and its synonyms, including *R. pekinensis*, such as the axillary flowers, solitary or several flowers clustered at the terminal of branchlets or formed into short racemes (Figs 1–6). c) the field survey in type localities of *R. pekinensis* and *R. crataegifolius* shows that they share characters of “branchlets angular, thinly pubescent when young, gradually glabrescent, with lanceolate or falciform prickles; leaves simple, middle-lower palmately 3–5-lobed, oblong-lanceolate, top entire lanceolate; stipules adnate to base of petiole, linear; flowers in branchlets axillary, others, terminal, short racemes or flowers several in a cluster; pedicel pubescent, sometimes equipped with aculeolus; calyx abaxially thinly pubescent, sepals narrowly ovate to ovate-oblong, apex acute to shortly acuminate, aggregate fruit red, glabrous” (Figs 5B–D, 6A–D, 7A–D). This is consistent with the protologue of Focke (1917: 104) and Bunge (1835: 98).

Based on the analysis above and the characters defined from the type specimen, we can confirm that *R. pekinensis*, together with *R. ampelophyllus*, *R. itoensis*, *R. ouensanensis* and *R. wrightii*, are conspecific with *R. crataegifolius*. As *R. pekinensis* was published later than *R. crataegifolius*, *R. pekinensis* should be a synonym of *R. crataegifolius*, according to nomenclatural priority.



Figure 6. Specimens of *R. pekinensis* collected from Miaofengshan.

Taxonomic treatment

***R. crataegifolius* Bunge** Mém. Acad. Imp. Sci. St.-Pétersbourg Divers Savans 2: 98. 1835.

R. crataegifolius Bunge Mém. Acad. Imp. Sci. St.-Pétersbourg Divers Savans 2: 98. 1835.

Types: China, Hebei (now Tianjin): Pan-schan, 1831, Bunge s. n. (Lectotype LE! LE 01015265 (designated here by Ti R. Huang); Isolectotypes LE!, LE01015266, LE01015267)

=*R. pekinensis* Focke Annuaire Conserv. Jard. Bot. Genève 20: 104. 1917, syn. nov.

Types: China, Beijing: Miaofangshan, May 1887, O. Warburg 6549 (Holotype B! B101154579).

=*R. ampelophyllus* H. Lév. Repert. Spec. Nov. Regni Veg. 5: 279. 1908. Types: Corea, Quelpaert, U. J. Faurie 302 (Holotype BM! BM000622269)

=*R. itoensis* H. Lév. & Vaniot Bull. Soc. Agric. Sarthe 60: 62. 1905. Types: Japan, Kiushu, circa Takeo, 13 April 1903, U.J. Faurie 5365 (Lectotype BM! BM 000622252 (designated here by Ti R. Huang); Isolectotype E! E 00010657)

=*R. ouensanensis* H. Lév. & Vaniot Bull. Soc. Agric. Sarthe 60: 62. 1905. Types: Corea, Ouen san, July 1901, U. J. Faurie 83 (Holotype E! E00010580; Isotypes A! A00040688 (Image of E00010580); G! G00437174; P! P00755199, P00755200)

=*R. wrightii* A. Gray Mem. Amer. Acad. Arts ser. 2, 6(2): 387. 1858. Types: Japan, Hokkaido, 1853, C. Wright s. n. (Lectotype GH! GH00040766 (designated here by Ti R. Huang); Isolectotypes K! K000737783; NY! NY00391934 US! US00095501)

Distribution and habitat. *R. crataegifolius* grows on slopes, broad-leaved evergreen forests on hills, coniferous forests, thickets and roadsides. Its elevation ranges from 500–1000 m. In China, it is distributed in Anhui, Fujian, Guangxi, Jiangsu, Jiangxi and Zhejiang and overseas, in Japan.

Phenology. Flowering from May to June and fruiting from July to August.

Taxonomic notes. *R. crataegifolius* is similar to *R. chingii* H.H. Hu, the differences being: the latter leaves suborbicular, always palmately 5-parted, rarely 3- or 7-parted; flowers solitary; aggregate fruit densely hairy.

Additional specimens examined. CHINA. Beijing. 20 June 1964, Anren Li et al., no. 77 (PE00169287); June 1956, Herbarium, s. n. (TIE 00033553); 11 May 1930, T.N. Liou, no. 6874 (PE00169338); 30 June 1930, W.W. Smith, no. 1081 (PE00169344); Heibei. 1934, C.W. Wang, no. 61702 (IBSC0323698); 24 July 1984, Wuxiu Zhang et al., no. 0241 (PE01546856); 12 October 1957, Shaoying Qin, no. 38 (PE01466183); Shandong. 3 June 2013, Zhiyun Zhang & Lei Xie, 2013-032 (PE01979816); 1 May 2004, Yuantong Hou, no. 42011 (HITBC0008947); s.n. no. 56047 (BNU002768); Shanxi. 29 May 1959, Kechien Kuan & Yilin Chen, no. 519 (PE00169384); 12 June 1959, Kechien Kuan & Yilin Chen, no. 684 (PE00169383); 6 July 1959, Kechien Kuan & Yilin Chen, no. 02128 (PE00169382 & PE00169385); 25 July 2014,



Figure 7. Specimens of *R. crataegifolius* collected from Panshan.

Dongmei Kong, no. k0483 (PE02035182); 18 July 1986, Lanbin Guo, no. CLB0544 (BJFC00027073); **Tianjin**. 10 June 1984, Shiyuan He, no. 34234 (BNU 002746); 3 July 1956, Kechian Kuan, no. 1902 (PE 00169272); 9 July 1976, Jiayi Liu & Cailing Wang, no. JI0217 (TIE 00014583); 17 May 1985, Cailing Wang & Yongli Yu, no. JIN01446 (TIE 00014579); **JAPAN**. 3 August 1983, H. Migo, s.n. (NAS00368819, NAS00368820); 27 July 1928, Inagaki Kanichi, s.n. (NAS00368832); 1861, Albrecht, s.n. (K000737782); 1916, U.J. Faurie, 2370 (P03375389, P03375390, P03375391).

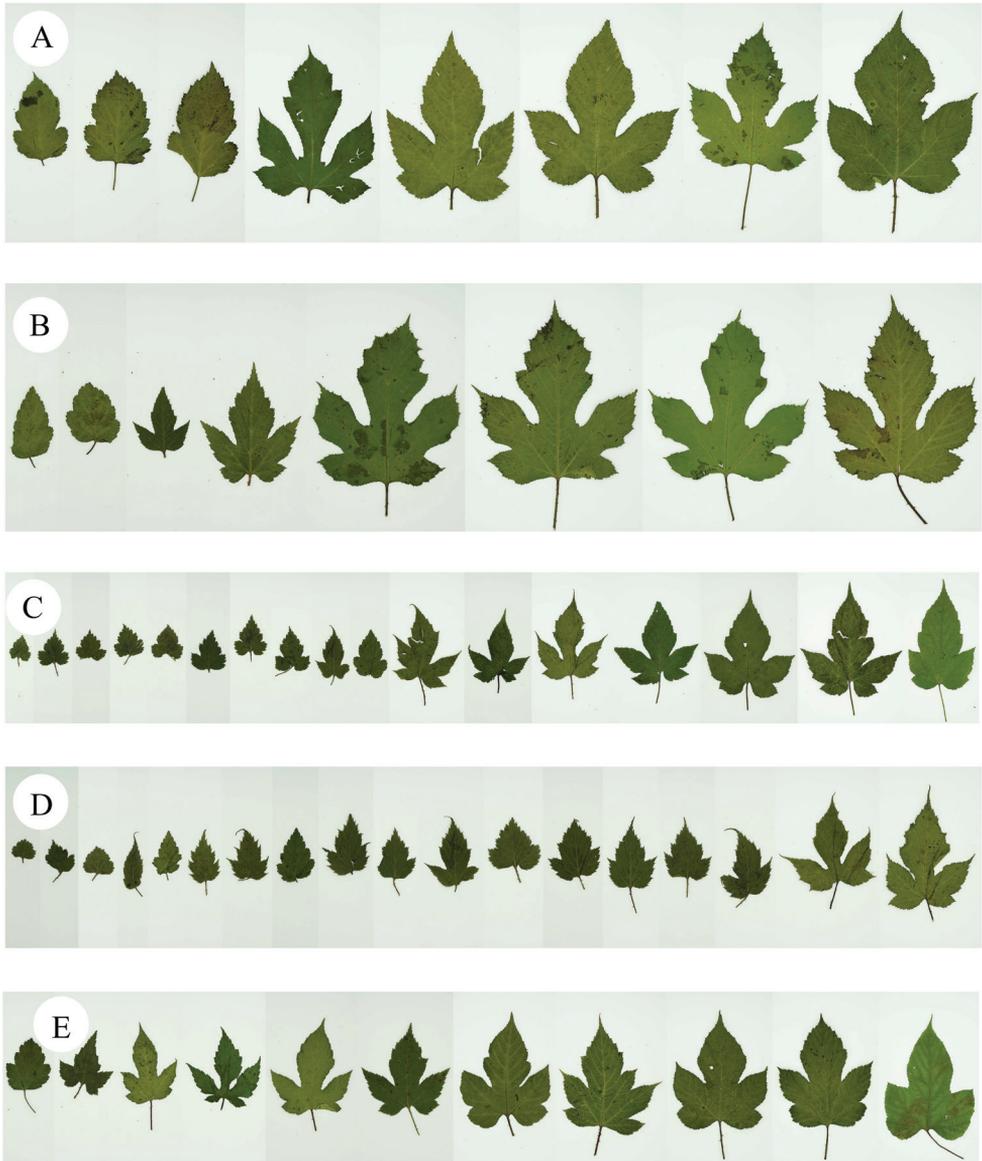


Figure 8. Morphological diversity of leaves **A, B** leaves of *R. crataegifolius*, collected from Panshan **C–E** leaves of *R. pekinensis*, collected from Miaofengshan.

Discussion

In the Flora of China, Lu and Boufford (2003: 284) indicated that *R. pekinensis* had been described from Peking (Beijing), China, but they have seen no specimens, and are therefore unable to treat it. Meanwhile, they stated that further revision of this species is necessary. In this paper, we carried out a field survey in the type locality from where morphological variations of *R. pekinensis* and *R. crataegifolius* in different populations were studied. There are many variations in the shapes of leaves both in *R. pekinensis* and *R. crataegifolius*, even in the same plant, where leaves simple, ovate, narrowly ovate, lanceolate or oblong-lanceolate, entire or palmately 3-lobed or 5-lobed, can be seen. Flowers solitary, clustered and short racemes sometimes co-existed in the same plant. This is consistent with the description of *R. crataegifolius* (Focke 1910, 1911, 1914). In further studies of *Rubus*, understanding the diversity of the species between different populations needs critical examination of specimens and field surveys, especially when a new species is being published.

Acknowledgements

We would like to express gratitude to Robert Vogt, curator of the Berlin herbarium (B), for providing Focke's type material of *R. pekinensis*, and other curators of the herbaria: A, BM, E, G, GH, K, LE, NY, P and US. We are grateful to an anonymous reviewer for valuable comments and to postgraduate Sun Mengtao and Zhang Junxin's help for specimen collection. This research was financed by the Beijing Municipal Education Commission under Grant KM201910020016.

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The history and typification of *Lilium brownii* A.Lemoinier (Liliaceae)

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Abstract

The Chinese *Lilium brownii* has been much confused with the Japanese endemic species *Lilium japonicum*. In this paper, it is shown that *L. brownii* was introduced to England at least four times between 1804 and 1819. The history of *L. brownii* is fully discussed and its taxonomy, nomenclature and relationships are examined. A neotype is designated for the name, its correct botanical authority is given and the correct place of its publication is provided. Lectotypes are also provided for the names *Lilium aduncum* Stapf, *Lilium australe* Stapf, *Lilium odorum* Planch., *Lilium brownii* var. *colchesteri* E.H.Wilson and *Lilium brownii* var. *ferum* Stapf.

Keywords

Brown nursery, China, Horticultural Society Kerr, Kew, *Lilium brownii*, *L. japonicum*, nomenclature, Reeves, typification

Introduction

Nine species names have been given to Chinese species of the genus *Lilium* L. that have infundibuliform or trumpet-shaped flowers (Liang and Tamura 2008). The first of these to be formally described with a Latin name, is currently accepted as *Lilium brownii* F.E.Brown ex Mieliez in 1841. This species with a widespread distribution across central and southern China has been known to the Chinese as an important medicinal and culinary plant under the name “pae hup” in Cantonese or “bai he” in Mandarin from as early as the Tang dynasty (618–907). This species is known today by the very similar sounding Cantonese name of Pak Hup 百合.

Since the species was first introduced to England from Canton [Guangzhou] China as an ornamental in 1804, it has been persistently confused with the Japanese endemic species *Lilium japonicum* Thunb. ex Houtt. and was, on its arrival, initially given that name (W.T.Aiton 1811: 240). This initial misidentification was a problem which persisted well into the late 19th century (e.g. Baker 1875: 230). The two species share some morphological similarities, but can readily be distinguished (see confusion with *L. japonicum* below). To add to this, several illustrations have been made of *Lilium brownii* by Japanese artists in Japan owing to the introduction of the species to that country and its subsequent cultivation there since at least the 16th century (Okubo et al. 2012).

Lilium brownii has also been confused with other trumpet-flowered species, in particular with *L. longiflorum* Thunb., a species native to Japan, the Ryukyu Islands and along the northern coast of Taiwan. The two species both have white, trumpet-shaped flowers, but *L. longiflorum* has no reddish-brown colouration on the outside of the corolla and the anthers carry bright yellow not reddish-brown pollen.

The French missionary botanist Julien Cavalerie's uncertainty of the distinctions between these Chinese trumpet-flowered lilies is exemplified by his description of *L. sulphureum* Baker ex Hook.f. (Hooker 1892: 351) under the name *L. brownii*. This misidentification was yet further exacerbated by his description of *L. brownii* immediately afterwards under the name *L. longiflorum* (Cavalerie 1911: 245). *Lilium brownii* has even been considered to be of hybrid origin, albeit without any evidence to support that suggestion (e.g. Franchet 1892: 312; R.Wallace 1932: 51). When Louis van Houtte made a comparison between *Lilium longiflorum* var. *suaveolens* and what he was calling *L. japonicum*, he specifically mentioned that his "*L. japonicum*" had violet-purple internal colouring and dark chocolate brown pollen (Van Houtte 1833: 182). He may have been referring to a hybrid with *L. brownii* as a putative parent.

At no stage in its botanical history has a type been allocated to the species. The liliophile Kew botanist John Gilbert Baker segregated *L. brownii* var. *viridulum* from (by implication) var. *brownii* on the shorter, wider, more oblanceolate leaves and paler greenish colouration on the outside of the corollas with less pronounced claret markings (Baker 1885: 131). Baker's statement "The leaves are much broader and shorter than in the type" is almost certainly intended to refer to what he regarded as the typical variety of *L. brownii*, a point further indicated by his citation of (Mielle) [i.e. Mieliez] as the author of the name and which was accompanied by a reference to the description and illustration in *Flore des Serres* by Charles Lemaire. The latter portrays a plant with linear-lanceolate leaves and a flower with reddish markings on the outside of the perianth (Lemaire 1845: t. 47). These references, however, do not constitute typification of the species. William Stearn regarded what he called *L. brownii* var. *brownii* as being based on *L. japonicum* var. *brownii* (Spae) Baker (Baker 1871: 709); however, he again did not indicate any type specimen or illustration (Stearn 1948: 5).

All authors prior to this paper have followed the Belgian botanist Dieudonné Spae (1819–1858) by wrongly attributing the name of the plants from which the species originated to F. E. Brown of the Slough nursery near Windsor, England (Spae 1845: 438; 1847: 12). It is shown here that, although the Slough nursery was indeed the

source of Spae's plants, no such member of the Brown family with those initials was ever involved therein. It is shown here too that Miellez, who was also credited with authorship of the name, never validly described the species.

William Kerr's introductions from Canton to Kew

The bulbs of the as yet unnamed *Lilium brownii* were first unloaded at the wharves of the East India Company's dockyard, Blackwall, London on 14 August 1804 (Hardy 1811: 222). They were part of a consignment of plants collected by William Kerr (1779–1814), a Scottish gardener at the Royal Garden at Kew who was sent to China's southern port of Canton in 1803 by Sir Joseph Banks, special advisor to King George III. His mission was to remain in Canton specifically to collect plants, which he did until 1812 (Goodman and Jarvis 2017: 269). Kerr's sending of his shipments from Canton to Kew ceased in 1810. The bulbs that were sent with his first shipment were put in a box at Canton on the 1200 ton Honourable East India Company's ship [HEICS] "Henry Addington", commanded by Captain John Kirkpatrick (1766–1816) for the long sea journey back to England. The Henry Addington set sail from Canton leaving the anchorage below the Second Bar Island, 12 miles (ca. 19 km) south of Whampoa Island [Pazhou island], Canton on 1 February 1804 for its homeward bound journey. This took the ship around the Cape of Africa with a stop off at the South Atlantic Ocean island of Saint Helena which was then under the governorship of the East India Company.

It is remarkable that the ship with the bulbs on board survived that journey. The "Henry Addington" was involved in the Battle of Pulo Aura [Pulau Aur] between the British and the French following the collapse of the Treaty of Amiens in 1803 and the reconvening of the Napoleonic Wars. The ship was part of a large convoy of British merchant ships that set off from China and sailed through the Straits of Malacca under the command of Sir Nathaniel Dance, commodore of the EIC fleet. This convoy encountered four roving French warships and a Dutch brig under the command of the French Contre-Admiral Charles comte de Linois on 15 February 1804 who, believing it to be a fleet of British warships, left the scene after only a skirmish (Hardy 1811: appendix 123).

The first written record of this shipment of plants was in the list put together by William Kerr in his "*Memorandum of Plants, Seeds & c. sent from China to the Royal Gardens, Kew*" which is now conserved in the library of the School of Oriental and African Studies (SOAS) in London (Kerr 1804). Included as the first part of Kerr's journal is a "*Catalogue of plants procured at Canton, China and sent to England on board the ship Henery Addington (sic) in a greenhouse or plant cabin prepared for the purpose. This ship with the whole China Fleet of the season sailed from the Second Bar Canton River Feb. 1st 1804*" (Kerr 1804: fol. 1). This "Memorandum" recorded the first of about a dozen shipments of plants that Kerr sent back to Kew from 1804 until 1810 (Kew Record Book 1804–1826).

William Kerr did not elucidate how or from where he had acquired the plants that he had put on board the EIC ship. During his time in Canton after his arrival

in late 1803, he frequently visited the garden nurseries at Fa-tee or Fati [Huadi] “flowery land” across the Pearl [Zhujiang] River and a little upstream from where he was compelled to reside and spend the majority of his time in the British factory (Fan 2003: 71). This building set back from, but facing the river was one of 17 elaborately-fronted foreign offices and warehouses all known as the “factories” along the river at Xiguan (Livingstone 1819: 126). From 1757, the Qianlong Emperor (1711–1799) closed China to all foreign trade, except that which was permitted from the ports of Canton and Macao. This restriction continued until the treaties that emerged as a result of the Anglo-Chinese opium wars (1839–1842). When Kerr arrived in China, trade with the Chinese within Canton by foreigners was severely restricted to within these factories and to the houses of the Chinese “Hong” merchants and was only permitted to take place during the winter months i.e. between October and March (Compton 2015: 265). This explains why it was that, as Kerr himself stated, he did not see these lilies when they came into flower in June or July. In his “Memorandum”, Kerr stated that all the plants on board the HEICS “Henry Addington” during the journey to England were carefully tended by his friend Mr. Allen (Kerr 1804 fol. 78). Kerr had clearly met and befriended John Allen, a Derbyshire miner who was passing through Canton on his way back to England from Australia (Kilpatrick 2007: 168).

Kerr’s entry for number nine on his list included the Chinese name “Pae-hup-fa” with “fa” meaning flower in Cantonese and, next to this entry, he placed four crosses (“xxxx”). Kerr does not indicate what these four crosses symbolised, but it would have been the level of desirability according to the code for desiderata designed by Sir Joseph Banks. These symbols relate to the list of Chinese plants and their corresponding illustrations in “The Book of Chinese Plants” which he had been lent by Banks (Goodman and Jarvis 2017: 266). Thus four crosses next to the name of a plant meant that it was an unknown plant of high desirability, reducing in the value of its desirability down to one cross x = known, but not seen living (Goodman and Jarvis 2017: 266). Kerr mentions in his “Memorandum” for his entry number one: “*T’hoi tong-fa* Begonia fig. 4 xxx in the Chinese Book of Drawings brought out by Mr Lance”. This book was a quarto book of Chinese plant illustrations which was most probably based on others undertaken previously by Chinese artists for John Bradby Blake’s visits to Canton. Blake was a supercargo [merchant] for the EIC from 1766 until his death in Canton in 1773 (Goodman and Jarvis 2017: 252, 266). The illustrated book was designed to aid in the identification of the Chinese plants so that those collected by Kerr were not duplicated.

Kerr also included in his “Memorandum” a square symbol (“□”) which meant that the plants were placed in a wooden box. The number of squares placed next to a plant’s name indicated the number of boxes loaded on board ship (Kerr 1804: fol. 1). Next to number nine in the “Memorandum”, Kerr added the script:

“9. *Pae-hup-fa* fig. 36 xxxx This is a bulbous rooted plant. The bulb resembles that of *Lilium bulbiferum*. I have neither seen the flowers nor leaves. Used in medicine as well as for ornament □ 1.”

Kerr's mention of "fig. 36" most probably refers to an illustration of this plant in "The Book of Chinese Plants" brought to him by Mr. David Lance who had been tasked to hold overall responsibility for Kerr's welfare in Canton. Lance, a friend of Sir Joseph Banks and a senior supercargo in Canton, had travelled out from England with Kerr along with the ship's surgeon and keen botanist John Livingstone on the HEICS "Coutts", commanded by Captain Robert Torin (Kilpatrick 2007: 165). The "Coutts" left The Downs in Kent on 6 May 1803 and did not arrive in Whampoa, Canton, until 1 October 1803 (Hardy 1811: 228). The ship survived a disastrous typhoon which destroyed both of the ship's masts and caused the loss of the anchors overboard, necessitating the ship to be towed into Canton (Kerr 1804 fol. 75). The "Book of Chinese Plants" must have been given into Lance's safe-keeping by Sir Joseph Banks and is now missing. There is also another entry on Kerr's "Memorandum" list: number 19 "Kuntan" xxx (= unknown and desirable) and the statement: "*Lilium?* I have not yet seen the leaves or flowers, the bulbs resemble those of *Lilium candidum*".

These were the only lilies that Kerr included in this, his first list delivered to the Royal Garden at Kew. Kerr's description of his number nine "having bulbs resembling *Lilium bulbiferum*" equates to the whitish bulbs of *L. brownii*.

In the Kew Record Book (1804–1826), which holds records of all the plants arriving into the Royal Gardens, there are a number of similar entries referring to the various dispatches of Kerr's plants from Canton. These entries are carefully cross-referenced by Kerr to correspond to the numbered plants in his *Memorandum* and to the illustrations in the "Book of Chinese Plants". On the first folio of the Kew Record Book, Kerr added some additional information regarding this first collection of his plants: "*As far as number 62 are all cultivated plants either for ornament or use*". Later he added: "*From number 62 are wild plants collected in Danes Island*". The significance of this statement is that his number nine "Pae-hup-fa" was a cultivated and not a wild plant. Danes Island [Changzhou Island] next to Whampoa held a Danish cemetery. In Kew Record Book 1804: fol. 5, the full entry for number nine states:

"9. *Pae-hup-fa* fig. 36 xxxx *A liliaceous and bulbous rooted plant, the roots resemble those of Lilium candidum. I have not yet seen either flowers or leaves. It is a very scarce plant here and is originally from Nan-Kin, the roots are used in medicine*"

It should be noted that Kerr's switching of the resemblance of the bulbs from *L. bulbiferum* L. in his "Memorandum" to *L. candidum* L. in the Kew Record Book is of little significance as the bulbs of both species are very similar. His reference to Nan-kin [Nanjing, Jiangsu Province] is unknown, but may refer to his belief that the lily had a more northern wild distribution.

Later in the Kew Record Book (1804–1826), there is a second reference to Kerr sending more bulbs of *Lilium brownii*. Kerr dispatched plants "*in the plant cabin aboard the HEICS Hope with Captain Pendergrass*". These were sent back from Canton on 23 February 1806 (Kew Record Book 1806: 47). The entry simply states: "*Number 27 Pa-hup Lillium sp. (sic.) 1 [box]*". The 1200 ton *Hope* arrived back in London on 7 September 1806 (Hardy 1811: 246).

First description of the Chinese trumpet-lily

The superintendent of the Royal Garden at Kew, William Townsend Aiton (1766–1849) was the first to describe the new Chinese lily as *Lilium japonicum* (Aiton 1811: 240). His description was based on the lily's first flowering in cultivation at Kew and appeared in the second volume of the second edition of *Hortus Kewensis*, the catalogue of the plants cultivated in the garden. Aiton called it the “White Japan Lily” stating that it had come from China in 1804, courtesy of William Kerr on the HEICS *Henry Addington* under Captain Kirkpatrick. The second of Kerr's Chinese lilies, i.e. his “kun-tan”, also flowered and was described under the name *L. tigrinum* Ker Gawl. (W.T.Aiton 1811: 241). This was almost certainly what is now recognised as *L. lancifolium* Thunb. Aiton added that this species had also been sent by Kerr with Captain Kirkpatrick to Kew in 1804.

Kerr's new lily introduction was once again fully described under the name *Lilium japonicum* by John Bellenden Ker-Gawler along with a coloured illustration by Sydenham Edwards (see Fig. 1) in Curtis's *Botanical Magazine*, volume 38 (Ker-Gawler 1813: t. 1591). Gawler added to the confusion by stating that the lily was native to both China and Japan. He cited *L. japonicum* Thunb. and thanked William Townsend Aiton for being able to depict the plant which had flowered for the first time at Kew in July 1812, although Aiton must have described it flowering before 1811 (Aiton 1811: 240). There is no doubt that this is *L. brownii*.

The Belgian nobleman and politician François de Cannart d'Hamale wrote a literary appraisal of all the previously-published works on the genus *Lilium* up to the 1860s (Cannart d'Hamale 1870). In this work, he stated that the lis du Japon (*L. japonicum* Thunb.) had taken some years to arrive in France following its introduction to Europe in 1804 by the directors of the [British] East India Company courtesy of Captain Kirkpatrick and that, in France, it had first flowered in the garden of Monsieur Dumont at Courset near Boulogne in 1809 (Cannart d'Hamale 1870: 394). This first flowering in France was also undoubtedly of *L. brownii*, based on that description and, if correct, had come into flower two years before the plants had first flowered at Kew.

Confusion with *Lilium japonicum* Thunb. ex Houtt.

Lilium japonicum, the Japanese bamboo lily or sasa-yuri was first validly, but rather poorly described by the Dutch botanist Maarten Houttuyn, accompanied by a far from convincing illustration of a single unopened trumpet-shaped flower (Houttuyn 1780: 245 t. 82, f. 2). According to Houttuyn, the depiction and description of the new lily was based on one of Thunberg's collections from Japan in 1775 and 1776 and Houttuyn stated that Thunberg called it the Japanese Lily. Thankfully, four years later, Thunberg himself added a more comprehensive description that diagnostically identified this species as having petiolate, lanceolate leaves and a campanulate white flower (Thunberg 1784: 133). There is an original specimen of this species conserved amongst the Thunberg collections in Uppsala (UPS-THUNB 8137, catalogue number V-008137 and another in Geneva G-00818143).



Figure 1. A good representation of *Lilium brownii* var. *brownii* A.Lemoinier, based on William Kerr's original collection published in "Curtis's Botanical Magazine" 38 t. 1591 (1813) and labelled as *Lilium japonicum*.

Lilium japonicum which is endemic to the southern parts of the Japanese islands does occasionally produce white flowers, but these are predominantly of a beautiful pale pinkish colour which would not have shown in dried herbarium material. The species consists of three accepted varieties: *Lilium japonicum* var. *japonicum* with leaves 5–10 cm long, with a pale rose-coloured infunduliform corolla with tepals 12–15 cm long; var. *abeanum* (Honda) Kitam., Acta Phytotax. Geobot. 14: 121 (1952) with corollas white or light pink 5–7 cm long and var. *angustifolium* (Makino) Makino, J. Jap. Bot. 1(5): 16 (1917), with pink corollas and leaves 11–20 cm long (Hayashi 2016: 117). *Lilium japonicum* var. *japonicum* occurs in damp woods of the central and western parts of Honshu, Kyushu and Shikoku; *Lilium japonicum* var. *abeanum* occurs only in Tokushima Prefecture on Shikoku Island and *Lilium japonicum* var. *angustifolium* occurs only in the wet forests of the Kii Peninsula in southern Honshu (Hayashi 2016: 117).

The lack of diagnostic characters in the original protologue undoubtedly muddled distinctions between Thunberg's *L. japonicum* and the arrival of *L. brownii*. *Lilium japonicum* frequently also has brown pollen, thus the initial confusion with *L. brownii* is more understandable. The petiolate and lanceolate tapering leaves of the endemic Japanese lily whose delicate flowers are carried on a narrow stem are characteristic and

show its superficial resemblance to bamboo; hence, its Japanese name. This contrasts with the more robust Chinese species with thicker lanceolate or oblanceolate leaves, absence of petioles and whose white flowers are purplish (rarely greenish) tinted only on the outside of the perianth and are especially dark streaked along the mid-rib of each tepal. In addition the margins of the nectary furrows on the perianth segments of *L. japonicum* are consistently glabrous, whereas those of *L. brownii* are frequently densely papillose. In addition, their native habitats do not overlap; *L. brownii* is endemic to China, whereas *L. japonicum* is restricted to the Japanese islands.

The description of *Lilium japonicum* by the French botanist Jean Poiret seems to refer to the true Japanese species, not to the Chinese species under that name, as he described petiolate leaves and he failed to mention the dark red colouration on the outside of the flower to be found on *L. brownii* (Poiret 1813: 456). There is a question regarding the basis of his description - did he describe plants that he had seen in cultivation or, more likely, was he merely repeating the description of it provided by Thunberg? Murmurings of doubt as to the identity of this species in cultivation seem to have occurred a year later in the *Supplément* to volume seven of the second edition of Dumont de Courset's "Le botaniste cultivateur" (Dumont de Courset 1814: 54). Dumont stated under the title "*Autres espèces cultivées: 1. Lis de Japon, Lilium japonicum Thunb. An L. sinense Hortul.? An L. concolor? Smith. Feuilles radicales, longues, lancéolées, pétiolées, acuminées, très-entières, glabres, bordées etc.*" [basal leaves long, lanceolate, petiolate, apices acuminate, margins completely entire, glabrous, veined etc.] and later "*Le Japon, où l'on cultive ce lis pour sa beauté. Fleurit en Juin*" (Dumont de Courset 1814: 54–55). The latter statement "in Japan where this lily is cultivated for its beauty" does not mention it as being a native of that country, yet might refer to either species.

Japan at that time was under strict *Sakoku* (locked in) without access to trade with all foreign nations, except with the Dutch until the opening of the country in the late 1850s. The Dutch were permitted to trade with the Japanese only from their little island of Dejima in Nagasaki Bay, but were in political upheaval at this time as a result of conflict with the British. The Kingdom of Holland, as a client state of the French during the Napoleonic wars (1803–1815), were the principal power in the Dutch East Indies. The presence of Dutch ships in the western Pacific Ocean inevitably involved the Dutch coming into confrontation with the British who took the Javanese city of Batavia [Jakarta] in 1811. The British did not return the island of Java to the Dutch until 1814 and consequently trade with the Dutch from Japan had more or less, then to Europe and ground to a standstill. Hitherto, all trade by the Dutch from Japan went first to Java which included the transportation of all Japanese plants. Is it, therefore, too far a leap to suggest that most (if not all) of the lilies cultivated at that time in Europe under the name *L. japonicum* were in fact *L. brownii* (An *L. sinense Hortul.?* of Dumont de Courset 1814: 54) and not the delicate Japanese species described by Houttuyn and Thunberg? Certainly the illustrations of plants in Europe named *L. japonicum* at that time all appear to represent *L. brownii*.

The question of the misidentification and misapplication of the name *L. japonicum* to *L. brownii* and the uncertainty surrounding the identity of the true *L. japonicum*

and its synonym *L. krameri* Hook.f., Bot. Mag. 99 t. 6058 (1873) was set to continue as subsequent introductions of both species arrived from China and Japan respectively throughout the later 19th Century (e.g. A.Wallace 1875: 292; Elwes 1877: t. 8; A.Wallace 1878: 505; Krelage 1878: 541).

John Reeves's introductions from Canton to the Horticultural Society

Two more introductions of the lily as *L. japonicum* were reported to have arrived in London from China in 1819 (Loddiges 1820: t. 438; Brookes 1822: 551). The lily from one of these introductions was described and painted by George Loddiges and engraved by George Cooke in "Loddiges Botanical Cabinet" (Loddiges 1820: t. 438). Loddiges mentioned that the plant that grew in China and Japan had been introduced by the Horticultural Society of London. This Society was founded in 1804 with Sir Joseph Banks as one of its founding members and it eventually became the Royal Horticultural Society after 1859. Loddiges did not specify the precise origin of the painted plant nor the exact date of its arrival in England, but his praise of Joseph Sabine for the distribution of plants from the Society indicated that it was Sabine who must have been the provider of this plant to his famous Hackney nursery.

The source of the lily in China would have been John Reeves (1774–1856) who was then the EIC Assistant Inspector of teas in Canton from 1812 to 1826, thence Chief Inspector to 1831. Reeves had been in China since 1812 following the loss of his wife Sarah Russell in 1810. In May 1816, Reeves returned to England to resuscitate his health from the subtropical heat and to marry his fiancée Isabella Andrew as his second wife (Bailey 2019: 83). For the next year, he was to work in India House for the EIC, returning to his duties in China in 1817 (for additional information on Reeves, see Bailey 2019). It was part of Sir Joseph Banks's request to Reeves as it had earlier been to Kerr to have Chinese plants illustrated. In this case, not for Kew, but for the Horticultural Society, in order for the Society to see and make a judgement on the merits of the plants prior to granting approval for their introduction. During his visit home, Reeves must have met Joseph Sabine, the Secretary of the Society to discuss the idea of commissioning Chinese artists to undertake the illustrations. The Horticultural Society's Council Minutes for 18 February 1817 recorded: "*That the proposal of John Reeves esq. to send plants and drawings from China for the use of the Society, be accepted with thanks and that the Secretary do offer to Mr. Reeves the advance of such sums as he may require towards the cost of the same*". On 1 April 1817, the Council Minutes simply stated "*£25 to Mr. Reeves for executing the said instructions*".

In 1817, the Horticultural Society did not possess a garden in which to put any plants arriving from abroad. Council Minutes 17 February 1818 reveal, however, that the Society was negotiating with a Mr. Sutton for the lease of ground for a garden in Kensington and had agreed to employ Charles Strachan as gardener. Council Minutes 29 April 1818 indicated the arrival of Chinese plants and their current lack of garden facilities: "*The Secretary reported that he had received advice of the arrival of some plants*

from China for the Society which Mr. Lee of Hammersmith had offered to take charge of for the Society, which offer was accepted with thanks". The famous Hammersmith nursery firm of Lee and Kennedy founded ca. 1745 was by this time under the management of the younger James Lee (1754–1824) and his partner John Kennedy (1759–1842).

On Tuesday 16 June 1818, the Council Minutes relate that: "*Mr Reeves's expenditure thus far on plants and drawings amounted to £25 and that an advance of a further £25 was to be made for next season*".

On 7 July 1818, the Council Minutes provided a comprehensive description of the arrival of two shipments of Chinese plants for the Society from Mr. Reeves in Canton and that these were sent to Mr. William Anderson, curator of the Botanic Garden in Chelsea [now Chelsea Physic Garden]. John Reeves had entrusted their care during the long journey from China into the hands of two ship's captains; Captain Archibald Hamilton of the 1242 ton HEICS "Bombay" and Captain Charles Mortlock of the 1507 ton HEICS "Lowther Castle". Council also thanked Mr. David Maclean of the Customs House for his care of the plants and drawings on their arrival in London. The fifth voyage of HEICS "Bombay" left the Second Bar, Canton on 22 November 1817 and arrived at Long Reach, Gravesend on 20 May 1818. The fourth voyage of HEICS "Lowther Castle" left the Second Bar on 19 December 1817 and arrived at The Downs on 2 June 1818 (Hardy 1820: 340). The two shipments, therefore, arrived within a fortnight of each other.

The same Minutes on 7 July 1818 stated that "*29 Chinese Drawings arrived having been directed by Mr. Reeves and these were examined and approved by Council*".

The RHS Lindley Library has two paintings of *Lilium brownii* under the name *L. japonicum* undertaken in China by Chinese artists working for John Reeves on behalf of the Society. These are catalogued as A/REE/SmV5/5 (small volume page 5) and A/REE/SmV5/114 (small volume page 114) and, due to their time of flowering i.e. June–July, would have been undertaken during the summer in the Company Factory House in Macao. There is no additional data on the arrival in England of the first of these, but it may have coincided with the introduction of bulbs of the Chinese species under the name *Lilium japonicum* that arrived during 1818. The second painting A/REE/SmV5/114 falls within the batch number 112–117 as HS [Horticultural Society] 143 listed in the The Society's Drawing Committee's Minutes as having arrived after 1822 (Charlotte Brooks, pers. comm.).

The Council Minutes recorded on 4 August 1818 included written verification that the lily was, by that time, in the Society's possession:

"Mr Sabine stated that he had presented to Sir Joseph Banks in the name of the Society, two bulbs of the Lilium japonicum, recently imported from China by the Society."

Whether these bulbs were donated to Banks by Joseph Sabine for Banks's own Spring Grove House garden in Isleworth or as an additional gift for the Royal Garden at Kew is not known.

Council Minutes for 19 January 1819 relate: "*The Chinese plants which had been entrusted to the care of Mr. William Anderson in the botanic garden Chelsea were ordered to be removed to the Society's garden and a letter of thanks extended*".

The Society's Garden Committee Minutes for 5 March 1819 included: "*Ordered that one pot of Lilium japonicum be presented to each of the nurserymen who are members of the Society*" (Helen Winning, pers. comm.). This statement implies that there were enough bulbs to spare for distribution to the nurserymen from their small rented garden at St. Mary Abbots Place, Kensington. It also confirms that bulbs of the lily will have been in one of Reeves's two consignments that arrived in 1818, the year before Samuel Brookes's consignment (see below).

Samuel Brookes's introduction from Canton

Samuel Brookes, a nurseryman of Ball's Pond Nursery, Newington Green near London, wrote another account of *Lilium japonicum* in a letter to the Horticultural Society on 2 August 1821, which was published in the fourth volume of the Society's Transactions (Brookes 1822: 551–553). In this letter, he stated that he and his late partner, Thomas Barr, had imported from China in 1819 a large consignment of the lily that had arrived on board the HEICS "Lady Melville". The "Lady Melville", 1263 tons, sailed from London on 16 April 1818 under the command of Captain John Stewart arriving at Whampoa, Canton on 14 September. The return voyage left the Second Bar anchorage, Canton on 25 November 1818 stopping at the south Atlantic island of St Helena for supplies on 3 March 1819 and arrived back in London's East India Docks on 6 May 1819 <https://discovery.nationalarchives.gov.uk/details/r/b23b1f48-af85-4375-8bf6-5c8ae0630ef5>. The consignment of bulbs would certainly have been included on board as "Private Trade". A later report on the difficulties of the transportation of Chinese plants to England written by the EIC surgeon at Canton, John Livingstone, mentioned that Brookes and his partner Barr had actually sent out a collector to Canton in 1819 to locate and bring back plants, although the name of the said collector was not mentioned (Livingstone 1822: 426). This would seem unlikely but may have referred to a possible collaboration with John Reeves.

Brookes reiterated that bulbs of the same lily had been originally sent from China to Kew on board the "Henry Addington" in 1804 and that one plant had flowered at Kew in July 1813, where it was figured by Sydenham Edwards for "Curtis's Botanical Magazine" as plate 1591. Aiton, however, had described it flowering before 1811 (Aiton 1811: 240). Significantly, Brookes went on to say that all those original plants had since died out, but that bulbs from his own introduction in 1819 and also from another consignment brought in by the Horticultural Society that had also arrived in 1819 were thriving.

Brookes's mention of the shipments from the Horticultural Society as having arrived in the year 1819 might also be correct as EIC ships may have brought plants including bulbs back that year; however, this was not recorded in the Society's Minute Book. The next sailing of the "Lowther Castle" did not arrive back from Canton until 9 April 1820, while that of the "Bombay" did not return to Long Reach until 29 September 1820 https://threedecks.org/index.php?display_type=show_ship&id=29088.

Chinese Illustrations of *Lilium brownii*

Samuel Brookes mentioned a drawing of the lily that was in the collection of the East India Company as drawing number 94 (Brookes 1822: 553). It was listed under the Chinese name of “Pa-kup”, a name very similar to the one listed by Kerr in 1804. This has been located in the William Kerr collection of Chinese paintings, now conserved in the archives at the Royal Botanic Gardens Kew. It is catalogued as Kerr Collection *Lilium longiflorum* number 94 (Fig. 2).

This illustration of *Lilium brownii* is numbered 94 in the top right hand corner and has two Chinese characters in ink 百合 representing “pa hup” and, in pencil at the bottom, *L. longiflorum* which may have been added later. The illustration is part of two dispatches totalling some 400 Chinese paintings of plants undertaken on behalf of Sir Joseph Banks for the East India Company. One set arrived in 1805 and the second in 1807. There is no indication as to which of these dispatches this illustration belongs. Kerr was tasked by Banks on behalf of the East India Company to find Chinese artists to paint a range of Chinese plants as a putative adjunct to the “Cabinet of Natural Productions” for the India Museum (Jordan Goodman, pers. comm.). This Museum was established in 1801 alongside East India House, the Company headquarters in Leadenhall Street (Goodman and Jarvis 2017: 270, 271). When the India Museum closed in 1879, the natural history drawings were sent uncatalogued to Kew.

The painting must have been undertaken by a Chinese artist under Kerr’s supervision in Macao during its flowering season sometime between June and August. It shows two flowering stems, one with a single bud, the other with two open flowers. There are dissections of the flower showing the six brownish-red stamens, the ovary with the style and stigma attached and the six individual perianth segments. There is also a complete subglobose bulb showing the white scales. Along the margin on one side, “*L. brownii?*” is faintly added in pencil, which must have been added many decades later.

The Asian and African Studies Print Room in the British Library also holds a collection of 309 watercolours of Chinese plants in six volumes that came from the East India Company (NHD52–57). The majority of these were on paper with the Whatman watermark dated 1794. There is no date on any of the watercolours, but there is a sheet of paper amongst the collection with meteorological data on it headed “*Monthly account of the fall of rain at Macao and Canton in China, from September 1807 to July 1809*”. The handwriting on this sheet closely resembles that of William Kerr (Joseph Richard pers. comm.). Two watercolours represent *Lilium brownii*. The first NDH52/14 has an inflorescence with a single open white flower without showing signs of the reddish colouration on the outside. The lanceolate leaves are bright green and there are individual dissections of the six brown stamens, the ovary with style and stigma attached and the six perianth segments. There is also depicted a squat white bulb and an individual white bulb scale. On the bottom right, in ink in Chinese characters is written “pae hup fa” 百合花 (also written on the reverse in English). In pencil is written “*Lilium japonicum*” and bottom left in ink “W.Ch”. The origin and purpose of these initials remains a mystery, but might refer to the Chinese name of the artist. The same initials were placed on 152 of the other paintings in the collection.



Figure 2. *Lilium brownii* Illustration number 94 (as *Lilium longiflorum*) of William Kerr's drawings for the East India Company conserved at the Royal Botanic Gardens Kew mentioned by Samuel Brookes in *Horticultural Transactions* vol. 4: 553 (1822).

The second illustration NDH56/25 also has a single inflorescence with one open white flower. This too has the six brown stamens, ovary, style and stigma and six white tepals showing a greenish tinge to the nectaries within. The leaves are shorter and more oblanceolate. There is no name written in pencil in English, but 百合 [“pa hup”] is written in ink in Chinese characters and again in English in pencil on the reverse. At the bottom left, it has the abbreviation “H.Sh.” written in ink. The significance of this is also unknown, but might again refer to the Chinese artist. These initials were placed on 129 of the other paintings in the collection. These two illustrations bear a number of similarities with the Kerr painting at Kew, in particular with respect to the execution of their anatomical dissections. They too must have been undertaken in Macao during the summer months when the plants were in flower.

In his letter on *Lilium japonicum*, Samuel Brookes stated that a painting of the lily had been prepared by Barbara Cotton in 1820 from the five plants that had flowered from his own consignment and that the painting had been given by him to the Horticultural Society (Brookes 1822: 551). The Society's Drawing Committee's Minutes from 1815–1824 included the information that Barbara Cotton (1794–1829), who from 1823 became Mrs Lawrence, had been commissioned to paint a series of paintings of *Lilium* from 1822 onwards. Perhaps, this series of paintings was inspired by the one given to the Society by Brookes? The painting of “*L. japonicum*”, being part of the Miscellaneous Drawings collection, was sold by the Society in 1859 (Charlotte Brooks, pers. comm.).

The spread of “*Lilium japonicum*”

In 1822, the current *L. brownii* was once again mentioned under the name *L. japonicum* as having first arrived in England from China in 1804 by Stephen Reynolds Clarke, although he does not mention from which introduction the description of his plants originated (Clarke 1822: 332). In November 1820, the Horticultural Society sent John Potts from the Society’s Kensington garden to Canton where he met John Reeves and, after a year collecting plants under Reeves’s aegis, he returned in August 1822 having sent back shipments of plants (Elliott 2004: 198). Unfortunately, Potts died shortly after his return, but the Society, undaunted by his death, sent John Damper Parks, this time from the newly-leased garden in Chiswick, out to Canton in April 1823 on the HEICS “Lowther Castle” (Elliott 2004: 200). Parks returned in May 1824 having made contact with John Reeves and having also sent back plants for the Society’s Chiswick garden. Neither the Society’s “Transactions” nor the Council Minute books refer to any lilies having been collected either by John Potts or by John Damper Parks.

The additional introductions of *Lilium brownii* brought back by Reeves and Brookes, however, soon led to the species becoming widely dispersed. The bulbs crossed the Atlantic to North America where, by 1822, William Prince’s Linnaean Botanic Garden nursery at Flushing, Long Island, New York listed on p. 30: “18. Japan white - *Lilium japonicum* for \$3. 25 cents each.

Meanwhile, in France, according to the French physician and botanist Jean-Louis-Auguste Loiseleur-Deslongchamps, the Chinese species (as *L. japonicum*) was in cultivation in the gardens of Monsieur Cels and Monsieur Boursault and had once again been painted (Loiseleur-Deslongchamps 1822: t. 375). Loiseleur-Deslongchamps mentioned that, although the species had been introduced to England in 1804, it had only recently arrived in France and had flowered for the first time on 10 July 1821. The first of the gardens he mentioned belonged to François Cels (1771–1832), the son of the famous nurseryman Jacques-Philippe-Martin Cels of Petit Montrouge which was then a village just south of Paris. Cels’s garden comprised some 18 acres full of rare plants. The second referred to the garden of the actor, theatre director and revolutionary Jean-François Boursault-Malherbe (1750–1842), whose country house at Yerres, Villeneuve-Saint-Georges, then a small village south-east of Paris, was equally renowned for the rare plants within it and especially for its roses. Both grew the Chinese lily under the name Lis du Japon or *L. japonicum* as depicted in the fine coloured illustration by Pancrace Bessa (Loiseleur-Deslongchamps 1822: t. 375).

A year later, the French botanist Jean Poiret was clearly referring to the Chinese lily under the name *Lilium japonicum* (Poiret 1823: 21). He stated that the flowers of this lily were larger than others that he had encountered and referred to the exterior of the flower as having a reddish flush. He also reiterated the occasion of its flowering for the first time in 1821 in the gardens of Messieurs Cels and Boursault.

Ten years later, evidence of the success of this lily in cultivation was again illuminated by the beautiful illustration of it as *L. japonicum* by Priscilla Susan Bury (Bury 1831: t. 2). Mrs Bury stated that the plants from which her painting was made had been growing in the Liverpool Botanic Garden for several years. This would have been

the garden of the polymath and abolitionist William Roscoe, founded in 1802 near Mount Pleasant, Liverpool, but which is now sadly lost to housing east of Abercromby Square. She also cited Samuel Brookes' apparent success with the lily by mentioning that his plants had produced three flowers per stem instead of what had previously been reported to be just one. The Liverpool plants she added, were clearly suited to their habitat and were stated to produce five or six flowers per stem. Her mention of Brookes implies that the lilies which he had introduced in 1819 might be the same as those that were still in cultivation in Liverpool by 1830.

The naming of *Lilium brownii*

The Chinese lily was finally recognized as distinct from *L. japonicum* and was named *Lilium brownii* in 1841 (Lemoinier 1841: 7). This was not, however, in the catalogue to an exhibition in Lille, which has been regularly cited to have been its first place of publication (Fig. 3). All reference works which are consistently cited in botanical and horticultural literature refer to the nurseryman F. E. Brown of Slough as being the source of the plant from which the name of the species was derived (e.g. Spae 1845: 138; Spae 1847: 12; Duchartre 1870: 347; Wilson 1925: 28; Woodcock and Stearn 1950: 161; Syngé 1980: 161). Also referred to by these writers as the author of the name is the French nurseryman Auguste-Joseph Miellez (1809–1860) of Esquermes-les-Lille, son of Louis Xavier Joseph Miellez (1777–1849), a founder of the Société National d'Horticulture de France in 1825. In neither case, the authority and place of publication is correct. It is correct, however, that the Chinese lily was first included as number 102 in the list of flowers exhibited by the Miellez nursery under the name *Lilium brownii* in the Société d'Horticulture de Lille (Nord) - 13th Exposition Juin 20, 21 and 22, 1841 (Fig. 3). Miellez's exhibit, with "(1841)" next to it, signified that 1841 was the first year in which he exhibited that plant, as he had for all other newly exhibited plants. It was entered in the Summer Exhibition in the Bourse [Stock Exchange] in Lille but, as the name lacked any accompanying description in the catalogue, it is a nomen nudum (Art. 38.1 ICN, Turland et al 2018). It was therefore not validly published in that catalogue in spite of the belief by many subsequent writers that it had been (e.g. by Spae 1845: 138; Spae 1847: 12; Duchartre 1870: 347; Wilson 1925: 28; Woodcock and Stearn 1950: 161; Syngé 1980: 161). The significance of the date "1841" in this exhibition catalogue means that the reference by the writers cited above to Miellez's catalogue will have been to the catalogue of Miellez's plants which were exhibited for the first time at the exhibition in Lille, not to the publication of the name in any nursery catalogue produced by Miellez. Moreover, there do not appear to be any extant Miellez nursery catalogues of that period despite a comprehensive search for them.

Six years later, Charles Morren, editor of "Annales de la Société royale d'agriculture et de botanique de Gand", reviewed the work of Dieudonné Spae praising his colleague (Spae 1845: 438, t. 41) for his full and accurate description of *Lilium brownii* (Morren 1847: 309). Morren went on to include a highly

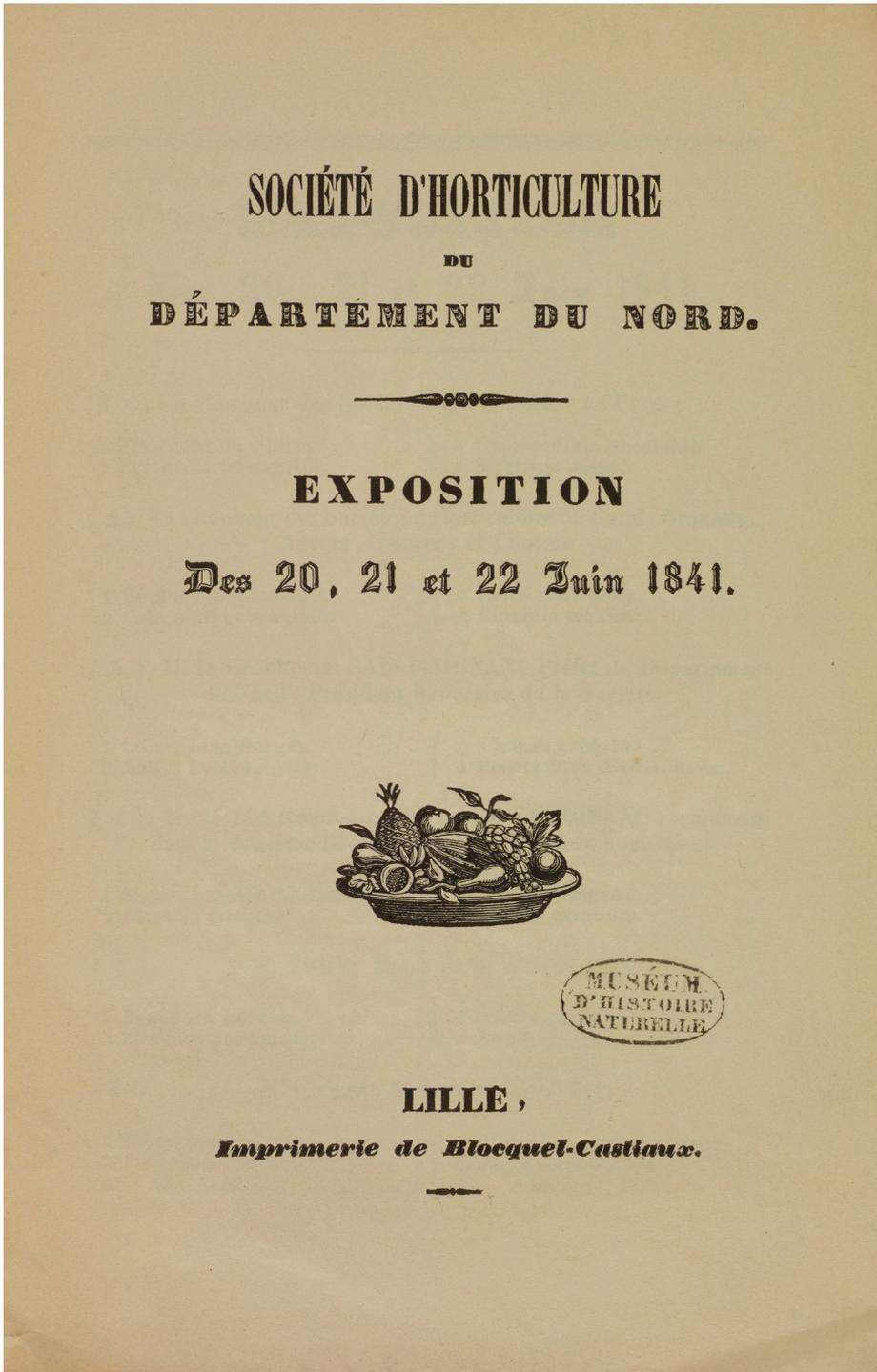


Figure 3. The frequently cited first place of listing of the name *Lilium brownii* as a nomen nudum in the Société d'Horticulture de Lille (Nord) - 13th Exposition Juin 20, 21 and 22, 1841.

critical note concerning the taking up of names published without any adequate description and in an unscientific manner. Morren made this point entirely with reference to the inadequate naming of plants in catalogues, such as in the one for the Lille Summer Exhibition in which *Lilium brownii* was merely listed (Morren 1847: 309). According to current rules of the ICN, however, Spae (1845) was beaten to it by an earlier description (Art. 11.3).

The first valid description of *L. brownii* was published four years before Spae's and was in the report of the Summer Exhibition in Lille in the first cahier (issue number 1) of the "Annales de la Société d'Horticulture du Département du Nord (Lille)" 13: 7 (1841) (Fig. 4). The description (translated from the French) is:

"Mr. Miellez has received recently the" *Lilium brownii*, majestic liliaceae, newly imported from Japan, whose stem was surmounted by two vast calyces placed horizontally, washed with brown on the outside and traversed longitudinally on this face by a brown stripe in the centre of each petal, the interior with a white background from which protrude large brown anthers; this magnificent plant exhaled, like most lilies, a very sweet scent."

That report, however, was unsigned, but according to the "Annuaire statistique du Département du Nord 14th Année" -1842 (Demeunynck and Devaux 1842: 379), the secrétaire-adjoint, who would also have been the editor [rédacteur] of the "Annales de la Société d'horticulture du Département du Nord" in 1841, was Auguste Lemoinier. The correct authorship and place of publication of this name is, therefore, *Lilium brownii* A.Lemoinier, Ann. Soc. Hort. Dép. N. 13: 7 (1841). Lemoinier was cited as secrétaire-adjoint for the following year in the "Annuaire statistique du Département du Nord 14th Année" -1842 (Lemoinier 1842: 379).

The name *Lilium brownii* becomes established

As the appeal of *Lilium brownii* spread across the Continent of Europe, it was inevitable that celebrated writers on all matters horticultural proceeded to describe and/or illustrate this highly ornamental species (e.g. Poiteau 1844: 496; Spae 1845: 438, tab. 41; Lemaire 1845: 257 + tab.; Van Houtte 1845: 22; Spae 1847: 12; Lemaire 1848: 74 + tab.; Duchartre 1870: 342).

Shortly after its first appearance under the unpublished name *L. brownii* by Miellez on the exhibition table in Lille, the lily was exhibited two years later as *L. brownii*. There was no description and it was listed as number 2569 by the nurseryman Jean van Geert of Gand [Ghent], in Belgium. Van Geert exhibited it in the Catalogue de l'Exposition de la Société Royale d'Agriculture et de botanique de Gand (Anon 1843: 42). In France, the botanist Pierre-Antoine Poiteau also recognised that a name change for the lily was required from the continued use of *L. japonicum* to *L. brownii*, publishing this proposal in the fifth volume of the influential *Revue Horticole* (Poiteau 1843: 406). The following year, the liliophile Belgian botanist Dieudonné Spae exhibited the lily under

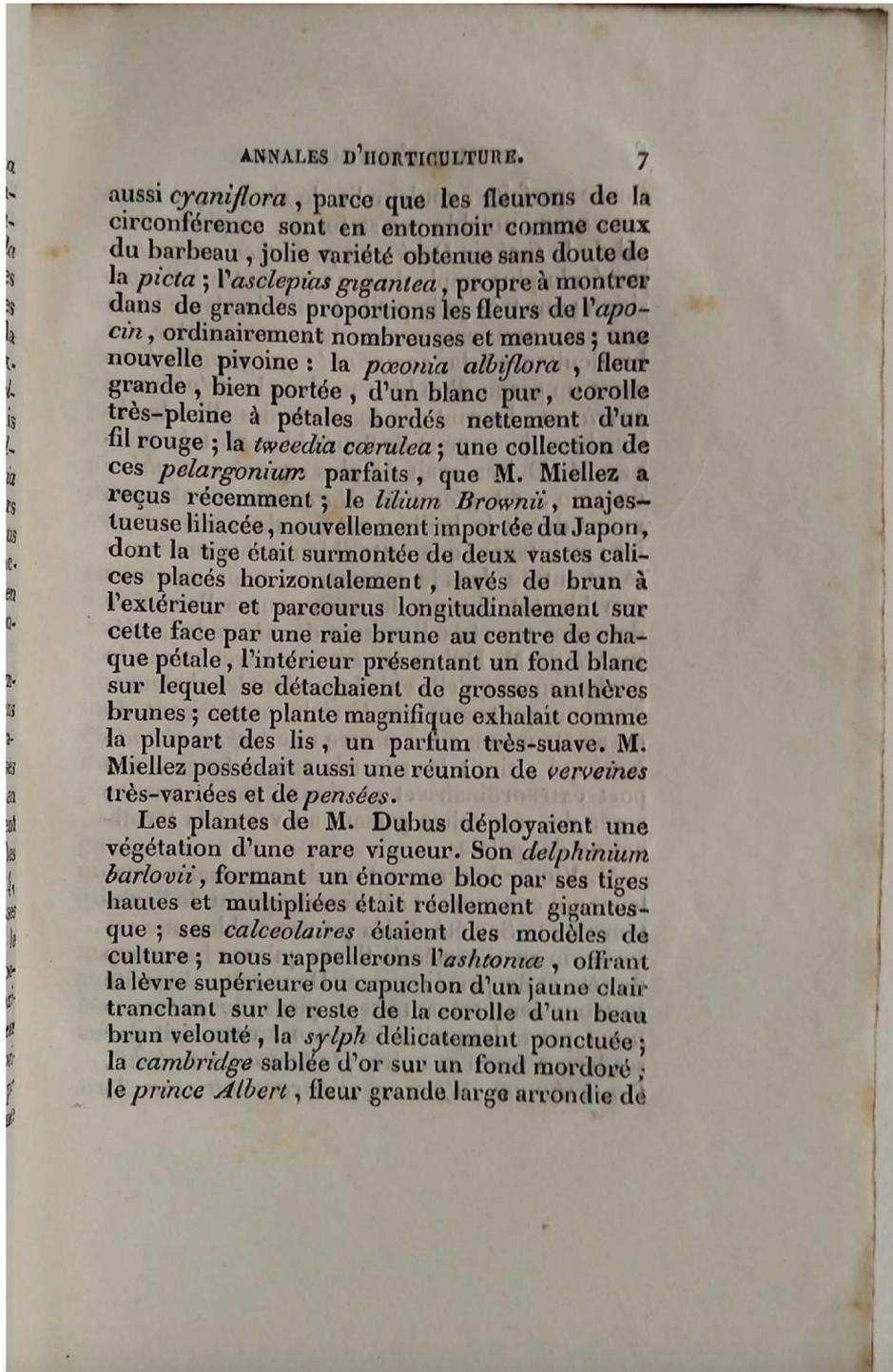


Figure 4. The first validly published description of *Lilium brownii* A.Lemoinier in *Annales de la Société d'Horticulture du Département du Nord* (Lille) 13: 7 (1841).

the name *L. brownii* with “(*L. japonicum*)” as its synonym, again without description, under exhibit number 1244 in the 76th Exposition de la Société Royale d’Agriculture et de Botanique de Gand in March 1844 (Anon 1844: 25). Three years later, another mention of the lily as *L. brownii* was by Pierre Denis Pépin who stated that the nurseryman Louis Thibault had sent to the Société d’horticulture a superb plant of this species with its white tubular flowers washed with purple on the outside (Pépin 1847: 345). Louis Thibault had only just formed a partnership with Jean-Baptiste Keteleer at Sceaux near Paris in order to grow many rare and exotic plants. The lily was by then, well established in cultivation and at long last was becoming recognised under the correct name.

Lilium brownii was eventually included by Henry John Elwes in his superb Monograph on the genus *Lilium*, accompanied by a beautiful illustration by Walter Hood Fitch (Elwes 1877: t. 8).

The Brown nursery of slough

Few accurate records exist of the Nursery known as Browns of Slough in the 18th and early 19th centuries because all documents relating to them were destroyed in a catastrophic fire in Thomas Brown’s house in 1840 (Dean 1885: 264). Census records, births, marriages and deaths in the National and Parish registers, wills in the National Archives and numerous articles in the horticultural and local press have helped to fill in some of the missing data presented here.

Thomas Brown (1748–1814) founded a nursery at Upton-cum Chalvey in 1774 on the fertile and well-drained soil of the Thames Valley alongside the Great West Road from London to Bristol (Fraser Maxwell 1973: 100). The nursery was just to the east of the small village of Slough, then in the county of Buckinghamshire and was a major exhibitor of plants to the Salt Hill Floral Society established in Slough in 1783. His son Thomas Harper Brown (1777–1817) married Elizabeth Penny (1780–1833) and, together, Thomas father and son and the son’s wife Elizabeth ran the nursery. Thomas Harper Brown and Elizabeth had several children of whom the oldest were Thomas (b. 1804), Edward, (b. 1805) and John (b. 1807).

Thomas Harper Brown died in 1817. According to the terms of his will, the nursery was to be left in the hands of his cousin Charles Brown (1796–1836) of Alpha Cottage, Slough in a partnership with Thomas’s widow Elizabeth. The partnership between Charles and Elizabeth was to remain in place until Elizabeth’s sons reached the age of 21. In 1833, Elizabeth Brown died leaving the nursery in the hands of Charles Brown who was joined in 1834 by his young cousin Thomas upon his reaching 21 and the following year by Thomas’s younger brother Edward (the youngest brother John having died in 1824). In 1836, Charles Brown died aged just 40 leaving the brothers Thomas and Edward as partners in the nursery business of Messrs Brown of Slough.

Charles Brown became a leading light in the nursery world, specialising in breeding and exhibiting dahlias, roses, heartsease and tulips. He was elected a member of the prestigious Horticultural Society of London on 6 July 1819 (Helen Winning

pers. comm.). In 1833, Charles Brown was awarded two Banksian medals from the Horticultural Society for his exhibits of heartsease and tulips (Bentham 1835: 534). Charles and his cousin's wife Elizabeth also exhibited as E & C Brown of Slough at the local Salt Hill Society's flower show and, encouraged by the florist George Glenny, also exhibited for the Metropolitan Society of Florists and Amateurs whose Patroness was Queen Adelaide. The latter was founded in 1832 as a rival to the Horticultural Society of London (Elliott 2001: 172). Charles Brown married Sarah Botham and the Brown nursery floral exhibits were regularly included in the Metropolitan Society shows held in the garden of Botham's Hotel, Salt Hill, Slough. This Hotel was one of the grandest along the fashionable Great West Road from London to Bath (Glenny 1834: 28; Fraser Maxwell 1973: 72).

After Charles's death in 1836 (Anon 1836: 5), Thomas and Edward Brown maintained a partnership at Slough and Salt Hill as Messrs Brown of Slough. The brothers also maintained a seed shop in the Egyptian Hall, Piccadilly, London until 1841 (Brown and Brown 1841: 762). In March 1842, Edward Brown announced that he was leaving the business and dissolving the partnership. There was a subsequent sale of the stock within the 14 acres of the Hencroft Nursery which belonged to Edward (Anon 1842: 138). Thomas Brown was, thenceforth, the sole owner (Brown 1842: 601) and was still listed under the category of nursery and seedsmen in Pigot's Directory (Slater 1844: 50). Edward Brown, meanwhile, seems to have capitalised on his property assets. No doubt as a result of his nursery credentials, he was chosen to be the secretary of a Testimonial fund in 1864 to Mr. Thomas Ingram, head gardener at the Royal Gardens, Frogmore (Brown 1864: 1129).

Thomas Brown was elected a Fellow of the Horticultural Society on 20 September 1836 (Helen Winning pers. comm.) and, less than a month later, he married Mary Ann Rhodes on 15 October 1836. Thomas Brown exhibited plants at the Horticultural Society's shows from 1838 to 1844 (e.g. Loudon 1838: 543–544; Brown 1843: 641; Anon 1844: 375). There is, however, an advert signalling the end of Thomas Brown's tenure at Slough due to his failing health which stated that he was submitting for sale his magnificent tulip collection at the Slough Nursery through his agent Mr George Glenny at the Gardener's Gazette Office (Glenny 1845: 282). He sold the contents of his house and advertised the contents of his nursery for sale in July 1845. It seems that Thomas Brown then leased the nursery to a partnership of William George Cutter and George Shanklie in 1845 which was then dissolved in 1848. Thomas Brown then sold the nursery which was, by then, known as the Royal Nursery Slough to his former foreman Charles Turner (1816–1885), in December 1848. Thomas emigrated with his wife Mary Ann and his three sons and a daughter to Hawaii where he served as Recorder of Deeds and died in Honolulu in 1886 (Robinson 1886: 598). Turner maintained the nursery into the mid- and later 19th century (Anon 1885: 12).

There clearly was never any F. E. Brown who was associated with this nursery and any attribution to a Mr F. E. Brown of Slough in relation to *Lilium brownii* is an error. It is probable that the original source of this error was Dieudonné Spae who wrote "*il a fleuri pour la première fois chez MM. F. E. Brown, à Slough près de Windsor*" (Spae 1845:

138). It is also clear from the many attributions to “Messrs Brown of Slough, Charles Brown of Slough and Thomas and Edward Brown of Slough” that these were, without doubt, the nurserymen for which the species was named. How then did *Lilium brownii* arrive at this nursery? Charles Brown was a fellow judge with Donald Munro, the head of the ornamental section of the Horticultural Society of London’s Chiswick garden when floral exhibits were held at their horticultural shows (Anon 1832: 4). There is a coincidence that occurred at a show in the Horticultural Society’s Hall in Regents Street, London on 2 July 1833. At that show, Charles Brown exhibited a large bunch of one of his roses ‘Brown’s Superb’ and a huge collection of 120 of his heartsease (*Viola* hybrids), while, in the same show, *Lilium japonicum* was also exhibited by Donald Munro on behalf of his employer, the Horticultural Society (Loudon 1833: 508). As there was no description of the exhibit, one can only assume that this was the Chinese lily and not the Japanese *L. japonicum*. It is not beyond the realms of possibility that Charles Brown could have acquired the lily from his colleague. *Lilium japonicum* was again exhibited the following year on 5 July 1834 in the Society’s Chiswick garden (Paxton 1834: 381).

Alternatively, perhaps it may have been acquired during the first year of the partnership of brothers Thomas and Edward Brown, following the death of Charles Brown in 1836? Thomas had been elected a Fellow of the Horticultural Society in 1836, so when *Lilium japonicum* was once again exhibited by Donald Munro for the Horticultural Society on 18 July 1837, did Thomas acquire the lily then (Loudon 1837: 478)? Thomas Brown was certainly known to have exhibited several species of *Lilium* at the Chiswick Horticultural Show on 4 July 1840, although neither the name *L. japonicum* nor any other lilies names were specifically mentioned (Marnock 1841: 60).

The French connection – Pépinières Miellez

It was widely reported that *Lilium brownii* was introduced to England circa 1835 or 1836, where it was acquired by Messrs Brown of Slough near Windsor (Spae 1845: 438; Spae 1847: 12; Duchartre 1870: 342; Van Eeden 1876: t. 63). This acquisition by Messrs Brown is unlikely to have been from an unverified later introduction of the species from Canton. It has been suggested that it was Thomas Brown who was responsible for the importation of the bulbs directly from China (Fraser Maxwell 1973: 99), but this is highly unlikely due to the stringent regulations imposed on foreign regimes by the Chinese at that time as have already been explained above.

In the Horticultural Society of London’s Council Minutes, dated 2 July 1830, there is the statement: “Ordered that Mr. Reeves be written to, to discontinue the importations and drawings now forwarded by him to the Society”. By 1831, the Society was in great financial difficulties and keen to save money in whichever way possible. One small way for them to do this was to stop the expense on the importation of plants and drawings. John Reeves left Canton to finally return to England in 1831, which coincidentally was only two years before the EIC lost its monopoly in China through the Charter Act

1833. John Reeves had been joined in Canton in 1824 by his son John Russell Reeves (1804–1877), who remained as the last EIC Tea Inspector in Canton until 1838 and was known to have sent some plants back to England. There is no evidence to suggest that *Lilium brownii* was amongst them, but that possibility cannot be ruled out.

According to the reports mentioned by Spae (1845) and van Eeden (1876), three bulbs of the lily were acquired in 1837 from Brown of Slough by Monsieur Auguste Miellez at Esquermes for his nursery which was, at that time, in a district just to the southwest of Lille in northeast France. Reports suggested that M. Miellez had imported them into Belgium the following year [1838?] under the name *L. brownii*, so named by him in honour of those who first flowered the species (Van Eeden 1876: t. 63). Thence, it seems the lily was communicated to Herman Shuurmans-Stekhoven (1757–1839), the head gardener of the Leiden Botanic Garden in The Netherlands (Spae 1847: 12).

The question then arises as to how Auguste Joseph Miellez pépinière [nurseryman] of Lille actually acquired the bulbs and how there may have been a link with the Slough nursery? Owing to the absence of reliable records following the disastrous fire in Thomas Brown's house in 1840, any suggestions as to how the lily bulbs might have crossed the Channel must be pure speculation. The nursery of Louis Xavier Joseph Miellez and his son Auguste Miellez was famous for the breeding and cultivation of roses. Charles Brown of Slough was also a well-respected breeder of roses as mentioned by John Claudius Loudon (Loudon 1831: 66) and by the rosarian and nurseryman Thomas Rivers of Sawbridgeworth, Hertfordshire (Rivers 1838: 17). Thomas Brown followed in his cousin Charles's footsteps after Charles's death in 1836, specialising in the breeding and exhibition of dahlias, but he also bred roses, heartsease, tulips and pinks.

It is possible that Auguste Miellez had heard of the lily via his nursery colleagues and had simply asked for them to be sent to him or, alternatively, he may have made a visit across the Channel on a 400 mile (ca, 640 km) return journey to Slough in search of new plants for his nursery. It is also possible that Charles Brown may, perhaps, have gone the other way offering the three bulbs and one of his roses in exchange for one of Miellez's fabulous French roses. That journey either way may have also taken place after Charles Brown's death in 1836 and during the tenure of the brothers ("les frères T & E") Thomas and Edward Brown. Any further evidence, if it still exists and comes to light, may fill in this small piece of the puzzle.

***Lilium odorum* Planch.**

A further complication arose with the history and description of the Canton lily under yet another species name *Lilium odorum* Planch. (Planchon 1854: 53 t. 876). The plant described by the French botanist Jules Emile Planchon under the name *L. odorum* has lanceolate leaves and white flowers stained with deep red externally and with deep red along the mid-ribs. There is no doubt this is *L. brownii*.

Planchon stated that two different species were known under the name *L. japonicum*, one was *L. japonicum* of Thunberg and the other was *L. brownii* Hort. which, at first

sight according to Planchon, was very similar to *L. odorum* (Planchon 1854: 53). He had examined a sheet of *L. japonicum* collected by Thunberg in Japan and which was conserved in the [Jules Paul] Benjamin Delessert Herbarium in Geneva. He noticed that the leaves on the Thunberg specimen were distinctly petiolate and concluded that the plant introduced by Captain Kirkpatrick of the East India Company and which was subsequently described by various authors under the name *L. japonicum* was not Thunberg's plant. He believed the latter, which was also figured in Loddiges Botanical Cabinet with a plate as *L. japonicum* (Loddiges 1820: t. 438), was identical with his *L. odorum*. Having made that statement, he added a short footnote "*Cette plante serait-elle le Lilium brownii? Mais les antheres plus courtes semblent la rapprocher davantage de notre L. odorum.*" [could this plant be *Lilium brownii*? The shorter anthers seem to bring it closer to our *L. odorum*]. Planchon added that *L. odorum* can be distinguished [from *L. brownii*] by the narrower leaves, less strongly scented flowers and the longer anthers (Planchon 1854: 53). The accompanying illustration of *L. odorum* by Louis Stroobant, painted from a specimen growing in Louis van Houtte's nursery, also includes as a synonym *L. japonicum* Lodd. (non Thunb.). The morphological distinctions described by Planchon, however, all fall within the range of *L. brownii* and no mention is made of the origin of the plant he described and had figured, although "Japan - châssis froid" [cold frame] is written on the illustration.

Eduard Regel in Zurich very quickly picked up on Planchon's new species name. In July that year, under the heading Neue Zierpflanzen [new ornamental plants], he stated that *L. japonicum* with its petiolate leaves is unlikely to still be in cultivation. He reiterated Planchon's point that *L. odorum* is the plant depicted in Loddiges Botanical Cabinet under the name *L. japonicum* (Loddiges 1820: t. 438) and that *L. brownii* is closely related, but the flower is comparatively odourless (Regel 1854: 234–235).

Taxa related to *Lilium brownii*

Taxonomists in the past have found difficulty in diagnosing the morphological differences between those species of *Lilium* with infundibuliform or funnel-shaped flowers (Duchartre 1870; Baker 1875; Elwes 1877; Franchet 1892; Cavalerie 1911). These scholars used such characters as leaf shape and length, perianth shape and colouring and glabrous or pubescent nectaries, filaments and style bases in order to delimit the taxa and found them to be variable and, therefore, inconsistent.

Several molecular DNA-based studies using both plastid and nuclear markers have helped resolve some of the relationships amongst these species (Nishikawa et al. 2001; Lee et al. 2011; Du et al. 2014; Gao et al. 2015; Huang et al. 2018; Givnish et al. 2020). These molecular studies have shown that the Asian species of *Lilium* with trumpet-shaped flowers belong in two clades: One comprises *Lilium brownii*, *L. formosanum* A.Wallace, *L. longiflorum* Thunb., *L. neilgherrense* Wight, *L. philippinense* Baker and *L. wallichianum* Schult. & Schult.f. These species all have bulbs with either white, ivory or yellow coloured bulb scales, which, on exposure to air, exhibit a pinkish or light brownish colour. In addition, the inner basal section of the corolla in all these species

is greenish-white or ivory-white, not yellow. Two more recently described Chinese species also share many of the same characters as *L. brownii* with white or pale yellow bulb scales. These are *L. anhuiense* D.C.Zhang & J.Z.Shao, Acta Phytotax. Sin. 29(5): 475 (1991) and *L. wenshanense* L.J.Peng & F.X.Li, Acta Bot. Yunnan., Suppl. 3: 33 (1990). *Lilium anhuiense* was distinguished from *L. brownii* by the foliar bracts at the apex of the inflorescence axis being curved as opposed to straight and by the style bases being pubescent as opposed to glabrous. These are, however, variable characters across the range of the species. *Lilium wenshanense* was distinguished from *L. brownii* by the bulbs having segmented rather than entire scales. This character too has been found to be inconsistent (Gao and Gao 2014: 102). These two species have also been shown to belong on the same clade as the other species with predominantly white or yellow bulb scales (Huang et al. 2018) and are, therefore, placed here into the synonymy of *L. brownii*.

The second clade comprises those species with pink to dark reddish-purple, sometimes almost blackish bulb scales when fresh and have corollas that are richly yellow within. These have been placed in *Lilium* sect. *Regalia* Baranova, *Novosti Sist. Vyssh. Rast.* 8: 94 (1971): *L. leucanthum* (Baker) Baker, *L. sulphureum* Baker ex Hook.f., *L. sargentiae* E.H.Wilson, *L. regalia* E.H.Wilson and *L. centifolium* Stapf.

Typification of *Lilium brownii*

At no stage in its botanical history has a type been allocated to the species. The liliophile Kew botanist John Gilbert Baker segregated *L. brownii* var. *viridulum* from (by implication) var. *brownii* on the shorter, wider, more oblanceolate leaves and paler greenish colouration on the outside of the corollas with less pronounced claret markings (Baker 1885: 131). Baker's statement "The leaves are much broader and shorter than in the type" is almost certainly intended to refer to what he regarded as the typical variety of *L. brownii*. This point is strengthened by his citation of (Mielle) [i.e. Mieliez] as the author of the name and was accompanied by a reference to the description and illustration in *Flore des Serres* by Charles Lemaire. The latter portrays a plant with linear-lanceolate leaves and a flower with reddish markings on the outside of the perianth (Lemaire 1845: t. 47). These references, however, do not constitute typification of the species. William Stearn regarded what he called *L. brownii* var. *brownii* as being based on *L. japonicum* var. *brownii* (Spae) Baker (Baker 1871: 709); however, he again did not indicate any type specimen or illustration (Stearn 1948: 5).

The neotype chosen here for the name *Lilium brownii* is a collection by Pierre Julien Cavalerie from Guizhou Province, China https://data.rbge.org.uk/search/herbarium/?specimen_num=956330&cfg=zoom.cfg&filename=E00934044.zip. Cavalerie was clearly confused as he described *L. brownii* var. *brownii* as a variety of *L. longiflorum*. He compared it to what he had already just referred to as *L. brownii*, but which, according to his description, "la tige bulbifere chez les jeunes sujets qui n'ont pas des fleurs" was in fact *Lilium sulphureum* Baker ex Hook.f.. His description of what he referred to as *Lilium longiflorum* but was described by Lévêille as *L. longiflorum* var. *purpureoviolaceum* (i.e. *L. brownii* var. *brownii*) included the statement "La fleur

un peu plus petite, plus ouverte, à divisions plus minces est intérieurement blanche et extérieurement d'un violet très variable bien que le blanc domine. Ce lis fleuret deux mois plus tôt que le *L. brownii*; il est commun au sud de Pin-Fa" [The somewhat smaller, more open flowers with narrower divisions is white internally and with very variable purple markings outside on a white background. This lily flowers two months earlier than *L. brownii*; it is common near Pin-Fa] (Cavalerie 1911: 245).

The neotype is based on one of two Cavalerie collections at E from this locality described by his friend Augustin Abel Hector Léveillé under the name *Lilium longiflorum* var. *purpureoviolaceum* H.Lév. in 1909. This sheet fits well with the protologue of the name. Lemoinier's mention of the large white flowers washed with brown externally and with a dark brown stripe along the mid-rib equate to the dark purplish colouring of the variety published by Hector Léveillé (Léveillé 1909: 264). In fact, the colouring lies somewhere between brown and purple. The lanceolate leaves which barely shorten up the inflorescence axis clearly refer to *L. brownii* var. *brownii* and not to the oblanceolate leaves that quickly shorten to obovate as they extend up the axis in *L. brownii* var. *viridulum* Baker.

There is a useful representative illustration of *L. brownii* var. *brownii* (Fig. 1) in Curtis's Botanical Magazine (Ker-Gawler 1813: t. 1591). The plant in the illustration exhibits leaves which are lanceolate and remain more or less consistent in length up the inflorescence axis and the corollas have a pronounced dark purplish flush on the three outer perianth segments. *Lilium brownii* var. *chloraster* (Baker) Baker has greenish corollas and lanceolate leaves, whereas *L. brownii* var. *viridulum* Baker, although having a distinct brownish-red flush on the outside of the corolla, has oblong-lanceolate leaves that decrease markedly to obovate in length up the inflorescence axis.

Note: The article in Gardeners' Chronicle, in which the names *Lilium aduncum*, *L. australe*, *L. brownii* var. *ferum* and *L. brownii* var. *primarium* were first published, was written by Elwes (Elwes 1921: 100–101). However, the key to the taxa, associated with *L. brownii* as well as the five additional adnotations, were quoted directly from a letter written to Elwes by Otto Stapf in Kew. According to Elwes, this letter was sent to him following his request for clarification on the status of one of Kew's specimens. The key and adnotes by Stapf are clearly indicated by the enclosing quotation marks within Elwes's paper. Stapf is, therefore, the author of these names within this article. As there are no specimens at K annotated by Stapf attributed to the name *Lilium brownii* var. *brownii* and he did not include that varietal name in his key, *L. brownii* var. *primarium* Stapf is considered to refer to typical *L. brownii* var. *brownii*. Moreover, with respect to a choice of type material, Stapf did not include the term "typus" or its equivalent (Art. 7.11). Gao and Gao (2014: 102) attempted to neotypify *L. brownii* on a Cipriano Silvestri specimen from Hubei *Silvestri* 199, July 1904 conserved in FI; however, they did not cite "designated here" or "hic designatus" and, therefore, being published after 2000, this putative typification also does not satisfy the requirements of Art. 7.11, hence the need for the neotype designation in this paper.

Further invalid names or later homonyms are:

- *Lilium brownii* Miellez, Cat. Exposition 20–22 Juin Société d'horticulture de Lille: 9. (1841) nom. nud.
- *Lilium brownii* Poit. & A.Vilm., Rev. Hort. Ser. 2(2) vol. 5: 495 (1844)

- *Lilium brownii* Spae, Ann. Soc. Roy. Agric. Gand 1: 437 (1845)
- *Lilium japonicum* var. *brownii* Siebold, Catalogue 1870–1871: 51 nom. nud.
- *Lilium japonicum* var. *colchesteri* Van Houtte, Fl. Serres Jard. Eur. 21: 73 (1875) nom. nud.

***Lilium brownii* A.Lemoinier, Ann. Soc. Hort. Dép. N. 13: 7 (1841).**

Neotype designated here. China, Guizhou Province, Pin-fa, 26 June 1907, *P.J. Cavalerie* s.n. (neo. E!) [E-00934044]. Note: this is also the holotype of *Lilium longiflorum* var. *purpureoviolaceum* H.Lév. See also above under typification. https://data.rbge.org.uk/search/herbarium/?specimen_num=956330&cfg=zoom.cfg&filename=E00934044.zip

Key to the varieties of *Lilium brownii*

- 1 Leaves linear to lanceolate, reducing only slightly in length towards the apex of the inflorescence axis.....**2**
- Leaves oblanceolate to obovate, reducing markedly in length towards the apex of the inflorescence axis; corollas tinged externally with only a faint dash of claret-brown on outer tepals***Lilium brownii* var. *viridulum***
- 2 Corollas ivory white tinged externally with claret-brown with a pronounced dark streak along the mid-ribs of each outer tepal
..... ***Lilium brownii* var. *brownii***
- Corollas ivory white tinged greenish externally especially along tepal mid-ribs ***Lilium brownii* var. *chloraster***

Lilium brownii* var. *brownii

- ≡ *Lilium japonicum* var. *brownii* (A.Lemoinier) Baker (as *L. japonicum* var. *brownii*), Gard. Chron. 1871(1): 709 (1871).
- ≡ *Lilium brownii* var. *primarium* Stapf in Elwes, Gard. Chron., ser. 3, 70: 101 (1921)
– See Note above under typification.
- = *Lilium odorum* Planch., Fl. Serres Jard. Eur. 9: 53 (1853–1854) Lectotype designated here [Icon]: t. 876 Fl. Serres Jard. Eur. 9 (1853–1854)
- ≡ *Lilium brownii* var. *odorum* (Planch.) W.Watson, The Garden 47: 97, (1895).
- = *Lilium longiflorum* var. *purpureoviolaceum* H.Lév., Repert. Spec. Nov. Regni Veg. 6: 264 (1909). Holotype: China, Guizhou, Pin-fa, 26 June 1907, *P.J. Cavalerie* s.n. (holo. E!) [E-00934044]; paratype: China, Guizhou, Pin-fa, 13 Feb 1902, *P.J. Cavalerie* 448, (para. K!).
- = *Lilium australe* Stapf in Elwes, Gard. Chron., ser. 3, 70: 101 (1921). Lectotype designated here from syntypes: China, Hong Kong, (as *Lilium longiflorum*) 1847, *Captain Champion* 23 (lecto. K!) [K-000464652]; isolectotype: China, Hong Kong, (as *Lilium longiflorum*) sheet labelled 23 (isolecto. K!) [K-000464653];

isolectotype: China, Hong Kong (as *Lilium longiflorum*) without collector, but with number “23”, without locality or date (isolecto. K!) [K-000464655] !); syntypes: China, Hong Kong, (as *Lilium longiflorum*) top of ridge, 28 June 1859 “Colonel Urquhart”, sheet labelled 200 (syn. K!) [K-000464654

- ≡ *Lilium brownii* var. *australe* (Stapf) Stearn, Lilies of the World: 165 (1950).
- = *Lilium brownii* var. *colchesteri* E.H.Wilson, Lilies East Asia: 30 (1925). Lectotype designated here: [Icon] Bot. Mag. 38: t.1591 (1813) as *L. japonicum* non Thunb.
- = *Lilium anhuiense* D.C.Zhang & J.Z.Shao, Acta Phytotax. Sin. 29: 475 (1991). Holotype: China, Anhui, Shitai, Guniujiang, 1800 m alt. 18 June 1983, *Shao Jian-Zhang* 8350111 (ANUB).

Description. A variable species with a wide distribution across central and southern China. Three varieties are recognisable.

Bulb subglobose frequently slightly flattened 2–5 × 2–7 cm, scales white, ovate, thick, sometimes articulated; *stem* 70–200 cm, green or reddish tinged, smooth or papillose, rooting at base when growing; *leaves* scattered, sessile, linear, lanceolate, (oblancoate or obovate-lanceolate in var. *viridulum*) (5) – 16 × (0.6) – 2 cm, glabrous, dark green, paler beneath, 3–7 veined, margins entire or undulate; *inflorescence* 1–7 flowered, subumbellate; *pedicels* 3–6 cm long, glabrous; *flowers* horizontal, slightly to strongly fragrant, tepals spreading gradually from the base, recurved at apex, ivory white within, externally suffused or finely speckled with reddish-purple, especially on the three outer tepals, often with pronounced reddish-purple colour along mid-ribs (greenish externally in var. *chloraster*) 13–18 × 2–4 cm; inner tepals 13–18 × 3.5–5 cm; nectaries linear, green, papillose or subglabrous along margins; *stamens* 10–13 cm long, slightly upwardly curving, glabrous or papillose at base, anthers versatile, linear, brown or orange-brown, pollen cinnabar to reddish-brown; *style* 9–11 cm long, glabrous or pubescent at base, stigma 6–8 mm across, trilobed, pale greenish-yellow; *capsule* 4–6 × 3–4 cm, cylindrical, six-ribbed.

Distribution. CHINA: Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, Yunnan, Zhejiang.

Ecology. Growing in open grassy meadows, rocky hillsides, open woods and amongst low scrub, 100 to 2200 m alt. Flowering in June to August.

Illustration. <http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000464654> (as *Lilium australe*)

***Lilium brownii* var. *chloraster* (Baker) Baker, Gardeners Chronicle ser. 3 vol. 10: 225 (22 August 1891)**

- ≡ *Lilium brownii* var. *chloraster* (Baker) Baker, Gardeners Chronicle ser. 3 vol. 10: 225 (22 August 1891)
- ≡ *Lilium chloraster* (Baker) E.H.Wilson, Journal of the Royal Horticultural Society vol. 42: 36 (1916)
- ≡ *Lilium leucanthum* var. *chloraster* (Baker) E.H.Wilson, Lilies East Asia: 41 (1925).

= *Lilium wenshanense* L.J.Peng & F.X.Li, *Acta Bot. Yunnan.*, Suppl. 3: 33 (1990).
 Holotype: China, Yunnan, Wenshan “in pratis 1000–2200 m” (Cultivated Kunming Botanic Garden), 30 June 1989, *L.J.Peng* 89-1 (holo. KUN!), KUN304310 [barcode KUN-1219367]; isotype: China, Yunnan, Wenshan (Cult.) (iso. KUN!), KUN304309 [barcode KUN-1219364].

Basionym. *Lilium longiflorum* var. *chloraster* Baker, *Gardeners Chronicle* ser. 3 vol. 10: 66 (18 July 1891) **Holotype:** China, Hubei, *A.Henry* s.n. (Cult. July 1891, RBG Kew, floral parts in two capsules) via Charles Ford in Hong Kong (holo. K!) [K-000464716]

Diagnosis. Differing from var. *brownii* by the greenish colouration on the outside of the corolla. *Lilium wenshanense* was differentiated by having articulated scales, a feature now found to vary across the range of the species.

Distribution. CHINA: Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, Yunnan, Zhejiang.

Ecology. Growing in open grassy meadows, rocky hillsides, open woods and amongst low scrub, 100 to 2200 m alt. Flowering in June to August.

***Lilium brownii* var. *viridulum* Baker, *Gard. Chron.* 24: 134 (1 August 1885)**

= *Lilium brownii* [unranked] *brevifolium* T.S.Ware ex Rob., *The Garden* 28: 115 (1 August 1885). Type not found.

= *Lilium brownii* var. *platyphyllum* Baker, *Gard. Chron.* ser. 3, 10: 225 (1891). Type not found: China, Hubei, *A.Henry* s.n.

= *Lilium aduncum* Stapf in Elwes, *Gard. Chron.*, ser. 3, 70: 101 (1921). Lectotype designated here from syntypes: China, Hubei, Ichang [Yichang] and immediate neighbourhood, San-ya-yang, May 1888, *A.Henry* 4160 (lecto. K!) [K-000464659]; syntype: China, Hubei, Ichang, “between the mountains and the hills” received March 1886, *A.Henry* 514 (syn. K!) [K-000464658].

= *Lilium brownii* var. *ferum* Stapf in Elwes, *Gard. Chron.*, ser. 3, 70: 101 (1921). Lectotype designated here from syntypes: China, Hubei, Ichang, “Nan-to and mountains northward”, February 1887, *A.Henry* 2047 (lecto. K!) [K-000464656]; syntype: China, Western Hubei, June 1907 to November 1909, *E.H. Wilson* 1447 (syn. K!) [K-000464657].

Holotype. ex Japan (cultivated), Thomas Softley Ware, Hale Farm Nursery, Tottenham, London, 22 July 1885 (holo. K!) [K-000464651]. Paratype: “Hort. Ware, July 1885” (para. K!).

Diagnosis. Differing from var. *brownii* and var. *chloraster* by the dark green obovate-lanceolate to oblanceolate leaves 5–7 × 1–2 cm (vs. linear to lanceolate 0.6–1 cm wide). The leaf size also decreases and becomes more sparse towards the apex of the inflorescence than in the other varieties. Corolla colour varies in the degree of colouration from finely chestnut brown markings externally to greenish.

Distribution. CHINA: Anhui, Fujian, Gansu, Guangxi, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shanxi, Sichuan, Yunnan, Zhejiang. It seems that var. *viridulum* does not occur in Guangdong Province.

Ecology. Growing along ravines on grassy slopes, in clearings of open forests and amongst low scrub, 100 to 1000 m alt. Flowering in June and July.

Illustration. <https://www.biodiversitylibrary.org/item/92598#page/82/mode/1up> (as *Lilium japonicum*).

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A new species and new records of *Solanum* (Solanaceae) from Colombia

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Abstract

We describe a new species of the Geminata clade of *Solanum* from Colombia and provide new distributional records for two additional *Solanum* species, recorded here for the first time in Colombia. *Solanum bobsii* J.D. Tovar, **sp. nov.** is morphologically similar to *S. chlamydogynum* Bitter from Venezuela of the *Solanum sessile* species group (Geminata clade). These two species can be distinguished by trichome morphology, as well as colour and density of the indumentum. In addition, we report new range expansions into Colombia for two species: *S. tanysepalum* S.Knapp (Geminata clade) known previously only from Venezuela and *S. verecundum* M.Nee (Brevantherum clade) from Ecuador and Peru.

Resumen

Describimos una nueva especie del clado Geminata de *Solanum* de Colombia y proveemos nuevos datos de distribución para otras dos especies de *Solanum*, registradas aquí por primera vez en Colombia. *Solanum bobsii* J.D. Tovar, **sp. nov.** es morfológicamente similar con *S. chlamydogynum* Bitter de Venezuela del grupo de especies de *Solanum sessile* (Geminata clade). Estas dos especies se diferencian por el tipo de tricomas, color y densidad de su indumento. Además, reportamos nuevos registros de dos especies que expanden sus rangos de distribución a Colombia: *S. tanysepalum* S. Knapp (clado Geminata) conocida previamente solo de Venezuela y *S. verecundum* M. Nee (clado Brevantherum) de Ecuador y Perú.

Keywords

distribution, nightshades, *Solanum* clade Brevantherum, *Solanum* section *Geminata*, tropical Andes

Palabras clave

Andes tropicales, distribución, *Solanum* clado Brevantherum, *Solanum* sección *Geminata*

Introduction

Solanum L., with ca. 1,250 species, is the largest genus in the Solanaceae and one of the 10 most species-rich genera of flowering plants (Frodin 2004; Gagnon et al. 2022). The genus has a worldwide distribution with a centre of diversity in South America concentrated in the Andes (Knapp 2002a). A total of 164 species, with 10 of them endemic, are found in Colombia alone, where *Solanum* species occur in all biogeographic regions of the country, from sea level to 4,200 m in elevation (Orozco et al. 2015).

Molecular studies have divided *Solanum* into 12 major clades (Bohs 2005; Weese and Bohs 2007; Särkinen et al. 2013; Gagnon et al. 2022) that have redefined the infrageneric classification of the genus and are being used as informal infrageneric groups in *Solanum*. The Brevantherum and Geminata clades are among the largest of the non-spiny neotropical clades of *Solanum* with ca. 100 and 150 species, respectively (Knapp 2008; Giacomini 2015). The two clades have similar distributions with centres of diversity and endemism in the Atlantic Forest of Brazil and the tropical Andes (see Knapp et al. 2015; Giacomini 2015). Both clades are unarmed and woody (shrubs or trees) but differ in their trichome type and inflorescence position and morphology. Species of the Brevantherum clade have mostly stout and highly branched inflorescences positioned in branch forks (i.e. terminal inflorescences in dichasially branching stems) and most species have stellate trichomes (Giacomini 2015). Members of the Geminata clade, in contrast, all lack stellate trichomes and have mostly monochasial branching with inflorescences that are leaf-opposed or rarely terminal or internodal (Knapp 2008; Knapp et al. 2015).

Here we describe a new species for the Geminata clade and provide new records of two species from the Brevantherum and Geminata clades of *Solanum* for Colombia with field photos. These discoveries were made during herbarium and fieldwork conducted for a broader phylogenetic study of the Geminata clade. The discovery of the new species from the Geminata clade is not surprising considering members of this clade have an intricate taxonomy due to similar morphology (Knapp 2008) and are often poorly known because they occur in forest understorey habitats, where they are often inconspicuous, locally rare and rarely collected (Knapp et al. 2015). In fact, most undetermined specimens of *Solanum* in tropical herbaria are from the Geminata clade and it is, hence, not surprising that a new species was found during our study of the clade in Colombia.

Methods

We examined specimens from the FAUC, HUA, HUQ and JBB Herbaria (acronyms from Index Herbariorum; <http://sweetgum.nybg.org/science/ih>). For the new species

here described, duplicates of paratypes are still awaiting distribution to other countries. Descriptions are based on field observations and herbarium specimens. Preliminary conservation status assessments were done using the IUCN Red List Categories and Criteria (IUCN 2017), based on extent of occurrence (EOO) and area of occupancy (AOO) calculated with the GeoCat tool (www.geocat.kew.org; Bachman et al. 2011). For the AOO calculation, a cell size of 2 km² was used. The morphological cluster species concept of Mallet (1995) was used in defining a species.

Taxonomic treatment

Solanum bobsii J.D. Tovar, sp. nov.

urn:lsid:ipni.org:names:77297478-1

Figs 1 and 2

Diagnosis. Like *Solanum chlamydogynum* Bitter, but with a translucent indumentum of unbranched or, at most, furcate trichomes restricted to the veins of the leaves (vs. dendritic, more abundant and ochraceous in *S. chlamydogynum*), cucullate calyx lobes (vs. non-cucullate in *S. chlamydogynum*) and glabrous ovaries (vs. densely pubescent in *S. chlamydogynum*).

Type. COLOMBIA. Risaralda: Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2100 m elev., 15 Nov 2020 (fl, fr), J.D. Tovar & A.F. Bohorquez 484 (holotype: FAUC [FAUC36396]; isotypes: COL, FMB, HUA).

Description. Shrubs or small trees, 2–7 m tall; stems winged, greenish-brown when young, turning brown with age, young stems pubescent with translucent simple or furcate 4–8-cellular trichomes. Sympodial units difoliate and geminate, leaves ovate to obovate, glabrous adaxially or with translucent trichomes along the mid-rib and secondary veins like those on stem, abaxially pubescent with trichomes in the mid-rib and along the secondary and tertiary veins; major leaves 18–35 (45) × 12–16 cm, with 12–15 pairs of main lateral veins, these often strongly parallel, the apex acute, base oblique and decurrent on to petiole; petioles 1–2 cm long, pubescent with translucent trichomes, like those on stem; minor leaves differing only in size, not in shape, 8–9.5 × 4–5 cm, with 6–9 pairs of main lateral veins, adaxially and abaxially pubescent along the mid-rib and main lateral veins like those on stem, the apex acute or rounded, base oblique and decurrent on to petiole; petiole 0.5–0.7 cm long, pubescent with translucent trichomes, like those on stem. Inflorescences leaf-opposed, forked and erect, 20–50 flowered, peduncles 1.5–2.5 cm, with unbranched and uniseriate trichomes, like those on stem, pedicel scars densely spaced, not overlapping, pedicels 0.5–1 cm long, deflexed, thickened at the apex and purple in live plants. Buds globose, with the corolla strongly exerted from the calyx tube prior to anthesis. Flowers 5–6-merous, all perfect; calyx tube cyathiform, 2–3 mm long, the lobes deltoid abruptly reduced and hooded at the apex, 1.5–2 × 1.8–2.2 mm, abaxially glabrous or densely pubescent with trichomes like those of the young stem and with a tuft of hairs at apex; corolla



Figure 1. *Solanum bohsii* J.D. Tovar **A** plant habit **B** flowers and buds **C** infructescence **D** sympodial units.

1.5–2 cm in diameter, white, fleshy, lobed ca. $\frac{3}{4}$ of the way to the base, the lobes 0.7–0.9 × 3–4 mm spreading or deflexed at anthesis, glabrous and cucullate at the tips; anthers 4–6 × 1.2–1.7 mm, poricidal at the tips, sagittate at the base; free portion of the filaments 0.2–1.2 mm long; ovary glabrous; style 0.5–0.7 mm long, terete, glabrous, stigma capitate, light green in live plants. Fruit a globose green berry, 1–1.2 cm in diameter, glabrescent or with a few scattered trichomes like those on stem and an apical scar, green at maturity; fruiting pedicels 1.5–2 cm long, erect, woody and somewhat rugose, distally enlarged, the calyx constricted and with lobes woody in fruit. Seeds ca. 80 per fruit, 2.5–3.5 × 2–2.5 mm, flattened-reniform, greyish when dry, the margins incassate, the surfaces minutely pitted. Chromosome number not known.

Distribution and ecology. *Solanum bohsii* is known only from three localities in the western slopes of the central Andean cordillera in Colombia in the Departments of Caldas, Quindio and Risaralda (Fig. 3) where it inhabits secondary forest edges between 1,900–2,300 m elevation, forming groups of up to 10 individuals.

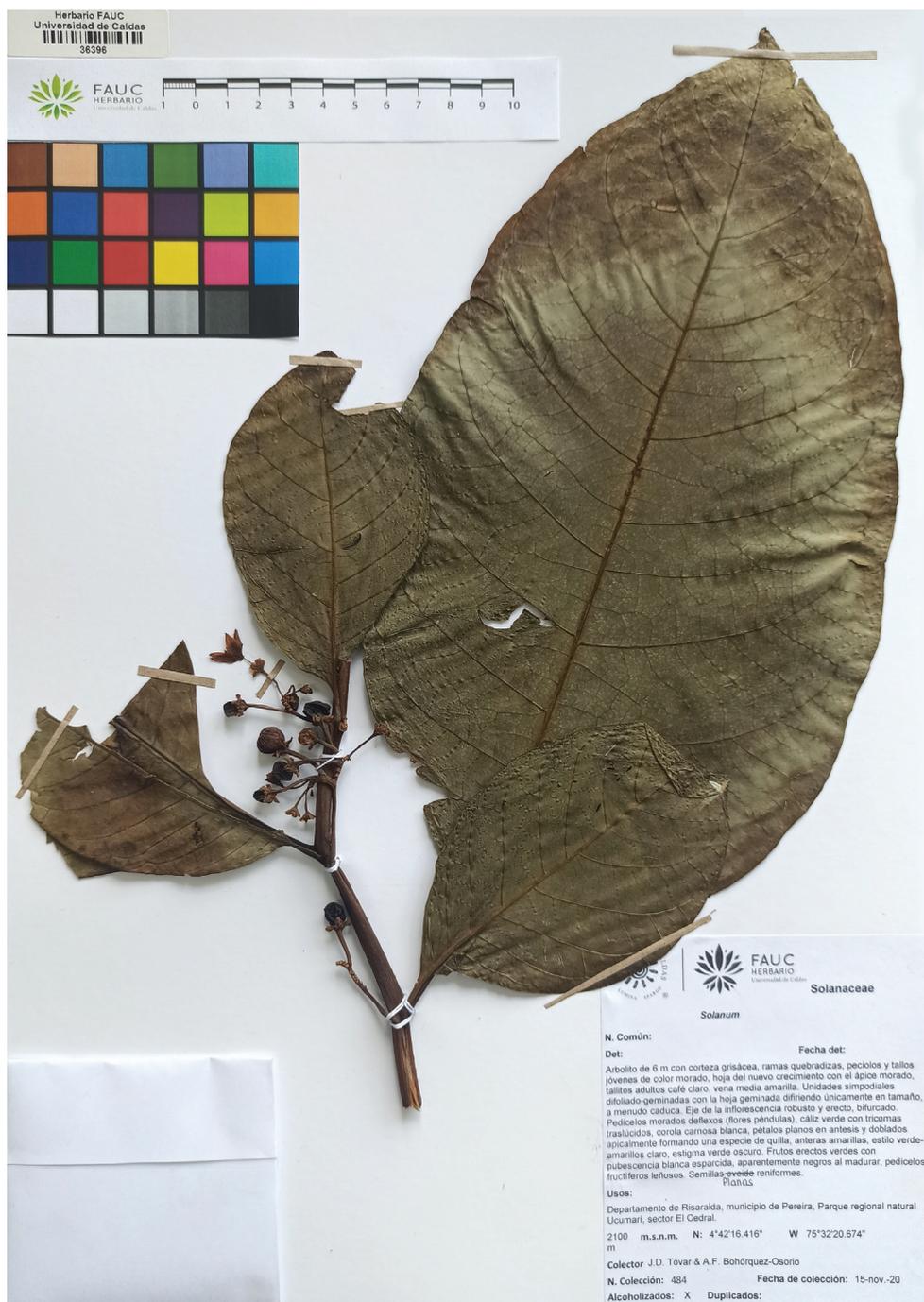


Figure 2. Holotype of *Solanum bohsii* J.D. Tovar [J.D. Tovar & A.F. Bohórquez 484 (FAUC 36396)].

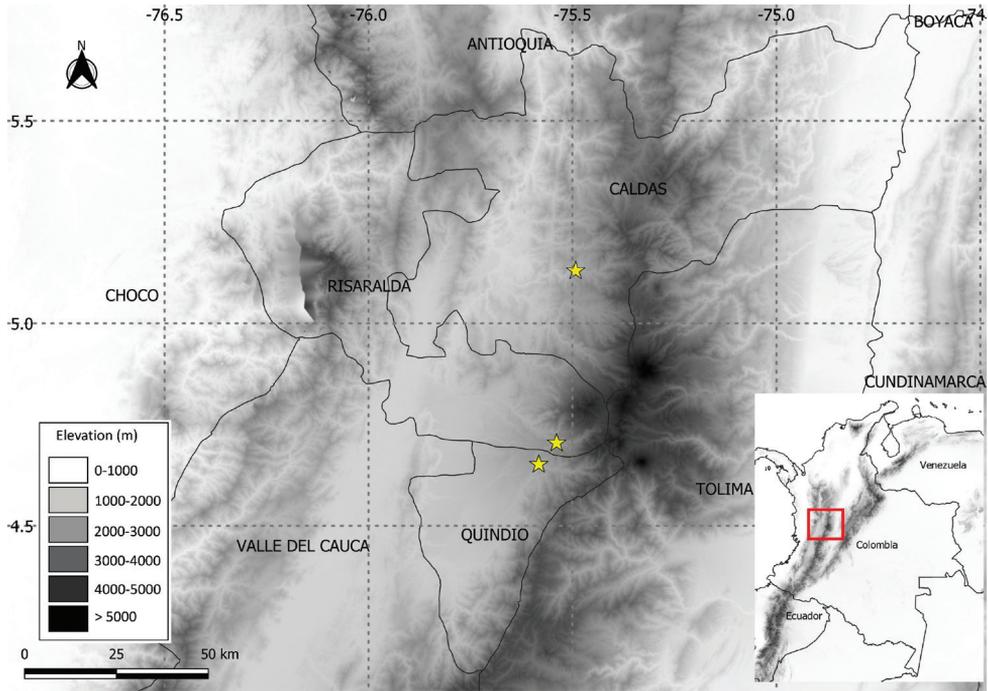


Figure 3. Distribution of *Solanum bohsii* J.D. Tovar (yellow stars).

Phenology. According to the collections studied, *S. bohsii* produces flowers and fruits throughout the year.

Etymology. The specific epithet honours Lynn Bohs, an American botanist and expert in the Solanaceae family, who has made great contributions to the understanding of systematics and evolution of the genus *Solanum* over the last 30 years.

Preliminary conservation status. Endangered [EOO (100 km²) and AOO (12 km²)]. We assign *S. bohsii* a preliminary IUCN Red List status of endangered (EN), based on assessment criteria B2 a, b (i,ii,iv) (IUCN 2017).

Notes. According to its morphology, *S. bohsii* is a member of the *Solanum sessile* species group (sensu Knapp 1991; 2002b) with difoliate and geminate sympodial units, large leaves and large branched inflorescences and ovoid-reniform seeds. It is easily distinguished from the other species in the group by its large leaves up to 45 cm long and the secondary veins strongly parallel in mature leaves. *Solanum bohsii* is morphologically similar to *S. chlamydogynum* from Venezuela with which it shares the winged stems, the fleshy large flowers with cucullate lobes and the presence of trichomes in both leaf surfaces. However, trichomes of *S. bohsii* are simple or, at most, furcate, translucent and less abundant compared to *S. chlamydogynum* which has dendritic ochraceous trichomes. Furthermore, inflorescence peduncles are less pubescent in *S. bohsii*, while densely pubescent with ochraceous trichomes in *S. chlamydogynum*.

Solanum bohsii has distinctive mature fruits in dry material with a network-like pericarp, resembling the venation of leaves. From the other species in the *Solanum sessile* species group, it is easily recognised either by the pubescence, the winged stems, the large leaves or the large flowers with cucullate lobes (see Knapp 1991; 2002b).

Paratypes. COLOMBIA. Caldas: Municipio de Neira, antigua fábrica de cementos Caldas, 2,026 m elev., 7 Jul 2001 (fl, fr), Camilo Rivera, Andres Castellanos, C. Arbelaez & W. Acosta 8 (FAUC). **Quindío:** Municipio de Salento, bosque frente al molino, 1,900–2,000 m elev., 9 May 1997 (fl, fr), W. Vargas 3820 (HUQ). **Risaralda:** Municipio de Pereira, cerca de la entrada del Parque Regional Ucumari, 1,800 m elev., 10 Aug 2014 (fl, fr), J.D. Tovar 114 (JBB); Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2,100 m elev., 15 Nov 2020 (fl), J.D. Tovar & A.F. Bohorquez 481 (FAUC); Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2,100 m elev., 15 November 2020 (fl), J.D. Tovar & A.F. Bohorquez 482 (FAUC); Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2,100 m elev., 15 Nov 2020 (fl, fr), J.D. Tovar & A.F. Bohorquez 483 (FAUC); Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2,100 m elev., 15 Nov 2020 (fl), J.D. Tovar & A.F. Bohorquez 485 (FAUC); Municipio de Pereira, Parque Regional Natural Ucumari, sector el cedral, 4°42'16"N, 75°32'20"W, 2,100 m elev., 15 Nov 2020 (fr), J.D. Tovar & A.F. Bohorquez 486 (FAUC). Municipio de Pereira, Santuario de flora y fauna Otún Quimbaya, 12 Feb 2016 (fl, fr). A. Orejuela et al. 2630 (JBB); Municipio de Pereira, Reserva Natural Ucumari, La Pastora, camino a Ceylan, 5°06'47"N, 75°53'16"W, 2,300 m elev., 30 Jul 2006 (fl, fr), F.J. Roldan et al. 4010 (HUA); Municipio de Pereira, Valle del río Otún, Parque Regional Ucumari, El Cedral, 4°02'N, 79°31'W, 1,980–2,000 m elev., 20 Feb 1990 (fl, fr), T.B. Croat & M.P. Galeano 70797 (HUA, MO).

New records for *Solanum* in Colombia

Solanum tanysepalum S. Knapp, *Brittonia* 38: 284. 1986

Type. VENEZUELA. Aragua: Parque Nacional Henri Pittier, Portachuelo to Pico Periquito trail, W of Estación Biológica Rancho Grande, premontane to montane rainforest, 1,100–1,400 m elev., 10°21'N, 67°42'W, 22 Oct 1984, S. Knapp & J. Mallet 6856 (holotype: MY; isotypes: BH, F! photo, K!, MO! photo, NY! photo, US! photo, VEN).

Notes. *Solanum tanysepalum* (Fig. 4a) is a species previously known only from the Cordillera de la Costa in Venezuela in cloud forest from 1,000 to 1,700 m elevation. Our new records presented here extend the distribution to the departments of Huila and Magdalena in Colombia (Fig. 5). The species belongs to the *Solanum arboreum* species group in the Geminata clade and can be easily recognised by the long-acuminate calyx lobes that are persistent and somewhat woody in fruit (Knapp 2002a, b). More information and a complete description are available at: <https://solanaceaesource.myspecies.info/solanaceae/solanum-tanysepalum>.



Figure 4. New records of *Solanum* from Colombia **A** *Solanum tanysepalum* S.Knapp (upper left corner: detail of calyx lobes; Geminata clade) **B** *Solanum verecundum* M.Nee (Brevantherum clade).

Specimens examined. COLOMBIA. Huila: Municipio de La Plata, reserva natural Meremberg, bosques contiguos al cementerio familiar, 2°13'09.6"N, 76°06'39.9"W, 2,300 m elev., 15 May 2019 (fl, fr), *J.D. Tovar 446* (JBB); Municipio de La Plata, reserva natural Meremberg, bosques contiguos al cementerio familiar, 2°13'09.6"N, 76°06'39.9"W, 2300 m elev., 15 May 2019 (fl, fr), *J.D. Tovar 447* (JBB); Municipio de La Plata, reserva natural Meremberg, bosques contiguos al cementerio familiar, 2°13'09.6"N, 76°06'39.9"W, 2,300 m elev., 15 May 2019 (fl, fr), *J.D. Tovar 448* (JBB); Municipio de La Plata, reserva natural Meremberg, bosques contiguos al cementerio familiar, 2°13'09.6"N, 76°06'39.9"W, 2,300 m elev., 15 May 2019 (fl, fr), *J.D. Tovar 449* (JBB). **Magdalena:** Sierra nevada de Santa Marta, in forest N of finca Cecilia, quebrada Indiana, 10°59'N, 73°58'W, 1,820 m elev., 31 Aug 1972 (fr), *J.H. Kirkbride Jr.* 2021 (NY).

***Solanum verecundum* M.Nee, Kurtziana 28: 137, 2000.**

Type. ECUADOR. Sucumbios: El Salado, colecciones en el sendero a la finca del Sr.

Segundo Pacheco, 1,400 m elev., 13 October 1990, J. Jaramillo, E. Grijalva & M. Grijalva 13285 (holotype: QCA!; isotype: NY! [00381798]).

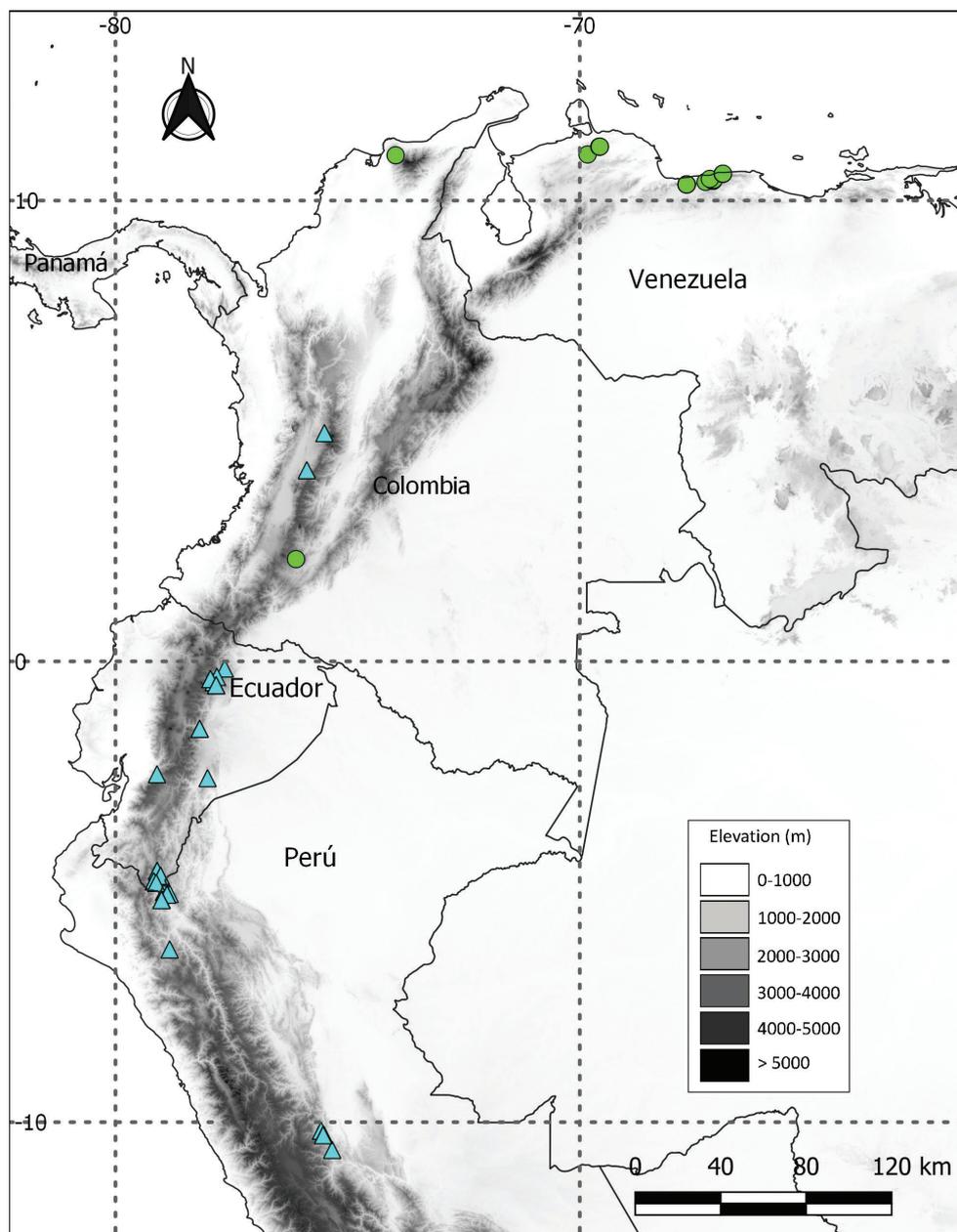


Figure 5. Distribution of *S. tanysepalum* S.Knapp (green circles) and *S. verecundum* M.Nee (cyan triangles).

Notes. *Solanum verecundum* (Fig. 4b) was previously known from montane forests in Peru and Ecuador (Giacomin 2015). Our new records presented here extend the distribution to Colombia to the Departments of Caldas, Quindío and Valle del Cauca (Fig. 5). The species belongs to the *Brevantherum* clade and is related to the species

traditionally included in section *Brevantherum* Seithe (Tovar et al. 2021). *Solanum verecundum* can be easily recognised by the membranaceous leaves, stellate-lepidote trichomes (with partially fused rays) in both leaf surfaces and puberulous orange-coloured fruits at full maturity. More information and a complete description are available at: <https://solanaceaesource.myspecies.info/solanaceae/solanum-verecundum>

Specimens examined. **COLOMBIA. Caldas:** Municipio de Villamaria, ruta del condor, carretera entre la Telaraña y La Guyana, 4°57'18.5"N, 75°30'03.3"W, 2,000 m elev., 29 Nov 2021 (fl,fr), *J.D. Tovar & M.A. Buitrago* 487 (FAUC). **Quindío:** Municipio de Génova, por la trocha que conduce a Pijao, camino al Cedral, 1,800 m elev., 11 Feb 2016 (fr), *A. Orejuela* et al. 2627 (JBB); Municipio de Génova, vereda La Esmeralda, borde de carretera, 1,750 m elev., 8 Jan 1993 (fr), *C.A. Lopez* 61 (HUQ). **Valle del Cauca:** Márgenes del río Bugalagrande, Calamar, 1,680 m elev., 28 Mar 1946 (fr), *J. Cuatrecasas* 20500 (F).

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A phylogenetic and morphological study of the *Tectaria fuscipes* group (Tectariaceae), with description of a new species

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Abstract

The fern species *Tectaria fuscipes* and morphologically similar species, which are common in tropical and subtropical mainland Asia, constitute a taxonomically confusing group. To better understand species boundaries and relationships within the *T. fuscipes* group, we conducted phylogenetic analyses of five plastid regions and morphological observations of herbarium specimens and living plants. As a result, we produced a generally well-resolved phylogeny of the *T. fuscipes* group and related species in Asia. The phylogenetic analyses supported the monophyly of the *T. fuscipes* group, which includes *T. dissecta*, *T. fuscipes*, *T. ingens*, *T. paradoxa*, *T. setulosa*, *T. subfuscipes*, *T. subsageniacea* and a new species, but excludes *T. kusukusensis*. However, *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* are almost indistinguishable in morphology, which form a complex characterised by the black linear-lanceolate stipe scales. The new species found in southern China and Vietnam is described here as *T. fungii*. It is similar to the *T. fuscipes* complex and *T. kusukusensis*, but differs from the former mainly by its brown-castaneous lanceolate stipe scales and from the latter by having nearly hairless laminae (versus frond axes abaxially bearing copious hairs).

Keywords

fern, mainland Asia, molecular phylogeny, morphology, taxonomy

Introduction

Tectaria Cav. is a pantropical and south-temperate fern genus of about 264 species (Hassler 2004–2022). It belongs to Tectariaceae of eupolypods I in the classification of PPG I (2016). *Tectaria* in the modern sense is based on molecular phylogenetic data (Ding et al. 2014; Wang et al. 2014; Chen et al. 2018), covering *Aenigmopteris* Holttum, *Cionidium* T. Moore, *Ctenitopsis* Ching ex Tardieu & C. Chr., *Heterogonium* C. Presl, *Psomiocarpa* C. Presl, *Tectaridium* Copel. and other satellite genera previously recognised. The species now included in *Tectaria* are morphologically very diverse, which makes *Tectaria* as a genus difficult to be distinguished from its allied genera *Hypoderris* R. Br. ex Hook. and *Triplophyllum* Holttum (Moran et al. 2014). For the majority of *Tectaria*, the diagnostic features include the rhizome and stipe covered with lanceolate scales, the less dissected and herbaceous fronds with their axes adaxially non-grooved and bearing ctenitoid hairs, anastomosing veins and discrete round sori on the abaxial surface of laminae (Ching 1931). According to recent studies (Ding et al. 2014; Zhang et al. 2017; Dong et al. 2018a), *Tectaria* phylogenetically includes four major clades, with one clade confined to the Neotropics and the other three in the Old World; but none of them was found having a synapomorphic morphology.

In tropical and subtropical Asia, *Tectaria fuscipes* (Wall. ex Bedd.) C. Chr. and morphologically similar species constitute a taxonomically confusing group. They are characterised by the fronds with basal pinnae basiscopically produced and veins being wholly free or, as in *T. fuscipes*, with veins anastomosing to form costal areoles in its sterile fronds (Holttum 1991). In the area from Taiwan Island westwards to South Asia, the species having the frond shape like that of *T. fuscipes* and free veins include *T. dissecta* (G. Forst.) Lellinger, *T. fuscipes*, *T. ingens* (Atk. ex C.B. Clarke) Holttum, *T. kusukusensis* (Hayata) Lellinger, *T. setulosa* (Baker) Holttum, *T. paradoxa* (Fée) Sledge, *T. subfuscipes* (Tagawa) C.M. Kuo and *T. subsageniacea* (Christ) Christenh. (Holttum 1988; Kuo 1997; Zink 2006; Lindsay and Middleton 2012 onwards; Xing et al. 2013; Fraser-Jenkins et al. 2018), as well as an unidentified taxon which was labelled as “*Tectaria* sp.1” by Dong et al. (2018a). Of these species, the most contentious species are *T. subfuscipes* and *T. subsageniacea*. *Tectaria subfuscipes* was described for the plants from Taiwan Island that are morphologically similar to *T. fuscipes*, but different in the free veins and monomorphic fronds (Tagawa 1939). When revising *Tectaria* species with free or partly anastomosing veins in Asia, Holttum (1988) treated *T. subfuscipes* as a synonym of *T. fuscipes*. However, both *T. subfuscipes* and *T. fuscipes* were accepted as distinct species in the fern flora of Taiwan (Kuo 1997; Knapp 2014). In southern China, *Aspidium subsageniaceum* Christ (1906) (= *T. subsageniacea*), *Ctenitopsis glabra* Ching & Chu H. Wang (Ching and Wang 1964) and *Ctenitopsis acrocarpa* Ching (Ching and Wang 1981) were proposed as morphologically similar species to *T. fuscipes*. The first author (Dong) agreed with a broad concept of *T. fuscipes* sensu Holttum (1988) and treated all these names for plants from southern China as synonyms of *T. fuscipes* (Dong et al. 2002; Dong 2017). However, the phylogenetic analyses by Zhang et al. (2017) showed that *T. subsageniacea* was in a different subclade from *T. fuscipes*.

The relationships between *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* had not been resolved in Zhang et al. (2017).

To better resolve the relationships between species and explore species boundaries within the *T. fuscipes* group, we conducted phylogenetic analyses of plastid sequences with an enlarged sampling and made morphological observations of herbarium specimens and living plants. Specifically, the purposes of this study are to construct a phylogeny of the *T. fuscipes* group and related species in mainland Asia, to detect morphological differences amongst *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* and to determine the identity of the “*Tectaria* sp.1” in Dong et al. (2018a).

Methods

For morphological comparisons, the first author (Dong) studied in person the specimens of *Tectaria* in these Herbaria: BM, BO, DACB, E, GAUA, HITBC, HN, HNU, IBK, IBSC, K, KUN, L, LAE, P, PE, PNH, PYU, SING and TAIF. In addition, we conducted morphological observations of living plants in the wild of Bangladesh, China and Vietnam. For both herbaria specimens and living plants, we focused on the states and the variation of stipe scales (shape and colour), lamina hairs, fronds dimorphism, venation and sori distribution, which were characters frequently used by previous authors (e.g. Holttum 1988; Shieh 1994; Kuo 1997; Wang 1999; Xing et al. 2013; Fraser-Jenkins et al. 2018) to recognise *T. fuscipes* and morphologically similar species.

The sampling for phylogenetic analyses in this study focused on species with free or relatively simple anastomosing species in the Old World which constitute one of four major clades in *Tectaria* (Ding et al. 2014; Zhang et al. 2017; Dong et al. 2018a). A total of 51 specimens were analysed, including three specimens of the unidentified taxon, 16 of *Tectaria fuscipes* s. l. (including *T. subfuscipes* and *T. subsageniacea*) and representatives of all known species with free veins in mainland Asia and adjacent islands, except for *T. hennipmanii* (Tagawa & K. Iwats.) S.Y. Dong, a very rare species and hitherto represented only by its type from Thailand. Of the 51 specimens, 23 were newly sequenced and analysed in this study (Appendix 1). The same five plastid regions (*atpB*, *ndhF* plus *ndhF-trnN*, *rbcL*, *rps16-matK* plus *matK* and *trnL-F*) used in previous studies (Ding et al. 2014; Dong et al. 2018a) were followed here to infer the phylogeny.

Genomic DNA of the 23 newly-added samples were extracted from silica-dried leaves, except for that of *T. paradoxa*, for which we instead used leaf fragments of herbarium specimens. The subsequent amplifications were carried out with the primers described in Ding et al. (2014) according to the standard protocols of PCR. The PCR products were sequenced using the BigDye Terminator Cycle Sequencing kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) on an ABI 3730XL automated sequencer. Newly-generated sequences and those from GenBank were aligned individually using MAFFT (Katoh et al. 2005) and subsequently adjusted manually in BioEdit version 7.2.0 (Hall 1999). We then concatenated the five regions of each sample into a combined matrix for the following analyses.

We analysed the matrix using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP), respectively. The software jModeltest2 (Posada 2008) was used to determine the best-fitting substitution models for the combined sequences and the results suggested GTR+G+I as the best-fitting model in both BI and ML analyses. The BI analysis was conducted with MrBayes 3.2.6 (Ronquist et al. 2012), using 10 million generations with one tree sampled every 1,000 generations; four runs with four chains were performed in parallel. The first 25% trees were discarded as burn-in. The ML analysis was conducted using raxmlGUI2.0 (Edler et al. 2020). A thorough tree search for the best ML tree was performed. The ML Bootstrap analysis was performed with 1000 replications; bipartition information from the bootstrap trees was drawn on the best ML tree. The MP analysis was conducted in PAUP* version 4.0d100 (Swofford 2002), with all characters weighted equally and gaps considered as missing data. One thousand heuristic replicated searches were carried out using random stepwise addition with branch swapping by tree bisection-reconnection (TBR), saving 100 trees per replicate. Bootstrap values (BS) were calculated with 1000 heuristic bootstrap replicates, one random sequence addition and TBR swapping.

Results

Scales

For the *Tectaria fuscipes* group, the colour of stipe scales can be determined as two basic states: brown and black. Scales in *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* are constantly black, with or without very narrow brown margins, whereas, in the unidentified taxon and other species, they are brown, sometimes brown-castaneous. The black scales are associated with a linear-lanceolate shape, which measures 0.5–1 mm wide (Fig. 1A–C). In contrast, the brown scales are generally broader, usually 1–1.5 mm or, as in *T. ingens* and *T. setulosa*, up to 3 mm wide (Fig. 1D–I).

Lamina hairs

Based on the abundance of hairs on the abaxial surface of costae, which are easily observable in herbarium specimens, the fronds can be generally recognised as either nearly hairless or obviously hairy for *T. fuscipes* and morphologically similar species. We observed fronds with dense hairs in *T. kusukusensis*, some collections of *T. fuscipes* from Taiwan and Bangladesh and some collections of *T. ingens* and *T. setulosa*. In the unidentified taxon and other species of the *T. fuscipes* group, the fronds are nearly hairless. The abundance of lamina hairs is variable in *T. fuscipes*, *T. ingens* and *T. setulosa*. We noticed that the fronds can be hairless or hairy even in a single population of *T. fuscipes*, such as *Lu 16213* (TAIF) from Bangladesh.

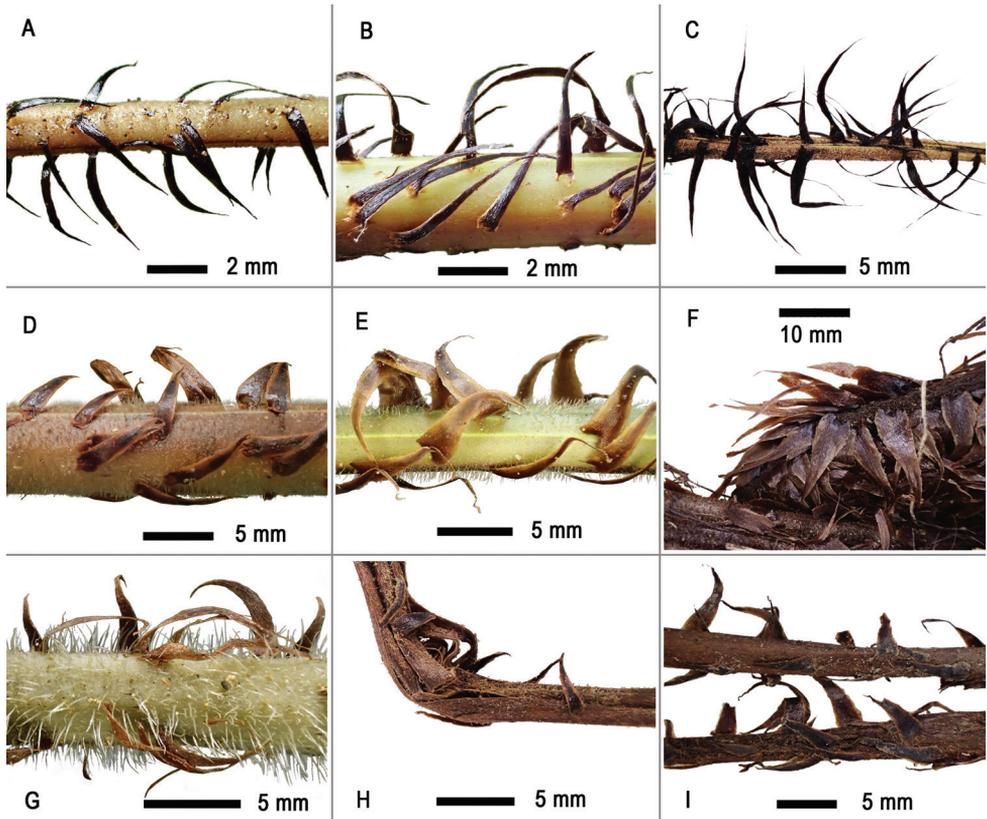


Figure 1. Comparison of stipe scales in *Tectaria fuscipes* and morphologically similar species **A** *T. fuscipes* (Dong 5194, IBSC) **B** *T. subsageniacea* (Dong 4270, IBSC) **C** *T. subfuscipes* (Chang 20140503021, TAIF) **D** *T. sp.* (Dong 5096, IBSC) **E** *T. setulosa* (Dong 4782, IBSC) **F** *T. ingens* (Miehe et al. 13093007, SING) **G** *T. kusukusensis* (Dong 4851, IBSC) **H** *T. paradoxa* (Fraser-Jenkins FN77, TAIF) **I** *T. dissecta* (Chang 20160125, TAIF).

Frond dimorphism

The fronds of all species in the *T. fuscipes* group are more or less dimorphic, i.e. a fertile lamina being contracted to a certain extent as compared with a sterile lamina in a population. Our observations showed that the obvious dimorphism of fronds is frequent in *T. fuscipes*, sometimes occurs in *T. subsageniacea*, but is scarce in other species of the *T. fuscipes* group including the unidentified taxon. However, it is difficult to determine the fronds as monomorphic or dimorphic for *T. fuscipes* and *T. subsageniacea* because the variation from monomorphic to dimorphic is gradual and continuous. We detected different variation tendencies of frond dimorphism instead of clear differences in these two taxa. Namely, the fronds tend to be dimorphic in *T. fuscipes*, but are mostly nearly monomorphic in *T. subsageniacea*. Notably, we observed three different states

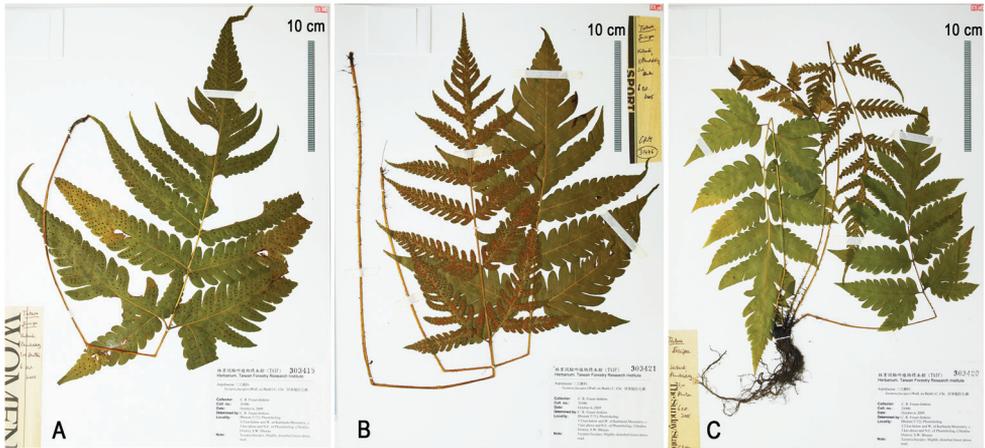


Figure 2. Three states of fronds' fertile-sterile dimorphism in one population of *Tectaria fuscipes* from Bhutan (*Fraser-Jenkins 31446*, TAIF) **A** monomorphic **B** subdimorphic **C** dimorphic.

in a single collection of *T. fuscipes* from Bhutan (*Fraser-Jenkins 31446*, TAIF) (Fig. 2), which indicates the instability of frond dimorphism in this species.

Venation

The venation in the *T. fuscipes* group can be recognised as three states: free (Fig. 3A), intermediate (Fig. 3B) and costal-veins-anastomosing (i.e. veins along costae regularly forming costal areoles) (Fig. 3C). The costal-vein-anastomosing venation was observed only in *T. fuscipes*; the intermediate venation was found in *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea*; and the free venation was found in the unidentified taxon and all species of the *T. fuscipes* group. The intermediate venation covers a variation range, being free on some pinnae of a frond, but forming several costal areoles (continuous or not) on other pinnae of the same frond. The intermediate venation occurs frequently in *T. subfuscipes* from Taiwan Island, *T. subsageniacea* in southern China and

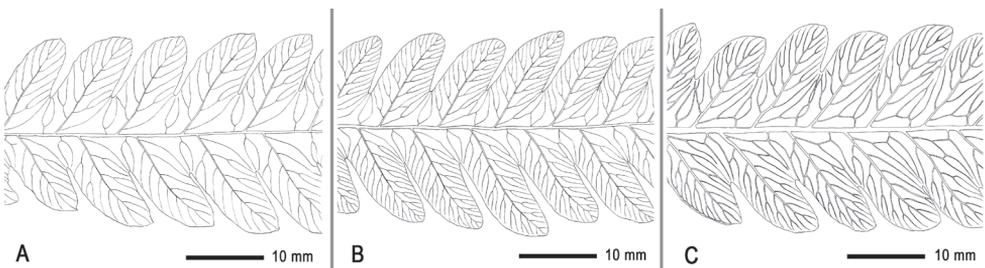


Figure 3. Three states of venation in the *Tectaria fuscipes* group **A** free as in *T. subsageniacea* (*Dong 3856*, IBSC) **B** intermediate (veins unstably forming areoles along costae) as in *T. subsageniacea* (*Dong 4585*, IBSC) **C** costal-vein-anastomosing (veins regularly forming areoles along costae) in *T. fuscipes* (*Dong 4686*, IBSC).

Indochina and *T. fuscipes* in north-eastern India and nearby regions of East Himalayas. Our statistics showed that, in East Himalayas, there are about 54.7% of herbarium specimens of *T. fuscipes* having the free or intermediate venation; for *T. subfuscipes* from Taiwan Island, there are about 15% of herbarium specimens having the intermediate venation. Notably, we found that the free venation and the costal-vein-anastomosing venation can simultaneously occur in a single collection from north-eastern India (e.g. *Fraser-Jenkins FN57, 174, 31446*, all in TAIF).

Sori arrangement

Sori are regularly arranged in two rows on the ultimate segments of pinnae in the *T. fuscipes* group. They are medial (positioned between mid-rib and margin) and are distributed nearly from base to tip on ultimate segments (Fig. 4A) in all species of this group, except *T. paradoxa*; in the latter, sori are mostly supramedial and restricted to the middle and apex of segments (Fig. 4B).

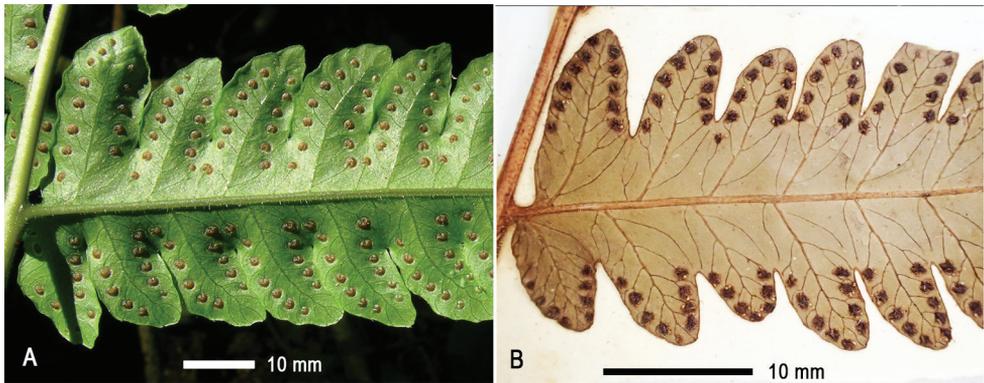


Figure 4. Two states of sori arrangement in the *Tectaria fuscipes* group **A** sori medial, borne on segments almost from base to tip as in *T. sp.* (*Dong 1589*, IBSC) **B** sori supramedial, borne on distal half of segments in *T. paradoxa* (*Thwaites 3061*, BO, TUB).

Molecular phylogeny

The concatenated alignment of the five plastid regions (*atpB*, *ndhF* plus *ndhF-trnN*, *rbcL*, *rps16-matK* plus *matK* and *trnL-F*) accounts for 5865 base pairs, including 64 indels. Of the total 5865 characters, 1027 are variable and 688 are parsimony informative. The length of the best MP trees is 1763 (consistency index = 0.633, retention index = 0.800). The likelihood score of the ML tree is -18708.288.

The topology resulted from the BI analysis is consistent with that of the ML analysis, while in the tree from the MP analysis, the samples are not so well resolved as in the BI or ML tree. There are no obvious conflicts between the topology of BI

(or ML) analysis and that of MP analysis, except for the position of *T. subglabra* (Holttum) S.Y. Dong, which was resolved as sister to *T. aurita* (Sw.) S. Chandra and *T. nayarii* Mazumdar in the MP tree with low support (MPBS = 68%), while as sister to *T. profereoides* (Christ) S.Y. Dong and allied species in the BI or ML tree with poor support (PP = 87%, MLBS = 57%). There is no conflict involving the position of the *T. fuscipes* group and species relationships within this group between the trees inferred from different analyses. Therefore, we adopt the topology resulting from the BI analysis (Fig. 5), where most samples were well resolved and 75% nodes received strong support (PP = 1.0 or 0.99, MLBS > 80%), as a base to describe and discuss the relationships involving the *T. fuscipes* group.

All in-group samples were resolved into two large clades and each clade generally corresponds to a geographical region. The samples from Malesia clustered into a clade

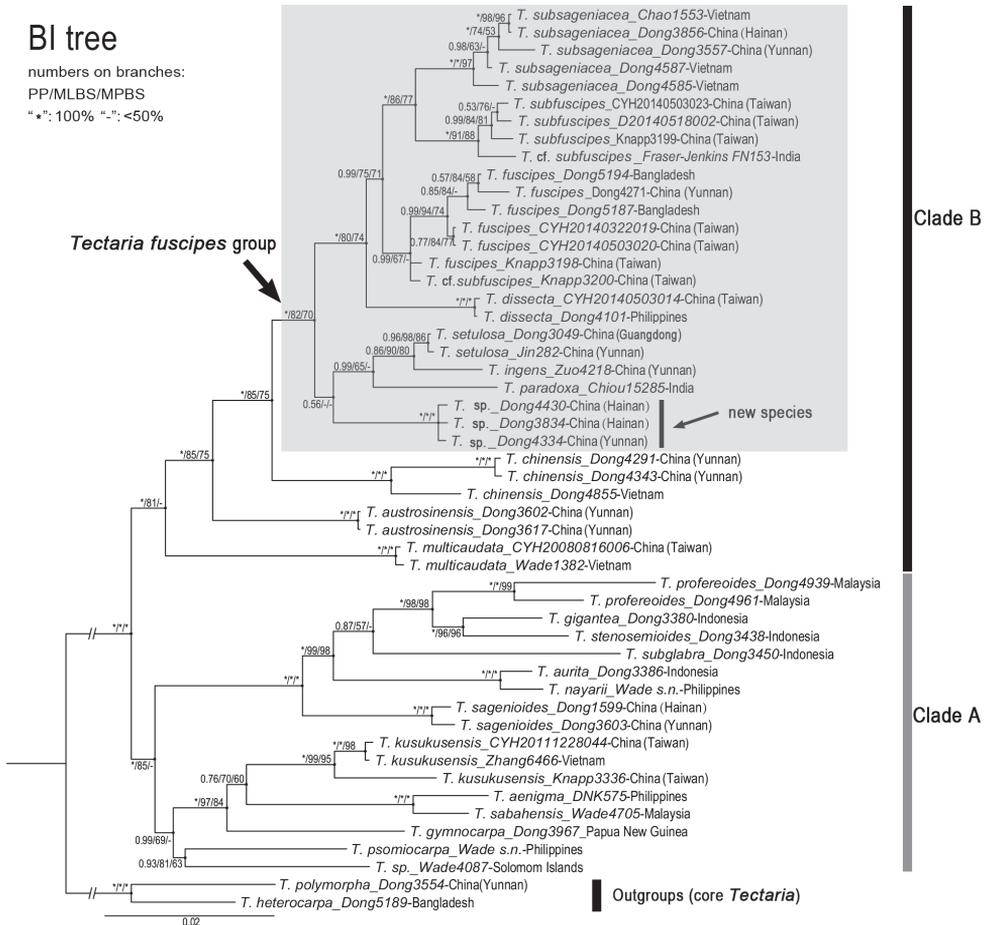


Figure 5. Bayesian consensus tree of the *Tectaria fuscipes* group and allied species with free or relatively simply anastomosing veins in the Old World, based on concatenated plastid regions of *atpB*, *ndhF* plus *ndhF-trnN*, *rbcL*, *rps16-matK* plus *matK* and *trnL-F*.

(Clade A) and those from mainland Asia and adjacent islands (except for *T. kusukusensis* and *T. sagenioides* (Mett.) Christenh.) clustered into another clade (Clade B) (Fig. 5). The *T. fuscipes* group was resolved as a monophyletic clade, forming a sister relationship with *T. chinensis* (Ching & Chu H. Wang) Christenh. Within the *T. fuscipes* group, all samples were further resolved into three clades: (1) the unidentified taxon represented by three specimens, (2) *T. paradoxa*, *T. ingens* and *T. setulosa* and (3) *T. dissecta*, *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea*. The unidentified taxon was resolved as sister to the *T. paradoxa* clade with poor support values.

The 16 specimens representing *Tectaria fuscipes* s. l. (including *T. subfuscipes* and *T. subsageniacea*) were well resolved into three clades. All specimens of *T. fuscipes* s. str., including one (*Knapp 3200*) with a morphology similar to *T. subfuscipes*, clustered together, forming a sister clade to the rest. All specimens of *T. subsageniacea* from mainland China and Vietnam were well resolved in a clade, forming a sister relationship with the clade containing three specimens of *T. subfuscipes* from Taiwan Island and one *T. subfuscipes*-like specimen from India.

Discussion

A new species supported by molecular and morphological evidence

The results of our morphological comparisons and phylogenetic analyses of plastid sequences support an undescribed species in the *Tectaria fuscipes* group. As shown in the phylogenetic tree (Fig. 5), three specimens of the undescribed species (*Dong 3834*, *4334*, *4430*) formed a relatively independent, well-supported subclade in Clade B. Its herbarium specimens were frequently misidentified as *T. fuscipes* sensu Holtum (1988) or *T. kusukusensis* (Ching 1938 as *Ctenitopsis kusukusensis* (Hayata) Ching). The new species differs from *T. fuscipes* s. l. (including *T. subfuscipes* and *T. subsageniacea*) mainly in the brown or castaneous and relatively broad stipe scales (Fig. 1D) (versus black and narrow-lanceolate, Fig. 1A) and differs from *T. kusukusensis* in its frond-axes (stipe, rachis and costae) bearing sparse and short hairs (ca. 0.5 mm) (versus dense and in 1–1.5 mm long, Fig. 1G). Additionally, the stipe scales appear somewhat different in the two species, being slightly lustrous and brown or castaneous in the new species (Fig. 1D), but dull brown in *T. kusukusensis* (Fig. 1G). The new species is formally described as *T. fungii* in the taxonomic treatment below.

Circumscription and interspecific relationships of the *Tectaria fuscipes* group

The morphological and phylogenetic analyses support the close affinity amongst *T. dissecta*, *T. fungii*, *T. fuscipes*, *T. ingens*, *T. paradoxa*, *T. setulosa*, *T. subfuscipes* and *T. subsageniacea*. These taxa constitute a natural group, namely *T. fuscipes* group (Fig. 5), which is morphologically characterised by the free venation (or sometimes costal-vein-anastomosing venation), fronds with proximal pinnae basiscopically divided with seg-

ment or pinnules elongated and sori being terminal on free veins and in two rows on ultimate segments. The most closely allied species to the *T. fuscipes* group is indicated to be *T. chinensis*, which differs in its high number of anastomosing veins (having costal areoles and additional areoles) and sori on anastomosed veins or non-apical on free veins (Dong et al. 2018b).

Tectaria kusukusensis has the characteristic morphology of the *T. fuscipes* group, but should not be considered as a member of this group. *Tectaria kusukusensis* agrees well with the free-veined *T. fuscipes* and *T. dissecta* in lamina dissection, the shape of basal pinnae, venation and sori arrangement, but differs in having copious hairs on fronds (Shieh 1994; Kuo 1997; Xing et al. 2013). It is distributed in tropical East Asia (Holtum 1988), falling within the distribution range of *T. fuscipes*. However, the phylogenetic analyses in this and previous studies (Zhang et al. 2017; Dong et al. 2018a) consistently resolved *T. kusukusensis* in a different clade from the *T. fuscipes* group. Its close relatives are suggested to be *T. aenigma* (Copel.) C.W. Chen & C.J. Rothf., *T. sabahensis* C.W. Chen & C.J. Rothf. and *T. gymnocarpa* Copel. (Fig. 5), which are all confined to Malesia. It is likely that *T. kusukusensis* had originally been derived in Malesia and later colonised in tropical East Asia.

Within the *T. fuscipes* group, *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* constitute a closely allied subgroup, which is supported by the black linear-lanceolate stipe scales. The closely allied species to them is suggested to be *T. dissecta*, which differs in its broad lanceolate stipe scales and much narrow fronds and is the only species in the *T. fuscipes* group mainly distributed in Malesia to the Pacific Islands (Holtum 1991). The remaining four species, *T. fungii*, *T. ingens*, *T. paradoxa* and *T. setulosa*, constitute another subgroup in the *T. fuscipes* group. Of the four, *T. ingens* and *T. setulosa* are closely allied to each other. The relationships amongst *T. ingens* plus *T. setulosa*, *T. paradoxa* and the new species *T. fungii* currently remain uncertain, which is probably due to the incomplete sequences of the only representative of *T. paradoxa* (*Chiou 15285*) analysed in this study. As shown in the tree produced by Dong et al. (2018a), where *T. paradoxa* was not sampled and complete sequences are available for all accessions in the *T. fuscipes* group, *T. fungii* (then named *Tectaria* sp.1) was well resolved as sister to *T. setulosa*. Therefore, it is expected that the relationships between the new species and other species will be well resolved when better DNA materials are available for *T. paradoxa*.

Indistinct morphology between *Tectaria fuscipes*, *T. subfuscipes* and *T. subsageniacea*

According to the current sampling, the phylogenetic analyses supported *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* as three different lineages (Fig. 5). However, we currently did not find a morphological character which can clearly distinguish one from the other two species. Especially for specimens from Taiwan Island and East Himalayas, they are difficult to be determined as either *T. fuscipes* or *T. subfuscipes*. *Tectaria subfuscipes* was regarded as distinguishable from *T. fuscipes* by the absence of costal areoles and the nearly monomorphic (versus dimorphic) fronds (Kuo 1997). In fact, our examinations showed that the free or anastomosing venation cannot be used

to group specimens from East Himalayas into different taxa because those two states of venation were observed occurring in a single collection there. Similarly, we found that about 15% of specimens from Taiwan Island cannot be identified as *T. fuscipes* or *T. subfuscipes*, based on the variation of venation. It is neither feasible to recognise the three species by the variation of frond dimorphism (monomorphic or dimorphic), because the fertile fronds are contracted to different extents as compared with the sterile fronds in all these species nor is it possible to draw a line between the two states of fronds. In our opinion, *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea* constitute a species complex which are currently indistinguishable in morphology.

We noticed three collections from the same locality (a forest valley in Gaoxiong, Taiwan Island), i.e. *Knapp 3198, 3199, 3200*, which exhibit gradually varied states of frond dimorphism and venation, but were resolved into two clades in the phylogenetic tree (Fig. 5). This result and the comparatively large size of *Knapp 3200* suggest possible hybridisations existed between *T. fuscipes* and *T. subfuscipes*. Unfortunately, we hitherto have very few cytological or reproductive data for these species. Only one specimen (*Kato et al. 2624*) from southern Yunnan was reported by Kato et al. (1992) having 80 ($2x$) somatic chromosome numbers and sexual reproduction. One specimen (*Dong 3557*) also from southern Yunnan was examined having the same number ($2n = 80$) (unpubl. data). To better understand the morphological variations amongst *T. fuscipes*, *T. subfuscipes* and *T. subsageniacea*, more cytological and reproductive data, as well as more sampling in phylogenetic analyses, are needed.

Taxonomic treatment

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

urn:lsid:ipni.org:names:77297479-1

Figs 1D, 4A, 6, 7

Type. China. Hainan: Lingshui, 3–20 May 1932, *H. Fung 20093* (holotype, two sheets, BM-000801750!, BM-000801751!; isotypes: E!, K!, US-01580253!, US-01580666!).

Diagnosis. *Tectaria fungii* is similar to the sympatric species *T. subsageniacea* and *T. kusukusensis*. It differs from *T. subsageniacea* in its broader (1–1.5 mm versus 0.5–1 mm), brown or obviously bicolor (castaneous with brown margins) (versus black) stipe scales and from *T. kusukusensis* by its nearly hairless laminae (versus frond axes abaxially bearing copious hairs).

Description. **Rhizome** short, erect. **Fron**ds slightly dimorphic, rarely obviously dimorphic. **Stipe** stramineous or dark brown, ca. 4 mm in diameter, 50–60 cm long, bearing copious scales towards base and fewer on upper part. **Scales** lanceolate, ca. 6–7 × 1–1.5 mm, brown or castaneous with pale margins. **Lamina** oblong, 55–78 × 30–45 cm, round at base, somewhat suddenly narrowed and acute towards apex, 1-pinnate-pinnatifid, free pinnae (6) 9–12 pairs; basal pinnae triangular, deeply lobed to 1-pinnate



Figure 6. Holotype of *Tectaria fungii* (Fung 20093, BM), sp. nov. **A** frond's upper half **B** frond's lower half **C** details of middle pinnae (abaxial view).

at base, (18) 24–26 × 13–18 cm, shortly stalked (0.5–2 cm), with basal basiscopic 2–3 pinnules free and markedly prolonged (up to 16 × 3.5 cm), pinnatifid upwards, acuminate at apex; suprabasal pinnae linear, 16–24 × 3.8–5 cm, sessile or very shortly stalked, deeply

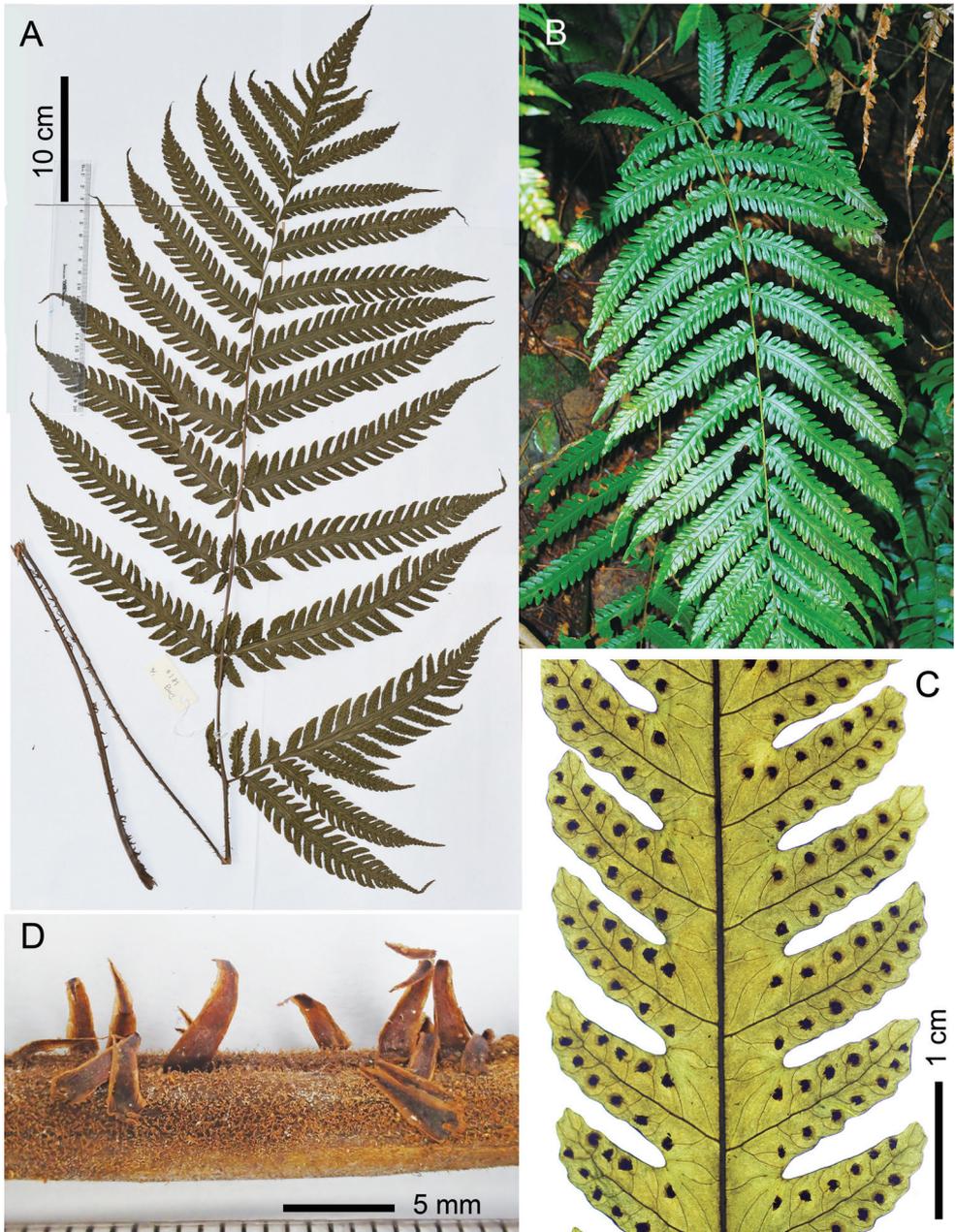


Figure 7. Morphology of *Tectaria fungii*, sp. nov. **A** paratype specimen (*Dong 3834*, IBSC), showing outline of a frond **B** habit in the wild **C** portion of a middle pinna, showing veins and sori **D** scales on basal stipe. (All from *Dong 3834*).

lobed 2/3–3/4 of the way to costae, with a pair or only the basal basiscopic lobes almost free; lobes or pinnules anadromous on basal pinnae and catadromous on pinnae above, basal acroscopic lobes slightly prolonged and parallel to rachis, basal basiscopic lobes obliquely

spreading, lobes entire (except for those on the base of lower pinnae, which are crenate to pinnatifid), obtuse or acute at apex, sterile lobes larger, usually $2\text{--}2.4 \times 0.8\text{--}0.9$ cm, fertile lobes $1.6\text{--}1.8 \times 0.6$ cm. **Veins** free, simple or mostly once forked. **Hairs** short, relative dense on adaxial surface and sparse on abaxial surface of costae; no hairs on abaxial surface between veins; with a few hairs on adaxial surface of lamina, especially on margin and at sinus between lobes. **Sori** terminal on simple veins or on the acroscopic branch of a forked vein, in one row on either side of mid-rib of lobes, medial between mid-rib and margin of lobes. **Indusia** round-reniform, ca. 1 mm in diameter, persistent, usually ciliate at margin.

Additional specimens examined. CHINA. **Hainan:** Baisha, *S.Y. Dong* 728 (PE); Baoting, *G.A. Fu* 2951 (IBSC); Ledong, *S.Y. Dong* 1589 (IBSC); Qiongzhong (Mt. Limushan), *S.Y. Dong* 832 (PE); Mt. Wuzhishan, *C. Wang* 35347 (IBK, IBSC, PE); *S.Y. Dong* 5096 (IBSC), *Wuzhishan Fern Survey* 036, 176, & 498 (PE); Mt. Yinggeling, *S.Y. Dong* 3834, 3842, 3867, 4430 (IBSC). **Yunnan:** Jinghong, *B.G. Li* 98162 (HITBC), *Q.J. Li* 42730 (HIBTC); Menghai, *W.M. Chu et al.* 15749 (GAUA, PYU), *H. Shang* SG2638 (CSH), *X.L. Zhou* 5727, 5731 (CSH); Mengla (Bubeng), *S.Y. Dong* 4307, 4334, 4825 (IBSC). VIETNAM. **Dak Nong:** Dak Plao, *L. Averyanov et al.* 5589 (HN), 5601 (HN, HNU).

Distribution and Habitat. China (Hainan, southern Yunnan) and Vietnam (Dak Nong); terrestrial in montane rainforest, occurring in dense-shady and wet slopes, elev. 600–1300 m, locally common.

Etymology. The specific epithet honours Mr. Hom Fung, who collected lots of plant specimens in Hainan and Guangdong, southern China in 1930s. This species was probably first collected by him from Hainan in 1932.

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Appendix I

List of *Tectaria* samples used for phylogenetic analyses in this study with voucher information (collection number, herbarium and locality) and GenBank numbers for *rbcL*, *atpB*, *rps16–matK* plus *matK*, *ndhF* plus *ndhF-trnN* and *trnL-F*. GenBank numbers for newly-generated sequences are in bold and a dash indicates data absent.

Tectaria aenigma (Copel.) C.W. Chen & C.J. Rothf., *DNK 575* (UC), Philippines, KY927533, –, KJ196548, –, KY927538. *Tectaria aurita* (Sw.) S. Chandra, *Dong 3386* (IBSC), Indonesia (Java), KJ196849, KJ196404, KJ196548, KJ196762, KJ196631. *Tectaria austrosinensis* (Christ) C. Chr., *Dong 3602* (IBSC), China (Yunnan), KJ196899, KJ196447, KJ196516, KJ196804, KF561670; *Dong 3617* (IBSC), China (Yunnan), KJ196847, KJ196446, KJ196517, KJ196803, KJ196629. *Tectaria borneensis* S.Y. Dong, *Dong 3438* (IBSC), Indonesia (Java), KJ196854, KJ196489, KJ196514, KJ196767, KJ196642. *Tectaria chinensis* (Ching & Chu H. Wang) Christenh., *Dong 4291* (IBSC), China (Yunnan), **MH542574, MH542584, MH542595, MH542606, MH542618**; *Dong 4343* (IBSC), China (Yunnan), MF623757, MF623685, MF623709, MF623733, MF623780; *Dong 4855* (IBSC), Vietnam, **MH542575, MH542585, MH542596, MH542607, MH542619**. *Tectaria dissecta* (G. Forst.) Lellinger, *CYH 20140503014* (IBSC, TAIF), China (Taiwan), **MH542570, MH542580, MH542591, MH542603, MH542614**; *Dong 4101* (IBSC), Philippines (Luzon), AWD73648, AWD73600, –, AWD73624, –. *Tectaria fungii* S.Y. Dong (sp. nov.), *Dong 3834* (IBSC), China (Hainan), KJ196826, KJ196505, KJ196591, KJ196751, KJ196703; *Dong 4334* (IBSC), China (Yunnan), **MH542571, MH542581, MH542592, MH542604, MH542615**; *Dong 4430* (IBSC), China (Hainan), **W795604**, –, **MW795617, MW795625**, –. *Tectaria fuscipes* (Wall. ex Bedd.) C. Chr., *CYH 20140322019* (IBSC, TAIF), China (Taiwan), **MH542568, MH542578, MH542589, MH542601, MH542612**; *CYH 20140503020* (TAIF), China (Taiwan), **MH542569, MH542579, MH542590, MH542602, MH542613**; *Dong 4271* (IBSC), China (Yunnan), **OL828756, OL828758, NA, OL963688, OL828760**; *Dong 5187* (IBSC), Bangladesh, –, –, –, **MW795618, MW795626**; *Dong 5194* (IBSC), Bangladesh, **MW795597, MW795605, MW795611, MW795619, MW795627**; *Knapp 3198* (P), China (Taiwan), KY937334, –, KY937227, –, KY937497. *Tectaria gigantea* (Blume) Copel., *Dong 3380* (IBSC), Indonesia (Java), KJ196853, KJ196403, KJ196530, KJ196737, KJ196660. *Tectaria gymnocarpa* Copel., *Dong 3967* (IBSC), Papua New Guinea (Kimbe), MF623765, MF623693, MF623717, MF623741, MF623786. *Tectaria heterocarpa* (Bedd.) C.V. Morton, *Dong 5189* (IBSC), Bangladesh, **MW795598, MW795606, MW795612, MW795620, MW795628**. *Tectaria ingens* (Atk. ex C.B. Clarke) Holttum, *Zuo 4218* (KUN), China (Yunnan), **MW795599, MW795607, MW795613, MW795621, MW795629**. *Tectaria kusukusensis* (Hayata) Lellinger, *CYH 20111228044* (IBSC, TAIF), China (Taiwan), MF623770, MF623698, MF623722, MF623746, MF623790; *Knapp 3336* (IBSC), China (Taiwan), **MH542573, MH542583, MH542594**, –, **MH542617**; *Zhang 6466* (CDBI, MO, VNMN), Vietnam, KP271079, –, KU605135, –, KP271096. *Tectaria multi-caudata* (C.B. Clarke) Ching, *CYH 20080816006* (IBSC, TAIF), China (Taiwan), **MH542572, MH542582, MH542593, MH542605, MH542616**; *Wade 1382* (IBSC), Vietnam, KJ196834, KJ196425, KJ196558, KJ196756, KJ196713. *Tectaria nayarii* Mazumdar, *Wade s.n.* (TAIF), Philippines (Luzon), KJ196823, KJ196405, KJ196594, KJ196722, KJ196699. *Tectaria paradoxa* (Fée) Sledge, *Chiou 15285*

(TAIF), India, **MW795600**, –, –, –, **MW795630**. *Tectaria polymorpha* (Wall. ex Hook.) Copel., *Dong 3554* (IBSC), China (Yunnan), KJ196889, KJ196477, KJ196524, KJ196794, KJ196657. *Tectaria profereoides* (Christ) S.Y. Dong, *Dong 4939* (IBSC), Malaysia, **MW795601**, **MW795608**, **MW795614**, **MW795622**, **MW795631**; *Dong 4961* (IBSC), Malaysia, **MW795602**, **MW795609**, **MW795615**, **MW795623**, **MW795632**. *Tectaria psomiocarpa* S.Y. Dong, *Chen s.n.* (IBSC), Philippines (Luzon), KJ196822, KJ196502, KJ196595, KJ196723, KJ196698. *Tectaria sabahensis* C.W. Chen & C.J. Rothf., *Wade 4705* (TAIF), Malaysia, KY927534, –, KY927535, –, KY927537. *Tectaria sagenioides* (Mett.) Christenh., *Dong 1599* (IBSC), China (Hainan), KJ196896, KJ196436, KJ196550, KJ196760, KJ196625; *Dong 3603* (IBSC), China (Yunnan), KJ196897, KJ196437, KJ196518, KJ196801, KF561672. *Tectaria setulosa* (Baker) Holttum, *Dong 3049* (IBSC), China (Guangdong), –, KJ196428, KJ196527, KJ196791, KJ196670; *Jin et al. 282* (IBSC, PE), China (Yunnan), –, KJ196427, KJ196557, KJ196757, KJ196714. *Tectaria subfuscipes* (Tagawa) C.M. Kuo, *CYH 20140503023* (IBSC, TAIF), China (Taiwan), **MH542566**, –, **MH542587**, **MH542599**, **MH542610**; *Deng D20140518002* (IBSC, TAIF), China (Taiwan), **MH542567**, **MH542577**, **MH542588**, **MH542600**, **MH542611**; *Knapp 3199* (P), China (Taiwan), KY937373, –, KY937273, –, KY937563. *Tectaria subglabra* (Holttum) S.Y. Dong, *Dong 3450* (IBSC), Indonesia (Java), KJ196807, KJ196406, KJ196532, KJ196738, KJ196676. *Tectaria sublageniacea* (Christ) Christenh., *Chao 1553* (IBSC), Vietnam, KJ196900, KJ196414, KJ196560, KJ196733, KJ196711; *Dong 3557* (IBSC), China (Yunnan), KJ196880, KJ196415, KJ196529, KJ196782, KJ196665; *Dong 3856* (IBSC), China (Hainan), MF623767, MF623695, MF623719, MF623743, MF623788; *Dong 4585* (IBSC), Vietnam, **MH542564**, **MH542576**, –, **MH542597**, **MH542608**; *Dong 4587* (IBSC), Vietnam, **MH542565**, –, **MH542586**, **MH542598**, **MH542609**. *Tectaria cf. subfuscipes*, *Fraser-Jenkins FN153* (TAIF), India (Meghalaya), **OL828757**, **NA**, **OL828759**, **OL963689**, **OL828761**; *Knapp 3200* (P), China (Taiwan), KY937374, –, –, –, KY937564; *Tectaria sp.*, *Wade 4087* (IBSC), Solomon Islands, **MW795603**, **MW795610**, **MW795616**, **MW795624**, **MW795633**.

Aster quanzhouensis (Asteraceae), a new riparian species from eastern China

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Abstract

Aster quanzhouensis **sp. nov.** (Asteraceae) from Fujian, eastern China, is described and illustrated. It grows on rocks in the riparian zone. Morphological, cytological and molecular investigations of *A. quanzhouensis* were carried out. The morphological data and phylogenetic analysis based on combined ITS, ETS and *trnL-F* dataset suggest that *A. quanzhouensis* is a separate species closely related to *A. tonglingensis*. The new species differs from the latter by the shorter stem length, leaf morphology, colour of phyllaries, number of ray florets, and achene shape. The cytological observation shows that the new species is diploid with a karyotype of $2n = 18$.

Keywords

Asteraceae, *Aster quanzhouensis*, new species, taxonomy

Introduction

The genus *Aster* L. in its recent circumscription is restricted to Eurasia and comprises ~150 species, of which 123 occur in China (Chen et al. 2011), a main diversity centre of *Aster* (Li et al. 2012). Recently, ten new *Aster* species have been described, and almost all these species have a narrow distribution pattern known from only one or two populations in different regions of China (Zhang et al. 2015, 2019; Li et al. 2017, 2020; Xiao et al. 2019a, b, 2020, 2021; Xiong et al. 2019).

Recently, Guo-Jiao Yan, a young amateur naturalist and one of the authors of this paper, collected some unique samples from the riversides of the Min and Jin rivers, Quanzhou city, Fujian, eastern China. The morphological, cytological and phylogenetic data show that the specimens represent an undescribed species, which is reported herein.

Materials and methods

Material collection

Specimens of the new taxon were collected in Dehua and Yongchun counties (Fig. 1), Fujian, China. We collected leaf material and dried it with silica gel for molecular experiments. The voucher specimens were deposited at the Herbarium of Hunan Normal University (HNNU) and Jiangxi Agricultural University (JXAU).

Morphological observations

The description of the new species is based on living material, dry specimens and FAA-fixed materials. Twenty-one individuals were examined. The morphological comparison with *Aster tonglingensis* G.J.Zhang & T.G.Gao is based on the study of herbarium specimens, from PE, HNNU and JAXU. We compared the shape and size of the leaves, length of stems, phyllaries, number of florets, and achenes.

Cytology

Excised root tips from the cultivated plants of the new species were pretreated with 0.1% colchicine at 10 °C for 4 h, then fixed in Carnoy's solution (95% ethanol and glacial acetic acid in 3:1 ratio) at 20 °C for 12 h. The root tips were then macerated in 1 M hydrochloric acid at 60 °C for 10 min, stained in Carbol fuchsin solution, washed in distilled water for 30 min and finally depigmented and squashed in 45% acetic acid (Li et al. 2011). Karyotype formulae were calculated based on measurements of mitotic metaphase chromosomes taken from photographs. The symbols used to describe the karyotypes followed Levan et al. (1964).

Taxon sampling, DNA extraction, PCR reaction and sequencing

Nuclear ribosomal DNA ITS and ETS sequences and plastid DNA *trnL-F* sequences of 66 species and varieties, representing major clades of the genus *Aster* and its relatives (Li et al. 2012, 2017, 2020; Zhang et al. 2015, 2019; Xiao et al. 2019a, b, 2020, 2021), were downloaded from GenBank (Appendix 1). Besides, eleven newly sequenced accessions are included from Dehua and Yongchun counties two individuals of *Aster quanzhouensis* (Appendix 1). The names of the taxa mentioned above follow Chen et al. (2011). *Grangea maderaspatana* (L.) Poir. and *Dichrocephala integrifolia* (L.f.) Kuntze were selected as outgroups following

Li et al. (2012). Voucher specimens of newly sequenced material were deposited in HNNU. Total DNA extraction, PCR and sequencing were carried out according to Li et al. (2012).

Phylogenetic analysis

Boundaries of the ITS, ETS and *trnL-F* regions were determined through comparison with previously published sequences (Li et al. 2012). DNA sequences were aligned initially using Clustal X1.83 (Jeanmougin et al. 1998), performed by MUSCLEv3.8.31 (Edgar 2004), and adjusted manually in PhyDE ver0.9971 (Müller et al. 2010). The optimal model of DNA substitutions was selected using the Akaike information criterion (Akaike 1973) as applied in jModelTest 2.1.4 (Darriba et al. 2012) prior to the maximum likelihood (ML) analyses and Bayesian inference (BI). The best fit models for ITS, ETS and *trnL-F* were GTR + G, GTR + I + G and TVM+I, respectively. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted using RAxML 7.2.6 and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Stamatakis 2006), respectively. For BI, four chains, each starting with a random tree, were run for 1,000,000 generations with trees sampled every 1000 generations. The average standard deviation of split frequencies (< 0.01) was used to assess the convergence of the two runs. After the first ca. 25% discarded as burn-in, the remaining trees were imported into PAUP* v.4.0b10 and a 50% majority-rule consensus tree was produced to obtain posterior probabilities (PP) of the clades. Before the datasets were combined, the incongruence length difference test (Farris et al. 1994) was performed on PAUP* v.4.0b10 (Swofford 2001).

Results

Aster quanzhouensis M.Tang, G.J.Yan & W.P.Li, sp. nov.

urn:lsid:ipni.org:names:77297480-1

Figs 1–3

Type. CHINA, Fujian province, Quanzhou city, Dehua county, Nancheng town, alt. ca. 500 m, 25°34.20'N, 118°29.65'E, 5 Oct 2021, Guo-Jiao Yan, YGJ2110003 (Holotype: HNNU!, isotypes: HNNU!, JXAU!) (Fig. 3).

Additional collection seen. CHINA. Fujian province, Quanzhou city, Yongchun county, alt. ca. 500 m, 25°24'N, 118°21'E, 30 Nov 2021, Guo-Jiao Yan, YGJ21113001 (HNNU!).

Diagnosis. *Aster quanzhouensis* differs from *A. tonglingensis* by its stems only 21–30 (60) cm (vs. 70–100 cm) long, narrowly lanceolate (vs. lanceolate) rosulate leaves, purplish-red (vs. green) apices of the phyllaries, 9–20 (40) (vs. more than 30) capitula, 7–11 (vs. ca. 15) ray florets, 11–14 × ca. 2 mm (vs. 7–10 × ca. 2 mm) lamina, two- or three-ribbed (vs. 4-ribbed) achenes and flowering period (Sep to early Dec vs. Jul) (Figs 2, 3, Table 1).

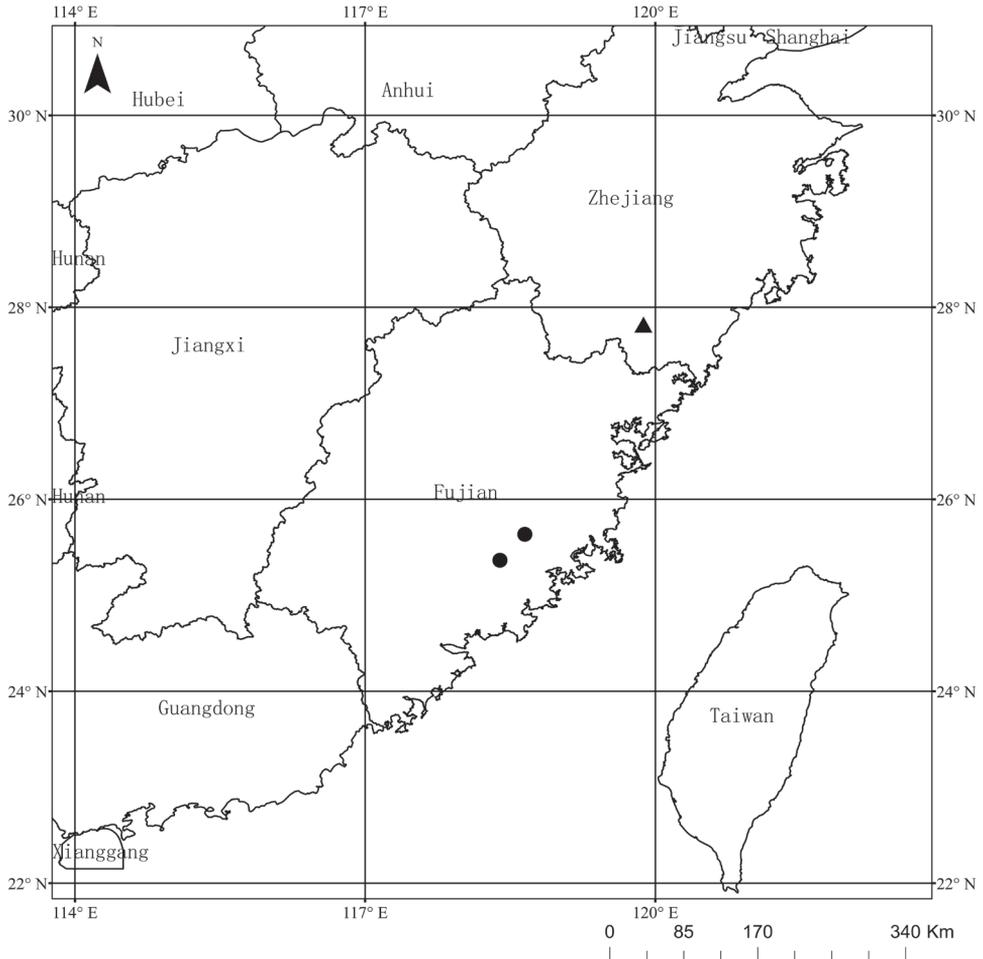


Figure 1. Distribution of *Aster quanzhouensis* (black circle) and *A. tonglingensis* (black triangle).

Description. Perennial herb, 21–30 (60) cm high. Rhizomes thin, with adventitious roots, stolons absent. Stem solitary, erect, unbranched except for inflorescence, glabrous or puberulent in upper part. Leaves slightly leathery, narrowly lanceolate, apex acute, base gradually narrowing, subclasping, abaxially light green, adaxially dark green and glossy, 3-veined, midvein abaxially prominent; rosulate leaves 4–13 × 0.4–1.7 cm, margin serrate, petiole 3–11 cm long, both surfaces glabrous; lower cauline leaves persistent at anthesis or rarely withered, 3–10 × 0.3–0.8 cm, sessile or with obscure petioles, margin entire or serrate, abaxially glabrous, adaxially sparsely strigose; middle cauline leaves sessile or with obscure petiole, 4–7 × 0.3–0.4 cm, margin entire or serrate, abaxially glabrous, adaxially sparsely strigose; upper leaves sessile, margin entire. Capitula 9–20 (40) in a terminal corymbose cyme, peduncle puberulent. Involucre campanulate, 5–8 mm in diameter; phyllaries in 5–7 rows, imbricate, lanceolate,

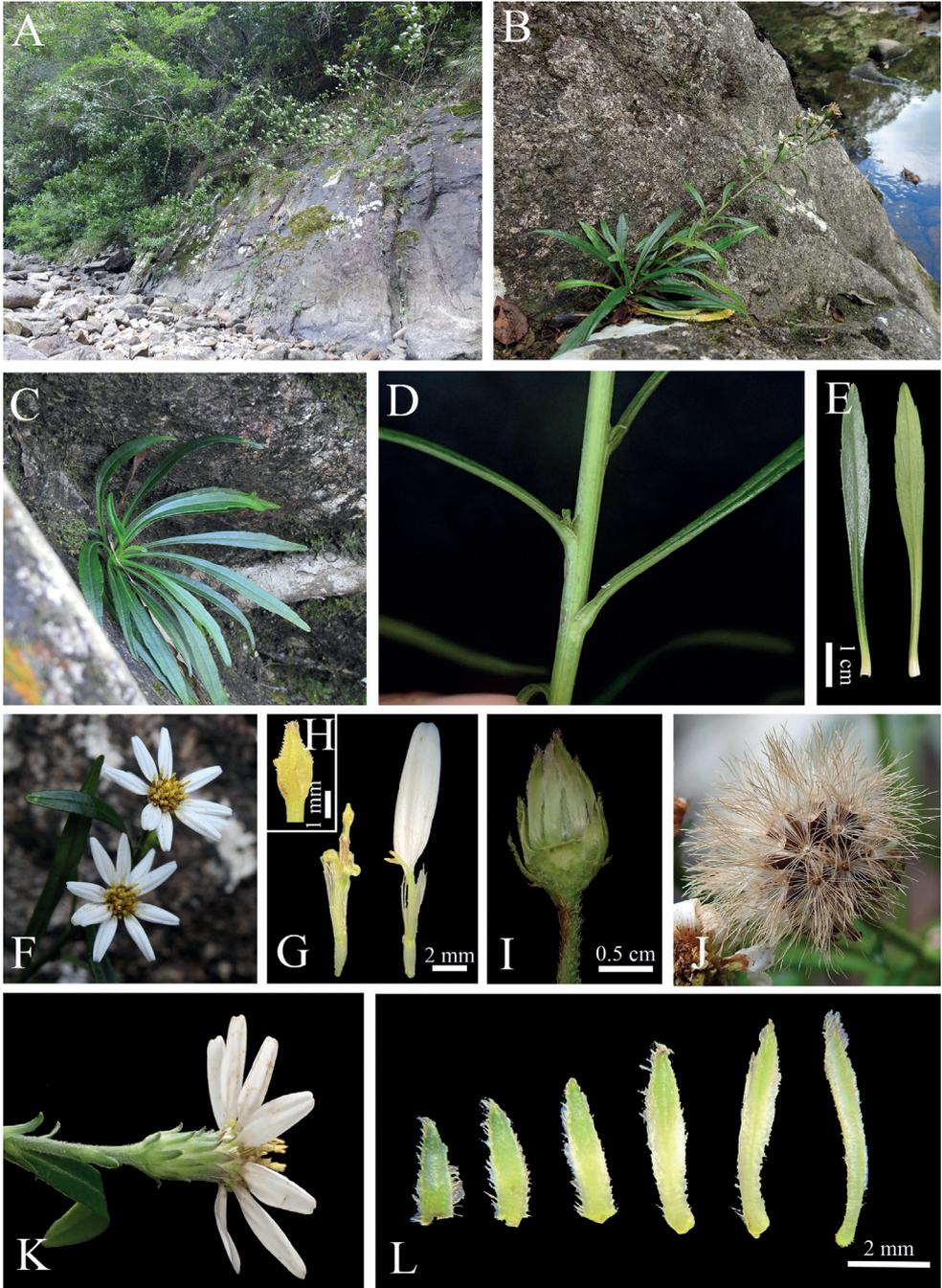


Figure 2. Images of living plants of *Aster quanzhouensis* **A** habitat **B** habit **C** seedling **D** stem **E** rosulate leaves **F** top view of the capitulum **G** a disk floret (left) and a ray floret (right) **H** style branches of a disk floret **I** receptacle **J** fruits **K** dorsal view of a capitulum **L** phyllaries (from outer to inner, left to right).



Figure 3. Holotype of *Aster quanzhouensis* M.Tang, G.J.Yan & W.P.Li.

the outer rows shorter than the inner ones, reflexed, densely pilose, with ciliate margin; outer phyllaries 3.2–6.2 × 1.1–2 mm; middle phyllaries 4.6–13 × 1.5–2.2 mm, with narrowly scarious margin, tip purplish-red; inner phyllaries 10.1–13.0 × 1.5–1.7 mm, with broadly scarious margin, tip purplish-red. Receptacles flat, alveolate. Ray florets 7–11, female, tube ca. 4 mm, glabrous, ligules whitish, lanceolate, 11–14 × ca. 2 mm, with four nerves, apex with two or three teeth. Disc florets (11) 18–24, hermaphrodite, yellow, tube puberulent, ca. 3 mm, thin but expanded at base, 5-lobed, lobes spreading to reflexed, narrowly triangular, unequal, 1.1–1.5 mm, glandular; anthers ca. 1.8 mm (excluding collar), apical appendage 0.35–0.45 mm long, narrowly lanceolate, anther collar ca. 0.4 mm long; style arm appendage lanceolate, ca. 2.5 mm, stigmatic lines 1.2–1.4 mm, equal to the sterile style tip appendages. Achenes 4.5–5.5 × 0.9–1.4 mm, narrowly oblong, strigose, eglandular, two- or three-ribbed. Pappus uniseriate, dirty white, 8–11 mm, nearly as long as disc corolla at anthesis.

Phenology. Flowering from September to early December and fruiting from October to December.

Etymology. The species is named after its type locality, Quanzhou city, Fujian province, China.

Vernacular name. quán zhōu zǐ wǎn (Chinese pronunciation); 泉州紫苑 (Chinese name).

Distribution and habitat. *Aster quanzhouensis* is known from Dehua and Yongchun counties, Quanzhou city, Fujian province, China. The new species grows on rocks in riparian habitats at an altitude of ca. 500 m a.s.l.

Conservation status. *Aster quanzhouensis* seems to be a narrowly distributed species, currently known only in rocky areas along two streams (Jin river and Min river) in Quanzhou city, and each population with ca. 150 (total < 1000) individuals were found. The habitat of *A. quanzhouensis* is easily disturbed or damaged. Further fieldwork is needed to evaluate the exact distribution of the species, and it is possible that other populations could be found in similar habitats of the Jin and Min rivers. Therefore, we only temporarily assign the species to the category DD (Data Deficient) according to the International Union for Conservation of Nature (IUCN 2022).

Table 1. Comparison of *Aster quanzhouensis* and *A. tonglingensis*. The data of the latter species were taken from Zhang et al. (2019).

Characters	<i>Aster quanzhouensis</i>	<i>A. tonglingensis</i>
Stem	21–30(60) cm, solitary	70–100 cm, solitary or two to three
Basal leaves	narrowly lanceolate, 4–13 × 0.4–1.7 cm	lanceolate, 4–18 × 0.8–2.5 cm
Capitula	9–20 (40)	More than 30
Phyllaries	5–7-seriate, apex purplish-red	5–7-seriate, apex green
Ray florets	7–11	ca. 15
Achenes	2–3-ribbed	4-ribbed
Pappus	8–11 mm	ca. 7 mm
Flowering period	Sep to early Dec	Jul

Cytology

The somatic chromosomes of the new species at metaphase are illustrated in Fig. 4. The two populations have a same karyotype formula, $2n = 18$, and Stebbins' 1A-type (Stebbins 1971), but differs in ratio of long to short arm of chromosomes (the former is 1.02–1.55, while the latter 1.06–1.45), the chromosomes length (the former is 1.49–2.72, while the latter 1.71–2.77), and the AI value (the former is 0.54, while the latter 0.57).

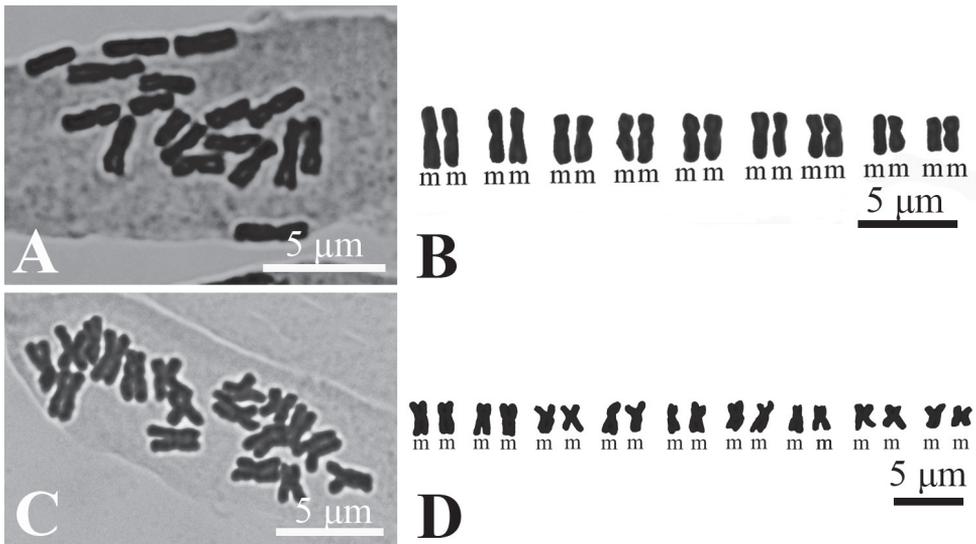


Figure 4. Micrographs of somatic metaphase chromosomes (**A**, **C**) and the karyotype (**B**, **D**) of *Aster quanzhouensis* from two different populations. (**A**, **B** Dehua county, Quanzhou, Fujian, China; **C**, **D** Yongchun county, Quanzhou, Fujian, China).

Molecular phylogeny

The aligned lengths of ITS, ETS and *trnL-F* are 647 bp, 568 bp and 957 bp, respectively, yielding a concatenated alignment of 2172 bp. Character state changes were equally weighted and gaps were treated as missing data. ML and BI analyses produced similar topology and only the ML tree was presented in Fig. 5, with ML bootstrap (LP), and PP values for each clade. The phylogenetic results showed that the two samples of the new taxon were grouped together with strong support (PP = 1.00, LP = 100%) and are closely related to *Aster tonglingensis* with strong support (PP = 1.00, LP = 99%). According to these results, *A. quanzhouensis* is nested within the core *Aster* clade (PP = 1.00, LP = 100%) that is the redefined genus *Aster* in Eurasia (Li et al. 2012; Nesom 2020a, b).

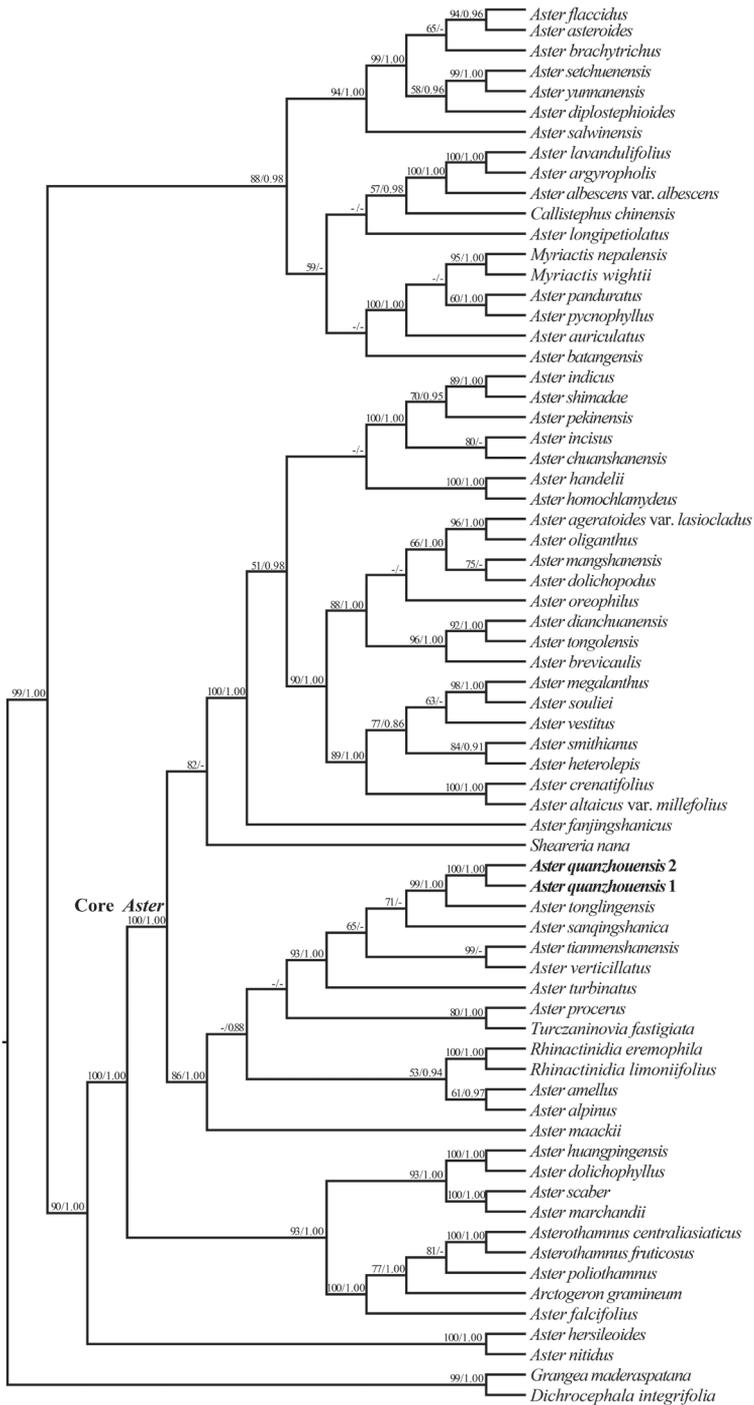


Figure 5. The phylogram of the maximum likelihood (ML) tree from the combined data (ITS, ETS and *trnL-F*), showing the phylogenetic position of *Aster quanzhouensis*. Bootstrap support values (1,000 replicates) for maximum parsimony (MP \geq 50%, left) and Bayesian posterior probabilities (PP \geq 0.90, right) are provided above the branches. The samples of *Aster quanzhouensis* are shown in bold.

Discussion

Morphological observations showed that *Aster quanzhouensis* has a perennial life form, lanceolate stigmatic appendage of disc florets, compressed fruits with two- or three-ribbed and uniseriate pappus (Figs 2, 3). All *Aster* species share these characters. In the phylogenetic tree (Fig. 5), *A. quanzhouensis* is deeply nested within the core *Aster* (Li et al. 2012). Morphological and phylogenetic analyses support that *A. quanzhouensis* is sister to *A. tonglingensis*. As mentioned above, the two species can be easily distinguished from each other (Figs 2, 3, Table 1).

Narrowly lanceolate leaves are rare in Eurasian *Aster* and can be found only in a few species, such as *A. huangpingensis* W.P.Li & Z.Li, *A. dolichophyllus* Y.Ling and *A. tonglingensis*. Our phylogenetic analyses (Fig. 5) revealed that the species with narrowly lanceolate leaves are nested in unrelated lineages of the genus *Aster* and are probably the result of convergent evolution. It is noteworthy to mention that they are all distributed in the same habitats confined to riparian rocks (Chen et al. 2011; Zhang et al. 2019; Li et al. 2020). The same leaf character may be related to their habitat. When the water level rises in some periods during the course of the year, these species were submerged and their narrowly lanceolate leaves may represent adaptation to water flowing in the rivers or streams.

Karyotype variation usually accompanies evolutionary divergence, a general phenomenon observed in plants and animals (Rieseberg 2001). Two populations of the *Aster quanzhouensis* were found with the same karyotype formula and Stebbins' type, with only slight differences in the karyotypic indexes, which might mean that *A. quanzhouensis* is a young species.

Aster quanzhouensis is known only from two populations (Dehua and Yongchun counties) restricted to Quanzhou, Fujian, China, while *A. tonglingensis* is restricted to Mt. Tongling Natural Reserve, Wencheng county, Zhejiang (Zhang et al. 2019). These two species occupy the same ecological conditions, but are geographically separated by a distance of 400 km.

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Appendix I

Table A1. Taxa sampled, vouchers and GenBank accessions. The newly sequenced samples are highlighted in bold.

Accessions	Voucher information or references	Accession number		
		ITS	ETS	<i>trnL-F</i>
<i>Aster quanzhouensis</i> M.Tang, G.J.Yan & W.P.Li 1	<i>Guo-Jiao Yan, YGJ2110001</i> , Dehua county, Fujian, China	ON055150	ON055152	ON055154
<i>Aster quanzhouensis</i> M.Tang, G.J.Yan & W.P.Li 2	<i>Guo-Jiao Yan, YGJ2112001</i> , Yongchun county, Fujian, China	ON055151	ON055153	ON055155
<i>A. sanqingshanica</i> J.W.Xiao & W.P.Li	Xiao et al. (2021)	MW419955	MW419952	ON055156
<i>A. marchandii</i> H.Lév.	Xiao et al. (2021)	MW419957	MW419954	ON055157

Accessions	Voucher information or references	Accession number		
		ITS	ETS	<i>trnL-F</i>
<i>A. dianchuanensis</i> J.W.Xiao & W.P.Li	Xiao et al. (2019b)	MK693180	MK693190	MK693202
<i>A. brevicaulis</i> W.P.Li	Xiao et al. (2019a)	MH638204	MH638209	MH638218
<i>A. tongolensis</i> Franch.	Xiao et al. (2019b)	MK693183	MK693193	JN543834
<i>A. ageratoides</i> var. <i>lasiocladus</i> (Hayata) Hand.-Mazz.	Li et al. (2012)	JN543781	JN543782	JN543783
<i>A. oliganthus</i> W.P.Li & Z.Li	Li et al. (2017)	KY428860	KY428852	MH638219
<i>A. mangshanensis</i> Y.Ling	Li et al. (2012)	JN543760	JN543761	JN543762
<i>A. oreophilus</i> Franch.	Li et al. (2012)	JN543826	JN543827	JN543828
<i>A. dolichopodus</i> Y.Ling	Li et al. (2012)	JN543775	JN543776	JN543777
<i>A. vestitus</i> Franch.	Li et al. (2012)	JN543769	JN543770	JN543771
<i>A. souliei</i> Franch.	Li et al. (2012)	JN543835	JN543836	JN543837
<i>A. megalanthus</i> Y.Ling	Xiao et al. (2019b)	MK693187	MK693197	MK693207
<i>A. smithianus</i> Hand.-Mazz.	Li et al. (2012)	JN543778	JN543779	JN543780
<i>A. heterolepis</i> Hand.-Mazz.	Li et al. (2012)	JN543823	JN543824	JN543825
<i>A. altaicus</i> var. <i>millefolius</i> (Vaniot) Hand.-Mazz.	Li et al. (2012)	JN543709	JN543710	JN543711
<i>A. crenatifolius</i> Hand.-Mazz.	Li et al. (2012)	JN543712	JN543713	JN543714
<i>A. fanjingshanicus</i> Y.L.Chen & D.J.Liu	Li et al. (2012)	JN543829	JN543830	JN543831
<i>A. pekinensis</i> (Hance) F.H.Chen	Li et al. (2012)	JN543718	JN543719	JN543720
<i>A. shimadae</i> (Kitamura) Nemoto	Xiao et al. (2020)	MT731682	MT731599	ON055158
<i>A. indicus</i> L.	Li et al. (2012)	JN543715	JN543716	JN543717
<i>A. incisus</i> Fisch.	Li et al. (2012)	JN543721	JN543722	JN543723
<i>A. chuanshanensis</i> W.P.Li	Xiao et al. (2020)	MT731676	MT731593	ON055159
<i>A. homochlamydeus</i> Hand.-Mazz.	Li et al. (2012)	JN543784	JN543785	JN543786
<i>A. handelii</i> Onno	Li et al. (2012)	JN543820	JN543821	JN543822
<i>A. maackii</i> Regel	Li et al. (2012)	JN543745	JN543746	JN543747
<i>A. turbinatus</i> S.Moore	Li et al. (2012)	JN543814	JN543815	JN543816
<i>A. verticillatus</i> (Reinw.) Brouillet	Li et al. (2012)	JN543706	JN543707	JN543708
<i>A. tianmenshanensis</i> G.J.Zhang & T.G.Gao	Zhang et al. (2015)	KP313677	KP313690	KP313703
<i>A. tonglingensis</i> G.J.Zhang & T.G.Gao	Zhang et al. (2019)	MH807119	MH807124	MH807126
<i>A. procerus</i> Hemsl.	Zhang et al. (2015)	KP313683	KP313696	KP313709
<i>A. amellus</i> Grierson	Li et al. (2012)	JN543742	JN543743	JN543744
<i>A. alpinus</i> L.	Li et al. (2012)	JN543817	JN543818	JN543819
<i>A. falcifolius</i> Hand.-Mazz.	Li et al. (2012)	JN543802	JN543803	JN543804
<i>A. poliothamnus</i> Diels	Li et al. (2012)	JN543763	JN543764	JN543765
<i>A. scaber</i> Thunb.	Li et al. (2012)	JN315934	JN315958	JN315910
<i>A. huangpingensis</i> W.P.Li & Z.Li	Li et al. (2020)	MH747070	MH747071	ON055160
<i>A. dolichophyllus</i> Y.Ling	Zhang et al. (2019); Li et al. (2020)	MH747068	MH747069	MH807108
<i>A. hersileoides</i> C.K.Schneid.	Li et al. (2012)	JN543787	JN543788	JN543789
<i>A. nitidus</i> C.C.Chang	Li et al. (2012)	JN543790	JN543791	JN543792
<i>A. salwinensis</i> Onno	Zhang et al. (2015)	KP313689	KP313702	KP313715
<i>A. diplostephioides</i> (DC.) Benth. ex C.B.Clarke	Li et al. (2012)	JN543847	JN543848	JN543849
<i>A. setchuenensis</i> Franch.	Li et al. (2012)	JN543850	JN543851	JN543852
<i>A. yunnanensis</i> Franch.	Li et al. (2012)	JN543853	JN543854	JN543855
<i>A. brachytrichus</i> Franch.	Li et al. (2012)	JN543838	JN543839	JN543840
<i>A. asteroides</i> (DC.) Kuntze	Li et al. (2012)	JN543841	JN543842	JN543843
<i>A. flaccidus</i> Bunge	Li et al. (2012)	JN543844	JN543845	JN543846
<i>A. batangensis</i> Bureau & Franch.	Li et al. (2012)	JN543859	JN543860	JN543861

Accessions	Voucher information or references	Accession number		
		ITS	ETS	<i>trnL-F</i>
<i>A. panduratus</i> Nees ex Walp.	Li et al. (2012)	JN543757	JN543758	JN543759
<i>A. auriculatus</i> Franch.	Li et al. (2012)	JN543754	JN543755	JN543756
<i>A. pycnophyllus</i> Franchet ex W.W.Sm.	Li et al. (2012)	JN543799	JN543800	JN543801
<i>A. longipetiolatus</i> C.C.Chang	Li et al. (2012)	JN315936	JN315960	JN315912
<i>A. lavandulifolius</i> Hand.-Mazz.	Li et al. (2012)	JN543796	JN543797	JN543798
<i>A. argyropholis</i> Hand.-Mazz.	Li et al. (2012)	JN543793	JN543794	JN543795
<i>A. albescens</i> (DC.) Wall. ex Hand.-Mazz. var. <i>albescens</i>	Li et al. (2012)	JN543862	JN543863	JN543864
<i>Sheareria nana</i> S.Moore	Li et al. (2012)	JN543703	JN543704	JN543705
<i>Arctogeron gramineum</i> (L.) DC.	Li et al. (2012)	JN315928	JN315952	JN315904
<i>Asterothamnus fruticosus</i> (C.Winkl.) Novopokr.	Li et al. (2012)	JN315929	JN315953	JN315905
<i>A. centraliasiatricus</i> Novopokr.	Li et al. (2012)	JN315930	JN315954	JN315906
<i>Callistephus chinensis</i> (L.) Nees	Li et al. (2012)	JN315931	JN315955	JN315907
<i>Myriactis wightii</i> DC.	Li et al. (2012)	JN315922	JN315946	JN315898
<i>M. nepalensis</i> Less.	Li et al. (2012)	JN315921	JN315945	JN315897
<i>Rhinactinidia limoniifolia</i> (Less.) Novopokr. ex Botsch.	Li et al. (2012)	JN543724	JN543725	JN543726
<i>Rh. eremophila</i> (Bunge) Novopokr. ex Botsch.	Li et al. (2012)	JN543727	JN543728	JN543729
<i>Turczaninovia fastigiata</i> (Fisch.) DC	Li et al. (2012)	JN543739	JN543740	JN543741
<i>Grangea maderaspatana</i> (L.f.) Kuntze	Li et al. (2012)	JN315920	JN315944	JN315896
<i>Dichrocephala integrifolia</i> (L.) Poir.	Li et al. (2012)	JN315919	JN315943	JN315895

Three new species of *Bredia* (Sonerileae, Melastomataceae) from the Sino-Vietnamese border area

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Abstract

Bredia bullata, *B. enchengensis*, and *B. nitida* (Sonerileae, Melastomataceae), three species occurring in Sino-Vietnamese limestone karst regions, are described as new. Molecular phylogenetic analyses and morphological divergence indicate that these species are well separated from their close relatives in *Bredia*, justifying their recognition as distinct species. *Bredia bullata* is unique in its interveinal areas prominently bullate each with an apical seta, a character otherwise never recorded in the genus. *Bredia nitida* resembles *B. malipoensis* in habit, leaf shape, and inflorescence morphology, but differs in the glabrescent and nitid adaxial leaf surface (vs. densely pubescent and subvelvety), ovate-elliptic or elliptic calyx lobes (vs. triangular to semiorbicular), and white petals (vs. purplish-red). *Bredia enchengensis* is closest to *B. longiradiosa*, but easily recognized by its prostrate habit (vs. erect), the yellowish-green, membranous and fragile leaves (vs. green or dark green, papery), and white anthers (vs. pink to purplish). These new discoveries show that further botanical exploration is warranted in the remote Sino-Vietnamese bordering region.

Keywords

Bredia, karst, Melastomataceae, phylogeny, taxonomy

Introduction

Karst is a kind of landscape characterized by a variety of closed surface depressions, a well-developed underground drainage system and a paucity of surface streams (Ford and Williams 2007). The complex terrains and variable climatic conditions on karsts provide numerous ecological niches that harbor a rich biodiversity (Clements et al. 2006). The vast karst terrain stretching across southern China and northern Vietnam connects two global biodiversity hotspots, viz. south-central China and Indo-Burma. It harbors remarkable biodiversity and a high level of endemism (Zhu 2007) and has been considered the model for karst studies (Sweeting 1978). As karst environments in these areas are often remote and under significant threats due to human activity, biodiversity survey and conservation are extremely urgent.

Bredia Blume (Melastomataceae) as currently circumscribed contains 24 species distributed from central and southern mainland China, Taiwan, northern Vietnam, to the Ryukyu Islands and Yakushima, Japan (Zhou et al. 2019a; Wen et al. 2019; Dai et al. 2020; He et al. 2020). Five species of *Bredia*, namely *B. latisejala* (C. Chen) R. Zhou & Ying Liu, *B. longearistata* (C. Chen) R. Zhou & Ying Liu, *B. longiradiosa* C. Chen ex Govaerts, *B. malipoensis* D. H. Peng, S. J. Zeng & Z. Y. Wen, and *B. reniformis* C. M. He, Y. H. Tong & S. J. Zeng, are restricted to limestone karst areas. These species share obvious resemblance in their isomorphic stamens and undulate petal margin ciliate with glandular hairs and thus are easily distinguished from the remaining species of the genus (Fig. 1). The only exception is *B. reniformis*, which does not have an undulate petal margin (Fig. 1D). Close relationships among the karst species were consistently recovered in previous phylogenetic studies based on nuclear ribosomal internal transcribed spacer (nrITS) and plastome sequences (Zhou et al. 2019a, b, c; Dai et al. 2020). From 2019 to 2021, multiple field expeditions were made to karst areas in southern Guangxi, southeastern Yunnan, and northern Vietnam. In the process, we encountered three species of *Bredia* with isomorphic stamens and undulate and ciliate petal margin that were morphologically distinct from limestone species. As shown in Fig. 2, the new taxa were found in three localities from Malipo County, Yunnan Province, China and Quan Ba District, Ha Giang Province, Vietnam (*B. bullata* J. H. Dai & Ying Liu; Figs 3, 4); in one locality from Daxin County, Guangxi Province, China (*B. enchengensis* J. H. Dai, Yan Liu & S. Y. Nong; Figs 5, 6); and in one locality from Hekou County, Yunnan Province, China (*B. nitida* J. H. Dai & Ying Liu; Figs 7, 8).

In this study, we inferred the phylogenetic position of the plants in question and then compared them with their close relatives in *Bredia* to evaluate their specific status.



Figure 1. Species of *Bredia* adapted to limestone karst habitats **A** *B. latisejala*, Ying Liu 557 (SYS) **B** *B. longiradiosa* var. *longiradiosa*, Ying Liu 486 (SYS) **C** *B. malipoensis*, Yunnan Expedition Team 1073 (PE), photographs by Bing Liu (PE) **D** *B. reniformis*, Ying Liu 748 (SYS).

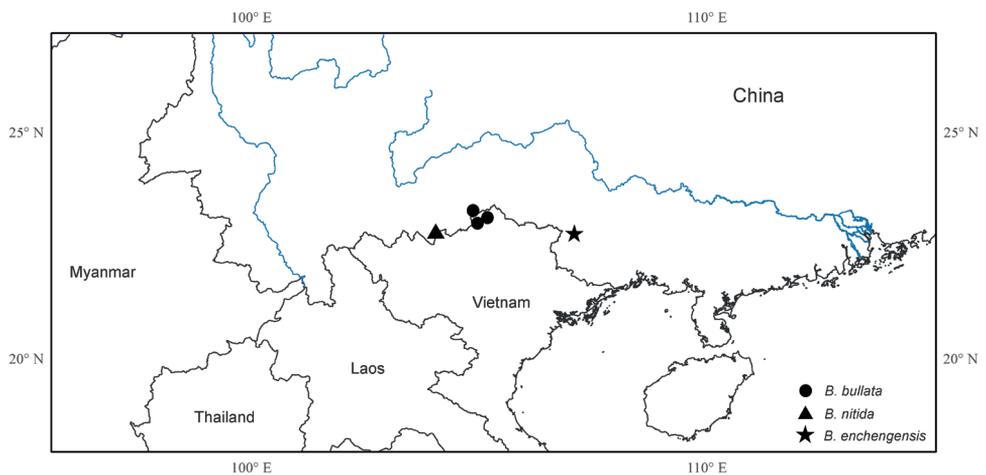


Figure 2. Distribution of *Bredia bullata* (solid circle), *B. nitida* (triangle), and *B. enchengensis* (star).

To this end, phylogenetic analyses were performed using sequence data of three nuclear markers (nrITS, *Dbr1*, and *SOS4a*) and one chloroplast intergenic spacer (*trnV-trnM*), sampling all species recorded in *Bredia*. The results confirmed our suspicion that these plants represented species of *Bredia* new to science. A key is provided for the karst species.

Materials and methods

Morphological data for the new species and previous recorded karst species were obtained through field expeditions, herbarium specimens (A, E, GXMI, IBK, IBSC, PE, SYS, VNMN) and literature (Chen 1984; Chen and Renner 2007; Wen et al. 2019; He et al. 2020) surveys as well as by observing living individuals in the facilities of Sun Yat-sen University.

To infer the phylogenetic position of *B. bullata*, *B. nitida*, and *B. enchengensis*, the type species of related genera (*Blastus* Lour., *Fordiophyton* Stapf, *Phyllagathis* Blume, *Tashiroea* Matsum. ex Ito & Matsum.), and all 24 species so far recorded in *Bredia* were included in the analyses. *Tashiroea yaeyamensis* Matsum. was selected as the outgroup according to Zhou et al. (2019a, b, c). The source of the materials and GenBank accession numbers are given in Suppl. material 1.

Total DNA was extracted from fresh leaves using the modified CTAB procedure (Doyle and Doyle 1987). For amplification and sequencing of *SOS4a*, we used two primers (*SOS4a*-F: 5'-CAAGAAGGTGAGATCATCCAAA-3', *SOS4a*-R: 5'-TAGTTTTGGCCTGCAATGCT-3') adapted from Reginato and Michelangeli (2016). Primers published in Zhou et al. (2020) were used for *Dbr1* and universal primers for nrITS and *trnV-trnM* (White et al. 1990; Hwang et al. 2000).

Sequences of four genes were aligned using MAFFT v.7.307 (Katoh and Standley 2013) and concatenated. Maximum likelihood (ML) analysis was performed in IQ-TREE v.2.1.4 (Nguyen et al. 2015). The optimal partitioning scheme and best-fitting model for each partition (Suppl. material 2) were selected using ModelFinder (Kalyaanamoorthy et al. 2017) under the Bayesian Information Criterion (BIC). Node support was evaluated by 1000 replicates of ultrafast bootstrap (UFBS) (Minh et al. 2013) and SH-aLRT test. For Bayesian inference (BI) analysis, we used PartitionFinder v.2.1.1 (Lanfear et al. 2017) for partitioning and model selection (Suppl. material 2). BI analysis was conducted in MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001). Two independent Markov chain Monte Carlo analyses (MCMC) were performed with four simultaneous chains of 2,000,000 generations sampling one tree every 100 generations. We verified that the average deviation of split frequencies had reached a value below 0.01 at the end of MCMC analyses. The first 25% of trees were discarded as burn-in and the remaining were used to construct a majority-rule consensus tree with Bayesian posterior probabilities (PP). Effective sample sizes (ESS) for all parameters and statistics were assessed using Tracer v.1.7.1 (Rambaut et al. 2018). Maximum parsimony (MP) analysis was carried out in PAUP v.4a165 (Swofford 2003). A heuristic

search strategy was conducted of 1000 random addition replicates, with the tree-bisection-reconnection (TBR) branch swapping algorithm and MultTrees on. Maxtree was set to 500. We evaluated node support (BSMP) by 1000 bootstrap replicates of 1000 random additions.

Results

The aligned sequence matrix contained 2536 characters. The optimal partitioning scheme contained three partitions, the statistics of which were summarized in Suppl. material 2. Trees from BI, ML, and MP analyses had identical topologies. The tree resulting from ML analysis is shown in Fig. 9, with PP, BSMP, UFBS, and support values from SH-aLRT test indicated at nodes. *Bredia bullata*, *B. nitida*, and *B. enchengensis* formed a strongly supported clade with the other 24 species of *Bredia* (PP = 1; BSMP = 99%; SH-aLRT test = 99%, UFBS = 98%). Within *Bredia*, the three new taxa formed a clade with the other karst species (karst clade, Fig. 9), although with low support (PP = 0.67; BSMP = 25%; SH-aLRT test = 0%, UFBS = 66%). *Bredia enchengensis* was recovered as sister to *B. longiradiosa* (PP = 1; BSMP = 100%; SH-aLRT test = 100%, UFBS = 97%), while *B. nitida* and *B. bullata* constituted a well resolved clade with *B. malipoensis* (PP = 1; BSMP = 100%; SH-aLRT test = 100%, UFBS = 100%).

Discussion

Phylogenetic data and morphology confirmed that *B. bullata*, *B. nitida*, and *B. enchengensis* belong in *Bredia*. All three species have cordate leaf blades, cymose inflorescences, isomorphic stamens, gibbous anthers and enlarged ovary crowns, all of which are typical of *Bredia* (Figs 3–8). In the present phylogenetic analyses (Fig. 9), the limestone species of *Bredia* formed a clade containing four subclades, viz. *B. reniformis*, *B. latisepala*-*B. longearistata*, *B. longiradiosa*-*B. enchengensis*, and *B. malipoensis*-*B. nitida*-*B. bullata*. Nevertheless, the karst clade is still weakly supported, as well as the relationships among its four subclades. Further molecular sampling is desired to improve these phylogenetic relationships.

Among the three species in question, *B. enchengensis* was well resolved as sister to *B. longiradiosa* (Fig. 9). It resembles *B. longiradiosa* in the somewhat broadly ovate leaf blade, inflorescence often an umbellate cyme, undulate petals with ciliate margin, and isomorphic stamens, but differs markedly from the latter in the prostrate habit (vs. erect), densely pubescent stem (vs. sparsely villous or glabrescent), yellowish-green, membranous and fragile leaves (vs. green or dark green, papery), and white anthers (vs. pink or purplish) (Figs 1B, 6). The remaining two species, namely *B. bullata* and *B. nitida*, formed another karst subclade in the genus with *B. malipoensis* (Fig. 9). *Bredia bullata* is distinct in its strongly sunken adaxial leaf veins with interveinal areas prominently bullate each with a short apical seta (Fig. 4E), a character otherwise

never recorded in the genus. *Bredia nitida* shares general similarities with *B. malipoensis* in leaf shape and morphology of the inflorescence, petals, and stamens, but is easily distinguished from the latter in the often glabrescent stem and leaves at maturity (vs. densely pubescent), nitid upper leaf surface (vs. subvelvety), ovate-elliptic or elliptic calyx lobes (vs. triangular to semiorbicular), and white petals (vs. purplish-red) (Figs 1C, 8). Based on the phylogenetic data and morphological divergence, *B. bullata*, *B. nitida*, and *B. enchengensis* should be recognized as distinct species in *Bredia*.

The Sino-Vietnamese limestone karst region provides a multitude of habitats, such as cliffs, caves, and shaded fissures/crevices (Schindler 1982; Xu 1995; Zhu 2007). For some calciphilous herbaceous plant groups with low vagility, such isolated habitats/microhabitats likely promote allopatric speciation and a steady accumulation of species over time, resulting in a high diversity of narrowly endemic species (Hughes and Hollingsworth 2008; Chung et al. 2014). *Aspidistra* Ker Gawl. (e.g., Liu et al. 2011), *Begonia* L. (e.g., Chung et al. 2014), *Impatiens* L. (e.g., Xue et al. 2020), and *Primulina* Hance (e.g., Kong et al. 2017) are among the most famous examples. The Sino-Vietnamese limestone areas, where seven species of *Bredia* have been recorded, is a diversification center for the genus. These species are capsule-fruited and disperse their seeds by raindrops and wind, often within a short distance from the mother plant. Current data indicate that geographic isolation is likely the primary mode of species diversification, in a group with limited distribution range or even site-endemics. The Sino-Vietnamese karst areas are hotspots of species richness and endemism and have been an important source of vascular plant novelties in the past 20 years (Du et al. 2020; Qian et al. 2020). The remote border regions should be further explored to fully unravel the rich biodiversity there.

Taxonomic treatment

Bredia bullata J. H. Dai & Ying Liu, sp. nov.

urn:lsid:ipni.org:names:77297481-1

Figs 3, 4

Type. CHINA. Yunnan Province: Malipo County, Ba-bu Town, Da-nong Village, 1,300 m, under forests, on limestone rocks, 30 May 2020, Jin-hong Dai and Ying Liu 849 (holotype: PE; isotypes: A, SYS).

Diagnosis. Distinguished in *Bredia* by its adaxially strongly sunken leaf veins (vs. veins not sunken), with interveinal areas prominently bullate each with an apical seta (vs. smooth, not bullate).

Description. Shrubs, 0.4–1.0 m tall. Stems erect and branched, terete, densely pubescent with 0.5–1 mm long, spreading, uniseriate to multiseriate hairs with or without a glandular head. Leaves opposite; petiole 3–12.5 cm long, puberulous with 0.5 mm long, spreading and often uniseriate hairs with or without a glandular head; blade ovate-cordate to elliptic-ovate, 4–22 × 2–12.5 cm, papery, secondary veins 2 or 3 on each side of midvein, all veins strongly sunken adaxially and prominent



Figure 3. Holotype of *Bredia bullata*, Jin-hong Dai and Ying Liu 849 (PE). Scale bar: 5 cm.

abaxially, with interveinal areas prominently bullate, each with an apical seta, adaxial surface green to dark green, sometimes with white zones along the midvein, sparsely puberulous with minute appressed uniseriate hairs, abaxial surface pale green to purplish, densely villous with uniseriate hairs, base cordate, margin ciliate and densely serrulate with each tooth having a terminal seta, apex acute or short acuminate.

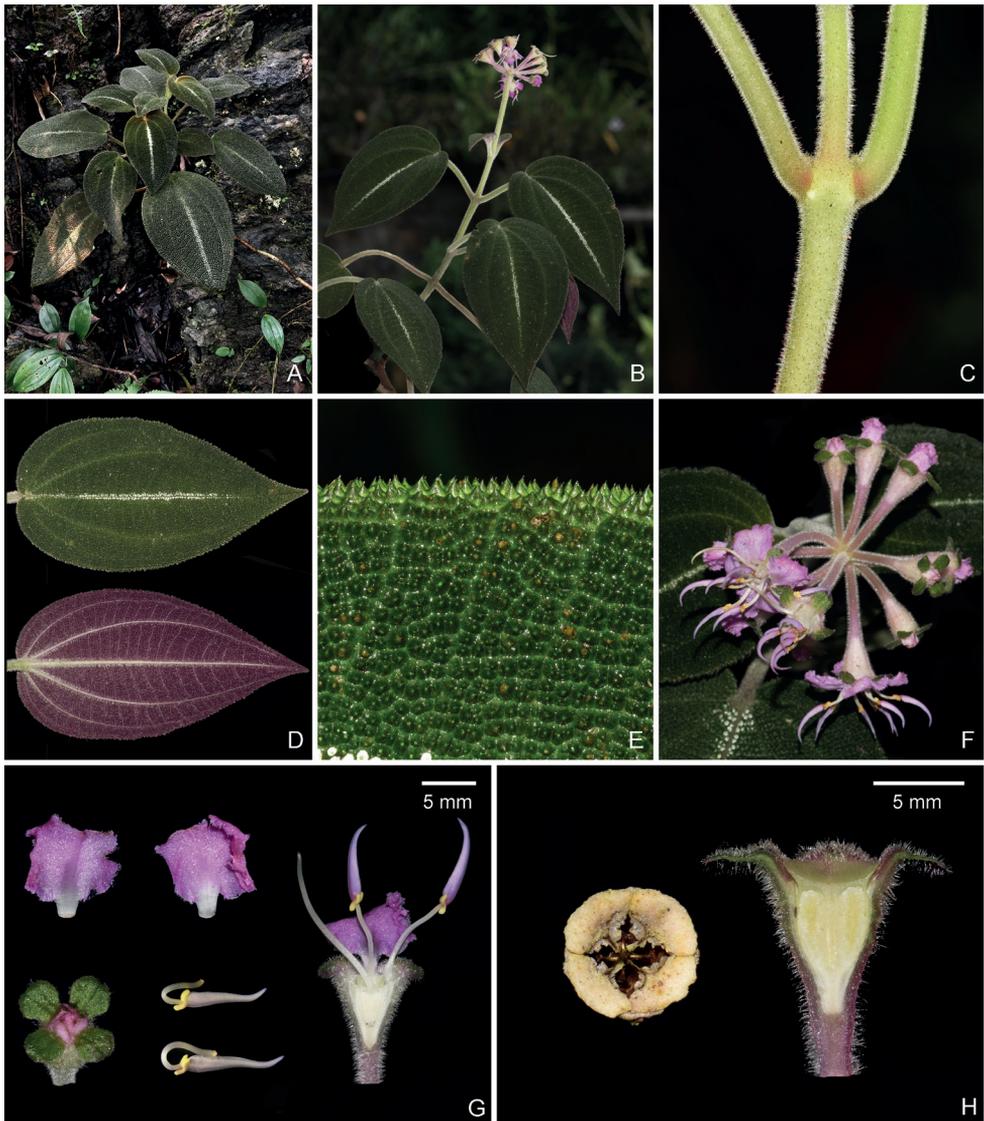


Figure 4. *Bredia bullata* **A** habit **B** a flowering branch **C** a branchlet showing spreading hairs with and without glandular head **D** adaxial (top) and abaxial (bottom) leaf surfaces **E** closeup of adaxial leaf surface showing interveinal areas prominently bullate, each bulla with an apical seta **F** flowering inflorescence **G** two petals (upper left and middle), bud showing rounded calyx lobes (lower left), inner and outer stamens (lower middle), and longitudinal section of flower (right) showing isomorphic stamens and ovary crown **H** top view of old capsule (left) and longitudinal section of young fruit showing enlarged ovary crown (right). Scale bars: 5 mm (**G**, **H**). All from Jin-hong Dai and Ying Liu 849 (A, PE, SYS).

Inflorescence terminal, a cyme or cymose panicle, 8–27-flowered, peduncle 3.5–6.5 cm long, densely puberulous. Flowers bisexual, radial but androecium slightly bilateral, 4-merous, pedicels, hypanthium and calyx lobes densely puberulous; pedicels 0.6–1.7 cm long; hypanthium yellowish-green to purplish, funnel-shaped, 4–7 × 4–6 mm;

calyx lobes 4, orbicular, 3 × 3 mm; petals 4, pink, broadly obovate to rounded, ca. 1.0 cm long, margin undulate and ciliate with glandular hairs, apex oblique; stamens 8 in two whorls, isomorphic, subequal in length with the outer whorl slightly longer than the inner one, filaments ca. 6–9 mm long, bent with the anthers to one side of the flower, anthers lanceolate, 6–8 mm long, purplish-pink, connective forming a 1 mm long, yellow dorsal spur and 2 yellow ventral lobes; ovary half inferior, locules 4, apex of ovary with membranous crown, crown margin ciliate with glandular hairs; style ca. 1.2 cm long, basally sparsely puberulous. Capsule 7 × 5 mm, funnel-shaped; placentation axial, placentas non-thready; seeds numerous, ca. 1 mm long, cuneate.

Phenology. Flowering May to June, fruiting June to August.

Etymology. The specific epithet is based on the bullate leaves.

Distribution. *Bredia bullata* is currently known from Malipo County, Yunnan Province, China and Quan Ba District, Ha Giang Province, northern Vietnam (Fig. 2), occurring in forests on limestone slopes near mountain tops and on cliffs of moist limestone caves at 1,000–1,400 m.

Additional specimens examined. VIETNAM. Ha Giang Province: Quan Ba District, Bat Dai Son Commune, Pai Chu Phin Village, Bat Dai Son Nature Reserve, 23.137864N, 104.999178E, 1,300 m, 5 June 2021, Do Van Truong DVT420 (VNMN); Tung Vai Commune, Kho My Village, Kho My limestone cave, 23.092797N, 104.905840E, 1,164 m, 6 June 2021, Do Van Truong DVT464 (VNMN).

***Bredia enchengensis* J. H. Dai, Yan Liu & S. Y. Nong, sp. nov.**

urn:lsid:ipni.org:names:77297482-1

Figs 5, 6

Type. CHINA. Guangxi Province: Daxin County, En-cheng Town, near Shang-ren Village, 234 m, on steep cliff of a limestone hill, 8 July 2021, Shi-yue Nong and Jin-hong Dai EC20210708001 (holotype: IBK; isotypes: A, PE, SYS).

Diagnosis. Resembles *B. longiradiosa* in leaf shape and morphology of the inflorescence, petals and stamens but differs in its prostrate habit (vs. erect), densely pubescent stem (vs. sparsely villous or glabrescent), yellowish-green, membranous and fragile leaves (vs. green or dark green, papery), and white anthers (vs. pink to purplish).

Description. Herbs, 8–20 cm tall. Stems to 80 cm long, branched, terete, densely pubescent with minute uniseriate hairs and 1 mm long, spreading, multiseriate glandular hairs, prostrate with adventitious roots at middle and lower parts, with the distal part (1 to 3 internodes) erect or ascending. Leaves opposite, equal to unequal; petiole 2.1–12.7 cm long, pubescent as the stem; blade broadly ovate-cordate to cordate-orbicular, 3–17 × 2.7–14 cm, membranous and fragile, pubescent on both surfaces, adaxial surface yellowish-green, abaxial surface pale green or reddish, secondary veins 3 or 4 on each side of midvein, base cordate, margin subentire, ciliate, apex acute. Inflorescence a terminal cyme, rarely cymose panicle, (1)3–13-flowered, peduncle 1.5–5.9 cm long, pubescent. Flowers bisexual, radial but androecium slightly bilateral, 4-merous, pedicels, hypanthium and calyx lobes pubescent; pedicels 0.6–2 cm; hypanthium



Figure 5. Holotype of *Bredia enchengensis*, Shi-yue Nong and Jin-hong Dai EC20210708001 (IBK). Scale bar: 5 cm.

light green, funnel-shaped, 4–6 × 3–4 mm; calyx lobes 4, broadly ovate to reniform, 2–3.5 × 3–5 mm, margin undulate; petals 4, white, sometimes pinkish at the apex, suborbicular, 2.5–7 mm long, margin undulate and ciliate with glandular hairs, apex oblique; stamens 8 in two whorls, isomorphic, equal in length, filaments 5–6 mm long, anthers lanceolate, 6–8 mm long, white, connective forming a yellow dorsal tubercle and 2 yellow ventral lobes; ovary half inferior, locules 4, apex of ovary with membranous

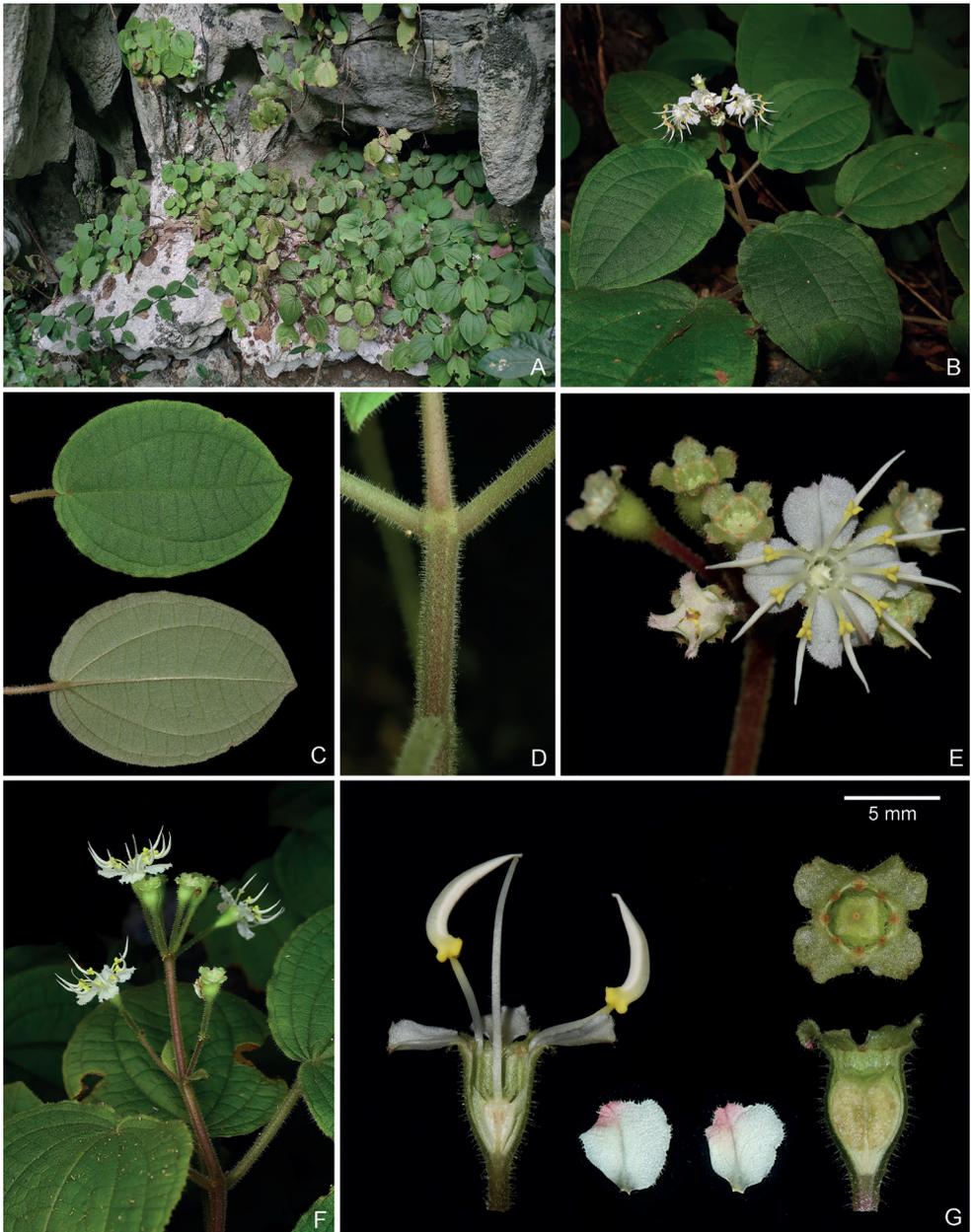


Figure 6. *Bredia enchengensis* **A** habitat and habit **B** flowering branch **C** adaxial (top) and abaxial (bottom) leaf surfaces **D** branchlet showing spreading glandular hairs **E** terminal cyme **F** cymose panicle **G** longitudinal section of flower (left) showing isomorphic stamens, two petals (lower middle), and top view (upper right) and longitudinal section (lower right) of young fruit showing broadly ovate to reniform calyx lobes and ovary crown. Scale bar: 5 mm (**G**). All from Shi-yue Nong and Jin-hong Dai EC20210708001 (A, IBK, PE, SYS).

crown, crown margin ciliate with glandular hairs; style 1.1–1.8 cm long, basally sparsely puberulous. Capsule 7 × 5 mm, funnel-shaped; placentation axial, placental column distally unhorned, placentas non-thready; seeds numerous, ca. 0.8 mm long, cuneate.

Phenology. Flowering June to July, fruiting July to August.

Etymology. The specific epithet is based on the name of the town, En-cheng, where *B. enchengensis* is discovered.

Distribution. *Bredia enchengensis* is currently known only from Daxin County, Guangxi Province, China (Fig. 2). It occurs in forests on steep, arid limestone cliffs at 234 m.

***Bredia nitida* J. H. Dai & Ying Liu, sp. nov.**

urn:lsid:ipni.org:names:77297483-1

Figs 7, 8

Type. CHINA. Yunnan Province: Hekou County, Nan-xi Town, Qin-cai-tang Village, 849 m, under forests, on limestone slope, 31 May 2020, Jin-hong Dai and Ying Liu 850 (holotype: PE; isotypes: A, SYS).

Diagnosis. Resembles *B. malipoensis* in leaf shape and morphology of the inflorescence, petal margin, and stamens but differs in the stem and leaves often glabrescent when mature (vs. densely pubescent), nitid upper leaf surface (vs. subvelvety), ovate-elliptic or elliptic calyx lobes (vs. triangular to semiorbicular), and white petals (vs. purplish-red).

Description. Shrubs, 40–65 cm tall. Stems erect and branched, terete, sparsely puberulous with spreading, minute uniseriate hairs when young, often glabrescent when mature. Leaves opposite, equal or unequal; petiole 2.1–9 cm long, sparsely puberulous when young; blade ovate-cordate to ovate, 3.2–12 × 1.5–8.8 cm, thin papery, adaxial surface green and nitid, sometimes with white, orbicular patches when young, sparsely puberulous, glabrescent when mature, abaxial surface pale green, puberulous on veins, secondary veins 2 or 3 on each side of midvein, base cordate to subrounded, entire, inconspicuously and sparsely ciliate, apex acuminate. Inflorescence a terminal cyme, 1–8-flowered, peduncle 0.5–2.5 cm long, sparsely puberulous. Flowers bisexual, radial but androecium slightly bilateral, 4-merous, pedicels, hypanthium and calyx lobes puberulous; pedicels 0.5–1.7 cm long; hypanthium white to purplish-red, funnel-shaped, ca. 6–7 × 4–5 mm; calyx lobes 4, ovate-elliptic or elliptic, 5.5–7 × 3–4 mm, adaxially with a thick basal protuberance; petals 4, white, orbicular, 0.5–1.0 cm long, margin undulate and ciliate with glandular hairs, apex oblique and retuse; stamens 8 in two whorls, isomorphic, equal in length, filaments 6–7 mm long, bent with the anthers to one side of the flower, anthers lanceolate, 7–8 mm long, purplish-red, connective forming a 1.5 mm long, yellow dorsal spur and 2 yellow ventral lobes; ovary half inferior, locules 4, apex of ovary with membranous crown, crown margin ciliate with glandular hairs; style 0.7–1.5 cm long, basally sparsely puberulous. Capsule 7–9 × 6–7 mm, funnel-shaped; placentation axial, placentas non-thready; seeds numerous, ca. 1 mm long, cuneate.

Phenology. Flowering June, fruiting late June to August.



Figure 7. Holotype of *Bredia nitida*, Jin-hong Dai and Ying Liu 850 (PE). Scale bar: 5 cm.

Etymology. The specific epithet is based on the nitid leaves.

Distribution. *Bredia nitida* is currently known from Hekou County, Yunnan Province, China (Fig. 2), occurring in moist forests on limestone slopes at 800–900 m at the Sino-Vietnamese border. Discovery of additional populations on the Vietnamese side is expected, as there are many lush limestone hills in the area.

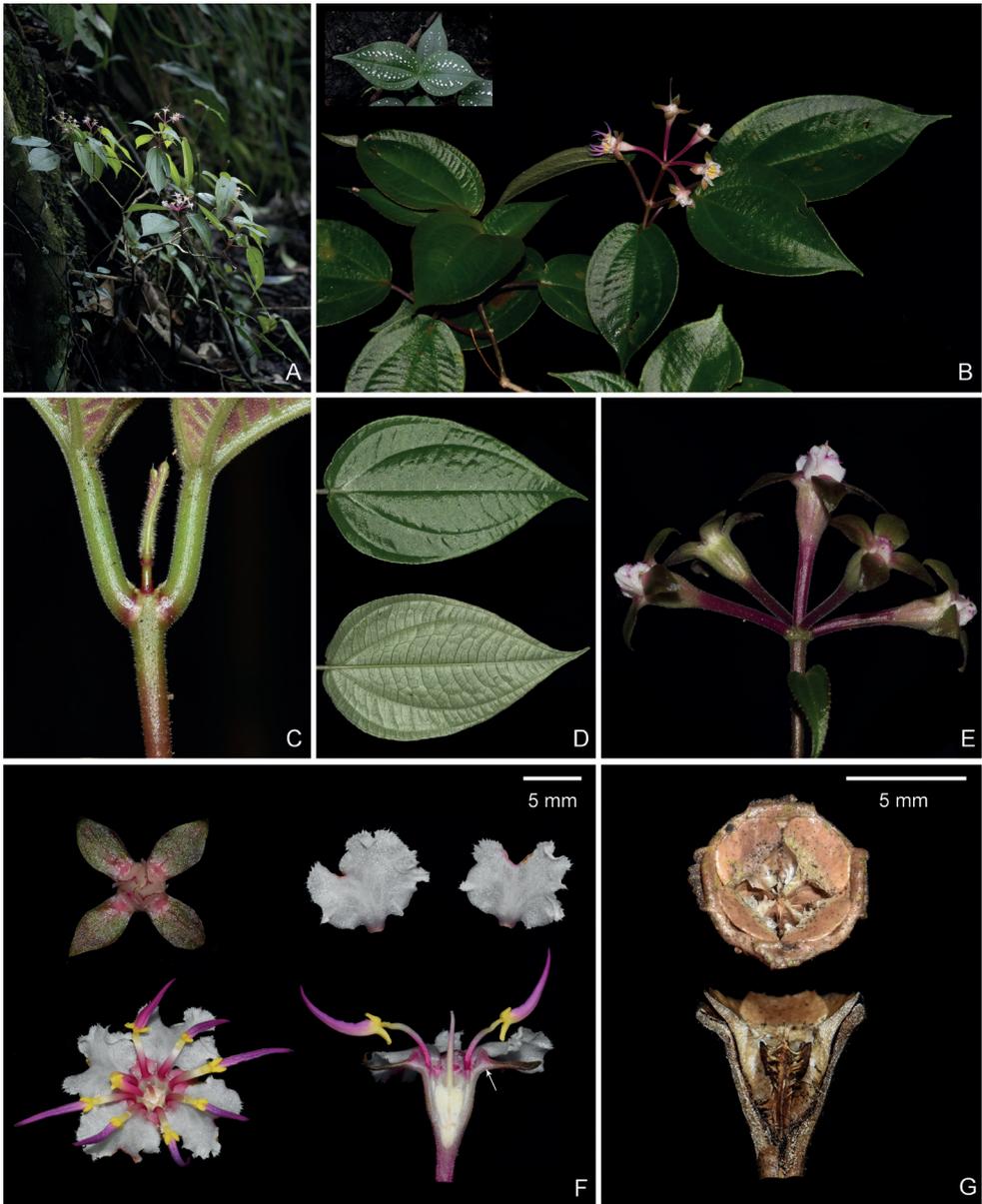


Figure 8. *Bredia nitida* **A** habit **B** young leaves with white patches (insert) and flowering branch **C** sparsely puberulous young branchlet with spreading minute hairs **D** adaxial (top) and abaxial (bottom) leaf surfaces **E** flowering inflorescence **F** top view of flower bud showing ovate-elliptic calyx lobes (upper left), two petals (upper right), top view of flower (lower left), and longitudinal section of flower (lower right) showing the isomorphic stamens and thick basal protuberance (indicated by arrow) on calyx lobe **G** top view (top) and longitudinal section (bottom) of old capsule showing enlarged ovary crown. Scale bars: 5 mm (**F**, **G**). All from Jin-hong Dai and Ying Liu 850 (A, PE, SYS).

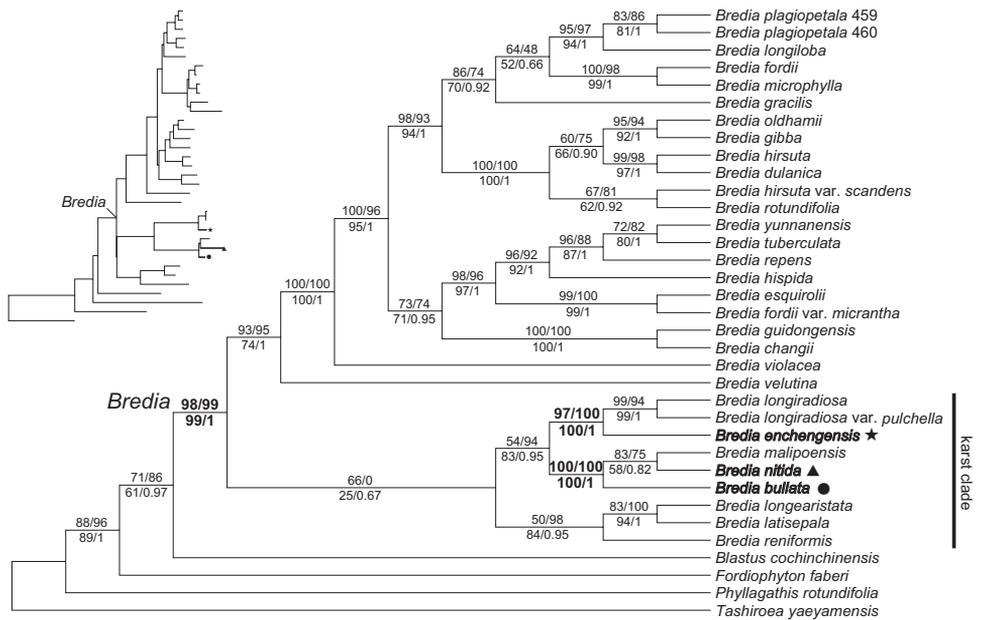


Figure 9. Phylogenetic position of *Bredia bullata*, *B. nitida*, and *B. enchengensis*. Maximum likelihood (ML) phylogenetic tree based on combined dataset of nrITS, *Dbr1*, *SOS4a*, and *trnV-trnM* sequences. Numbers above branches are ultrafast bootstrap (left) and SH-aLRT test (right) obtained from ML analysis, and those below branches are Bayesian posterior probabilities (right) and bootstrap values (left) resulting from maximum parsimony analyses. The new species are noted in bold.

Key to karst species of *Bredia*

- 1 Interveinal areas prominently bullate, each bulla with an apical seta ... ***B. bullata***
- Interveinal areas flat 2
- 2 Petal margin entire; stamens ≤ 3 mm long ***B. reniformis***
- Petal margin undulate; stamens > 5 mm long 3
- 3 Stem prostrate at least basally 4
- Stem erect 5
- 4 Blade broadly ovate-cordate to cordate-orbicular, membranous and fragile, densely pubescent adaxially; petals white ***B. enchengensis***
- Blade elliptic, oblong-elliptic, ovate to oblong-ovate or ovate-elliptic, papery, sparsely puberulous and strigose adaxially; petals pink ***B. longearistata*/*B. latisejala***
- 5 Stem broadly sulcate ***B. longiradiosa* var. *pulchella***
- Stem not sulcate 6
- 6 Hypanthium setose, hair multiseriate and basally inflated ***B. longiradiosa* var. *longiradiosa***
- Hypanthium puberulous, hairs uniseriate, not inflated basally 7

- 7 Stem and leaves densely pubescent; calyx lobes triangular to semiorbicular; petals purplish-red *B. malipoensis*
- Stem and leaves glabrescent when mature; calyx lobes ovate-elliptic or elliptic; petals white..... *B. nitida*

Acknowledgements

We thank Ping Yang (IBK) and the staff of the Forestry and Grassland Bureau of He-kou County and Malipo Laoshan Provincial Natural Reserve for their kind assistance during the field survey and Dr. Bing Liu (PE) for providing photos of *B. malipoensis*. This work was supported by the National Natural Science Foundation of China (grants 32170220, 31770214), Natural Science Foundation of Guangdong Province (grant 2021A1515011214), and partly by the Ministry of Planning and Investment, Vietnam, and the Vietnam Academy of Science and Technology under the project code UQĐTCB.06/22–23 to TVD.

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Supplementary material I

Table S1

Authors: Jin-Hong Dai, Shi-Yue Nong, Xi-Bin Guo, Truong Van Do, Yan Liu, Ren-Chao Zhou, Ying Liu

Data type: Table

Explanation note: Source of materials studied and GenBank accession numbers for nrITS, *Dbr1*, *SOS4a*, and *trnV-trnM*.

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Link: <https://doi.org/10.3897/phytokeys.195.83934.suppl1>

Supplementary material 2

Table S2

Authors: Jin-Hong Dai, Shi-Yue Nong, Xi-Bin Guo, Truong Van Do, Yan Liu, Ren-Chao Zhou, Ying Liu

Data type: Table

Explanation note: Summary statistics of the optimal partition scheme and best-fitting model for each partition in phylogenetic analyses.

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Link: <https://doi.org/10.3897/phytokeys.195.83934.suppl2>

Chimonobambusa sangzhiensis (Poaceae, Bambusoideae), a new combination supported by morphological and molecular evidence

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Abstract

This study elucidates the taxonomic position of *Indosasa sangzhiensis* in considering whether it belongs to *Indosasa* or *Chimonobambusa*. Based on morphological and molecular phylogenetic evidence, our results explicitly indicated that *I. sangzhiensis* should be a member of *Chimonobambusa*, rather than *Indosasa*, and is a distinct species closely related to *C. communis*, *C. opienensis* and *C. puberula*. Thus, the new combination *Chimonobambusa sangzhiensis* (B.M.Yang) N.H.Xia & Z.Y.Niu is made. A detailed description as well as two color plates of this species are also provided.

Keywords

bamboo, morphology, new combination, phylogeny

Introduction

Chimonobambusa Makino (1914), belonging to the subtribe Arundinariinae of the tribe Arundinarieae (Poaceae, Bambusoideae) (Zhang et al 2020), is characterized by leptomorph rhizomes, diffuse culms, basal internodes that are 4-angled and frequently with a ring of root thorns, internodes with 2 longitudinal ridges

and 3 grooves just above branching points at the node, three branches at each mid-culm node, small, triangular or subulate culm leaf blades (usually less than 1 cm long), pseudospikelets, and 3 stamens in each floret (Makino 1914; Xue and Zhang 1996; Li and Stapleton 2006). It contains about 42 species mainly distributed in China, Japan, Myanmar and Vietnam (Vorontsova et al. 2016). There are 38 species known, mainly distributed in Central, South and Southeast China (Li and Stapleton 2006; Vorontsova et al. 2016). Recent phylogenetic studies that focused on Arundinariinae have suggested that *Chimonobambusa* should be defined in a broad sense, including *Qiongzhusa* Hsueh & T.P.Yi (Hsueh and Yi 1980; Xue and Yi 1996), and thus it can be accepted as a monophyletic group, except for *C. sichuanensis* (T.P.Yi) T.H.Wen (Li and Stapleton 2006; Peng et al. 2008; Zhang et al. 2012; Guo et al. 2021).

Indosasa sangzhiensis B.M.Yang was described based on a collection (Vegetation Survey Group 00549) from Badagongshan, Sangzhi Xian, Hunan Province, China. In the protologue, Yang (1989) stated that this species resembled *I. glabrata* C.D.Chu & C.S.Chao (Chao and Chu 1983), but can be distinguished by its densely pubescent culms, solid distal internodes but hollow basal ones, few foliage leaf oral setae, as well as small and adaxially pubescent foliage leaf blades. However, after examination of the type specimen and protologue of *I. sangzhiensis*, we found that this species is characterized by small, erect and triangular culm leaf blades, which falls within the circumscription of *Chimonobambusa* rather than *Indosasa*.

During fieldwork at the type locality of *I. sangzhiensis* (Badagongshan, Sangzhi), we collected a bamboo with leptomorph rhizomes, diffuse culms and three branches at mid-culm nodes. After comparison of the specimens we collected and possibly related species, we found that it matches the type of *I. sangzhiensis* in both having culm internodes with densely white pubescence, solid distal internodes but hollow basal ones, ovate culm buds, small, erect and triangular culm leaf blades, glabrous culm leaf sheaths, 2–3 leaves per ultimate branches, developed foliage leaf oral setae and leaf blades with length of 9–19 cm and width of 1.2–2 cm. Therefore, we concluded that the specimens we collected are *I. sangzhiensis*. In addition, we found that this species is characterized by internodes with two longitudinal ridges and three grooves above the branching point, slightly 4-angled basal internodes, flat or only slightly prominent nodes, small, erect and narrowly triangular or subulate culm leaf blades, which is not typical of *Indosasa* species as far as we know, but conforms well with the known morphology of *Chimonobambusa*. Therefore, we conclude that *I. sangzhiensis* should be a member of *Chimonobambusa*, rather than *Indosasa*. After checking records of *Chimonobambusa* species from the Flora of China, we found that *I. sangzhiensis* is closely related to three species, viz., *Chimonobambusa communis* (Hsueh & T.P.Yi) T.H.Wen & Ohrnb., *C. opienensis* (Keng f.) T.H.Wen & Ohrnb. and *C. puberula* (Keng f.) T.H.Wen & Ohrnb (Hsueh and Yi 1980, 1983; Hsueh et al. 1996). In order to elucidate the relationship between *I. sangzhiensis* and related species, a detailed comparison of vegetative morphological characters and phylogenetic analyses were conducted in this study.

Materials and methods

Morphological observation

The main morphological characters of *I. sangzhiensis* and three species of *Chimonobambusa*, viz., *C. communis*, *C. opienensis* and *C. puberula*, were compared based on protologues and descriptions from floras. Some detailed characters, such as indumentum of the culm leaf ligule, were observed with a stereomicroscope (Mshot MZ101). Measurements were taken using a ruler or micrometer.

Taxon sampling for phylogenetic analyses

Two different molecular regions including complete chloroplast genome (cpDNA) and nuclear ribosomal DNA (nrDNA) were utilized to reconstruct the phylogenetic relationships of *I. sangzhiensis*. A total of 23 species representing 7 genera from Arundinarieae were utilized to reconstruct the plastid tree, for which *Bambusa emeiensis* L.C.Chia & H.L.Fung and *B. sinospinosa* McClure were set as the outgroup taxa. For nrDNA, 16 species representing 5 genera from Arundinarieae were utilized to reconstruct the nrDNA tree, for which *B. sinospinosa* and *B. multiplex* (Lour.) Raeusch. ex Schult.f. were set as the outgroup taxa. The generic type of *Indosasa* McClure, *I. crassiflora* McClure, was added here to clarify the systematic position of *I. sangzhiensis*. All voucher information and accession number of cp genomes are listed in Table 1. Our sample information for nrDNA and its sequence matrix can be found in the supplementary material (Suppl. material 1: Table S1).

DNA extraction, sequencing, assembly and annotation

Total genomic DNA was isolated from silica-dried leaves following manufacturer specifications TIANGEN Genomic DNA Extraction Kit (TIANGEN, Beijing, China). DNA samples of concentration up to standard ($\geq 1 \mu\text{g}$) were randomly sheared into fragments using Covaris M220 (Covaris, Woburn, MA). Insert size of 350 bp fragments were enriched by PCR, and the paired-end ($2 \times 150 \text{ bp}$) libraries were constructed on NovaSeq 6000 platform. As a result, about 20 G genome skimming data were generated.

To improve assembly accuracy and efficiency, Trimmomatic v 0.39 were utilized to filter out unpaired and low-depth reads from clean data using default parameters (Bolger et al. 2014). The whole cpDNA and nrDNA were assembled using the software GetOrganelle v 1.7.4 pipeline (Jin et al. 2018), with five k-mer sets of 45, 65, 85, 105, 125 bp. The filtered reads were transferred to Bandage (Wick et al. 2015) for plastid and ribosomal DNA scaffolds connection. Two opposite plastid scaffolds exported from Bandage were aligned with the reference *I. shibataeoides* (MF066251), and the one matching the reference was annotated using the PGA software (Qu et al. 2019) based on the annotation of *I. shibataeoides*. The sequences

Table 1. Voucher information of 22 complete chloroplast genomes used in this study.

Taxon	Voucher information	GenBank accession
Ingroup		
<i>Acidosasa purpurea</i> (Hsueh & T.P.Yi) Keng f.	Zhang08023 (KUN)	HQ337793
<i>Chimonobambusa angustifolia</i> C.D.Chu & C.S.Chao	Wu20210053(YAFG)	OK040768
<i>C. hejiangensis</i> C.D.Chu & C.S.Chao	GACP (NMGU)	MT884004
<i>C. hirtinoda</i> C.S.Chao & K.M.Lan	Not provided by the author	MT576658
<i>C. purpurea</i> Hsueh & T.P.Yi	LW20200602-01 (CAAF)	MW030500
<i>C. tumidissinoda</i> Ohrnb.	MPF10083 (KUN)	MF066244
<i>C. utilis</i> (Keng) Keng f.	Not provided by the author	OK040769
<i>Indocalamus sinicus</i> (Hance) Nakai	ZMY037 (KUN)	MF066250
<i>I. tongcheuensis</i> K.F.Huang & Z.L.Dai	Not provided by the author	MW279198
<i>Ravenochloa wilsonii</i> (Rendle) D.Z.Li & Y.X.Zhang	MPF10146 (KUN)	JX513421
<i>Indosasa crassiflora</i> McClure	BH58 (IBSC)	OK558536
<i>I. gigantea</i> (T.H.Wen) T.H.Wen	HNJ36052 (JXAU)	MN917206
<i>I. sangzhiensis</i> B.M.Yang	NZY109 (IBSC)	OM867788
<i>I. shibataeoides</i> McClure	MPF10028 (KUN)	MF066251
<i>I. sinica</i> C.D.Chu & C.S.Chao	MPF10034 (KUN)	MH394381
<i>Oligostachyum shiuyingianum</i> (L.C.Chia & But) G.H.Ye & Z.P.Wang	DZL09122 (KUN)	JX513423
<i>O. sulcatum</i> Z.P.Wang & G.H.Ye	Not provided by the author	MW190089
<i>Pleioblastus amarus</i> (Keng) Keng f.	Zhang Yu-QuC373 (SANU)	MH988736
<i>P. maculatus</i> (McClure) C.D.Chu & C.S.Chao	MPF10161 (KUN)	JX513424
<i>P. triangulata</i> (Hsueh & T.P.Yi) N.H.Xia, Y.H.Tong & Z.Y.Niu	NZY040 (IBSC)	OK323193
Outgroup		
<i>Bambusa emeiensis</i> L.C.Chia & H.L.Fung	Zhang08019 (KUN)	HQ337797
<i>B. sinospinosa</i> McClure	Li043 (KUN)	MK679807

were finally checked manually in Geneious 9.1.4 (Kearse et al. 2012). The assembled nrDNA sequences were annotated directly using Geneious. Illustration of the newly sequenced plastome of *I. sangzhiensis* was drawn by OGDRAW with default settings (Greiner et al. 2019).

Phylogenetic analysis

To determine the systematic position of *I. sangzhiensis*, maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted. A total of 22 complete cp and 16 nrDNA genomes were aligned with MAFFT v 7.450 (Kato and Standley 2013). Maximum likelihood (ML) analysis was generated by RAxML v 8.0.0 (Stamatakis 2014). Rapid bootstrap analysis and GTRGAMMAI were set as the best-fit algorithm and model. The number 12345 was specified as the random seed of parsimony tree inference with 1000 replicates performed. Bayesian inference (BI) was conducted using MrBayes v 3.2.6 (Ronquist et al. 2012). The model of SYM+G was defined by MrModeltest v 2.4 (Nylander 2004). The rates of variations across sites were trimmed as gamma. At least 6,000,000 generations were run to ensure average standard deviation of split frequencies (ASDFs) < 0.01 with sampling frequency set as 100 generations. After rejecting the first 25% burn-in samples, the optimized topology was printed.

For the tandemly repeated nrDNA, our *de novo* assembly obtained 5,799 bp sequences comprising 18S (1,811 bp), 5.8S (164 bp), and 26S (3,391 bp) ribosomal RNA gene along with two internal transcribed spacer I (ITS1) (215 bp) and ITS2 (217 bp) in the middle (Fig. 1B).

Morphological comparison

Indosasa sangzhiensis resembles *C. communis*, *C. opienensis* and *C. puberula* in having slightly 4-angled basal culm internodes, flat or slightly prominent culm nodes without root thorns, deciduous culm leaf sheaths, very small, erect and narrowly triangular or subulate culm leaf blades and well-developed foliage leaf oral setae; but can be easily distinguished from the latter three species by the morphological characters shown in Table 2. Specifically, it can be distinguished from *C. communis* in having culm internodes with dense white pubescence (Fig. 2B–E) (vs. glabrous internodes), culm leaf ligule margins with dense cilia (Fig. 3C) (vs. glabrous margin) and culm leaf blades broadly extending to join the sheath apex (Fig. 3B) (vs. slightly narrowed at the junction with the sheath apex). It differs from *C. opienensis* in having culm internodes with dense white pubescence (Fig. 2B–E) (vs. glabrous), 3 branches per mid-culm nodes (Fig. 2D, E) (vs. 2–3 branches per mid-culm node), culm leaf sheaths of glabrous adaxial surface (Fig. 3A) (vs. with sparse brown setose), culm leaf ligule margins with dense cilia (Fig. 3C) (vs. glabrous) and 1–3 foliage leaves per ultimate branch (Fig. 3D) (vs. 1(–2) foliage leaves). It differs from *C. puberula* in having glabrous rhizome internodes (Fig. 3E) with densely brown-setose parts below the nodes and glabrous rhizome internodes sheath scars (Fig. 2C) (vs. with densely brown setose);

Table 2. Morphological comparison of *Indosasa sangzhiensis* and three related species.

Morphology	<i>I. sangzhiensis</i>	<i>C. communis</i>	<i>C. opienensis</i>	<i>C. puberula</i>
Rhizome				
Surface of infranodes	Glabrous	Glabrous	Glabrous	Densely brown setose
Culm				
Height (m)	1–3	3–7	2–7	4–5
Diameter (mm)	0.5–1.5	1–3	1–5.5	1.5–2.5
Surface of internodes	Densely pubescent	Glabrous	Glabrous	Densely pubescent
Sheath scar	Glabrous	Glabrous	Glabrous	Densely brown setose
Branches				
Number per mid-culm node	3	3	2 or 3	3
Culm leaf				
Abaxial surface of sheaths	Glabrous	Glabrous	Sparsely brown setose	Densely brown setose
Apex of ligule	Densely ciliate	Glabrous	Glabrous	Glabrous
Base of blade	Extending outward to join sheath apex	Slightly narrowed to join sheath apex	Extending outward to join sheath apex	Extending outward to join sheath apex
Foliage leaf				
Number per ultimate branch	1–3	1–3	1(–2)	2–4
Shoots				
Phenology	April to June	May	April to May	October

culm leaf sheaths with glabrous abaxial surface (Fig. 3A) (vs. with densely brown setose surface); culm leaf ligule margins with dense cilia (Fig. 3C) (vs. glabrous margin) and new shoots produced during April to June (vs. October).

Phylogenetic analysis

The topologies based on ML and BI methods did not indicate any conflict between the cpDNA and nrDNA phylogenetic analyses, thus only ML cladograms are shown here (Figs 5, 6), with BP and PP values noted at each node. The plastid and nrDNA phylogenetic trees both strongly supported the case that *I. sangzhiensis* is distantly related to *I. crassiflora* (the type of *Indosasa*) (BS = 100% & PP = 1.00), but forms a sister clade with members of *Chimonobambusa* (BS = 95% & PP = 1.00) (Fig. 5). Our results also indicate that all samples of *Chimonobambusa* formed a monophyletic clade while *Indosasa* was found to be polyphyletic, as the samples of *Indosasa* and those of two other genera, viz. *Acidosasa* and *Oligostachym*, were intermixed within a clade.

Discussion

Indosasa was published by McClure (1940) based on a collection of flowering material in Vietnam. It is conventionally defined by having leptomorph rhizomes, diffuse culms, three branches per mid-culm node, prominent nodes, pseudospikelets and six stamens per floret (McClure 1940; Zhu and Stapleton 2006). Until now, it was thought that there were 18 species of *Indosasa* mainly distributed in South, South-west and East China, Vietnam and Laos (Zhu and Stapleton 2006; Vorontsova et al. 2016; Niu et al. 2021). However, recent molecular evidence based on chloroplast genome and ddRAD data indicated that *Indosasa* is a highly polyphyletic group placed in the subtribe Arundinariinae of the tribe Arundinarieae (Ma et al. 2017; Zhang et al. 2020), and the phylogenetic relationships among *Indosasa* and several closely related genera with similar vegetative characters, such as *Acidosasa*, *Oligostachyum*, *Sinobambusa*, etc., have not been resolved (Guo et al. 2021; Niu et al. 2021). However, a broad concept was proposed for *Chimobambusa* based on recent molecular evidence (Li and Stapleton 2006; Peng et al. 2008; Zhang et al. 2012; Guo et al. 2021), and hence this genus has been assumed to be monophyletic so far. Although *Chimonobambusa* resembles *Indosasa* in having leptomorph rhizomes, diffuse culms, three branches at each mid-culm node and pseudospikelets, it can still be distinguished from *Indosasa* in having slightly 4-angled basal internodes, internodes with two longitudinal ridges and three grooves above the branching point, basal nodes often with a ring of root thorns, small, triangular or subulate culm leaf blades (usually less than 1 cm long) and each floret with 3 stamens. In this study, based on the results of morphological and phylogenetic analyses, we consider that *I. sangzhiensis* should be a distinct species of *Chimonobambusa*, rather than *Indosasa*, and thus, a new combination in *Chimonobambusa* is proposed.

Taxonomic treatment

***Chimonobambusa sangzhiensis* (B.M.Yang) N.H.Xia & Z.Y.Niu, comb. nov.**

urn:lsid:ipni.org:names:77297484-1

Figs 2–4

Basionym. *Indosasa sangzhiensis* B.M.Yang (1989: 333); Yang (1993: 90); Yi et al. (2021: 244).

Type. CHINA. Hunan: Sangzhi Xian, Badagongshan, el. 1570 m, 23 September 1965, Vegetation Survey Group 00549 (holotype: HNNU!).

Note. After examining the type specimen and other specimens collected from the type locality, we are able to provide a revised description of the morphology of this species below.

Description. Small sized bamboo. Rhizomes leptomorph; internodes cylindrical, 4-angled when dry, 1.5–4 cm long, 5–8 mm in diameter, hollow, glabrous, walls ca. 1 mm thick; nodes flat, 1–3 roots at each node, glabrous; rhizome buds broad-ovate to subrounded, glabrous. Culms diffuse, erect, 1–3 m tall and 0.5–1.5 cm in diameter; internodes terete or base slightly 4-angled, with two longitudinal ridges and three grooves above branching points, 15–20(–24) cm long, densely white puberulent, especially below nodes, mid-culm internodes hollow, walls 1.5–3 mm thick, upper and basal internodes nearly solid; basal nodes without root thorns; supranodal ridges flat or slightly prominent at unbranched nodes, sheath scars prominent, corky, glabrous, intranodal regions 2–5 mm long, glabrous. Primary buds solitary, ovate, yellowish green, 5–8 × 3–5 mm, glabrous. Mid-culm branch complement with 3 branches, erect, subequal, inclined at an angle of 15°–45° with the culm, internodes 1.5–10 cm long, glabrous; supranodal ridges prominent; branch leaf sheaths thinly leathery, shorter than internodes, abaxially glabrous. Culm leaf sheaths long triangular, caducous, thinly leathery, 10–15 × 3.5–6 cm, 1/2–3/4 as long as internodes, pale brown, abaxially glabrous, longitudinal ribs conspicuous, upper parts of margins sparsely ciliate, deciduous when old; auricles absent; oral setae several, curly, scabrid, pale brown, 2–5 mm long, deciduous when old; ligules arcuate to truncate, 0.5–1.5 mm long, entire, densely ciliate; culm leaf blades erect, narrowly triangular or subulate, 4–11 × 1.5–4 mm, middle and upper parts involute, base extending broadly outward to join sheath apex, both sides glabrous, longitudinal ribs conspicuous. Foliage leaves 1–3 per ultimate branch; foliage leaf sheaths 3–4 cm long, initially purplish red, later becoming green, abaxially pubescent, glabrescent, margins ciliate, longitudinal ribs conspicuous; auricles absent; oral setae 3–5, curly, scabrid, 2–5 mm long; ligule truncate, ca. 1 mm long, entire or sparsely ciliate; pseudo-petioles 2–5 mm long, sparsely ciliate; foliage leaf blades lanceolate, papery, 9–19 × 1.2–2 cm, base widely cuneate, apex acute, abaxially glabrous, adaxially sparsely pubescent, glabrescent, margins serrulate, longitudinal secondary veins 3–5 pairs, transverse veins conspicuous. Inflorescence unknown.

Phenology. New shoots produced during April to June.

Vernacular names. Lěng Zhú (Chinese pronunciation), 冷竹 (Chinese name).



Figure 2. The holotype of *I. sangzhiensis* B.M. Yang (HNNU).

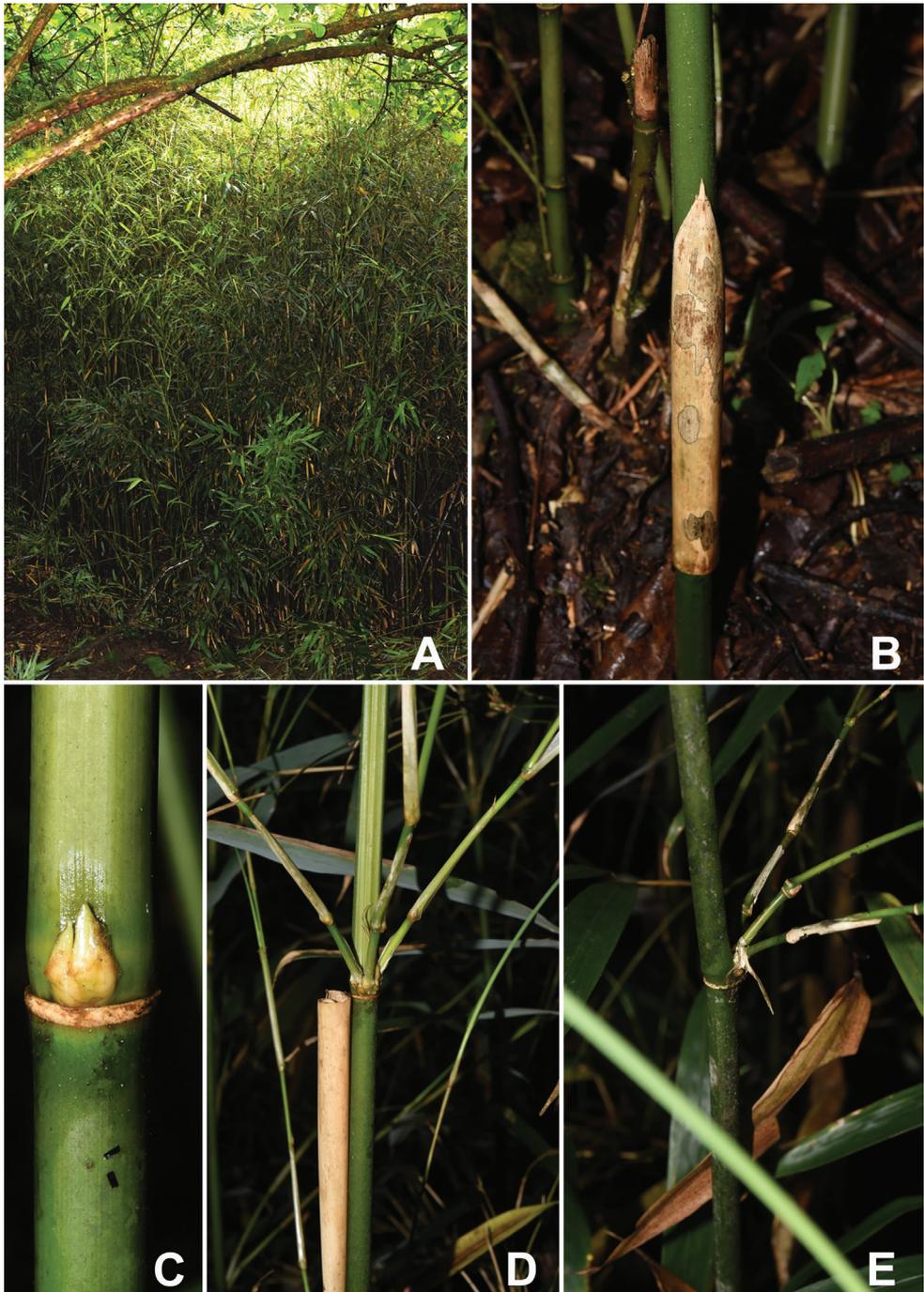


Figure 3. Morphological characters of *I. sangzhiensis* **A** habit **B** culm leaf sheath on internodes covered with dense white pubescence **C** culm bud, node and sheath scar **D** two longitudinal ridges and three grooves above branches **D, E** branches at mid-culm nodes, young **D** and old **E** culm. All photos by Z.Y.Niu.

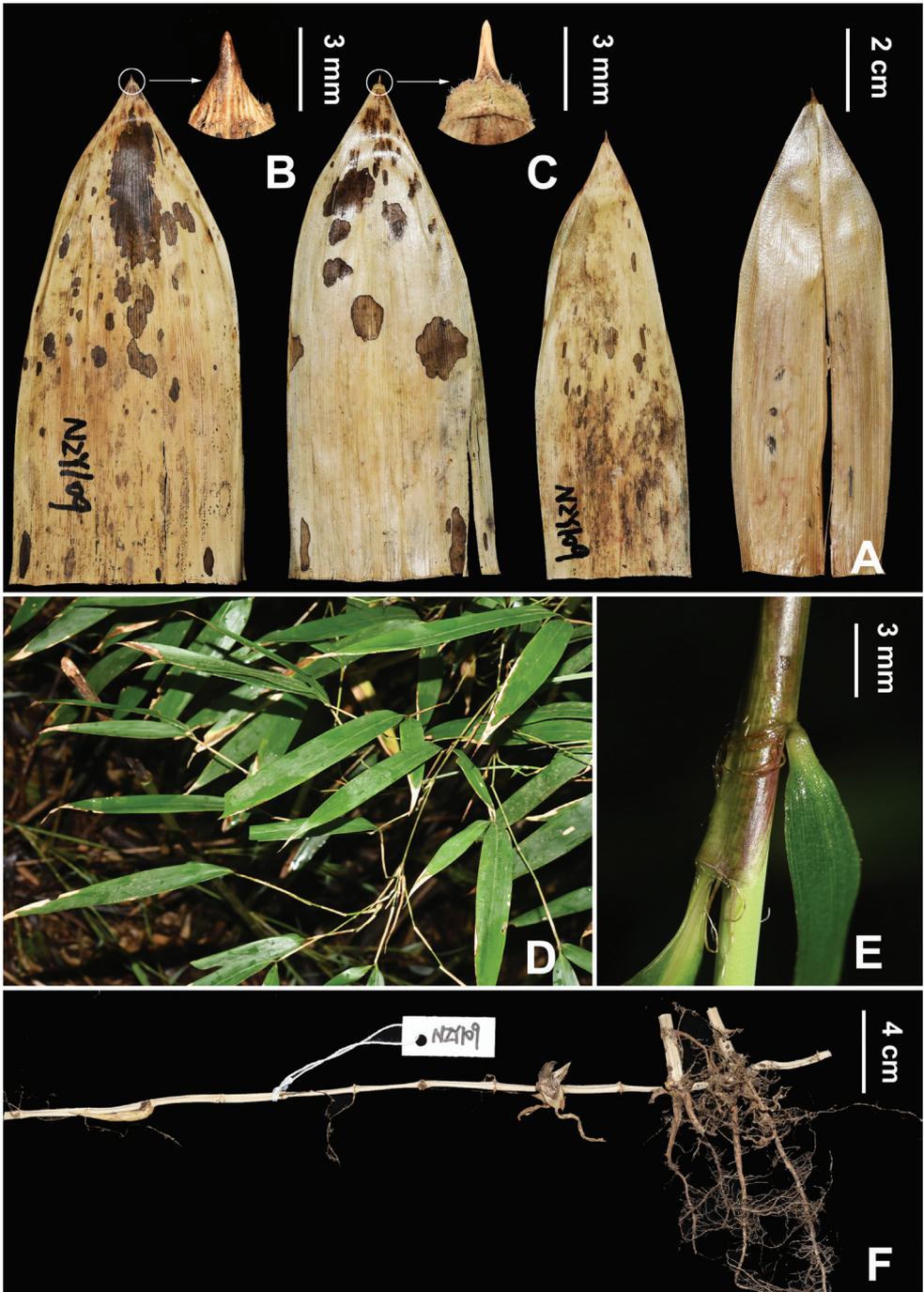


Figure 4. Morphological characters of *I. sangzhiensis* **A** culm leaf, detached **B** culm leaf blade **C** culm leaf ligule **D** ultimate leafy branches **E** foliage leaf oral setae **F** rhizome with partial young culms. All photos by Z.Y.Niu.

Distribution and habitat. The species has only been found at its type locality so far and is rather common on mountains between elevations of 1600 m to 1900 m. It prefers a cold and moist environment and often grows under forest cover.

Additional specimen examined. The same locality as the type, along the forest path, 29°40'43"N, 109°44'60"E, 1610 m, 29 June 2021, Z. Y. Niu NZY109 (IBSC).

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Supplementary material 1

Table S1

Authors: Zheng-Yang Niu, Nian-He Xia

Data type: Sample information.

Explanation note: Number and voucher of 16 nrDNAs.

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Link: <https://doi.org/10.3897/phytokeys.195.83004.suppl1>

Supplementary material 2

Chimonobambusa sangzhiensis nrDNA

Authors: Zheng-Yang Niu, Nian-He Xia

Data type: nrDNA sequences (fasta. file).

Explanation note: nrDNA sequences of 16 species sampled in this study.

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Link: <https://doi.org/10.3897/phytokeys.195.83004.suppl2>

Re-establishment of *Silene neglecta* Ten. (Caryophyllaceae) with taxonomic notes on some related taxa

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Abstract

Silene neglecta has been misunderstood and confused with *S. nocturna*, although several morphological characters (petal shape, calyx indumentum, hairiness of stamen filaments, seed size, seed-coat surface and shape) allow separation of these species. Moreover, *S. mutabilis* (which has been considered conspecific with *S. neglecta*) and *S. martinolii* (an alleged endemic species to south-western Sardinia) are considered here as taxonomic synonyms of *S. nocturna* and *S. neglecta*, respectively. These taxonomic conclusions are strongly supported by multivariate morphometric analyses of 21 characters.

Keywords

Mediterranean Basin, morphometrics, nomenclature, taxonomy

Introduction

Silene L. is one of the large genera in *Caryophyllaceae*, comprising around 850 taxonomic species (Jafari et al. 2020). The genus is widely distributed in temperate regions mainly in the Northern Hemisphere, with the centre of its diversity is in western Asia and the Mediterranean Basin.

Silene sect. *Silene* is one of the largest sections of the genus, as classified by Jafari et al. (2020). It comprises about 93 species mainly distributed in the Mediterranean. It is characterised by monochasial (sometimes dichasial) inflorescence, usually non-auriculate petal claws and often excavate or flat seeds with long and narrow testa cells (Jafari et al. 2020). *Silene nocturna* L., *S. neglecta* Ten. and *S. mutabilis* L. are annual species grouped in this section (Jafari et al. 2020; Mesbah et al., in prep.).

Tenore (1826) described *Silene neglecta* from southern Italy, whereas twelve years later (Tenore 1838), illustrations of the species which revealed its key characteristics were published. The taxon has been recognised at different levels, either as a separate species (Maire 1963; Pignatti 1982, 2017; Talavera 1990) or at subspecies level within *S. nocturna* (Arcangeli 1882; Fiori and Paoletti 1896; Chater et al. 1993). In a taxonomic study of the *S. nocturna* complex in Italy, Peruzzi and Carta (2013) proposed the species rank for *S. neglecta*, by providing morphological and karyological data and typifying the name. Later, in a study of original materials of some Linnaean names currently included within *Silene*, Peruzzi et al. (2014) concluded that the first available name at species level for the plants called *S. neglecta* was *S. mutabilis*. However, the application of the name *S. mutabilis* remains uncertain at present, since some authors have continued to use the name *S. neglecta* (Pignatti 2017; Bosch et al. 2019). Furthermore, *S. martinolii* Bocchieri & B.Mulas, an alleged endemic species to islets of south-western Sardinia, is morphologically very close to *S. neglecta* (Bocchieri and Mulas 1988), so it is advisable to clarify its taxonomic position.

This study aims to provide distinction between these taxa (*Silene neglecta*, *S. nocturna*, *S. martinolii* and *S. mutabilis*), based on macromorphological features and Scanning Electron Microscopy (SEM) observations of hairs and seeds.

Material and methods

Plant material

The present morphological and comparative study is based on the examination of specimens in the field and in herbarium/laboratory and on the analysis of the protologues. The names of the specimens were applied *a priori* following Bocchieri and Mulas (1988) and Pignatti (2017) for *Silene martinolii*; Linnaeus (1753) for *S. mutabilis*; Talavera (1990) and Pignatti (2017) for *S. neglecta* and Talavera (1990), Peruzzi and Carta (2013) and Pignatti (2017) for *S. nocturna*. Morphological characters, recognised as taxonomically discriminant within the *Silene nocturna* complex (e.g. Talavera 1990; Peruzzi and Carta 2013; Bacchetta et al. 2014; Peruzzi et al. 2014 and our own ob-

servations), were scored either in the field or in the herbarium specimens (BC, BCN, CAG, GB, ENSA, HJBS, JACA, LINN, MA, NAP, PI and WAG; acronyms according to Thiers (2022 [continuously updated])). Digital images from online databases for the Herbaria MPU, K, P and US were also examined. Morphological observations of materials were carried out under a binocular stereoscopic microscope Zeiss Stemi DV4 with eyepiece micrometer. Micromorphology was observed on calyces and mature seeds which were glued directly to aluminium stubs, coated with 40–50 nm gold and examined with a scanning electron microscopy (Hitachi 2300-SEM) at 20 kV. Given that detailed information on the seeds of *S. nocturna* has been provided in recent works (Peruzzi and Carta 2013; Peruzzi et al. 2014), seed data, based on SEM, provided for this species refers only to specimens that were misidentified as *S. neglecta* or *S. mutabilis*.

Data analysis

A total of 21 characters were selected and scored in 71 specimens. From the total 21 morphological characters, 15 were quantitative, three were calculated ratios and three were qualitative (Table 1). A non-metric multidimensional scaling (NMDS; Kruskal 1964), which represents the relationships amongst individuals in a reduced dimension scatterplot and Cluster Analysis (CA) using the average linkage method (UPGMA; Michener and Sokal 1957), which allows the classification of individuals by similarity, were performed with PAST 4.08 (Hammer et al. 2001). The similarity matrix was calculated using the Gower coefficient, suitable for mixed data (Gower 1971).

Table 1. Morphological variables used in morphometric analyses.

Abbreviation	Character name	Type
RL/W	Ratio length/width of longest stem leaf	Calculated ratio
EHLS	Length of longest eglandular hair on lower part of stem (mm)	Quantitative
EHMS	Length of longest eglandular hair on middle part of stem (mm)	Quantitative
GHMS	Length of longest glandular hair on middle part of stem (mm)	Quantitative
EHI	Length of longest eglandular hairs on inflorescence axis (mm)	Quantitative
GHI	Length of longest glandular hairs on inflorescence axis (mm)	Quantitative
RF/cm	Ratio number of flowers/cm	Calculated ratio
CLLF	Length of calyx of lowest flower (mm)	Quantitative
TL	Length of longest calyx tooth (mm)	Quantitative
TW	Width of longest calyx tooth (mm)	Quantitative
RCL/TL	Ratio calyx length/ calyx tooth length	Calculated ratio
EHCL	Length of longest eglandular hair between calyx veins (mm) ¹	Quantitative
GHCL	Length of longest glandular hair between calyx veins (mm) ¹	Quantitative
EHCLV	Length of longest eglandular hair on calyx veins (mm) ¹	Quantitative
GHCLV	Length of longest glandular hairs on calyx veins (mm) ¹	Quantitative
MHC	Main type of hairs on calyx ¹	Qualitative
PL	Petal limb incision ²	Qualitative
SH	Stamen filament pubescence ³	Qualitative
GL	Gonophore length (mm)	Quantitative
SL	Largest seed length (mm)	Quantitative
SW	Largest seed width (mm)	Quantitative

¹0: mainly eglandular; 1: approximately equal amounts of eglandular and glandular hairs; 2: mainly glandular.

²1: incision > 50% of limb length; 0 < 50% length of limb length.

³0: all stamens glabrous; 1: 50% stamens hairy; 2: all stamens hairy.

Results and discussion

The variation, based on morphometric analysis (NMDS and CA) and the morphological characters of *Silene neglecta*, *S. nocturna*, *S. martinolii* and *S. mutabilis*, are described and the taxonomic value of the characters is discussed.

Morphometric analysis

The NMDS, performed with three dimensions, yielded a value of stress of 0.10 corresponding to a good ordination result (Clarke 1993). The scatterplot showed two clearly defined groups, where *Silene martinolii* is intermingled with *S. neglecta* and both are separated from *S. nocturna* (Fig. 1). The UPGMA dendrogram (Fig. 2) yielded two well-defined clusters, one formed by individuals of *S. neglecta* and *S. martinolii* and a second one formed exclusively by individuals of *S. nocturna*. The cophenetic correlation coefficient was 0.98, indicating a good fit between the cophenetic value matrix and the similarity matrix.

Comparative morphology

Habit and hairiness

All the studied species are annuals, except some specimens of *Silene neglecta* from maritime sands in north-western Tunisia (Tabarka) which are biennials (monocarpic forming a leaf rosette in the first year). Concerning all the other characters studied (and also molecular data, Mesbah et al., in prep.), these Tunisian plants are perfectly identified as *S. neglecta*. Close to Tabarka, populations of typical annual plants of *S. neglecta* can be found. The middle and upper parts of the stems of *S. neglecta* and *S. martinolii* are usu-

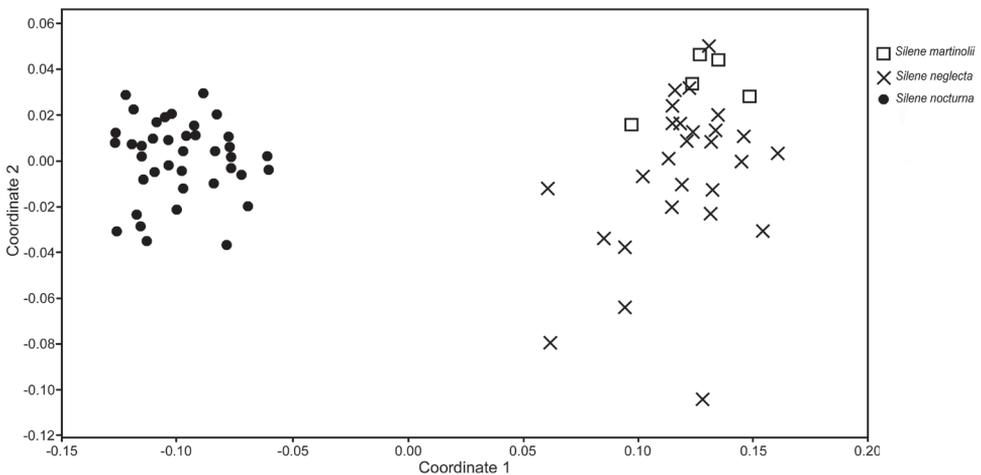


Figure 1. Non-metric multidimensional scaling scatterplot showing the first two dimensions of the analysis.

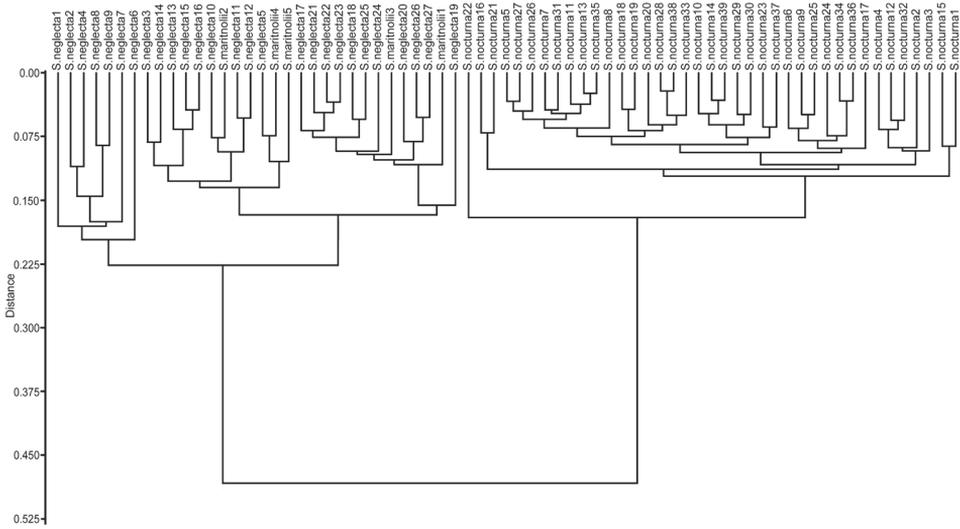


Figure 2. Hierarchical clustering of individuals of *Silene martinolii*, *S. neglecta* and *S. nocturna* using paired group algorithm (UPGMA) and Gower Similarity Index.

ally densely covered by glandular hairs up to 1.3 mm long, intermixed with eglandular hairs up to 1.9 mm long, whereas in *S. nocturna* (including the lectotype of *S. mutabilis*), the glandular hairs are up to 0.3 mm long. Peruzzi & Carta (2013) documented some differences in the hairiness of the basal portion of the stem between *S. neglecta* and *S. nocturna* (hairs longer than 1 mm in *S. neglecta* vs. hairs less than 1 mm long in *S. nocturna*). However, our observations indicate that individuals of *S. nocturna* with hairs longer than 1 mm long (up to 2.5 mm long) in the basal portion of the stem are not rare [Algeria: Crête Rouge (ENSA); Batna (GB); Croatia: Paklenica (PI); France: Garlaban (BC); Spain: Villareal (BC), Maresme (BC), Hospitalet (BC)].

Leaves

Leaf outline in *Silene neglecta*, *S. martinolii* and *S. nocturna* varies from spatulate to linear, usually tapering more or less progressively from the base towards the inflorescence. *Silene neglecta* has usually wider leaves than *S. nocturna*, but there is a notable variability with respect to this character so that it cannot be used for identification purposes. *Silene nocturna* sometimes has narrowly linear leaves in the middle and upper part of the stem (e.g. the lectotype of *S. mutabilis*), whereas in *S. neglecta*, they are spatulate to lanceolate or linear-lanceolate.

Pedicels

The pedicels are accrescent and their length is somewhat variable within a single taxon. Our study reveals that this character presents much more variability than has been

documented so far. The presence of long pedicels has been attributed to *Silene neglecta* (Peruzzi and Carta 2013) and, based on this character, *S. neglecta* and *S. mutabilis* were later considered as synonyms (Peruzzi et al. 2014). Indeed, *S. neglecta* can have remarkably long lowermost pedicels, up to twice the length of the calyx (Italy, MPU300592). However, there are specimens of *S. neglecta* with lowermost pedicels equal to or shorter than the calyx [Algeria: Kabylie de Collo (P), Bône (P); Spain: Roca del Barret (LS7707), Tunisia: Tabarka (L. Sáez, herb. pers.)] (Fig. 3) and specimens of *S. nocturna* with pedicels longer (up to 22 mm long) than calyx [Spain: Sant Julià (BC), Formentera (BC), Camí Geganta (BC), Hospitalet (BC), Unzue (BC), Lluç (HJBS); Tunisia: Melloula, Tabarka, Bizerta (L. Sáez, herb. pers.)]. The pedicels of *S. martinolii* are usually shorter than to equal to the calyx length (Bocchieri and Mulas 1988), although in some specimens (Sa Corona su Crabi, CAG), the pedicel is longer than the calyx, even without being the lowermost flower. Therefore, this character cannot be used for taxonomic purposes.

The inclination of the lowest flower's pedicel (in fruiting period) has been used by Peruzzi and Carta (2013) as a character to separate *Silene neglecta* (up to 90°) from *S. nocturna* (up to 40°). Although this relatively strong inclination is certainly observed in some specimens of *S. neglecta* (including the lectotype), there are also several specimens of this species with the lowest flower's pedicel suberect or erect-patent (Spain: LS7707, Fig. 3; Tunisia: Tabarka). Bocchieri and Mulas (1988) attributed erect-patent pedicels to *S. martinolii*. Although this is true in most cases, in two specimens (S'Aquasa Cana and Sa Corona su Crabi, CAG), several clearly patent lowest flower's pedicels (ca. 90°) are observed.

Gonophore

Both species have puberulent gonophores, being longer in *Silene neglecta* and *S. martinolii* than in *S. nocturna* (Table 2). Our study has revealed the existence of longer gonophores (up to 2.6 mm long) in *S. neglecta* than previously documented (Peruzzi and Carta 2013).

Calyx

As noted by Peruzzi and Carta (2013), *Silene neglecta* is distinct from *S. nocturna* by its larger calyx and larger calyx teeth. Our study reveals that there is a wide overlap in the calyx length (Table 2), so this character does not allow an unequivocal separation of both taxa. Bocchieri and Mulas (1988) attributed a higher maximum value of calyx length to *S. martinolii* (9–13 mm). However, we have observed specimens of *S. neglecta* from Spain that also reach 13 mm in length (this measurement refers to calyces that do not correspond to the lowermost flower). The length of the calyces and calyx teeth of the lectotype of *S. mutabilis* is 8.8–11.2 mm and 1.4–2.0 mm, respectively.

The hairiness type of the calyx has taxonomic value. Two types of hairs were identified: eglandular and glandular hairs. The eglandular hairs (unicellular or pluricellular, the



Figure 3. Field photos of *Silene neglecta* **A** inflorescence **B** flower with subentire petals **C** flower with emarginate petals **D** flower in lateral view **E** lowermost pedicel. Spain, Gavà, Roca del Barret, 29 Apr 2021, L. Sáez (herb. pers.).

latter with up to nine cells) are progressively tapering towards the apex. These hairs show striated or verrucate walls. The eglandular hairs are usually short and antrorse in *Silene nocturna* and in the lectotype of *S. mutabilis*. These hairs are somewhat longer on the veins of the calyx (Table 2), whereas between the veins, they rarely exceed 0.2–0.3 mm

Table 2. Morphological comparison of *Silene martinolii*, *S. neglecta* and *S. nocturna*. Quantitative values are expressed as 10–90 percentile intervals, with minimum and/or maximum in brackets and as mean \pm standard deviation.

	<i>S. martinolii</i>	<i>S. neglecta</i>	<i>S. nocturna</i>
Ratio length/width of longest stem leaf	(2.11)2.20–3.10(3.11) 2.60 \pm 0.43	(1.66)1.71–4.21(5.55) 2.92 \pm 1.00	(1.05)3.06–9.13(11.0) 5.77 \pm 2.42
Length of longest eglandular hair on lowest part of stem (mm)	(2.14)2.20–2.65(2.75) 2.42 \pm 0.23	(1.95)2.05–2.80(3.10) 2.38 \pm 0.31	(0.22)0.31–2.03(2.53) 1.09 \pm 0.64
Length of longest eglandular hair on middle part of stem (mm)	1.20–1.67(1.78) 1.44 \pm 0.24	(0.70)0.84–1.64(1.80) 1.21 \pm 0.32	0.20–0.42(0.61) 0.28 \pm 0.10
Length of longest glandular hair on middle part of stem (mm)	(0.43)0.53–0.97(1.02) 0.75 \pm 0.22	(0.32)0.49–1.0(1.15) 0.68 \pm 0.19	(0.14)0.17–0.22(0.23) 0.19 \pm 0.02
Length of longest eglandular hairs on inflorescence axis (mm)	(0.35)0.37–0.55(0.58) 0.49 \pm 0.09	(0.25)0.30–0.74(1.10) 0.51 \pm 0.19	(0.14)0.18–0.28(0.42) 0.21 \pm 0.05
Length of longest glandular hairs on inflorescence axis (mm)	(0.68)0.72–0.82(0.84) 0.78 \pm 0.06	(0.57)0.60–0.83(1.20) 0.74 \pm 0.13	(0.14)0.18–0.23(0.30) 0.20 \pm 0.02
Ratio number of flowers/cm	(0.37)0.45–0.78(0.86) 0.62 \pm 0.17	(0.30)0.45–1.0(1.09) 0.68 \pm 0.18	(0.30)0.43–0.84(1.38) 0.64 \pm 0.21
Calyx length of lower flower (mm)	(10.50)10.70– 11.54(11.70) 11.10 \pm 0.44	(10.3)10.5–11.62(12.0) 11.0 \pm 0.46	(8.0)10.44–12.6(12.6) 11.12 \pm 0.83
Longest calyx tooth length (mm)	(2.50)2.58–2.92(3.0) 2.76 \pm 0.18	(2.5)2.7–3.14(3.4) 2.95 \pm 0.21	(1.5)1.7–2.3(2.4) 2.0 \pm 0.20
Longest calyx tooth width (mm)	1.60–1.70 1.64 \pm 0.05	(1.0)1.36–2.0(2.1) 1.7 \pm 0.28	(1.1)1.5–1.82(2.2) 1.68 \pm 0.21
Ratio calyx length/calyx tooth	3.90–4.15(4.20) 4.02 \pm 0.12	(3.38)3.50–3.98(4.12) 3.73 \pm 0.19	(4.77)5.16–6.24(6.76) 5.59 \pm 0.46
Length of longest eglandular hairs on calyx limb (mm)	(0.24)0.26–0.78(0.90) 0.49 \pm 0.26	(0.18)0.21–0.69(0.88) 0.44 \pm 0.20	(0.20)0.21–0.31(0.51) 0.25 \pm 0.06
Length of longest glandular hairs on calyx limb (mm)	(0.50)0.58–0.77(0.78) 0.70 \pm 0.11	(0.30)0.31–0.70(0.71) 0.55 \pm 0.14	< 0.1
Length of longest eglandular hairs on calyx veins (mm)	(0.43)0.48–1.04(1.22) 0.72 \pm 0.31	0.3–1.1(2.0) 0.83 \pm 0.41	(0.20)0.22–0.76(0.92) 0.46 \pm 0.21
Length of longest glandular hairs on calyx veins (mm)	(0.72)0.76–0.92(0.93) 0.85 \pm 0.09	(0.27)0.39–1.16(1.97) 0.80 \pm 0.27	0.10–0.14(0.15) 0.11 \pm 0.02
Dominating type of hairs on calyx	glandular or eglandular + glandular	glandular or eglandular + glandular	eglandular
Petal limb incision	< 30% of limb length	< 30% of limb length	> 50% of limb length
Number of hairy stamen filaments	5–10	5–10	0
Gonophore length (mm)	2.0–2.26(2.3) 2.14 \pm 0.13	2.0–2.34(2.6) 2.2 \pm 0.15	(0.8)1.0–1.4(1.5) 1.18 \pm 0.14
Largest seed length (mm)	(0.92)0.93–1.01(1.03) 0.97 \pm 0.04	(0.90)0.91–1.02(1.05) 0.96 \pm 0.04	(0.65)0.69–0.77(0.81) 0.72 \pm 0.04
Largest seed width (mm)	(0.78)0.79–0.87(0.88) 0.83 \pm 0.04	(0.76)0.80–0.89(0.91) 0.83 \pm 0.04	(0.55)0.58–0.68(0.70) 0.61 \pm 0.04

in length (Table 2, Figs. 4–5). The hairs of the calyx of the lectotype of *S. mutabilis* are eglandular, short (up to 0.2 mm long) and antrorse (Fig. 5). This morphology matches the typical calyx hairiness of *S. nocturna*. In *S. neglecta* and *S. martinolii*, the eglandular hairs are longer, patent or antrorse. These hairs are somewhat longer on the veins of the calyx if compared to those located between the veins (Table 2).

The glandular hairs are formed by a gland and stalk consisting of 1–8 cells. These glandular hairs show striated walls. The glandular hairs are found in *Silene neglecta* and *S. martinolii*, while they are usually absent in *S. nocturna* and the type material of

S. mutabilis. Very rarely, as was noted by Talavera (1990), very short glandular hairs can be observed in some specimens of *S. nocturna* (when occurring, a stalk consisting of 1–2 cells). Within *S. neglecta*, the hairiness of the calyces is somewhat variable. Some populations from Spain (Gavà, Bruguers, can Riera, L. Sáez, herb. pers.), Italy (Vulcano, L. Sáez, herb. pers.) have calyces covered with exclusively or mostly glandular patent hairs. In Tunisia (Tabarka, L. Sáez, herb. pers.) and southern Italy [Torregaveta (MA), Vigneti del Vesuvio (BC) and the type material of *S. neglecta*], the calyces are usually densely covered by eglandular hairs mixed with sparse glandular hairs. However, plants with both main types of calyx indument can be found within a single location [Italy, Isola Elba (PI); Tunisia: Tabarka (L. Sáez, herb. pers.)].

Corolla

Flower opening in *Silene nocturna* is mainly nocturnal, while in *S. neglecta* and *S. martinolii*, it is diurnal. The petals of *S. nocturna* and *S. mutabilis* are bifid (see also Linnaeus, 1753; 1756). In cleistogamous variants of *S. nocturna*, the petal limbs are very short (usually included) or even absent. On the contrary, the limb of the petals of *S. neglecta* and *S. martinolii* is subentire to emarginate (Fig. 3); the sinus of the limb can reach almost a third of its length. The lobes of *S. neglecta* and *S. martinolii*, when present, are much wider than those of *S. nocturna*, which are narrowly oblong to sublinear. After the description of *S. neglecta*, Tenore (1838) provided detailed illustrations of the species, showing the presence of pink petals with broad, not bifid limbs. The study of the lectotype of *S. neglecta* reveals that the limb of the petals is emarginate.

The colour of the corolla of *Silene neglecta* and *S. nocturna* varies from pale pink (rarely white) to pinkish-purple. The petal colouration is variable within *S. nocturna*, even in the same population. The petals can be white (sometimes greenish in the abaxial surface), pale rose and even tinged with pink-purple (mainly on the abaxial side). This is remarkable, since this colouration was invoked by Linnaeus (1756) to describe his *S. mutabilis* (“*petalis post florescentiam extus purpurescentibus*”). We have observed specimens in populations from Spain (Barcelona Province) with this colouration attributed by Linnaeus to *S. mutabilis* and that perfectly fits the current concept of *S. nocturna* (Fig. 4).

Stamens

The hairiness of the stamen filaments is of taxonomic significance and, based on our observations, it is always related to the calyx hairiness (see above). The filaments are glabrous in *Silene nocturna*, whereas in *S. neglecta* and *S. martinolii*, alternate filaments are hairy at base (sometimes all are hairy at base). In those specimens in which all the stamen filaments are hairy (plants from Tabarka, Corona su Crabi, Torre gaveta, Campania and Vulcano), the hairy portion of the filament is noticeably shorter in those stamens adjacent to the petals. Possibly, the difficult observation of this character can explain why it has gone unnoticed so far.

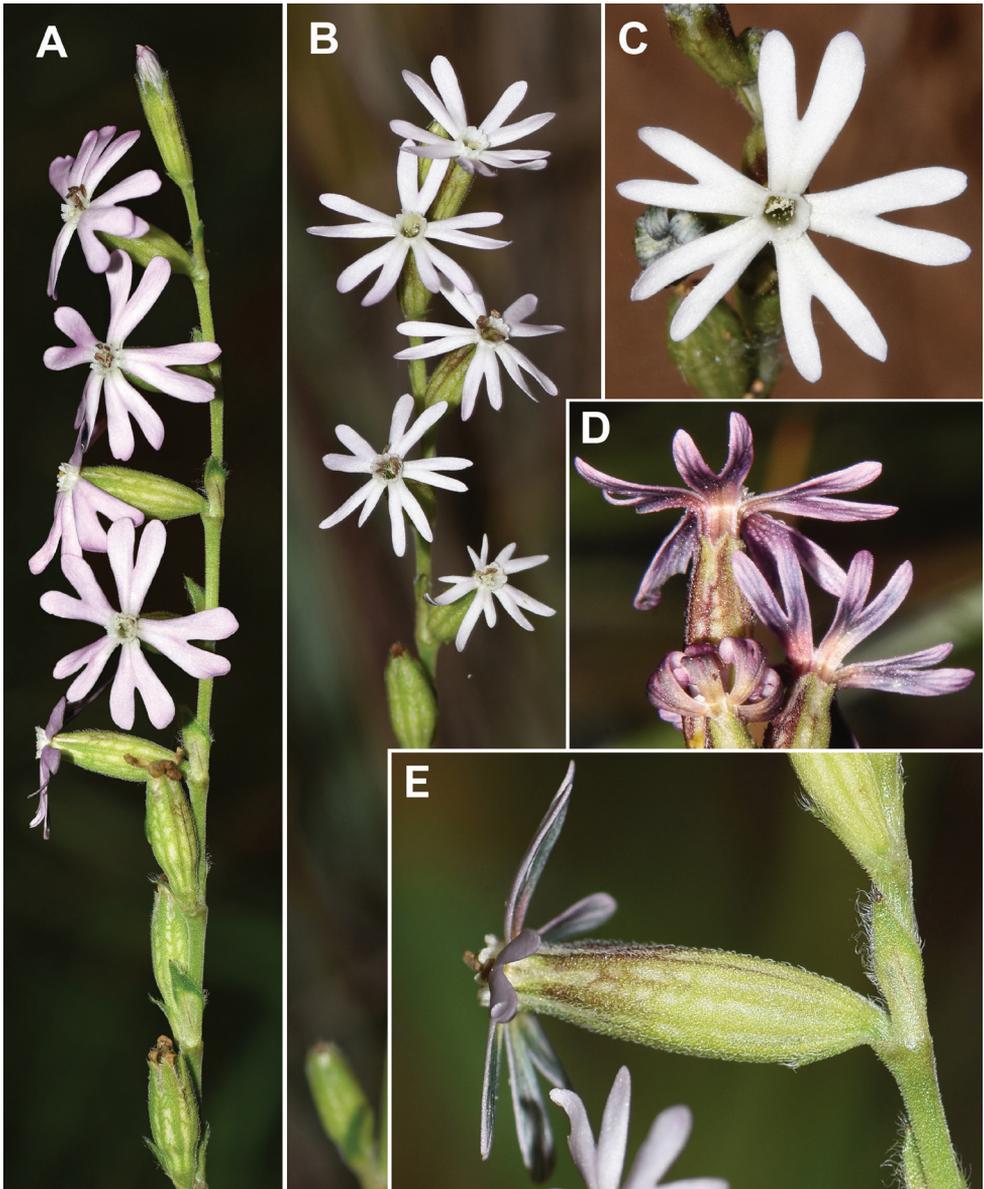


Figure 4. Field photos of *Silene nocturna* **A, B** inflorescences **C, D** flowers **E** flower in lateral view. Spain, Sant Feliu de Codines, 29 May 2021, L. Sáez (herb. pers.).

Seeds

The seeds are reniform with excavate lateral faces and a dorsal furrow. The seeds of *Silene nocturna* are somewhat smaller than those of *S. neglecta* and *S. martinolii*. There are differences in colouration (grey or greyish-brown in *S. nocturna*, blackish to dark-

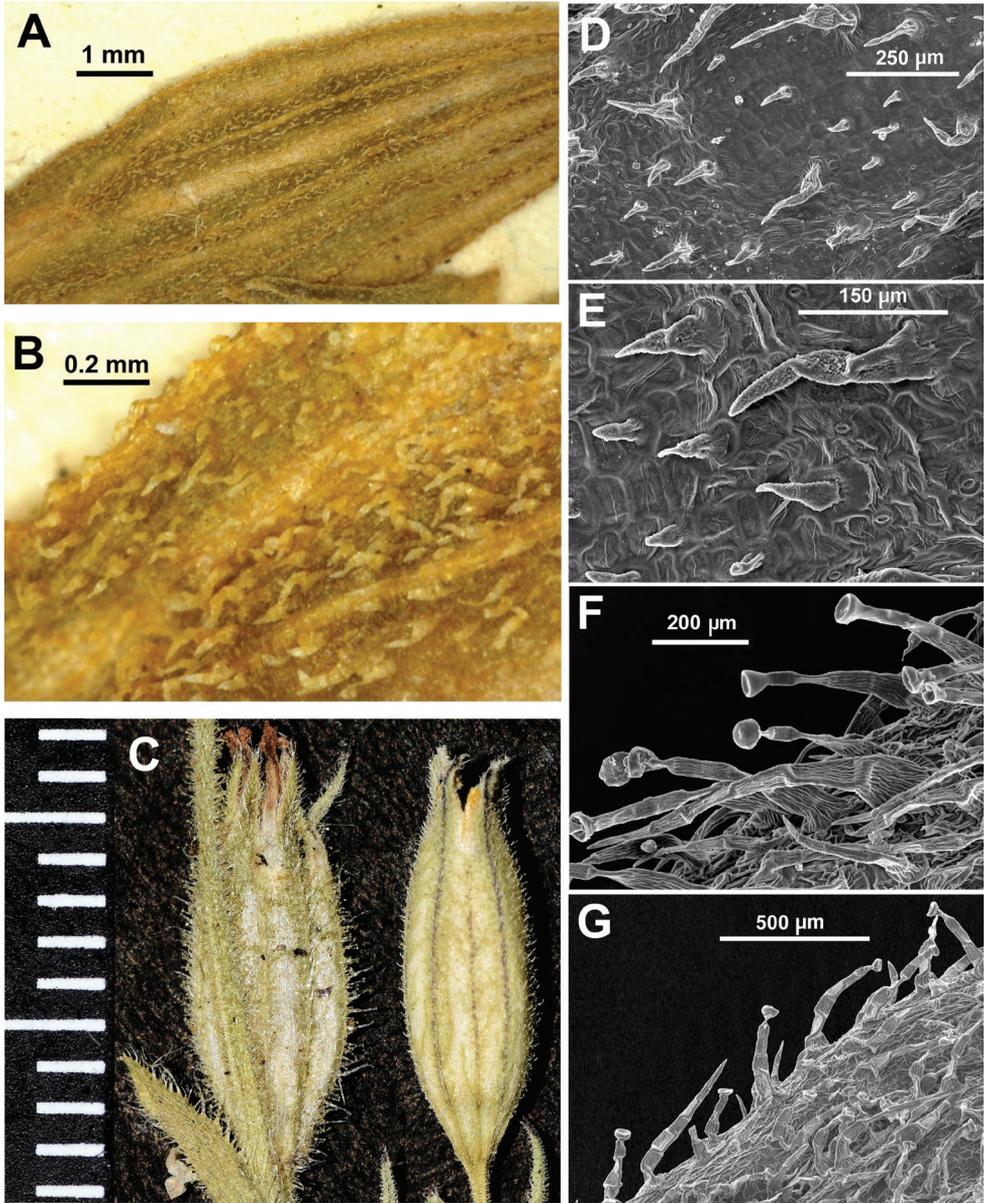


Figure 5. **A, B** indumentum of the lectotype of *Silene mutabilis* (LINN 583.8) **C** calyxes of *S. neglecta* (left, Isola d'Elba, PI) and *S. nocturna* (Livorno, PI) **D, E** calyx with eglandular hairs of *S. nocturna* (**D** Tunisia, L. Sáez herb. pers. **E** Livorno PI) **F, G** calyx with glandular and eglandular hairs of *S. neglecta*. (Tunisia, Tabarka, L. Sáez herb. pers.).

brown in *S. neglecta* and *S. martinolii*). Peruzzi et al. (2014) stated that the lectotype of *S. mutabilis* has polygonal (star-shaped) dorsal cells. However, based on our observations, *S. neglecta* has elongate (more or less polygonal), not star-shaped dorsal cells

(Fig. 6). The surface of the dorsal cells of *S. neglecta* has a more or less prominent central tubercle (up to 30 μm long), while in *S. nocturna*, these tubercles, when present, are not very prominent (less than 15 μm long). The pattern of morphological variability of *S. nocturna* seeds is complex and requires further study.

Taxonomic treatment

After a critical macro- and micromorphological analysis and detailed studies of the protologues, we conclude that *Silene mutabilis* is not conspecific with *S. neglecta*. *S. mutabilis* shows characters which fall within the range of morphological variation of the currently recognised *S. nocturna*. Further research is needed to identify the existence of taxonomic units within *S. nocturna*, which could be a polyphyletic species. Our study shows that *S. neglecta* and *S. nocturna* are distinct, based on macro-features (leaf shape and petal limb shape) and micromorphological characters (calyx and stamens indumentum and SEM analysis of seeds). An amended description is here also provided for *S. neglecta*, based on herbarium and live specimens collected from North Africa, Italy, Sardinia, Sicily and Spain. Based on this macro- and micromorphological

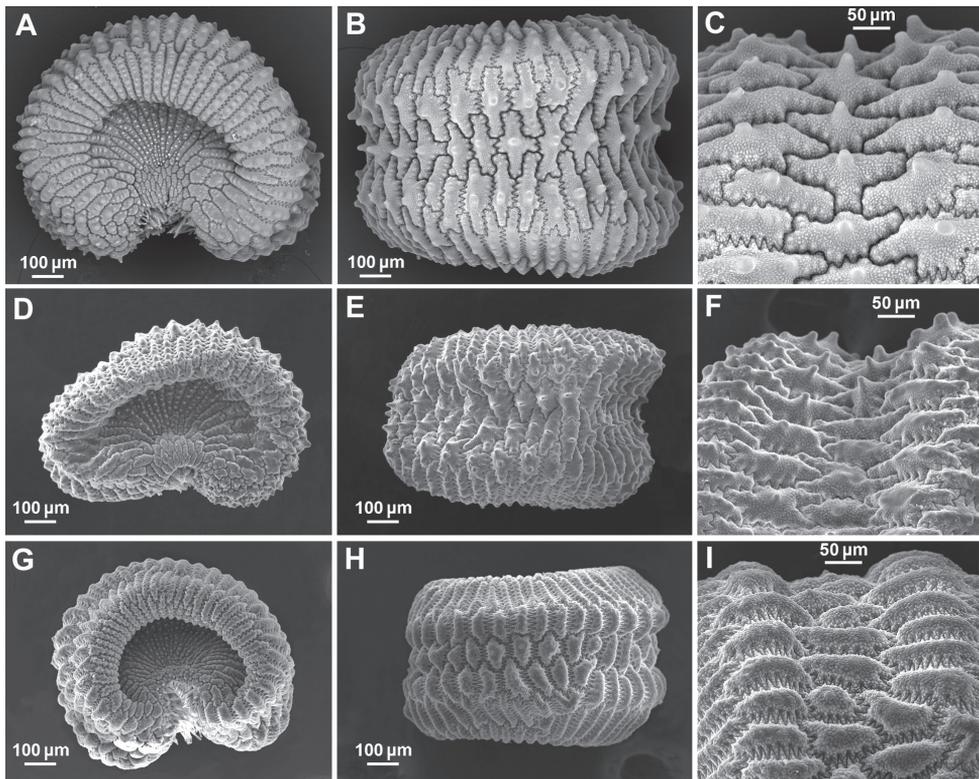


Figure 6. Seed micromorphology for *Silene neglecta* from Spain, Gavà, Roca de Barret (L. Sáez herb. pers.) (A, B, C), Tunisia, Tabarka (L. Sáez herb. pers.) (D, E, F) and *S. nocturna* Italy, Livorno (PI) (G, H, I). Lateral view (A, D, G); dorsal view (B, E, H); dorsal furrow (C, F, I).

evidence, in addition to morphometric analysis, we can conclude that *S. martinolii* falls within the variation of *S. neglecta*. Our preliminary phylogenetic results (Mesbah et al., in prep.) suggest that *S. neglecta* is not related to *S. nocturna*, but more closely related to *S. gallica* L., the type species of the genus *Silene*.

***Silene neglecta* Ten., Fl. Neapol. Prodr. App. 5: 13. 1826.**

≡ *Silene nocturna* subsp. *neglecta* (Ten.) Arcang. Comp. Fl. Ital.: 88. 1882. Lectotype (designated by Peruzzi & Carta, 2013: 45): [ITALY] Campania: Persano, s.d., *Tenore s.n.* (NAP!).

= *Silene martinolii* Bocchieri & B.Mulas, Boll. Soc. Sarda Sci. Nat. 26: 301. 1988; syn. nov. Holotype (Bocchieri & Mulas, 1988: 301): [ITALY. Sardinia] Isola il Toro, 22 May 1988, *Bocchieri & Mulas s.n.* (CAG!).

Description. Annual herb (rarely biennial) green or greyish-green, hairy. Stems (10–)15–50(–70) cm, more or less erect (rarely prostrate-ascending), unbranched to much-branched, densely hairy, usually viscid above. Lower leaves spatulate to obovate; cauline leaves obovate to linear-lanceolate. Flowers 2–9(–10) in raceme-like monochasial cymes; lowermost pedicels shorter or longer than calyx (0.45–2.00 times as long as calyx in fruit), erect to patent, pubescent-glandular. Calyx 8–13 mm long, subcylindrical to cylindrical-ovoid in flower, attenuate, becoming subovoid to ovoid in fruit, usually densely hairy, covered by long eglandular hairs (up to 1.9 mm long) and glandular hairs (up to 2 mm long); calyx teeth triangular to linear-lanceolate or linear, acute; veins anastomosing. Petals pale pink to pinkish-purple, rarely white; limb 4.5–6.0 mm, subentire or emarginate; coronal scales 1.2–2.2 mm, whitish to pink. Stamens with alternate filaments hairy at base, sometimes all hairy at base; anthers 1.0–1.5 mm long, lilac to purple, exserted from corolla mouth. Gonophore (1.8–)2.0–2.4(–2.6) mm long, puberulent. Capsule 6.0–8.5 mm, subcylindrical, enclosed within the calyx. Seeds (0.90–)0.91–1.02(–1.05) × (0.76–)0.80–0.89(–0.91) blackish to dark brown; faces deeply concave, tuberculate; back wide, slightly canaliculate.

Flowering time. Between March (exceptionally at the beginning of February) and May.

Chromosome number. $2n = 24$ (Peruzzi and Carta 2013; Bosch et al. 2019).

Habitat. Rocky places, maritime sands and grassland, usually on siliceous substrata, 0–1700 m a.s.l.

Distribution. Central and southern Italy, south-western Sardinia, Sicily, northern Algeria and Tunisia and north-eastern Spain.

Specimens examined. Algeria: Terrains siliceux, berges maritimes sur la route de Cap de Garde, au-dessous de la colline des Caroubiers à Bône, 5 March 1867, *Tribout* (P05032571); Bône, rochers maritimes, 12 Apr 1867, *Tribout* (P05033844); subénaire, près de Bessombourg, 500–600 m alt., Kabylie de Collo, 17 May 1944, *L. Faurel* (P05110031, P05135518); subénaire, des crêtes de Boudra, près de Bessombourg, 600 m alt., Kabylie de Collo, 26 May 1944, *L. Faurel* (P05050465); pentes rocheuses

du flanc sud du Djebel Tababort, 1700 m alt., (Dt. de Constantine), 29 May 1952, *L. Faurel* (P05110033); près du Col de Terras, 800 m alt., environ 5 km à l'ouest de Zitouna (ex Bessombourg), Kabylie de Collo, 8 Juin 1984, *A. Dubuis* (MPU286766); **Italy**: Campania: Ischia, Oct 1835, *Gussone* (K00728586); Campania, vigneti del Vesuvio, 150 m alt., 29 Apr 1911, *G. Pellanda* (BC8940, MA 31093, US1272504); Campi Flegrei, prope Capo Miseno, 100 m alt., solo siliceo, 15 May 1913, *M. Guadagno* (BC8939); Torre Gaveta, May 1913, *M. Guadagno* (MA31092, MA31096, MPU300592); Sardinia: Isola il Toro, 22 May 1988, *E. Bocchieri* & *B. Mulas* (CAG, holotype *S. martinolii*); Isola Rossa, Golfo di Teulada, 10 July 1988, *E. Bocchieri* (CAG, sub *S. martinolii*); Isola la Vacca, 18 Feb 1990, *E. Bocchieri* (CAG, sub *S. martinolii*); S'Aqua sa Canna, Isola di S. Antioco, 12 Apr 1992, *L. Mossa* (CAG, sub *S. martinolii*); Sa Corona su Crabi, isola di S. Antioco, 20 May 1993, *L. Mossa* (CAG, sub *S. martinolii*); Sicily: Lipari, Apr 1902, *Ross 317* (WAG); Vulcano Island, Apr 2017, *D. Carrera* (grown from seed by J. López & L. Sáez, May 2018, L. Sáez, herb. pers.); Tuscany: Insula Igilium (Giglio), 17 May 1894 *Sommier* (MPU772254); Insula Igilium (hod. Giglio), prope Portum, 5 Apr 1899, *Sommier* (MA31097); Insula Inarime, in campis aridis, May 1905, *M. Guadagno* (US548489); Isola d'Elba (Tuscan Archipelago), Miniera del Ginevro, 27 Apr 2017, *B. Pierini* (PI, sub *S. mutabilis*). **Spain**: Barcelona: Bruguers, 25 May 1984, *C. Blanché* (BCN 41111); Garraf massif, Gavà, Ermita de Bruguers, siliceous rocks, 265 m alt., 6 May 2015, *L. Sáez LS-7626* (L. Sáez, herb. pers.); Cervelló, Can Riera, 350 m alt., 9 May 2015, *D. Carrera* & *L. Sáez LS-7629* (L. Sáez, herb. pers.); Torrelles de Llobregat, Roca Plana NE from Turó de la Bruguera, 280 m alt., 23 Apr 2016, *L. Sáez LS-7706* (L. Sáez, herb. pers.); Gavà, Roca del Barret, 23 Apr 2016, *L. Sáez* (L. Sáez, herb. pers.); ibidem, 26 May 2016, *L. Sáez LS-7707* (L. Sáez, herb. pers.); ibidem, 29 Apr 2021, *L. Sáez* (herb. pers.); between castell d'Eramprunyà and Bruguers, 26 May 2016, *L. Sáez* (herb. pers.). **Tunisia**: Tabarka, maritime sands, March 2017, *R. El Mokni* (L. Sáez, herb. pers.; R. El Mokni, herb. pers.).

Silene nocturna L.

Specimens examined. **Algeria**: Batna: Campus Batna II, 14 Apr 2019, *F. Bekdouche* (GB); Bouira: Crête Rouge, 23 Apr 2017, *M. Mesbah* (ENSA); Bechloul devant la porte du lycée (Bouira), 17 Dec 2019, *M. Mesbah* (GB); W Sétif, a 800 m de Tizi N'Bechar, Apr 2019 *F. Bekdouche* (GB); Tlemcen, May 2018, *B. Babali* (ENSA); forêt de Remchi - Tlemcen, 1 June 2018, *B. Babali* (GB); Bejaia, Melbou 16 Apr 2019, *M. Mesbah* (GB). **Croatia**: Starigrad, Paklenica, 2 m alt., 27 Apr 2013, *L. Peruzzi* (PI). **France**: Bouches du Rhône: Marseille, 9 May 1866, *Linder* (BC135145); Garlaban, 19 May 1971, *R. Roncart* (BC803193); Corsica: Corse-du-Sud: Bonifacio, sémaphore de Pertusato, along the path, 85 m alt., sandy beach, 28 Apr 2008, *B. Frajman* & *P. Schönswetter* (GB); Hérault: Maguelone, Montpellier, lungo la strada che porta alla cathédrale Saint-Pierre-et-Saint-Paul de Maguelone, 2 m alt., 11 June 2016, *F. Roma-Marzio 379/2*, *G. Astuti*, *M. D'Antraccoli* & *L. Peruzzi* (PI); **Greece**: Nomos Fokidas, About 5 km W Galaxidia, 5 May 1984 *B. Oxelman* & *L. Tollsten* (GB); Nomos Arkadias, Mount Par-

non, 600–700 m alt., 29 Apr 1985, *B. Oxelman & L. Tollsten* (GB); Delfi, roadside in Parnassidos Province of Fokidos, 6 May 1984, *B. Oxelman* (GB-0194077). **Iran:** Gilan Province, *Mozaffarian* (TUH6771). **Italy:** Basilicata: Potenza in pascuis, 50–850 m alt., 13 May 1928, *Gavioli* (BC8926); Calabria: Tarsia (Cosenza), 156 m alt., May 2017, *G. Fasano* (PI, sub *S. mutabilis*); Campania: Parco Archeologico di Carbonara, Aquilonia, 600–675 m alt., 3 June 2015, *G. Astuti, L. Peruzzi & F. Roma-Marzio* (PI); Tuscany: Monte Pisano, Annunziata, 25 m alt., 11 May 2010, *L. Peruzzi, B. Pierini & G. Bedini* (PI); Isola di Capraia, 32TNN67.67, 242 m alt., 1 Apr 2012 *L. Peruzzi & G. Gestri* (PI); Rosignano Marittimo (Livorno), Castiglione, 15 June 2016, *L. Peruzzi* (PI, sub *S. mutabilis*). **Morocco:** Tangier-Tetouan: pr. Zinnat, inter Tandja & Tetouan, 28 May 1930, *Font Quer* (BC110896). **Spain:** Balearic Islands: Cabrera, camino de sa Vicaria, 27 July 1947, *Palau Ferrer* (MA31038); Cabrera, Es Penyal Blanc, 3 Apr 1948, *P. Ferrer* (BC104070); Formentera, La Mola, 1918, *Gros* (BC110894); Eivissa, Santa Eulàlia, 2 June 1918, *Gros* (BC 110897); Pla de Vila, 15 May 1919, *Font Quer* (BC110902); Mallorca, Bellver, pr. Palma, 12 May 1920, *Font Quer* (BC110901); Mallorca, Lluc, Clot d'Albarca, s.d., [Bonafè] (HJBS 1243); Mallorca, Palma towards Secar de la Real, 45 m alt., 2 June 2013, *L. Sáez, LS-7382* (*L. Sáez*, herb. pers.); Menorca, Ciutadella, Montefi, en el km 43 de la carretera, 31TEE7328, 40 m alt., 30 Apr 1951, *P. Montserrat* (JACA36851); Ciutadella, Torre del Ram, 31TEE6929, 40 m alt., 16 Apr 1957, *P. Montserrat* (JACA297519); Menorca, Trbaluger, bajando de Son Olivar, 60–70 m alt., 22 Apr 1957, *P. Montserrat* (JACA297520, JACA 297521); Menorca, Algaiarens, 23 Apr 1993, *P. Fraga* (*L. Sáez*, herb. pers.); Menorca, Arenal de Salomó, 11 June 1993, *P. Fraga* (*L. Sáez*, herb. pers.); Barcelona: Hospitalet de Llobregat, 26 Apr 1872, *A.C. Costa* (BC614253); Maresme, Camí Geganta, 7 Apr 1936, *P. Montserrat* (BC609200); Turó de Santa Margarida, Cànoves i Samalús, 350 m alt., 11 Apr 2015, *L. Sáez LS-7604* (*L. Sáez*, herb. pers.); Sant Feliu de Codines, towards Sot de l'Ullar, 460 m alt., grassland, siliceous rocks, 22 Apr 2016, *L. Sáez* (*L. Sáez*, herb. pers.); Viladecans, davant Parc de Can Guardiola, 31TDF1574, 3 May 2021, *A. Salvat* (*L. Sáez*, herb. pers.); Sant Feliu de Codines, Roques d'en Pere Pericó, 550 m alt., 29 May 2021, *L. Sáez LS-7910* (*L. Sáez*, herb. pers.); Castelló: Camí de Villareal, May 1954, *M. Caldusch* (BC128966); Girona Province, Sant Julià de Llor, 31 May 1920, s.r. (BC8927); Jaén: Albandes, márgenes del camino a Torres, 7 June 1925, *Cuatrecasas* (BC8897); Huesca: Salto de Roldan, YM1581, 1000 m alt., 19 July 1980, *J.M. Martí* (BC922231); La Almunia del Romeral, YM2476, 620 m alt., 3 May 1981, *J.M. Montserrat* (BC922230 sub *S. nocturna* subsp. *neglecta*); Málaga: plaza Adnana, waste land, 15 Apr 1968, *Strandhede & al.* 45 (GB); Navarra: Unzue, Puerto del Carrascal, 590 m alt., 22 May 1988, *I. Aizuru & P. Catalán* (BC834596); Tarragona: Coll de Balaguer, Hospitalet de l'Infant, 27 Apr 1974, *R. Folch & E. Velasco* (BC627072). **Tunisia:** Bizerta, 5 March 2017, *R. El Mokni* (*L. Sáez*, herb. pers.; *R. El Mokni*, herb. pers.); Melloula, 7 March 2017, *R. El Mokni* (*L. Sáez*, herb. pers.; *R. El Mokni*, herb. pers.); Monastir, 25 March 2017, *R. El Mokni* (*L. Sáez*, herb. pers.; *R. El Mokni*, herb. pers.); Tabarka, 7 March 2017, *R. El Mokni* (*L. Sáez*, herb. pers.; *R. El Mokni*, herb. pers.); Tabarka, Kroumiria, 1 May 2018, *R. El Mokni* (*L. Sáez*, herb. pers.; *R. El Mokni*, herb. pers.). **Turkey:** Mugla: road Mugla-Marmaris, 12 km N of Marmaris, 4 May 1988, *B. Oxelman* (GB). **Unknown origin:** Herb. Linn. No. 583.8 (LINN; lectotype).

Acknowledgements

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Appendix I

List of specimens included in morphometric analyses.

S. martinolii

Italy: Sardinia, Isola il Toro, 22 May 1988, *E. Bocchieri* & *B. Mulas* (CAG); Isola Rossa, Golfo di Teulada, 10 July 1988, *E. Bocchieri* (CAG); S'Aqua sa Canna, Isola di S. Antioco, 12 Apr 1992, *L. Mossa* (CAG) [2 specimens]; Sa Corona su Crabi, Isola di S. Antioco, 20 May 1993, *L. Mossa* (CAG).

S. neglecta

Italy: Insula Igilium (hod. Giglio), prope Portum, 5 Apr 1899, *S. Sommier* (MA 31097); Campania, vigneti del Vesuvio, 150 m alt., 29 Apr 1911, *G. Pellanda* (BC 8940, MA 31093) [2 specimens]; Campi Flegrei, prope Capo Miseno, 100 m alt., solo siliceo, 15 May 1913, *M. Guadagno* (BC8939); Torre Gaveta, May 1913, *M. Guadagno* (MA 31092, MA 31096) [2 specimens]; Italy, Isola d'Elba (Tuscan Archipelago), Miniera del Ginevro, 27 Apr 2017, *B. Pierini* (PI) [3 specimens]; Sicily, Vulcano Island, Vulcano Island, April 2017, *D. Carrera* (grown from seed by J. López & L. Sáez, May 2018, L. Sáez, herb. pers.) [2 specimens]. **Spain:** Barcelona Province, Cervelló, Can Riera, 350 m alt., 9 May 2015, *D. Carrera* & *L. Sáez LS-7629* (L. Sáez, herb. pers.); Torrelles de Llobregat, Roca Plana NE from Turó de la Bruguera, 280 m alt., 23 Apr 2016, *L. Sáez LS-7706* (L. Sáez, herb. pers.); Gavà, Roca del Barret, 23 Apr 2016, *L. Sáez* (L. Sáez, herb. pers.) [4 specimens]; ibidem, 26 May 2016, *L. Sáez* (L. Sáez, herb. pers.); between castell d'Eramprunyà and Bruguers, 26 May 2016, *L. Sáez* (herb. pers.) [2 specimens]. **Tunisia:** Tabarka, maritime sands, March 2017, *R. El Mokni* (L.

Sáez, herb. pers.; R. El Mokni, herb. Pers.) [4 specimens]; Tabarka, Kroumiria, 1 May 2018, *R. El Mokni* (L. Sáez, herb. pers.; R. El Mokni, herb. pers.) [3 specimens].

S. nocturna

Algérie: Crête Rouge, 1 June 2017, *M. Mesbah* (GB); forêt de Remchi - Tlemcen, 1 June 2018, *B. Babali* (GB); Campus Batna II (Batna), 14 Apr 2019, *F. Bekdouche* (GB); Melbou (Bejaia), 16 Apr 2019, *M. Mesbah* (GB); A 800 m de Tizi N. Bechar (W Sétif), Apr 2019, *F. Bekdouche* (GB); Bechloul devant la porte du lycée (Bouira), 17 Dec 2019, *M. Mesbah* (GB). **Croatia:** Starigrad, Paklenica, 2 m alt., 27 Apr 2013, *L. Peruzzi* (PI). **France:** Marseille, 9 Mai 1866, *Linder* (BC135145); Bouches du Rhône, Garlaban, 19 May 1971, *R. Roncart* (BC803193); Corsica, Corse-du-Sud: Bonifacio, sémaphore de Pertusato, along the path, 85 m alt., sandy beach, 28 Apr 2008, *B. Frajman & P. Schönswetter* (GB); Maguelone, (Montpellier), lungo la strada che porta alla cathédrale Saint-Pierre-et-Saint-Paul de Maguelone, 2 m alt., 11 June 2016, *F. Roma-Marzio 379/2, G. Astuti, M. D'Antraccoli & L. Peruzzi* (PI). **Greece:** Nomos, Fokidas, About 5 km W Galaxidia, limestone cliffs, 5 May 1984, *B. Oxelman & L. Tollsten* (GB); Nomos, Arkadias, Mount Parnon, 600–700 m alt., 29 Apr 1985, *B. Oxelman & L. Tollsten* (GB); Delfi, roadside in Parnassidos Province of Fokidos, 6 May 1984, *B. Oxelman* (GB). **Italy:** Potenza in pascuis, 50–850 m alt., 13 May 1928, *Gavioli* (BC 8926); Monte Pisano, Annunziata, 25 m alt., 11 May 2010, *L. Peruzzi, B. Pierini & G. Bedini* (PI) [2 specimens]; Isola di Capraia, 32TNN67.67, 242 m alt., 1 Apr 2012 *L. Peruzzi & G. Gestri* (PI) [2 specimens]; Rosignano Marittimo (Livorno), Castiglionello, 15 June 2016, *L. Peruzzi* (PI, sub *S. mutabilis*) [2 specimens]; Calabria, Tarsia (Cosenza), 156 m alt., May 2017, *G. Fasano* (PI, sub *S. mutabilis*). **Spain:** Balearic Islands, Ciutadella de Menorca, Torre del Ram, 31TEE6929, 40 m alt., 16 Apr 1957, *P. Montserrat* (JACA297519); Menorca, Trabaluger, bajando de Son Olivar, 60–70 m alt., 22 Apr 1957, *P. Montserrat* (JACA297520); Menorca, Ciutadella, Montefi, en el km 43 de la carretera, 40 m alt., 30 Apr 1951, *P. Montserrat* (JACA36851); Mallorca, Palma towards Secar de la Real, 45 m alt., 2 June 2013, *L. Sáez, LS-7382* (L. Sáez, herb. pers.); Barcelona Province, Sant Feliu de Codines, towards Sot de l'Ullar, 460 m alt., 22 Apr 2016, *L. Sáez* (L. Sáez, herb. pers.); Sant Feliu de Codines, Roques d'en Pere Pericó, 550 m alt., 29 May 2021, *L. Sáez LS-7910* (L. Sáez, herb. pers.); Viladecans, davant Parc de Can Guardiola, 31TDF1574, 3 May 2021, *A. Salvat* (L. Sáez, herb. pers.); Jaén Province, Albandes, márgenes del camino a Torres, 7 June 1925, *Cuatrecasas* (BC8897); Huesca Province, La Almunia del Romeral, YM2476, 620 m alt., 3 May 1981, *J.M. Montserrat* (BC922230); Málaga Province, plaza Adnana, waste land, 15 Apr 1968, *Strandbæde & al.* 45 (GB); Navarra Province, Unzue, Puerto del Carrascal, 590 m alt., 22 May 1988, *I. Aizuru & P. Catalán* (BC834596); Tarragona Province, Coll de Balaguer, Hospitalet de l'Infant, 27 Apr 1974, *R. Folch & E. Velasco* (BC627072). **Tunisia:** Bizarte, 5 March 2017, *R. El Mokni* (L. Sáez, herb. pers.; R. El Mokni, herb. pers.); Melloula, 7 March 2017, *R. El Mokni* (L. Sáez, herb. pers.; R. El Mokni, herb. pers.). **Turkey:** Mugla: road Mugla-Marmaris, 12 km N of Marmaris, 4 May 1988, *B. Oxelman* (GB).

Two new *Halamphora* (Bacillariophyta) species from the marine coasts off Livingston Island, Antarctica

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Abstract

During a survey of the marine benthic diatom flora on the coasts off Livingston Island (South Shetland Islands, Maritime Antarctic Region), two *Halamphora* species that could not be identified based on the currently available literature, were observed. Detailed light and scanning electron microscopy observations and thorough comparison with similar taxa in the literature revealed that both taxa should be described as new species. The first taxon, *Halamphora kenderoviana* **sp. nov.**, was most likely misidentified in past Antarctic studies, and included within the range of another taxon, *Halamphora coffeaeformis*. Analysis of literature data showed that the second new taxon, *Halamphora moncheviana* **sp. nov.**, has been previously reported from the Antarctic Continent (but as an unidentified species). The new taxa are compared with similar *Halamphora* taxa worldwide. Data on their ecology and distribution are also provided.

Keywords

Amphora sensu lato, diatoms, marine benthos, taxonomy

Introduction

In the past two decades considerable effort has been undertaken to improve our understanding of the diversity, species identities and distribution of the terrestrial and freshwater diatoms (Bacillariophyta) in the Antarctic realm (Zidarova et al. 2016 and

references therein). In contrast, the marine benthic diatom flora of the region remained far less studied. Although in the past 30 years three new marine benthic diatom genera have been described from islands in the Southern Ocean [*Tabulariopsis* D.M. Williams (Williams 1988), *Brandinia* L.F. Fernandes (in Fernandes et al. 2007), and *Australoneis* J.M. Guerrero & Riaux-Gob. (in Guerrero et al. 2021)], and several new species have been recognized and described within existing genera [including *Berkeleya* Grev. (Medlin 1990), *Cocconeis* Ehrenb. (Al-Handal et al. 2008, 2010), *Gomphonemopsis* Medlin (Al-Handal et al. 2018), *Nitzschia* Hassall (Al-Handal et al. 2019), *Melosira* C. Agardh (Fernandes and de Souza-Mosimann 2001), *Pteroncola* R.W. Holmes & Croll (Almandoz et al. 2014) and *Rhoicosphenia* Grunow (Ligowski et al. 2014)], a recent study on the marine benthic diatom flora from South Bay (Livingston Island, South Shetland Islands) showed that a large number of the recorded taxa could not be identified with certainty up to species level, with some of these taxa most likely being new to science (Zidarova et al. 2022).

The present paper describes two new marine species in the genus *Halamphora* (Cleve) Levkov, observed in several recently collected samples from the coasts off Livingston Island, part of the archipelago of the South Shetland Islands (Maritime Antarctic Region).

The genus *Halamphora* (Cleve) Levkov, originally described in 1895 by Cleve as a subgenus of *Amphora* Ehrenb. ex Kütz., was raised to genus level in 2009 (Levkov 2009). The characteristic features of the genus include a moderately to strongly dorsiventral valve outline, an eccentric raphe system, uni- to biseriate striae composed of round to elliptical and even transversally elongated areolae, internally occluded by hymenes, and a girdle composed of numerous open copulae. *Halamphora* species can be found both in freshwater and marine ecosystems (Levkov 2009). In the Antarctic Region, Van de Vijver et al. (2014) revised the freshwater *Halamphora* species, describing several new taxa, but in the marine realm data on *Halamphora* (or former *Amphora*) species are scarce. One of the observed species in the present study has most likely been misidentified and reported in the earlier Antarctic literature under the name of another, presumably widespread marine *Halamphora* species, *H. coffeaeformis* (C. Agardh) Kütz., whereas the second taxon is unknown. Following extensive light and scanning electron microscopy observations and comparisons of their morphology with similar taxa from all over the world and from the Antarctic Region, both taxa are described as new species: *Halamphora kenderoviana* sp. nov. and *Halamphora moncheviana* sp. nov. A survey of the Antarctic literature with iconographic material provided additional information on their basic ecology and Antarctic distribution.

Materials and methods

Livingston Island is the second largest of the South Shetland Islands, located ca. 130 km north of the Antarctic Peninsula (Fig. 1). Samples were collected in December 2018 and February 2020 from the epilithon of small pools on coastal rocks at the southern coasts of the island, including Hannah Point area and the eastern shores of South Bay (Fig. 1). These pools are small water basins, formed during low tide on or

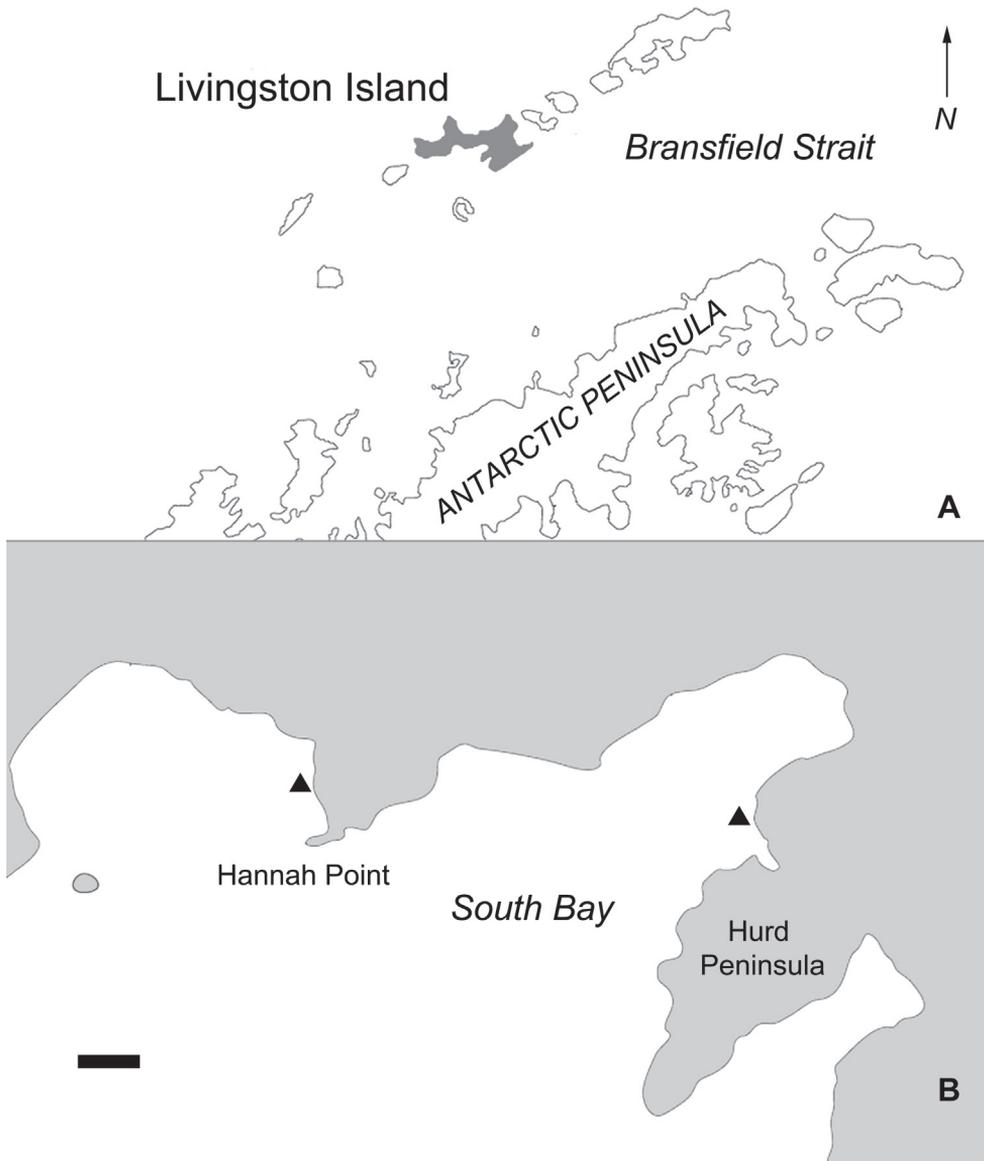


Figure 1. Map showing the position of Livingston Island relative to the Antarctic Peninsula (**A**), and the locations of the sampling sites (**B**, triangles). Map outlines are based on OpenStreetMap contributors (www.openstreetmap.org), edited and arranged using Adobe Illustrator and Adobe Photoshop. Scale bar: 2 km (**B**).

between larger rocks on the coasts, and having variable water temperature and salinity levels (Zidarova et al. 2022 and references therein). The biofilm covering the bottom or sides of the pools was collected using a toothbrush and preserved with 3% formaldehyde *in situ*. Samples, with their environmental parameters measured with a handheld multi-parameter meter WTW3410 during sample collection, are listed in Table 1.

Table 1. Samples containing the new taxa, with their environmental parameters.

Sample	Date	pH	Salinity, PSU	Conductivity, mS/cm	O ₂ , %	O ₂ , mg.L ⁻¹	Water T, °C
MO'	21/12/2018	8.4	33.1	52.2	n/a	n/a	5.5
LT10	04/02/2020	n/a	6.5	11.5	126.0	11.6	18.8

For light microscopy (LM), diatom samples were prepared following the method of Hasle and Fryxell (1970). Cleaned material was mounted in Naphrax. LM observations were conducted using an Olympus BX51 light microscope at 1000× magnification (N.A. 1.30), equipped with Differential Interference Contrast (DIC) optics and Olympus digital imaging system. For scanning electron microscopy (SEM), part of the suspension was filtered through 5 µm Isopore polycarbonate membrane filters (Merck Millipore), after air-drying pieces of which were affixed with double sided carbon stickers (Agar Scientific Carbon Tabs) on 12.7 mm Ø aluminium stubs (Agar Scientific Ltd), coated with a platinum layer of 20 nm and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 2 kV. Slides and stubs are stored at the BR-collection (Meise Botanic Garden, Belgium). Plates with the microphotographs of the species were prepared using Adobe Photoshop. For stria number, measurements were done starting from the valve middle. Terminology for taxa descriptions follows Round et al. (1990), Levkov (2009), and Stepanek and Kociolek (2018). In a search for more ecological and distributional data for the taxa we describe, a survey of the earlier Antarctic literature was conducted, including the major older works (e.g. Van Heurck 1909; Peragallo 1921; Frenguelli and Orlando 1958; Simonsen 1992), as well as more recent reports with iconographic material, such as Roberts and McMinn (1999), Cremer et al. (2003), and others.

Results

Descriptions of new species

Systematics follows the adopted in DiatomBase (Kociolek et al. 2022)

Phylum Bacillariophyta Haeckel

Class Bacillariophyceae Haeckel

Family Amphipleuraceae Grunow

Genus *Halamphora* (Cleve) Levkov

Halamphora kenderoviana Zidarova, P.Ivanov, Dzhebekova, M.de Haan & Van de Vijver, sp. nov.

Fig. 2A–M

Holotype. Slide BR-4681, Fig. 2D represents the holotype, Meise Botanic Garden, Belgium. Phycobank (<http://phycobank.org/103140>).

Isotype. Slide 401, University of Antwerp, Belgium.

Type locality. ANTARCTICA, Livingston Island, South Bay, Mongolian (Reserve) Port, small pool on a coastal rock during low tide, epilithon. 62°38'50"S, 60°22'26"W. Sample MO', *leg.* R. Zidarova, coll. date 21 Dec. 2018.

Description. LM description (Fig. 2A–I). Valves semi-lanceolate to narrowly semi-elliptic, with a straight ventral and distinctly convex dorsal margin. Valve dimensions ($n = 27$): length 13.5–20.5 μm , width 3.5–4.5 μm . Apices slightly ventrally bent, in larger valves protracted, rostrate to almost subcapitate (Fig. 2B), in smaller valves weakly protracted, subrostrate (Fig. 2F–I). Raphe branches straight. Central raphe endings expanded, slightly dorsally bent (Fig. 2A, C, I). Distal raphe fissures not discernible in LM. Axial area narrow. Central area on the dorsal side very small to usually absent, on the ventral side clearly enlarged. Dorsal striae parallel to weakly radiate in the middle, becoming more radiate towards the apices, 18–20 in 10 μm . Occasionally, one or two striae in the valve middle shortened (Fig. 2E, G), forming a very small dorsal central area. Ventral striae discernible in LM, interrupted in the valve middle (Fig. 2C–F, I), 27–28 in 10 μm .

SEM description (Fig. 2J–M). Externally, valves possess a distinct raphe ledge, running along the entire length of the valve, clearly widened in the valve middle, truncated and slightly expanded at the apices (Fig. 2J, M). Central raphe endings positioned relatively close together, slightly bent towards the dorsal side and pore-like enlarged (Fig. 2J, M). Terminal raphe fissures hooked to the dorsal side (Fig. 2J, M). Dorsal striae biseriate, composed of rounded poroids, the latter 60–65 in 10 μm . Striae continuing on the mantle, following a narrow dorsal ridge (Fig. 2M), where often reduced to a single, large areola (Fig. 2J). Striae on the ventral side short, composed of only one or two rounded areolae, often fused to form a single elongated areola (Fig. 2J). Internally, central raphe endings terminating onto a fused central helictoglossa (Fig. 2K). Terminal raphe endings finishing onto small helictoglossae (Fig. 2K). Striae internally located between narrow, quite prominently raised virgae (costae). Areolae internally occluded by individual hymenes (Fig. 2L).

Etymology. The new species is named after our colleague Dr Lyubomir Kenderov, hydrobiologist at the Faculty of Biology, University of Sofia, with whom RZ shared two Antarctic seasons, and who was often a helping hand during field work in Antarctica.

Ecology, Antarctic distribution and associated diatom flora. *Halumphora kenderoviana* was typically observed in tidal pools (Zidarova et al. 2022, as *Amphora* sp.5), but only found in abundance (17.5% of the counted valves) in the type locality, a tidal pool with alkaline water and a salinity level of 33.1 PSU (Table 1). Other common taxa in the sample are *Parlibellus rhombicus* W.Greg., *Tabulariopsis australis* (Perag.) D.M.Williams, and several *Navicula* species, including *N. aff. perminuta* Grunow and *N. glaciei* Van Heurck. So far, *H. kenderoviana* is known to be present with certainty on the marine coasts of the South Shetland Islands (Livingston Island). Earlier, Cremer et al. (2003) reported a very similar taxon as *Amphora coffeaeformis* (Cremer et al. 2003, fig. 13) from sediment cores in Windmill Island, East Antarctica. SEM observations will be needed to confirm that it is conspecific with *H. kenderoviana*, but it seems likely that at least some of the records of *A. coffeaeformis*, transferred to *Halumphora* by Levkov (2009) as *H. coffeaeformis* (Kütz.) Levkov, from saline waters in Antarctica might represent *H. kenderoviana*.

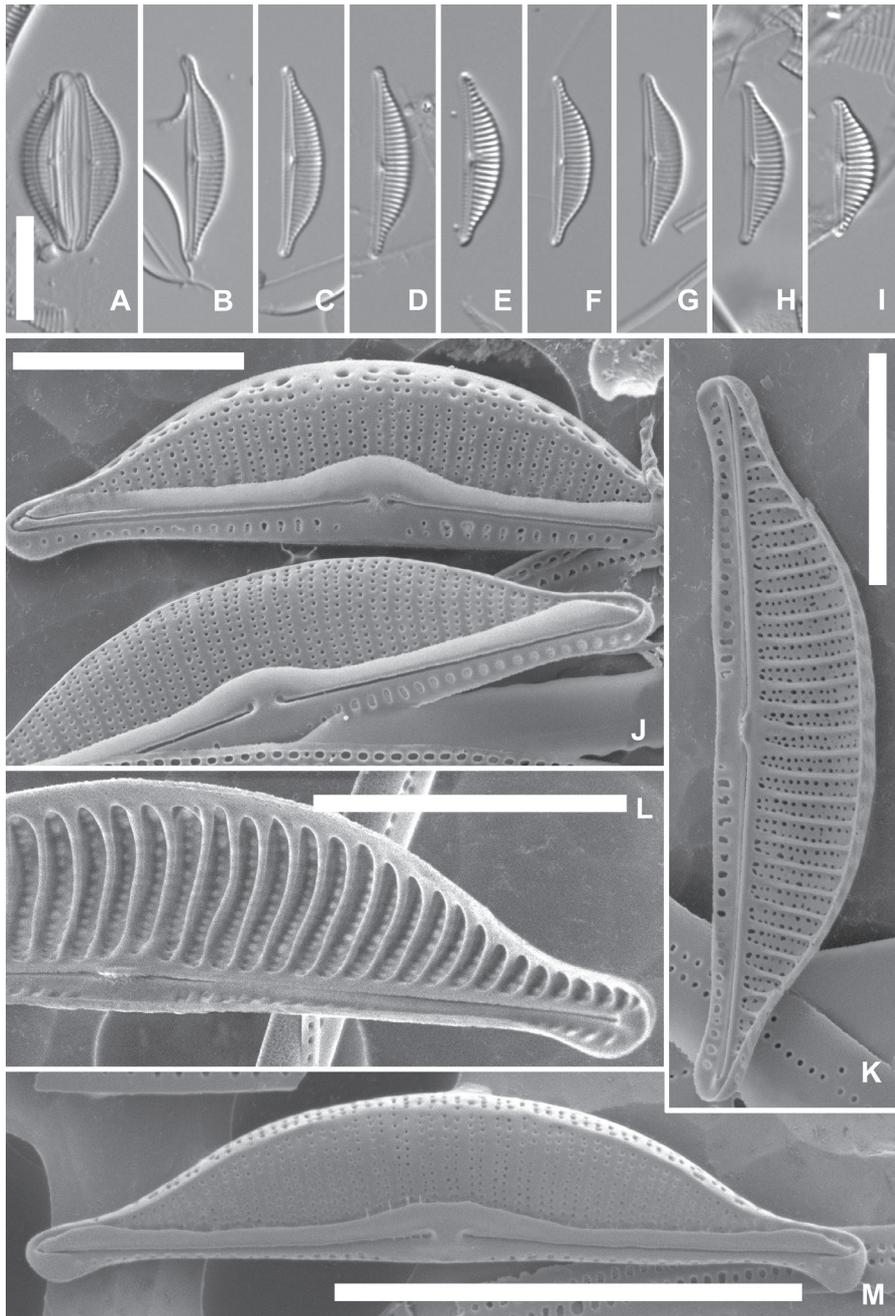


Figure 2. *Halamphora kenderoviana* sp. nov., valves from the type population from South Bay, Livingston Island **A–I** valves under LM, with fig. A showing an entire frustule **D** represents the holotype **J** two valves under SEM externally with details of the central raphe endings and mantle areolae **K** SEM of an entire valve internally **L** SEM of a valve internally, showing the prominent raised costae between the striae and the internal areolar occlusions **M** SEM of an entire valve externally, with a view on the mantle and the dorsal ridge. Scale bars: 10 μm (**A–I**, **M**); 5 μm (**J–L**).

***Halamphora moncheviana* Zidarova, P.Ivanov, Dzhembekova, M.de Haan & Van de Vijver, sp. nov.**

Fig. 3A–M

Holotype. Slide BR-4682, Fig. 3G represents the holotype, Meise Botanic Garden, Belgium. PhycoBank (<http://phycobank.org/103141>).

Isotype. Slide 400, University of Antwerp, Belgium.

Type locality. ANTARCTICA, Livingston Island, Hannah Point, small pool on a coastal rock north of the penguin rookeries, epilithon. 62°38'30"S, 60°36'32"W. Sample LT10, *leg.* R. Zidarova, coll. date 04 Feb. 2020.

Description. *LM description* (Fig. 3A–H). Valves weakly silicified, broadly semi-elliptic, with a more or less straight ventral and distinctly convex dorsal margin. Apices protracted, subcapitate in larger valves (Fig. 3A), becoming only weakly protracted, rostrate in smaller valves (Fig. 3F–G). Valve dimensions ($n = 23$): length 16.0–27.5 μm , width 5.0–7.0 μm . Raphe straight. Central raphe endings straight, enlarged (Fig. 3A, F–H). Terminal raphe fissures not discernible in LM. Axial area narrow, central area absent. Dorsal striae parallel to weakly radiate in the middle, becoming more radiate towards the apices, 24–27 in 10 μm , crossed by several undulating longitudinal lines (Fig. 3A–H).

SEM description (Fig. 3I–M). Externally, valves show a narrow, but distinct raphe ledge, slightly elevated and running on the entire length of the valve (Fig. 3I, M). Central raphe endings relatively close together, weakly dorsally bent, indistinct (Fig. 3M) to weakly enlarged (Fig. 3I). Terminal raphe fissures shortly hooked to the dorsal side (Fig. 3I, M). Dorsal striae on the valve face composed of usually 3–5 transapically elongated, sometimes almost rectangular areolae with recessed finely porous foramina (Fig. 3J). Areolae forming longitudinal rows (Fig. 3I, M). On the mantle, areolae get smaller (Fig. 3I). Distinct marginal dorsal ridge lacking (Fig. 3I, M). Internally, central raphe endings terminating onto fused helictoglossae. Terminal raphe endings finishing onto small helictoglossae (Fig. 3L). Areolae internally rectangular, arranged in regular transverse and longitudinal rows between raised virgae and vimines, possessing finely porous recessed foramina (Fig. 3K, L). Ventral striae only internally observed on the valve face, 33–34 in 10 μm , composed of a single elongated areola (Fig. 3L).

Etymology. The new species is named after Prof Dr Snejana Moncheva, phycologist and former Director of the Institute of Oceanology at the Bulgarian Academy of Sciences, to thank her for considering our (RZ, NDzh) employment and career possibilities at the Institute.

Ecology, Antarctic distribution and associated diatom flora. *Halamphora moncheviana* was most abundant in the epilithon of a small coastal pool, having a relatively low salinity (6.5 PSU, sample LT10, Table 1), where it was found together with *Craspedostauros laevisimus* (W.West & G.S.West) Sabbe and several *Nitzschia*, *Melosira* and *Navicula* species. Roberts and McMinn (1999, Pl. 1, figs 10–11) recorded the same taxon as *Amphora* sp. d from the Vestfold Hills on the Antarctic Continent, although their reported valves were slightly larger (length 30–35 μm , width 5–8 μm)

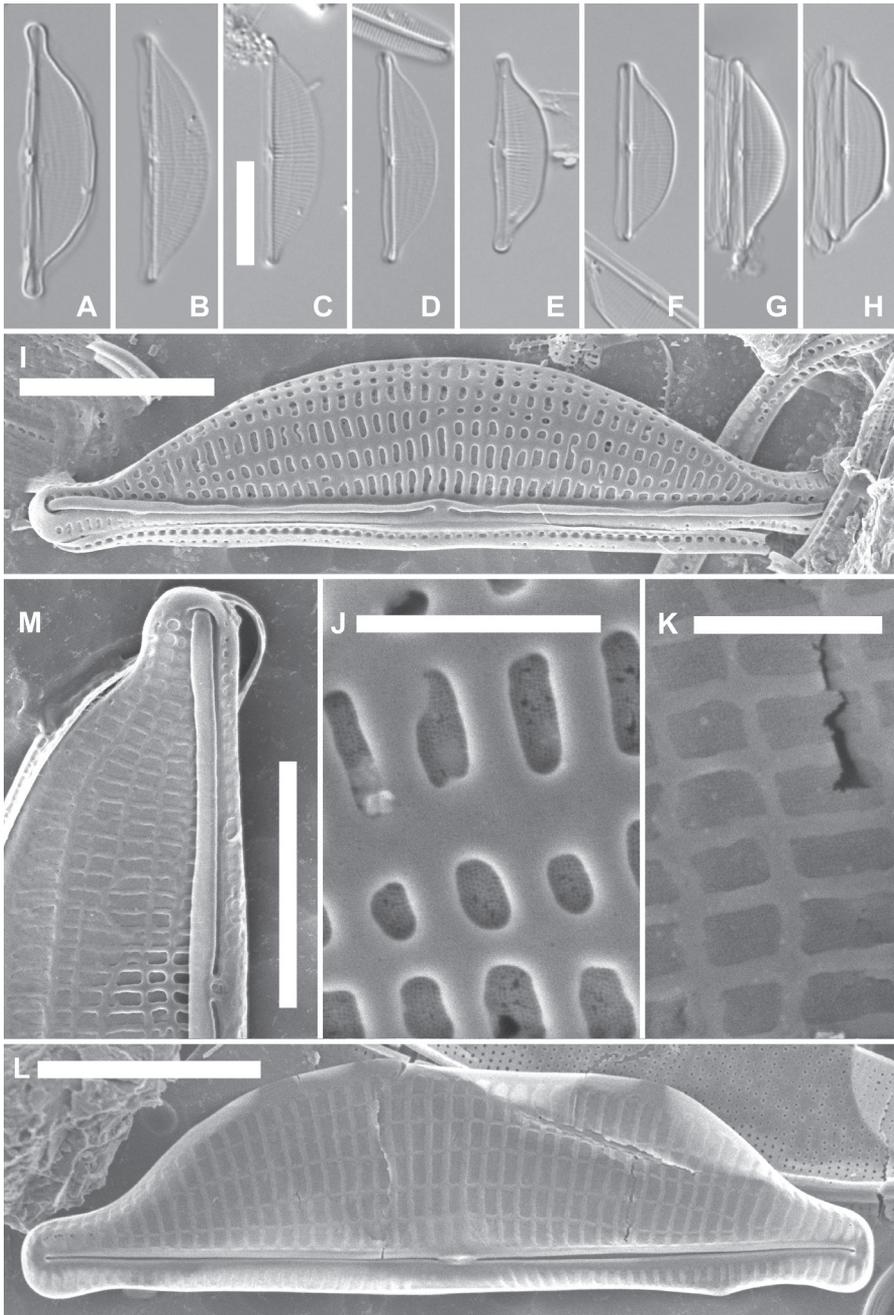


Figure 3. *Halamphora moncheviana* sp. nov., valves from the type population from South Bay **A–H** LM views of several valves **G** represents the holotype **I** SEM of an entire valve externally, showing the dorsal striae and the raphe endings **J** SEM, detail of the areolae externally, showing the recessed porous foramina **K** SEM, detail of the striae and areolae internally, showing the porous internal areolar foramina **L** SEM of an entire valve internally **M** SEM, external view of a valve with areolae arranged in longitudinal lines, most likely in a state of development. Scale bars: 10 μm (**A–H**); 5 μm (**I, L, M**); 1 μm (**J, K**).

and with a slightly coarser striation of “approximately” 22 striae in 10 μm . Nevertheless, the SEM photo of the species, identified as *Amphora* sp. d in Roberts and McMinn (1999, plate 1, fig. 11), presenting a valve externally with striae, composed of a few transapically elongated areolae on the dorsal side and forming irregular longitudinal lines on the valve face, confirms the conspecificity between the species observed on the Antarctic Continent, and *H. moncheviana*. Roberts and McMinn (1999) reported the species from hypersaline lakes. Based on their and our findings, *H. mocheviana* is apparently a very tolerant species to changes in salinity. Likely the same taxon was also depicted by Priddle and Belcher (1981, fig. 3l, as *Amphora* sp.), which they observed in the epilithon of a large, shallow pool (Pool 7) situated near the sea, together with several species of marine origin, including *Craspedostauros laevisissimus* (reported as *Tropidoneis laevisissima* W. West & G.S. West).

Discussion

Based on the observed set of morphological features of *H. kenderoviana* and *H. moncheviana*, both these species from the coasts of Livingston Island clearly belong to the genus *Halamphora*, as defined in Levkov (2009). The morphological analysis also showed that the combination of features in both taxa is sufficiently unique to justify their description as new species.

Halamphora kenderoviana is one of the many *Halamphora* species, having a valve outline with protracted apices and biseriate striae, similarly to *Halamphora coffeaeformis* and its related taxa (Stepanek and Kociolek 2018). The morphology of *H. coffeaeformis*, an often-misinterpreted species, was studied in detail by Archibald and Schoeman (1984), and later discussed in Levkov (2009) and in Stepanek and Kociolek (2018). In contrast to *H. coffeaeformis*, where the biseriate dorsal striae continuing onto the mantle are interrupted by more or less developed dorsal ridge, the mantle striae in *H. kenderoviana* are often reduced to only a single enlarged areola after the narrow dorsal ridge. Moreover, the biseriate striae in *H. coffeaeformis* are composed of very small (fine) and closely positioned areolae (see for instance figs 102, 105 and 108 in Archibald and Schoeman 1984, and Pl. 46 in Stepanek and Kociolek 2018), compared to the relatively large and distantly spaced areolae in the dorsal striae of *H. kenderoviana* (Fig. 2J). In *H. coffeaeformis* the two rows of areolae in each stria are also positioned very close together (e.g. figs 108, 116, 121, 142, 155, etc., in Archibald and Schoeman 1984, and Pl. 46, figs 6, 7 in Stepanek and Kociolek 2018), leaving wide virgae between the striae, whereas the two rows of areolae in the biseriate striae in *H. kenderoviana* are clearly widely spaced, leaving narrower virgae between the striae (e.g. Fig. 2J, M). *Halamphora coffeaeformis* is also a larger taxon, with a width of usually above 5 μm , and all populations, considered to be identical with the type, show a more or less arched raphe (e.g. Pl. 91, figs 1–14 in Levkov 2009 and Pl. 45, figs 1–8 in Stepanek and Kociolek 2018). Two other *Halamphora* species with biseriate striae, *H. aponina* (Kütz.) Levkov and *H. isumiensis* Stepanek et al. possess fine, rounded

areolae, with biseriate striae continuing onto the mantle (Levkov 2009, Pl. 233, figs 2, 7 and Stepanek and Kociolek 2018, Pl. 53, fig. 1, respectively). *Halamphora aponina* has slightly longer valves ($> 23 \mu\text{m}$) with a slightly finer striation of 20–22 striae in $10 \mu\text{m}$, and an almost linear raphe ledge, only expanded near the apices (Levkov 2009), but not in the valve middle, whereas *H. isumiensis* has a much finer striation on the ventral side (ca. 36 striae in $10 \mu\text{m}$ vs. 27–28 in *H. kenderoviana*), and a very distinct dorsal marginal ridge (Stepanek and Kociolek 2018), not observed in *H. kenderoviana*. *Halamphora kenderoviana* also differs from all the above-mentioned species with the presence of prominently raised, though narrow internal costae.

Kellogg and Kellogg (2002), who compiled a list of diatom taxa, recorded in the Antarctic by the year 2000, listed *Halamphora coffeaeformis* and some (earlier considered) infraspecific taxa of the latter, showing a similar valve outline and comparable valve dimensions to *H. kenderoviana*, such as *Amphora coffeaeformis* var. *borealis* (Kütz.) Cleve. *Amphora coffeaeformis* var. *borealis* is treated as a synonym of *A. borealis* Kütz. (see Kellogg and Kellogg 2002, p. 71 and references therein), a species now transferred to *Halamphora* as *H. borealis* (Kütz.) Levkov (Levkov 2009). However, *H. borealis* has uniseriate dorsal striae (Levkov 2009), contrary to the biseriate striae in *H. kenderoviana*. Other, and more recently described taxa, similar in valve outline and possessing biseriate dorsal striae (as both *H. coffeaeformis* and *H. kenderoviana*), include *H. bistriata* Stepanek & Kociolek (Stepanek and Kociolek 2018), *H. tumida* (Hustedt) Levkov (Sar et al. 2004; Levkov 2009) and *H. americana* Kociolek (Kociolek et al. 2014). When compared to *H. kenderoviana*, they all generally have a larger valve width of above $4 \mu\text{m}$ and biseriate striae in only part of the valve face dorsally.

The most similar taxa in LM in terms of valve outline and striation pattern include *Halamphora nagumoi* Stepanek et al. and *H. banzuensis* Stepanek et al. However, under SEM, *H. nagumoi*, described from the Pacific coasts, presents very closely positioned central raphe endings and a prominent dorsal ridge (Stepanek and Kociolek 2018, Pl. 55, fig. 1), contrary to *H. kenderoviana*, lacking these features. *Halamphora banzuensis* from the Banzu flat near Tokyo (Japan) has a much finer striation on the ventral side (40–43 vs. 27–28 striae in $10 \mu\text{m}$ in *H. kenderoviana*), a well-developed dorsal ridge, and striae dorsally are separated by raised virgae on the valve exterior (Stepanek and Kociolek 2018, Pl. 70, figs 3, 4), features not present in *H. kenderoviana*. Finally, based on valve outline and striation, *Amphora cognata* Cholnoky is also morphologically similar, but the latter has a slightly finer striation on the dorsal side (22–24 striae vs. 18–20 striae in $10 \mu\text{m}$ in *H. kenderoviana*), and a slightly larger width ($5\text{--}6 \mu\text{m}$ vs. up to $4.5 \mu\text{m}$ in *H. kenderoviana*) (Cholnoky 1966). As SEM observations are lacking for *A. cognata*, it is not possible to compare the ultrastructure of both species at present. They, however, differ in ecology. *Amphora cognata* was described from warm springs in South Africa (Cholnoky 1966), a very different habitat, compared to the cold-water Antarctic marine coasts where *H. kenderoviana* was discovered, and it is rather unlikely that the two taxa are conspecific. The Argentinean *Amphora* (*Halamphora*) *capitellata* Freng., whose original description and drawing are provided by Sar et al. (2009), is larger (valve width $5\text{--}6 \mu\text{m}$), and based on the drawing in Sar et al. (2009, p. 49),

possesses a less convex dorsal side and a small central area on the dorsal side, contrary to *H. kenderoviana*.

Halamphora moncheviana, showing internally rectangular areolar openings arranged in regular rows, and externally irregular longitudinal lines on the dorsal side in LM, can hardly be confused with any other *Amphora* or *Halamphora* taxa. In valve outline, with its shortly protracted subcapitate apices, it bears only a slight resemblance to the South American brackish species *Halamphora mira* (Krasske) Levkov. The latter is a much larger taxon, with a length exceeding 35 μm , a width above 9 μm , and with a strongly arched raphe (Lange-Bertalot et al. 1996, as *Amphora mira* Krasske; Levkov 2009), contrary to *H. moncheviana*. The smaller *H. miroides* Levkov also presents a strongly arched raphe, and finely punctate striae. Moreover, it is a typically freshwater (and not marine) species, known mostly from Africa (Levkov 2009). Both the Antarctic *Halamphora lateantarctica* Van de Vijver et al. and *H. vyvermaniana* Van de Vijver et al. possess a distinct arched raphe, and a distinct dorsal marginal ridge (Van de Vijver et al. 2014), in contrast to *H. moncheviana*. Moreover, they both lack the internal stria structure of *H. moncheviana*, composed of rectangular areolae, arranged in regular rows (e.g. Van de Vijver et al. 2014, fig. 9K and fig.11E). *Amphora* (*Halamphora*) *eunotia* Cleve var. *striolata* Freng. from Argentina has larger valves with a much coarser striation of 14–18 striae in 10 μm (Sar et al. 2009). *Halamphora siqueirosii* López-Fuerte et al. from hypersaline waters in Mexico has semi-lanceolate valves with a cut in the raphe ledge in the valve middle (López-Fuerte et al. 2020), a feature not observed in *H. moncheviana*, and lacks the arrangement of the elongated areolae in irregular longitudinal rows on the dorsal side externally. The freshwater species *Halamphora coloradiana* Stepanek & Kociolek is a smaller taxon, with a width of only 2.5–4.5 μm (vs. > 5 μm in *H. moncheviana*), with a very dense striation near the apices (> 29 striae in 10 μm), and lacks the clearly rectangular internal areolar openings (Stepanek and Kociolek 2013, fig. 85), present in *H. moncheviana*. Finally, the valves shown on the original drawing of *Amphora kuehniae* Schoeman in the diatom collection files of the Academy of Natural Sciences, Philadelphia (Potapova et al. 2022), show some similarity to *H. moncheviana* in their striation pattern; however, the valves from Lesotho, which were examined and depicted under LM by Levkov (2009), who also transferred the species to the genus *Halamphora* (as *H. kuehniae* (Schoeman) Levkov), differ from *H. moncheviana* with their strongly dorsiventral valves with coarse, but more rounded and not arranged in longitudinal rows areolae. *Halamphora kuehniae* is also a much larger taxon with an arched (and not straight) raphe (Levkov 2009).

Two other marine taxa, *Amphora antarctica* Hust. (Hustedt 1958) and *A. barrei* Manguin (Manguin 1960), were described from Antarctica. They share a similar valve outline with strongly protracted apices, overlapping dimensions and a very fine striation pattern, suggesting they might be conspecific, as noted earlier by Hargraves (1968). Both, however, clearly differ from *H. moncheviana* based on their dense and almost indiscernible striation pattern in LM and the presence of much more protracted apices (see also figs 5, 6 in Cremer et al. 2003 for *A. antarctica*), excluding all conspecificity.

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