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Hemipilia avisoides (Orchidaceae), a new species from Sichuan Province, China

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Abstract

A new orchid species, *Hemipilia avisoides*, is described from Songpan County and Maoxian County, Sichuan Province, China. Morphologically, *H. avisoides* is most similar to *H. hui*, but can be distinguished by the combination of its involute middle lip lobe that is smaller than the lateral lobes, floral bracts that are 5 mm long and are always shorter than the ovary, a leaf that is appressed to the substrate and is adaxially green with white lines along 7–9 principal veins and the subterranean stem with a solitary sheath at its base. The floral morphology of *H. avisoides* is presented by utilising *in vivo* micro-CT scanning and 3D visualisation.

Keywords

Arid valley, Minjiang River Valley, Orchidinae, taxonomy

Introduction

The genus *Hemipilia* Lindl. *sensu stricto* (Orchideae, Orchidaceae) comprises ca 10 species that are characterised by a protruding, tongue-like rostellum (Luo and Chen 2000; Chen et al. 2009b). Nevertheless, molecular phylogenies revealed *Hemipilia s.s.* as monophyletic, but nested deeply within a strongly-supported clade that also included several species from closely-allied genera, i.e., *Amitostigma hemipilioides* (Finet) Tang & F.T.Wang, *A. thailandicum* Seidenf. & Thaithong, *Ponerorchis brevicalcarata*

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(Finet) Soó, *P. limprichtii* (Finet) Soó and *Hemipiliopsis purpureopunctata* (K.Y.Lang) Y.B.Luo & S.C.Chen (Luo 1999; Bateman et al. 2003; Jin et al. 2014, 2017; Tang et al. 2015; Lai et al. 2021). This clade was named "the *Hemipilia* Clade" by Tang et al. (2015). In addition, the recently published species *Hemipilia galeata* Y.Tang, X.X.Zhu & H.Peng and *H. yajiangensis* G.W.Hu, Jia X.Yang & Q.F.Wang, both of which do not possess a protruding rostellum, were also recovered as closely related to *Hemipilia s.s.* in the *Hemipilia* Clade (Tang et al. 2016; Yang et al. 2022).

Given the fact that *Ponerorchis* Rchb.f. and *Amitostigma* Schltr. are paraphyletic to several taxa, including *Hemipilia s.s.*, Jin et al. (2014) formally combined the monotypic *Hemipiliopsis* with *Hemipilia* and expanded the circumscription of *Ponerorchis*. Tang et al. (2015) formally proposed *Hemipilia sensu latissimo*, lumping ca 65 species into a single broadly circumscribed and monophyletic genus. Under the latter treatment, seven sections that correspond to the seven major clades in nuclear trees were also established and *Hemipilia s.s.* and those basally divergent species were included in *H.* sect. *Hemipilia* (Tang et al. 2015, 2016; Yang et al. 2022).

When transferring *Ponerorchis limprichtii* to *Hemipilia sensu latissimo*, Tang et al. (2015) proposed a replacement name *H. occidensichuanensis* Y.Tang & H.Peng because of the existence of the name *H. limprichtii* Schltr. based on a different type. Unfortunately, the name *H. occidensichuanensis* turns out to be illegitimate. Schuiteman (2022) pointed out the problem and made a new combination and the correct name *Hemipilia hui* (Tang & F.T.Wang) Schuit. to replace *P. limprichtii* in *Hemipilia*. Coincidently, this particular species is the one most morphologically similar to the new taxon described in this study.

During the field trip in 2013 to collect *Hemipilia physoceras* (Schltr.) Y.Tang & H.Peng in Minjiang River Valley, Songpan County, Sichuan Province, China, one of the authors (Y. Tang) collected another orchid that morphologically fits into the category of *Hemipilia sensu latissimo*. It had been temporarily identified as *Ponerorchis* cf. *limprichtii* in the previous study by Tang et al. (2015). However, this taxon in Songpan not only differs in the morphology of the labellum and leaf but also diverges in DNA sequences, both of which suggest it is a potential new species (Tang et al. 2015). Here, we describe it in *Hemipilia* sect. *Hemipilia sensu* Tang et al. (2015) and present its floral morphology by using an *in vivo* micro-CT method.

Methods

Material collection

During our field investigation to Minjiang River Valley, Songpan, Sichuan, China in 7–9 June 2022, two populations of the new taxon with 12 flowering individuals were found. One population (ZJG) occurs at the same locality that was visited in 2013 by one of the authors (Y. Tang) and the other (JPY) is ca 11.2 km southwards in the Valley.

One living individual from the ZJG population and three from the JPY population with intact flowers were collected, each was packaged with soils and EPE pearl

cotton in a plastic bottle and transported by air to the Key Laboratory of Stratigraphy and Paleontology, Ministry of Natural Resources for *in vivo* micro-CT scanning. After scanning, these individuals were pressed and conserved as dried specimens.

The leaf material of one individual from the JPY population was collected and dried with silica gel for DNA sequencing.

To compare the new taxon with morphologically similar species, one population of *Hemipilia hui* in Kangding, Sichuan, China was investigated in 18 June 2022. The population was found under shrubs at the elevation of ca 3470 m. Five blooming individuals with intact flowers were observed, which showed some variations in morphology but generally fit well with the description in Flora of China (Chen et al. 2009a). One individual of *H. hui* from this population was collected as a reference specimen. Digital images of herbarium specimens of *H. hui* at A, AMES, CDBI, IBSC, KUN, PE, SZ and WUK were examined.

All voucher specimens collected as part of this study were deposited at the Herbarium of Sichuan University (SZ).

Morphological observations

The morphological description of the new taxon was mainly based on living materials. The length and width of leaves and the height of the inflorescence were measured on seven living, flowering plants in the field. The morphology of subterranean parts was described based on the four plants collected (see Material collection). The morphology of a single flower was described mainly based on the 3D mesh model reconstructed by micro-CT data.

Micro-CT scanning and 3D Visualisation

X-ray Computed Tomography (CT) was completed at the Key Laboratory of Stratigraphy and Paleontology, Ministry of Natural Resources. The individual collected from the ZJG population (see Material collection) was finally selected for scanning and was then chosen as the holotype of the new taxon. Its inflorescence with the uppermost three flowers was scanned *in vivo* in a NIKON XTH 225ST CT scanner at a resolution of 18.6 μm and X-ray of 90 kV and 70 μA.

The 3D reconstructions were performed in the software VGSTUDIO MAX 3.0 with STL files being exported. For the 3D model of inflorescence, however, only the uppermost two flowers were reconstructed due to the trade-off between resolution and computing time. Acquired 3D mesh models were visualised and processed by the software GOM INSPECT PRO in GOM SUITE 3.1.1109.0.

Taxon sampling, DNA sequencing and phylogenetic analyses

Based on previous studies (Tang et al. 2015, 2016; Jin et al. 2017; Lin et al. 2021; Peng et al. 2022; Yang et al. 2022), a total of 66 accessions, representing 55 taxa and all seven sections of *Hemipilia sensu latissimo*, were selected to examine the phylogenetic position of

the new taxon. Two species of the genus *Brachycorythis* Lindl. were chosen as outgroups. Voucher information and GenBank accession numbers are provided in Appendix 1.

Genomic DNA extraction, primer synthesis, PCR reactions and Sanger sequencing were completed by Tsingke Biotechnology Co., Ltd. (Chengdu, China). Four DNA markers, including one nuclear (nrITS) and three plastid markers (*matK*, *trnL-F* and *trnS-trnG*), were used in this study. The primer pairs for these regions were 17SE/26SE (Sun et al. 1994), 390F/1326R (Cuénoud et al. 2002), c/f (Taberlet et al. 1991) and trnS/trnG (Hamilton 1999), respectively. All regions were sequenced for both DNA strands. Contig sequences were assembled with SEQMAN 7.1.0.

Phylogenetic reconstruction was carried out using Bayesian inference (BI) and maximum likelihood (ML) analyses. Data for the plastid regions were combined, whereas the nrITS and combined plastid DNA datasets were analysed separately according to the results of Tang et al. (2015). Each region was individually aligned with MAFFT 7.313 (Katoh and Standley 2013) in PHYLOSUITE 1.2.2 (Zhang et al. 2020) using the "L-INS-I" strategy. Alignments were then manually adjusted in PHYDE 0.9971 (Müller et al. 2010) and ambiguously aligned characters in the *trnL-F* and *trnS-trnG* datasets were excluded prior to downstream analyses. ModelFinder (Kalyaanamoorthy et al. 2017) in PHYLOSUITE 1.2.2 (Zhang et al. 2020) was used to select the best-fit model for each dataset using the Bayesian information criterion (BIC) scores. Plastid regions were finally concatenated with PHYLOSUITE 1.2.2 (Zhang et al. 2020).

The best-fit models for BI are GTR+F+I+G4 (nrITS and *matK*) and GTR+F+G4 (*trnL-F* and *trnS-trnG*) and for ML analyses they are GTR+F+I+G4 (nrITS), K3Pu+F+R3 (*matK*), K3Pu+F+R2 (*trnL-F*) and K3Pu+F+G4 (*trnS-trnG*).

Partitioned BI analyses were conducted using MrBayes 3.2.7a (Ronquist et al. 2012) on XSEDE on the CIPRES Gateway (Miller et al. 2010). The Markov chain Monte Carlo (MCMC) analyses were run for 30,000,000 generations, sampling one tree every 1,000th generation. Convergence of runs was accepted when the average standard deviation of split frequencies (ASDSF) fell below 0.01. The initial 25% of sampled trees were discarded as burn-in. Partitioned ML analyses were conducted with IQ-TREE 2.1.2 (Nguyen et al. 2015) on XSEDE on the CIPRES Gateway (Miller et al. 2010) for 5,000 ultrafast (Minh et al. 2013) bootstraps. For the combined plastid dataset, each region was allowed to have its own evolution rate ("-spp"). TREEGRAPH 2.15.0-887 BETA (Stover and Muller 2010) was used to visualise the resulting trees with node support values. Nodes with a Bayesian posterior probability (BPP) ≥ 0.95 and/or a maximum likelihood bootstrap support (BS_{ML}) ≥ 80 were considered as strongly supported.

Data availability

The 3D mesh model of the uppermost two flowers on an inflorescence and photos of the corresponding micro-CT-scanned individual of *Hemipilia avisoides* are available on Zenodo via DOI: https://doi.org/10.5281/zenodo.6832154.

Results

Phylogenetic reconstruction

Trees reconstructed from the nrITS and combined plastid datasets in this study are similar to those of previous studies (e.g., Tang et al. 2015; Jin et al. 2017). Sequences of the accession "*Hemipilia avisoides* [Tang, Wang & Zhu 236]" generated in this study are nearly identical to those of the accession "*Ponerorchis* cf. *limprichtii*" identified and sequenced by Tang et al. (2015). The latter accession was labelled "*Hemipilia avisoides* [Tang 151]" in this study. The new species, represented by these two accessions, is revealed as a member of *H.* sect. *Hemipilia sensu* Tang et al. (2015). Both the nrITS and combined plastid trees recover the new species and *H. hui* as sister taxa with strong supports (Fig. 1: BPP = 1, BS_{ML} = 99; Fig. 2: BPP = 1, BS_{ML} = 95).

Taxonomic treatment

Hemipilia avisoides **Y.Tang, X.M.Wang & H.Peng, sp. nov.**
urn:lsid:ipni.org:names:77307801-1
Figs 3A–D, 4, 5, 6A; see also Data availability

Type. China, Sichuan Province, Aba Tibetan and Qiang Autonomous Prefecture, Songpan County, 9 June 2022, *Y. Tang, X.-M. Wang & Y.-T. Zhu 235* (holotype: SZ!); ibid., 9 June 2022, *Y. Tang, X.-M. Wang & Y.-T. Zhu 236* (paratype: SZ!); Maoxian County, 1 June 1958, *S.-Y. Chen, Z. He, M.-F. Zhong et al. 5078* (paratype: SZ!).

Diagnosis. Similar to *Hemipilia hui* (Tang & F.T.Wang) Schuit., from which *H. avisoides* can be distinguished by a set of characteristics: mid-lobe involute, sub-oblong in apical view, 2.2 × 1.2 mm, trapeziform when flattened, smaller than lateral lobes; floral bracts 5 mm long, always shorter than ovary; leaf appressed to the substrate, adaxially green with white lines along 7–9 principal veins; stem subterranean, with one sheath at the base (Table 1).

Description. Terrestrial, erect herbs, 8.5–31 cm tall. Tubers oblong, 2.5 cm long, 0.8 cm in diameter, neck with few roots. Stem subterranean, 2.7–5 cm long, 0.2 cm in diameter, with one sheath at the base. Sheath tubular, membranous, 1–2 cm long, pale yellow. Leaf appressed to the substrate, solitary, cordate, ovate or elliptic, 3–6.5 × 2–5.5 cm, apex acute, slightly fleshy, glabrous, abaxially purple, adaxially green with white lines along 7–9 principal veins, sometimes also with purple spots. Inflorescence terminal, erect, 3–14 cm long, 1–21-flowered, glabrous, dark purple. Flowers not secund, plum or purple plum, fragrant; floral bracts connivent to ovary, elliptic, 5 × 2.6 mm, shorter than ovary, apex acuminate, glabrous, dark purple; ovary curved, cylindrical, 10.5 mm long including pedicel, 1 mm in diameter, glabrous, dark purple. Dorsal sepal erect, oblong, cymbiform, 4.5 × 2.6 mm, apex rounded, sometimes concave at each side of central vein below middle, glabrous; lateral sepals spreading, obliquely ovate, cymbiform, 5.6 × 3.6 mm, apex obtuse, glabrous. Petals

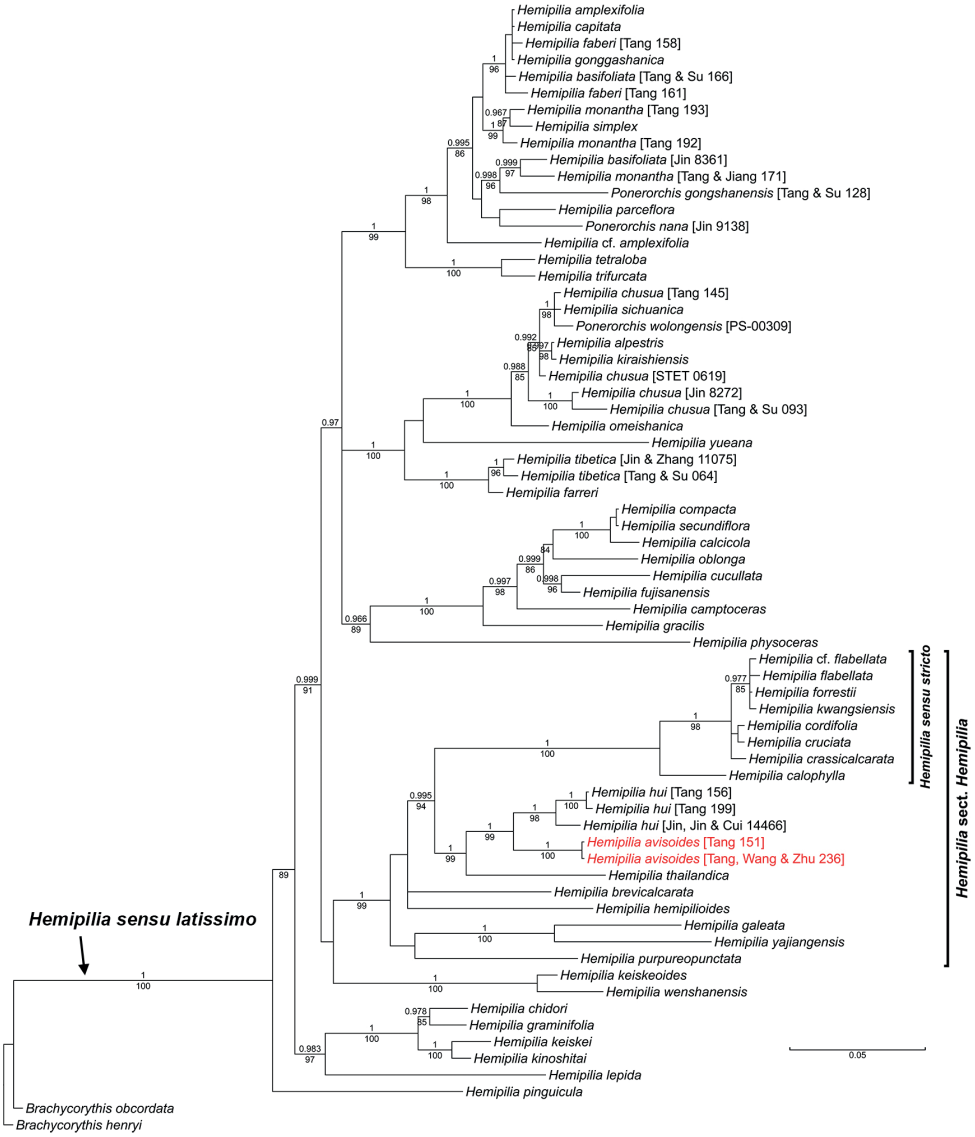

Figure 1. Phylogenetic placement of *Hemipilia avisoides* sp. nov. in the Bayesian analysis of the nrITS dataset. Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap supports (BS_{ML}) are displayed above and below the branches, respectively. Only BPP ≥ 0.95 and BS_{ML} ≥ 80 are considered as strong supports and are shown. The scale bar denotes the estimated number of substitutions in Bayesian analysis.

erect, connivent with dorsal sepal and forming a hood, apex bending similar to holding a fist in the other hand, obliquely ovate, 4 × 2.8 mm, apex obtuse, glabrous. Labellum spreading, broadly ovate when flattened, 7.1 × 5.4 mm, 3-lobed below middle, spurred, base collar-like raised on each side of spur entrance, glabrous, tinged white at

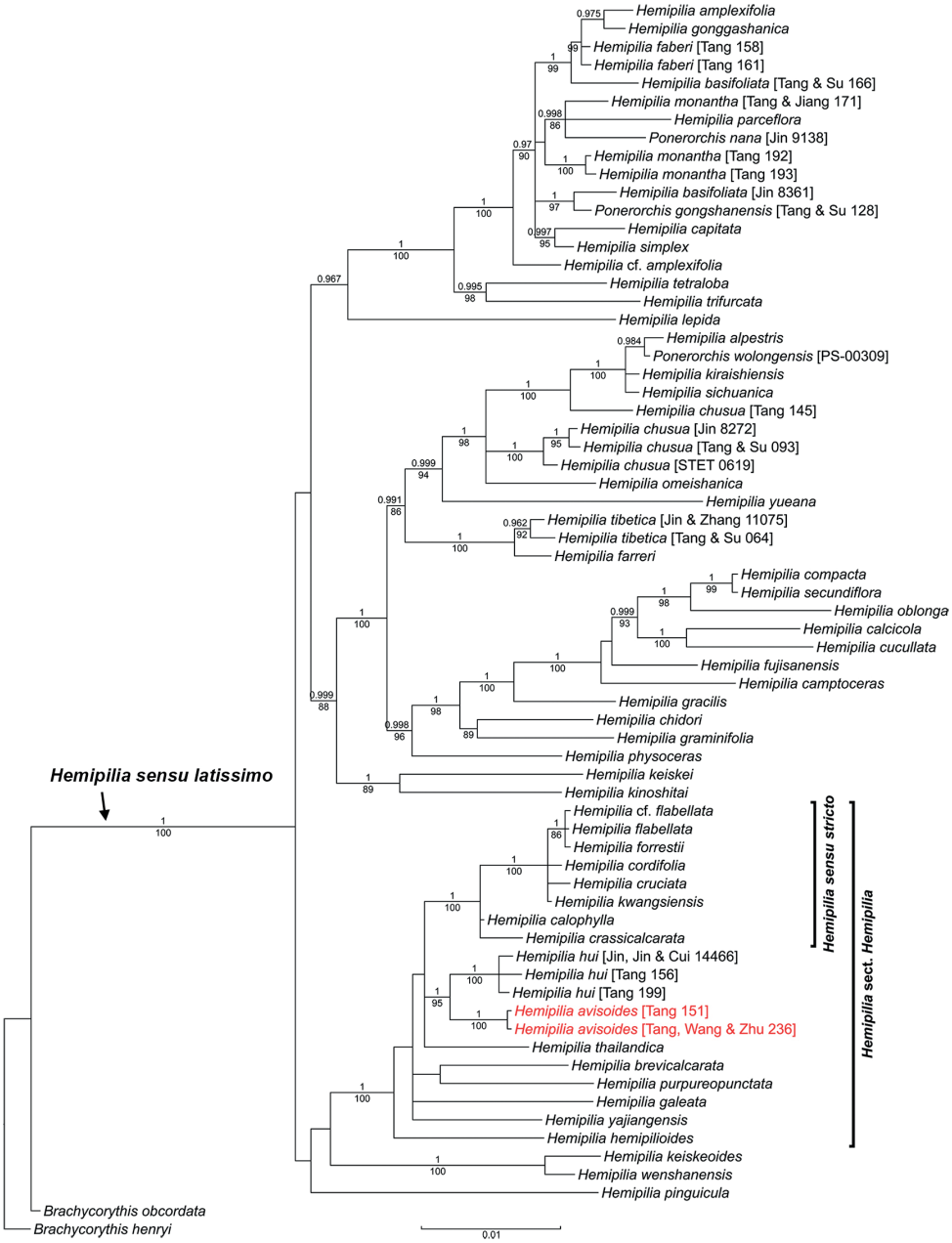

Figure 2. Phylogenetic placement of *Hemipilia avisoides* sp. nov. in the Bayesian analysis of the combined plastid dataset. Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap supports (BS_{ML}) are displayed above and below the branches, respectively. Only BPP ≥ 0.95 and BS_{ML} ≥ 80 are considered as strong supports and are shown. The scale bar denotes the estimated number of substitutions in Bayesian analysis.

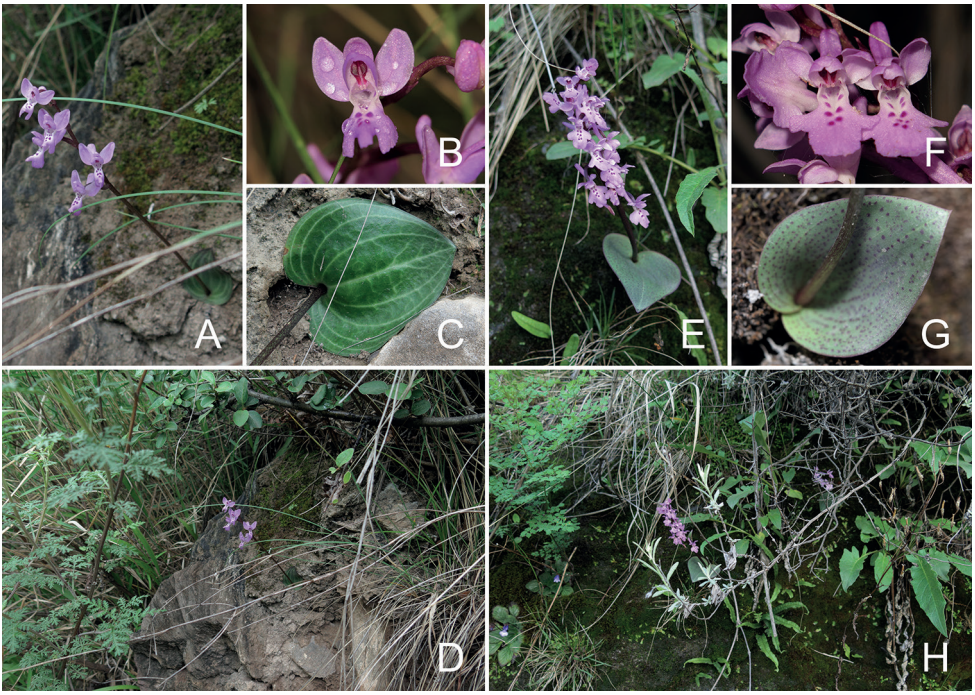

Figure 3. Comparisons between living plants of *Hemipilia avisoides* sp. nov. and *H. hui* in the wild **A–D** habit, flowers, leaf and habitat of *H. avisoides* **E–H** habit, flowers, leaf and habitat of *H. hui*. Photographs **A–H** by Y. Tang.

base, disc dotted with purple; lateral lobes pendulous, rhombic, 3.4 × 2.5 mm, apex truncate, margin slightly undulate; mid-lobe horizontal, involute, suboblong in apical view, 2.2 × 1.2 mm, trapeziform when flattened, apex rounded or sometimes apiculate; spur horizontal, straight or curved upwards, cuneate, 9 mm long, ventrally carinate along central axis, entrance 2.5 mm wide, apex swollen, obtuse, 2.7 mm wide; anther reclined, 2.8 mm long, 2-locular, locules parallel and closely spaced, aubergine; pollinia 2, sectile, ovate, 1.2 × 0.7 mm; caudicles cuneate, 1.2 mm long; viscidia 2, closely spaced, oblong, transparent, each enclosed within a separate bursicle; bursicles formed by folding of rostellar arms, oblong, 0.6 × 0.3 mm; rostellum median lobe triangle, 0.7 mm long, lateral lobes grooved; stigma ventral, lobes 2, divergent, lamelliform, 1.2 × 0.5 mm, with hairs at base; auricles 2, each placed laterally at base of anther and behind collar of labellum base, 0.5 mm long.

Flowering. Peaking in early June.

Distribution and habitat. *Hemipilia avisoides* is currently known from two localities in Songpan County, which are ca 11.2 km apart along the Minjiang River Valley and one locality in Maoxian County according to the collection by S.-Y. Chen et al. in 1958. Individuals of the new taxon occur under arid-valley shrubs and on moss-covered rocks (see Discussion).

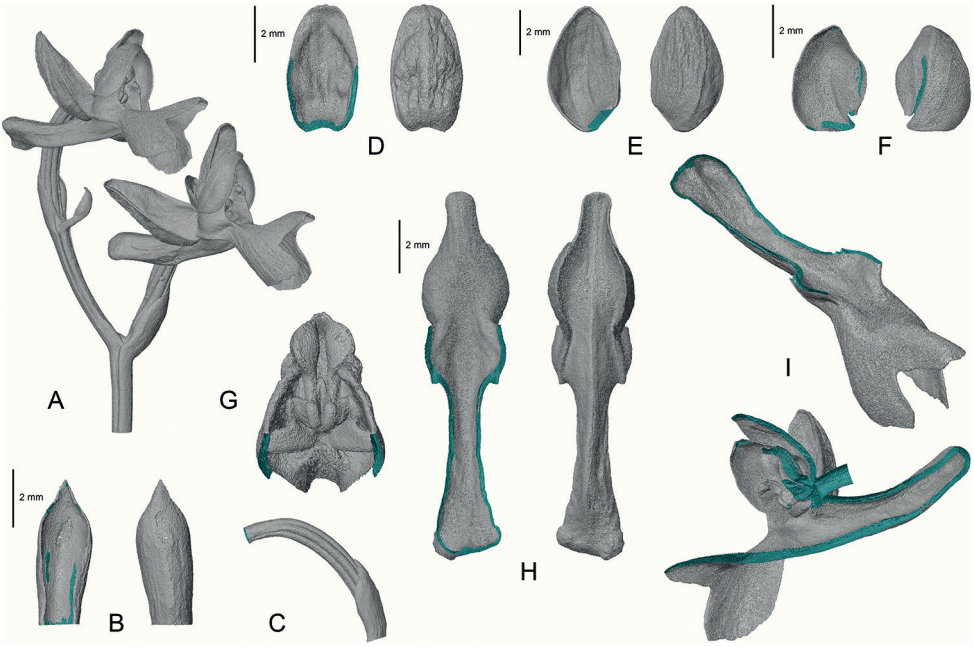

Figure 4. Floral morphology of *Hemipilia avisoides* sp. nov., based on 3D mesh model reconstructed by micro-CT data **A** inflorescence with uppermost two flowers **B** ventral and dorsal views of floral bract **C** lateral view of ovary, with floral bract at base **D** ventral and dorsal views of dorsal sepal **E** ventral and dorsal views of lateral sepal **F** ventral and dorsal views of petal **G** ventral view of gynostemium **H** ventral and dorsal views of labellum **I** lateral views of labellum. The 3D model in STL format is available on Zenodo (https://doi.org/10.5281/zenodo.6832154).

Etymology. Latin *avis*, bird, and suffix *-oides*, similar, alluding to appearance of flower arrangement simulating flying birds with flapped wings.

Chinese name. 雁字舌喙兰 (Chinese Pinyin: yànzì shéhuìlán).

Additional specimens examined. *Hemipilia avisoides*: **China, Sichuan Province, Aba Tibetan and Qiang Autonomous Prefecture**, Songpan County, 30 June 2013, *Y. Tang 151* (KUN!). *Hemipilia hui*: **China, Sichuan Province, Ganzi Tibetan Autonomous Prefecture**, Kangding City, 18 June 2022, *Y. Tang, X.-M. Wang, W.-Q. Yuan & Y.-T. Zhu 237* (SZ!); ibid., 17 June 2017, *Y.-L. Peng, Q. Yu & L.-L. Li THP-KD-1390* (CDBI!); ibid., 13 June 2014, *Y. Tang 199* (KUN!); ibid., 28 May 1981, *Z.-J. Zhao, J.-B. Shi & D.-G. Fan 114262* (SZ!); Luhuo County, 12 August 2005, *D. E. Boufford, J.-H. Chen, K. Fujikawa, S. L. Kelley, R. H. Ree, H. Sun, J.-P. Yue, D.-C. Zhang & Y.-H. Zhang 34770* (A!); Xiangcheng County, 15 July 2004, *D. E. Boufford, J.-H. Chen, S. L. Kelley, J. Li, R. H. Ree, H. Sun, J.-P. Yue & Y.-H. Zhang 30764* (A!); Daofu County, 10 June 1996, *J.-S. Yang 91-270* (IBSC!; PE!); Xinlong County, 28 June 1974, *Z.-S. Qin 06383* (CDBI!); ibid., 27 June 1974, *Z.-S. Yu 06409* (CDBI!); Yajiang County, 15 June 1961, *S. Jiang 05196* (KUN!). **China, Sichuan Province, Aba Tibetan and Qiang Autonomous Prefecture**, Xiaojin County, 2 July 2013, *Y. Tang 156* (KUN!);

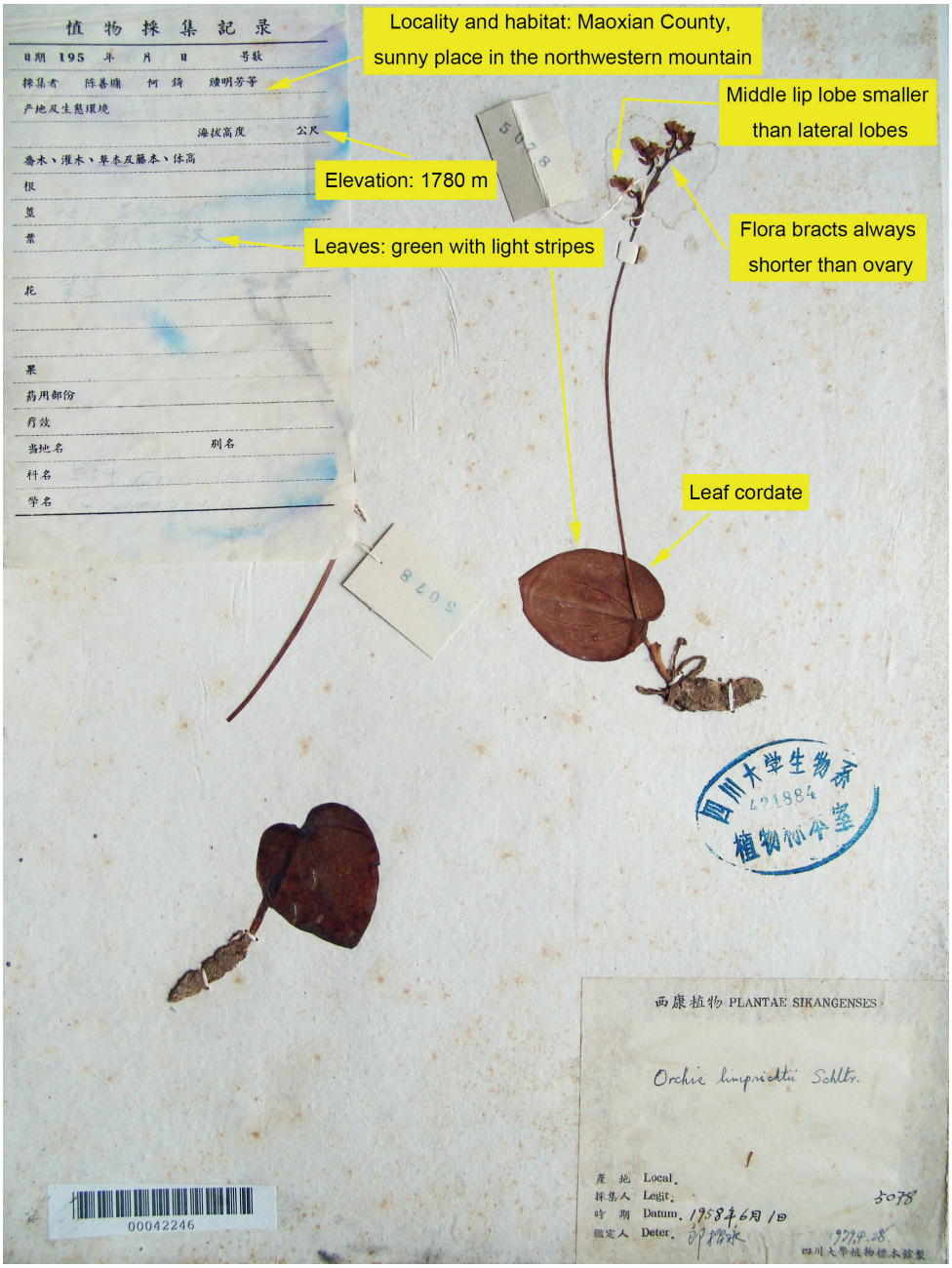

Figure 5. Specimen of *S.-Y. Chen, Z. He, M.-F. Zhong et al. 5078* (SZ!) identified as *Hemipilia avisoides* in this study. Key features, which would facilitate the identification of this specimen, are highlighted and arrowed in yellow.

ibid., 21 May 1959, *Xiaojin Zu 0130* (SZ!); ibid., 21 May 1957, *J. Zhou 34* (IBSC!); Maerkang City, 16 May 1957, *X. Li 71047* (PE!; SZ!). **China, Gansu Province, Longnan City**, Wenxian County, 12 May 2007, *Baishuijiang Caijidui 4839* (PE!); ibid.,

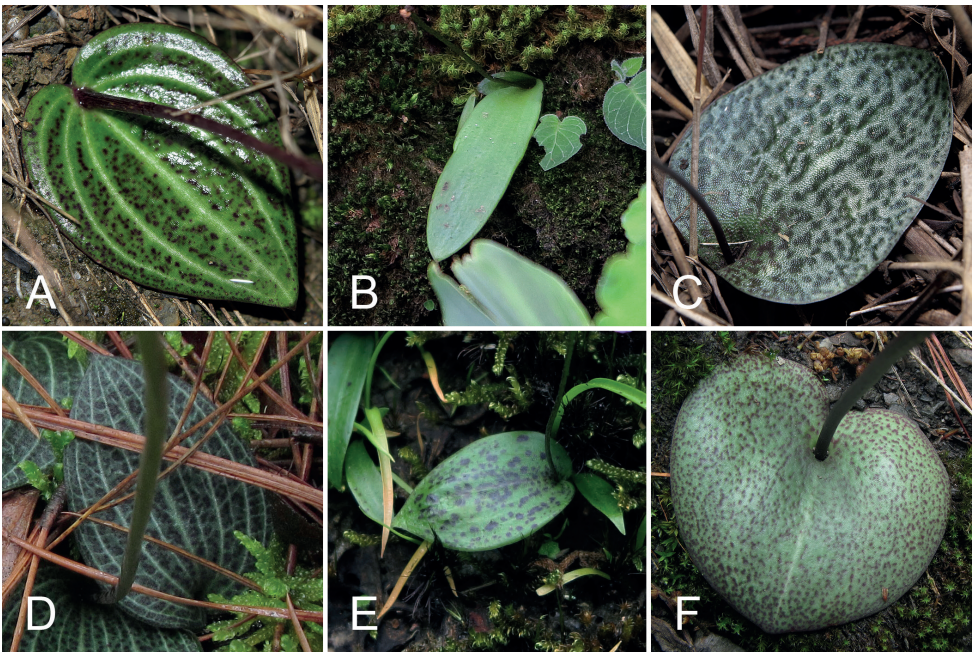

Figure 6. Leaves of *Hemipilia avisoides* sp. nov. and some related species in *H.* sect. *Hemipilia sensu* Tang et al. (2015) **A** *H. avisoides* **B** *H. thailandica* **C** *H. hemipilioides* **D** *H. brevicalcarata* **E** *H. galeata* **F** *H. cordifolia*. Photographs **A–F** by Y. Tang.

Table 1. Comparisons in morphology between *Hemipilia avisoides* sp. nov. and *H. hui*. Diagnostic characteristics of *H. avisoides* are in bold.

Species	*Hemipilia avisoides*	*H. hui*
Stem shape	**Subterranean, with 1 sheath at the base**	Partly subterranean, with 1 or 2 (or 3) sheaths at the base
Leaf position	**Appressed to the substrate**	Sub-basal
Leaf colour adaxially	**Green with white lines along 7–9 principal veins**, sometimes also with purple spots	Usually green with purple markings, sometimes green with white, reticulate venation or nearly uniformly green
Flora bract shape	Elliptic, **5 mm long, always shorter than ovary**	Lanceolate or ovate-lanceolate, lower ones nearly as long as ovary, gradually smaller upwards to shorter than ovary
Dorsal sepal shape	Oblong, apex rounded, sometimes concave at each side of central vein below middle	Suboblong, apex subacute
Lateral lip lobe shape	Pendulous, rhombic	Usually horizontal, auricular or transversely suboblong
Middle lip lobe shape	**Involute, suboblong in apical view, 2.2 × 1.2 mm, trapeziform when flattened, smaller than lateral lobes**, apex rounded or sometimes apiculate	Usually open and flat, subsquare, 4–5 × 3–4 mm, larger than lateral lobes, apex obtuse-rounded, sometimes slightly emarginate or shortly apiculate

9 May 2007, *Baishuijiang Caijidui 4514* (PE!); Wudu District, 15 June 1959, *Z.-Y. Zhang 4390* (WUK!); ibid., 5 June 1959, *Z.-Y. Zhang 3379* (WUK!); ibid., 30 May 1959, *Z.-Y. Zhang 3180* (PE!; WUK!). **China, Gansu Province, Gannan Tibetan Autonomous Prefecture**, Zhouqu County, 27 May 1999, *Bailongjiang Exped. 1408* (PE!). **China**, sine loc., 1959, *Chuan Jing A 0130* (KUN!); sine loc., July 1907, *E. H. Wilson 1762* (the second individual from left on the sheet: AMES!).

Conservation status. *Hemipilia avisoides* seems narrowly distributed within the arid valley in the upper reaches of Minjiang River (see Discussion), with few populations and individuals being found. The habitat of *H. avisoides* could be easily disturbed by development as it is close to roads and villages. According to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022), for *H. avisoides*, the area of occupancy (AOO) is 8 km^2, the number of locations is one and the area, extent and/or quality of habitat are likely to decline due to disturbances. Moreover, the number of mature individuals is less than 50. Therefore, *H. avisoides* is here tentatively assigned to the IUCN category CR B2ab (Critically Endangered).

Discussion

The new species *Hemipilia avisoides* has oblong tubers, two erect anthers, two stigmas that are beneath the rostellum and two sectile pollinia with viscidium each enclosed within a bursicle. These characteristics fit well into the category of *Hemipilia sensu latissimo* (Tang et al. 2015). *Hemipilia avisoides* has also a solitary, slightly fleshy leaf that is appressed to the substrate, which is quite reminiscent of *Hemipilia s.s.* (Chen et al. 2009b). However, the median rostellum lobe of *H. avisoides* never protrudes between anther cells like that of *Hemipilia s.s.*

Molecular phylogenies did, however, reveal a close relationship between *Hemipilia s.s.* and *H. avisoides* in a clade (Figs 1, 2), namely, the *Hemipilia* Clade according to Tang et al. (2015). The sister relationship between *H. avisoides* and *H. hui* is strongly supported in both the nuclear and plastid trees, while they are most similar in morphology (see Taxonomic treatment and below). Moreover, *H. avisoides* and *H. hui* each occupies a relatively long branch in molecular trees (Figs 1, 2). It is also notable that one accession of *H. hui* ("Jin, Jin & Cui 14466") shows obvious DNA sequence divergences from the other two accessions, though they still cluster into a monophyletic clade.

Amongst the specimens of *Hemipilia hui*, one collection by S.-Y. Chen et al. in 1958 caught our attention for it was gathered from Maoxian County at an elevation of 1780 m, this being close to the localities where we discovered *H. avisoides*. The environment of that region differs from the alpine habitat that *H. hui* usually favours. After careful examination, we believe that this specimen represents *H. avisoides* here described, although it was initially identified as *H. hui*. We highlighted in Fig. 5 the key features, which facilitated our identification of the specimen. Nevertheless, rather than on living plants, some subtle features could faintly be observed on pressed specimens of *H. avisoides* and its similar species. For example, the three-dimensional structure of flowers would collapse once pressed and the colours of leaves would fade away when drying. This might obscure the discrepancies between *H. avisoides* and *H. hui* or even other more distantly related species like *H. chusua* (D.Don) Y.Tang & H.Peng. We

hope that the 3D mesh model reconstructed in this study (see Data availability) would become helpful for recognising *H. avisoides* in future research.

According to the spatial delimitation of the arid valley in the upper reaches of Minjiang River (Zheng et al. 2017), of the two *Hemipilia avisoides* populations we discovered, JPY is distributed within the range of the arid valley and ZJG is closely situated next to the arid valley. The locality of the collection by S.-Y. Chen et al. in 1958 was not precisely recorded, but the elevation of 1780 m implied that the specimen was collected from the range of the arid valley in that region. However, it is notable that, as climate changes, the spatial range of the arid valley varies (Zheng et al. 2017). Focusing on vegetation type, based on our field observations, the vegetation where our collections of *H. avisoides* occur could be classified into Form. *Sophora davidii* (Franch.) Skeels, Form. *Prunus tangutica* (Batal.) Korsh. and/or Form. *Ostryopsis davidiana* Decaisne, which are typical of arid-valley shrubs and span an elevation between ca 1700 and 2500 m (Yang 2007). To sum up, the habitats of *Hemipilia avisoides* are mostly within the arid valley in the upper reaches of Minjiang River. To our knowledge, other orchids in the same and sympatric habitats include *Hemipilia physoceras*, *Habenaria acianthoides* Schltr. and *Cephalanthera erecta* Blume, although each of these species is more widely distributed overall. We believe the ecological characteristics of these orchids, including *Hemipilia avisoides*, are worthy of future study.

The morphologically similar species *Hemipilia hui* is also distributed in Gansu Province, which is north of Sichuan Province. According to the vegetation regionalisation of China (Zhang 2007), the information of specimens (see Additional specimens examined) and the online photos (see below), the habitats of *H. hui* in Gansu probably range from arid-valley shrubs to deciduous broadleaved forests at an elevation between 1250 and 1850 m. Besides herbarium specimens, there are some photo records of *H. hui* in Gansu on the websites of Plant Photo Bank of China, PPBC and China Field Herbarium, CFH (all in Chinese; see http://ppbc.iplant.cn/tu/5920959 [by R.-B. Zhu in Zhouqu County in 21 May 2016], http://ppbc.iplant.cn/tu/5919232 [by R.-B. Zhu in Wenxian County in 16 May 2016], http://ppbc.iplant.cn/tu/5919279 [by R.-B. Zhu in Wenxian County in 16 May 2016], http://ppbc.iplant.cn/tu/7885080 [by X.-J. Liu in Chengxian County in 4 May 2021], http://ppbc.iplant.cn/tu/11011659 [by Z.-F. Bai in Wenxian County in 28 April 2021] and the remaining photos in each album and http://www.cfh.ac.cn/a5074ffa-60e3-4bf6-83a9-eb7cff45b0df.photo [by J.-H. Wang in Wenxian County in 23 April 2015]). The plants shown in those photos have a subsquare mid-lobe that is larger than the lateral lobes; therefore, we recognised them as *H. hui*. Surprisingly, their leaves are green with white, reticulate venation, which mainly resemble those of *H. brevicalcarata* Finet and *H. yajiangensis* in *H.* sect. *Hemipilia*.

A few individuals of *Hemipilia avisoides* were observed in the field to possess conspicuously purple spots, along with white lines along 7–9 principal veins, on their leaves (Fig. 6A). However, *H. avisoides* lacks reticulate venation that is distinct in *H. brevicalcarata*, *H. yajiangensis* and the Gansu populations of *H. hui*. Occasionally, *H. hui* has a nearly uniformly green leaf as shown in another online photo (see

http://hengduan.huh.harvard.edu/fieldnotes/specimens/search/specimen_detail.zpt?specimen_id=21330&full_image=skelley04179 [by S. L. Kelley in Luhuo County, Sichuan in 12 August 2005]). Despite the variation of leaf-colour patterns within each species, *H. avisoides* could be distinguished from *H. hui* as their characteristics do not overlap.

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Appendix 1

Table A1. Voucher information and GenBank accession numbers of taxa included in phylogenetic reconstruction. Sequences generated in this study are marked with asterisks (*). Missing data are indicated with "–".

Accession	Voucher/Reference	ITS	*matK*	*trnL-F*	*trnS–trnG*
Brachycorythis henryi	Jin et al. 2017	MF944262	MF945438	MF945234	–
B. obcordata	Jin et al. 2017	MF944263	MF945500	MF945301	–
Hemipilia alpestris	Tang et al. 2015	KM651221	KM651385	KM651545	KM651627
H. amplexifolia	Tang et al. 2015	KM651222	KM651386	KM651546	KM651628
H. avisoides [Tang 151]	Tang et al. 2015	KM651296	KM651462	KM651621	KM651699
H. avisoides [Tang, Wang & Zhu 236]	Tang, Wang & Zhu 236	OP597820*	OP595696*	OP595697*	OP595698*
H. basifoliata [Jin 8361]	Jin et al. 2017	MF944399	MF945455	MF945251	–
H. basifoliata [Tang & Su 166]	Tang et al. 2015	KM651223	KM651387	KM651547	KM651629
H. brevicalcarata	Tang et al. 2015	KM651285	KM651449	KM651611	KM651689
H. calcicola	Tang et al. 2015	KM651279	KM651440	KM651605	KM651684
H. calophylla	Tang et al. 2015	KM651269	KM651433	KM651595	KM651674
H. camptoceras	Tang et al. 2015	KM651275	KM651439	KM651601	KM651680
H. capitata	Tang et al. 2015	KM651224	KM651388	KM651548	KM651630
H. cf. *amplexifolia*	Tang et al. 2015	KM651225	KM651415	KM651549	KM651631
H. cf. *flabellata*	Jin et al. 2017	KJ460050	KJ452806	MF945327	–
H. chidori	Tang et al. 2015	KM651287	KM651451	KM651612	KM651690
H. chusua [Jin 8272]	Jin et al. 2017	MF944401	MF945460	MF945257	–
H. chusua [STET 0619]	Jin et al. 2017	KJ460034	KJ452786	MF945189	–
H. chusua [Tang & Su 093]	Tang et al. 2015	KM651288	KM651452	KM651616	KM651694
H. chusua [Tang 145]	Tang et al. 2015	KM651290	KM651453	KM651615	KM651693
H. compacta	Jin et al. 2017	JN696455	KJ452796	MF945321	–
H. cordifolia	Jin et al. 2017	MF944329	MF945454	MF945250	–
H. crassicalcarata	Tang et al. 2015	KM651270	KM651434	KM651596	KM651675
H. cruciata	Jin et al. 2017	MF944330	MF945462	MF945259	–
H. cucullata	Tang et al. 2015	KM651276	KM651442	KM651604	KM651683
H. faberi [Tang 158]	Tang et al. 2015	KM651229	KM651391	KM651553	KM651635
H. faberi [Tang 161]	Tang et al. 2015	KM651230	KM651389	KM651554	KM651636
H. farreri	Tang et al. 2015	KM651231	KM651392	KM651555	KM651637
H. flabellata	Tang et al. 2015	KM651271	KM651435	KM651597	KM651676
H. forrestii	Jin et al. 2017	KJ460049	KJ452805	MF945326	–
H. fujisanensis	Tang et al. 2015	KM651280	KM651444	KM651606	KM651685
H. galeata	Tang et al. 2016	KT183499	KT183498	KT183500	–
H. gonggashanica	Tang et al. 2015	KM651233	KM651394	KM651557	KM651639
H. gracilis	Tang et al. 2015	KM651235	KM651397	KM651559	KM651641
H. graminifolia	Tang et al. 2015	KM651294	KM651458	KM651619	KM651697
H. hemipilioides	Tang et al. 2015	KM651238	KM651400	KM651562	KM651644
H. hui [Jin, Jin & Cui 14466]	Jin et al. 2017	MF944398	MF945425	MF945220	–
H. hui [Tang 156]	Tang et al. 2015	KM651297	KM651463	KM651622	KM651700
H. hui [Tang 199]	Tang et al. 2015	KM651298	KM651461	KM651623	KM651701
H. keiskei	Tang et al. 2015	KM651239	KM651401	KM651563	–
H. keiskeoides	Tang et al. 2015	KM651240	KM651402	KM651564	KM651645
H. kinoshitai	Tang et al. 2015	KM651241	KM651403	KM651565	KM651646
H. kiraishiensis	Jin et al. 2017	MF944403	MF945445	MF945241	–
H. kwangsiensis	Tang et al. 2015	KM651272	KM651436	KM651598	KM651677
H. lepida	Tang et al. 2015	KM651242	KM651404	KM651566	KM651647
H. monantha [Tang & Jiang 171]	Tang et al. 2015	KM651243	KM651405	KM651569	KM651650
H. monantha [Tang 192]	Tang et al. 2015	KM651244	KM651407	KM651567	KM651648
H. monantha [Tang 193]	Tang et al. 2015	KM651245	KM651406	KM651568	KM651649
H. oblonga	Tang et al. 2015	KM651281	KM651445	KM651607	KM651686

Accession	Voucher/Reference	ITS	*matK*	*trnL-F*	*trnS–trnG*
H. omeishanica	Tang et al. 2015	KM651299	KM651464	KM651624	KM651702
H. parceflora	Jin et al. 2017	KJ460052	KJ452808	KM651571	–
H. physoceras	Tang et al. 2015	KM651248	KM651410	KM651573	KM651654
H. pinguicula	Tang et al. 2015	KM651250	KM651413	KM651576	KM651657
H. purpureopunctata	Jin et al. 2017	KJ460051	KJ452807	MF945328	–
H. secundiflora	Jin et al. 2017	MF944406	MF945458	MF945254	–
H. sichuanica	Jin et al. 2017	KJ460059	KJ452815	MF945334	–
H. simplex	Tang et al. 2015	KM651253	KM651416	KM651578	KM651659
H. tetraloba	Tang et al. 2015	KM651255	KM651418	KM651580	KM651661
H. thailandica	Tang et al. 2015	KM651256	KM651419	KM651581	KM651662
H. tibetica [Jin & Zhang 11075]	Jin et al. 2017	MF944412	MF945449	MF945245	–
H. tibetica [Tang & Su 064]	Tang et al. 2015	KM651257	KM651421	KM651582	KM651663
H. trifurcata	Jin et al. 2017	KJ460055	KJ452811	KM651583	–
H. wenshanensis	Tang et al. 2015	KM651258	KM651422	KM651584	KM651665
H. yajiangensis	Yang et al. 2022	OM009240	OM009241	OM009241	OM009241
H. yueana	Tang et al. 2015	KM651259	KM651423	KM651585	KM651666
Ponerorchis gongshanensis [Tang & Su 128]	Tang et al. 2015	KM651226	KM651395	KM651550	KM651632
P. nana [Jin 9138]	Jin et al. 2017	MF944404	MF945475	MF945273	–
P. wolongensis [PS-00309]	Peng et al. 2022	MZ098270	–	–	–

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RESEARCH ARTICLE

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Morphological and molecular evidence gives insight into the taxonomic position of *Peucedanum pubescens* (Apiaceae, Selineae)

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Abstract

In this study, morphological and molecular evidences were combined to determine the taxonomic position of *Peucedanum pubescens* Hand.-Mazz. Morphologically, *Peucedanum pubescens* is similar to the species of the genus *Ligusticopsis* in having fibrous remnant sheaths at the stem base, pinnate and linear coexisted bracts, strongly compressed dorsally mericarps, filiform median and lateral ribs, winged marginal ribs, numerous vittae in each furrow and commissure, but can also be easily distinguished from members of *Ligusticopsis* by its hispid fruit and linear-lanceolate bracteoles. Molecular phylogenetic analyses based on the single-copy protein-coding sequences (CDS) of plastomes and internal transcribed space (ITS) region showed that *Peucedanum pubescens* nested in the genus *Ligusticopsis*. As both morphological and molecular data supported the inclusion of *Peucedanum pubescens* within *Ligusticopsis*, the species is here transferred and the new combination, *Ligusticopsis pubescens* (Hand.-Mazz.) J.J.Deng, C.K.Liu & X.J.He, made.

Keywords

Apiaceae, *Ligusticopsis pubescens*, new combination, *Peucedanum pubescens*

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Introduction

Peucedanum sensu lato was previously characterized by dorsally compressed mericarps with slightly prominent dorsal ribs, narrowly winged lateral ribs, as well as a broad commissure (Sheh 1992; Spalik et al. 2004; Sheh and Watson 2005). As such it was one of the largest genera of Apiaceae, comprising 100–120 species with 33 endemics to the territory of China, and with a distribution in Eurasia, South Africa, and sometimes Australia (Spalik et al. 2004; Sheh and Watson 2005; Cieśla et al. 2009). However, *Peucedanum* sensu lato has been a taxonomically confusing genus due to its great heterogeneous characteristics (Solov'eva et al. 1985; Reduron et al. 1997; Downie et al. 2000, 2010; Winter et al. 2008; Zhou et al. 2014), exhibiting a wide variety of life forms, leaf and fruit structures, and chemical compositions (Shneyer et al. 2003). Moreover, several molecular phylogenetic studies based on DNA fragments and plastomes indicated that *Peucedanum* sensu lato was not a monophyletic group (Downie et al. 2000; Spalik et al. 2004; Valiejo-Roman et al. 2006; Feng et al. 2009; Zhou et al. 2009, 2020; Liu et al. 2022). Consequently, the circumscription of the genus has been greatly reduced with *Peucedanum* sensu stricto, recognized by ternate leaves, linear-subulate or filiform bracteoles, one vitta in each furrow and two vittae on commissure in mericarp (Kadereit and Bittrich 2018) and several members of *Peucedanum* sensu lato were separated to restitute or establish genera or transfer into other genera (Reduron et al. 1997; Winter et al. 2008; Ostroumova et al. 2016; Pimenov et al. 2016; Pimenov 2017). However, the previous studies mainly focused on those species distributed in Europe and South Africa, and the taxonomic position of Chinese endemic species of this genus was still unresolved.

Ligusticopsis Leute was described by Leute in 1969 with *Ligusticopsis rechingeriana* Leute as its type species. The taxonomy of genus has been controversial since its establishment, due to its close morphology to *Ligusticum* (Zhou et al. 2008, 2009; Sun et al. 2010); e.g. Flora Reipublicae Popularis Sinicae and Flora of China treated *Ligusticopsis* as the synonym of *Ligusticum* and "*Ligusticum* in the broad sense", respectively (Sheh 1992; Sheh and Watson 2005), whereas the genus was recognized by Pimenov et al. (2001, 2003). Recently, a phylogenetic study based on morphological and molecular data confirmed the monophyly of *Ligusticopsis* and nine "true species of *Ligusticopsis*" were recognized (Li et al. 2022); the members of the genus are characterized by the following diagnostic characters: stem base clothed in fibrous remnant sheaths, pinnate bracts, pinnate bracteoles longer than rays of umbellule, mericarps strongly compressed dorsally, median and lateral ribs filiform or keeled, marginal ribs winged, and numerous vittae in each furrow and commissure.

Peucedanum pubescens Hand.-Mazz. (1933: 728) was described based on a collection (E00002620) from Yunnan, China, and was an endemic species to China (Sheh and Watson 2005; Pimenov 2017). Due to dorsally compressed mericarps with slightly prominent dorsal ribs and narrowly winged lateral ribs, *P. pubescens* was recognized as a member of *Peucedanum* sensu lato (Handel-Mazzetti Heinrich 1933). However, after examination of the type specimen and protologue, field observation, and morphological and micro-morphological research into it, we found this species was characterized

by stem base clothed in fibrous remnant sheaths, pinnate leaves, linear and pinnate co-existed bracts, strongly dorsally compressed fruits, numerous vittae in each furrow and commissure, and these features are significantly similar to members of *Ligusticopsis*. To determine the taxonomic position of *Peucedanum pubescens*, we performed morphological and molecular analyses.

Materials and methods

Morphological observation

The morphological features of *Peucedanum pubescens* were observed in field. Then, mericarp of this species was observed and photographed using a stereomicroscope, Nikon SMZ 25 (Japan). Furthermore, morphological diagnoses of nine "true species of *Ligusticopsis*" were obtained from type specimens from K, P, E, WU, BM, GH, KUN, and HNWP, Flora of China (Sheh and Watson 2005), and analysis performed by Li et al. (2022). The Herbarium code refers to Thiers (2015).

DNA extraction, ITS amplifying and sequencing

Total genomic DNA was extracted from silica-dried leaves with plant genomic DNA kit (Cwbio Biosciences, Beijing, China). The universal primers ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3', White et al. 1990) were used to amplify the entire internal transcribed sequences (ITS). Amplification was undertaken using a volume of 30 µl with 15 µl 2 × Taq MasterMix (CWBIO, China), 10 µl ddH2O, 1.5 µl forward primer, 1.5 µl reverse primer, and 2 µl total DNA. The amplification of the ITS region was obtained by initial denaturation for 3 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 70 s at 54 °C, and 90 s at 72 °C, and then a final extension of 10 min at 72 °C. All PCR products were separated using a 1.5% (w/v) agarose TAE gel and sent to Sangon (Shanghai, China) for sequencing.

Plastome sequencing and assembly

The extracted total DNA was fragmented into 400 bp to construct the pair-end library, following the manufacturer's protocol (Illumina, San Diego, CA, USA). The DNA libraries were sequenced on the Illumina NovaSeq platform at Personalbio (Shanghai, China). Quality control of the raw reads was performed using fastP v0.15.0 (-n 10 and -q 15) (Chen et al. 2018), produced at least 5GB clean reads per species. De novo genome assembly from the clean data was accomplished utilizing NOVOPlasty v2.6.2 (Dierckxsens et al. 2017), with a kmer length of 39 bp and a sequence fragment of the *rbcL* gene from *Ligusticopsis brachyloba* (Franch.) Leute (Genebank no. MN204661) as the seed sequence. The assembled complete plastome was annotated initially by using PGA (Qu et al. 2019) and then examined using Geneious v9.0.2 (Kearse et al. 2012).

Phylogenetic analyses

To confirm the phylogenetic position of *Peucedanum pubescens*, phylogenetic trees were reconstructed based on single-copy protein-coding sequences (CDS) of 34 plastomes and 36 ITS sequences (Table 1). *Chamaesium mallaeanum* Farille & S. B. Malla and *Chamaesium viridiflorum* (Franch.) Wolff ex Shan were selected as outgroups according to the result of a previous study (Li et al. 2022). Plastome CDs and ITS sequences were respectively aligned using MAFFT v7.221 (Katoh and Standley 2013), and then manually adjusted in MEGA7.0 (Kumar et al. 2016) to obtain plastome CDs and ITS datasets. The two alignments were subjected to Maximum-Likelihood (ML) analyses and Bayesian Inference (BI). For ML analyses, the software RAxML v8.2.8 (Stamatakis 2014) was used to construct the phylogenetic trees with the GTR+G+I model and 1000 bootstrap (BS) replicates. Bayesian inference (BI) analyses were conducted by MrBayes version 3.2.7 (Ronquist et al. 2012) with the best-fit substitution model (GTR+G+I) determined by Modeltest v3.7 (Posada and Crandall 1998). Markov Chain Monte Carlo (MCMC) search was performed for 1×10^6 generations, sampling every 100 generations. The first 25% of trees were discarded as burn-in and the remainder was used to generate the consensus tree. Results of phylogenetic analyses were visualized and edited in FigTree v1.4.2 (Rambaut and Drummond 2015).

Results

Morphological comparison

Peucedanum pubescens is similar to the species of *Ligusticopsis* in having fibrous remnant sheaths at the stem base (Fig. 3B), pinnate leaves (Fig. 3A, C–E), linear and pinnate coexisted bracts (Fig. 3F), strongly dorsally compressed fruits (Fig. 3G, H), numerous vittae in each furrow and commissure (Fig. 3H), but can be easily distinguished from the latter by the morphological characters shown in Table 2. In detail, *Peucedanum pubescens* is different from *L. rechingeriana* Leute, *L. involucrata* (Franch.) Lavrova and *L. hispida* (Franch.) Lavrova et Kljuykov in that *P. pubescens* has a triangular-ovate leaf blade in outline (Fig. 3C, D) (vs. oblong-ovate or lanceolate), obovate ultimate segments (Fig. 3C) (vs. ovate or linear), pinnate and linear coexist bracts (Fig. 3F) (vs. pinnate), linear-lanceolate bracteoles (vs. pinnate) (Fig. 3F), ovate to obovate mericarp shape (Fig. 3G) (vs. elliptic to ovate or elliptic), hispid mericarp surface (Fig. 3G, H) (vs. smooth) and 2–3 vittae in each furrow (Fig. 3H) (vs. 1–3). *Peucedanum pubescens* can be distinguished from *L. integrifolia* (H. Wolff) Leute, *L. brachyloba* and *L. modesta* (Diels) Leute in having linear-lanceolate bracteoles (Fig. 3F) (vs. pinnate and linear coexist or pinnate), hispid mericarp surface (Fig. 3G, H) (vs. smooth), ovate or obovate to orbicular mericarp shape (Fig. 3G) (vs. elliptic to ovate or elliptic) and 2–3 vittae in each furrow (Fig. 3H) (vs. 1–3 or 3–4 in each furrow). *Peucedanum pubescens* differs from *L. capillacea* Leute and *L. scapiformis* (H. Wolff) Leute in having triangular-ovate

Table 1. Voucher details and GenBank accession numbers of taxa used in this study. A n-dash (–) indicates unavailable information.

Species	Voucher information	Locality	GenBank accession number	
			Plastome	ITS
Angelica cartilaginomarginata	13E-39-3, SZ2006071804	USA, NIH; China, Yunnan	NC029393	EU647210
Angelic decursiva	13Q-02-1; v20060825 (SZ)	Korea, Cheongju-si; China, Sichuan	KT781591	EU418375
Angelica gigas	13E-39-3; SZ744110	USA, NIH; China, Sichuan	NC029393	GU395156
Angelica laxifoliata	–; 2006071804 (SZ)	China, Sichuan; China, Yunnan	NC040122	EU647210
Angelica nitida	–; 2006080501 (SZ)	China, Qinghai; China, Sichuan	MF594405	EU418378
Bupleurum chinense	–; C.Q. Feng	China, Sichuan	MN893666	EU001334
Bupleurum commelynoideum	–; 2008082002 (SZ)	China, Sichuan	MN893666	GU269874
Chamaesium mallaeanum	–; Strain NLM	China, Sichuan; China, Xizang	MT162552	KY74426
Chamaesium viridiflorum	–; Strain HB	China, Yunnan	MN119373	KY744260
Glehnia littoralis	–; SZ666775	China, Sichuan	KU921430	GU395183
Hansenia forbesii	–; SZ666939	China, Sichuan; China, Yunnan	NC034645	GU390407
Hansenia oviformis	–; F22	China, Sichuan	MT843761	MT337430
Hansenia weberbaueriana	–; J18091701	China, Sichuan	MN049520	MN049520
Ligusticopsis brachyloba	L081401 (SZ); L0814 (SZ)	China, Chongqing	MZ491174	MZ497218
Ligusticopsis capillacea	RT2019100601 (SZ); XB	China, Yunnan	NC049051	MT974023
Ligusticopsis hispida	RT2019100301 (SZ); L08110501 (SZ)	China, Yunnan	NC049052	OL600824
Ligusticopsis integrifolia	RT2019100202 (SZ); L081003 (SZ)	China, Yunnan	NC049055	MZ497219
Ligusticopsis involucrate	PC2018101905 (SZ); DB14	China, Yunnan	NC049054	MT974014
Ligusticopsis modesta	L081903 (SZ); L08190301 (SZ)	China, Yunnan	OL547615	OL600822
*Ligusticopsis pubescens*1	LCK2020817001	China, Yunnan	ON872189	ON870396
*Ligusticopsis pubescens*2	LCK2020817002	China, Yunnan	–	ON870397
*Ligusticopsis pubescens*3	LCK2020817003	China, Yunnan	–	ON870398
Ligusticopsis rechingeriana	L081103 (SZ); L081103 (SZ)	China, Yunnan	MZ491175	MZ497220
Ligusticopsis scapiformis	RT2019082001 (SZ); CT9	China, Sichuan	NC049057	MT974012
Ligusticopsis wallichiana	LD081506 (SZ); LD08150601 (SZ)	China, Xizang	OL547616	OL600823
Ligusticum delavayi	RT2019100301 (SZ); L08110501 (SZ)	China, Yunnan	NC049052	OL600824
Ligusticum jeholense	–; LGB1	China, Liaoning; China, Beijing	MT561037	KJ999437
Ligusticum sinense	–; Ge131139	China, Sichuan; China, Guangdong	NC038088	MH712648
Ligusticum tenuissimum	13I-08; JKTM-1-000065	Korea, Cheongju-si; Korea, Anyang-myeon	NC029394	KP058314
Ligusticum thomsonii	RT2019082301 (SZ); CJ (SZ)	China, Sichuan	MT409619	MT974009
Meeboldia yunnanensis	–; G18071908	China, Yunnan	MK993275	MN688997
Peucedanum ampliatum	JQP19082505 (SZ); NASLQX022	China, Shanxi; –	OK336475	JF977799
Peucedanum delavayi	SZ YY 062105; YY062105 (SZ)	–	EU418386	MT843765
Peucedanum japonicum	LCK2020001 (SZ); sb1	China, Sichuan; –	OK336477	EU224273
Peucedanum medicum	LCK2020004 (SZ); SZ66876	China, Guangxi; –	OK336473	HQ256686
Peucedanum praeruptorum	–; SZ ZXM 001	–	MN016968	EU418383

Table 2. Comparison of morphological characteristics between *Peucedanum pubescens* and the species of *Ligusticopsis*.

Characteristics	*P. pubescens*	*L. rechingeriana*	*L. involucrata*	*L. hispida*	*L. integrifolia*	*L. brachyloba*	*L. modesta*	*L. capillacea*	*L. scapiformis*	*L. wallichiana*
Stem (base)	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant	Fibrous remnant
Leaves	Pinnate, triangular-ovate	Pinnate, oblong-ovate	Pinnate, oblong-ovate	Pinnate, lanceolate	Pinnate, oblong-ovate	Pinnate, triangular-ovate	Pinnate, oblong-ovate	Pinnate, oblong-ovate	Pinnate, oblong-lanceolate	Pinnate, broadly ovate
Ultimate segments of leaves	Obovate	Ovate	Linear	Linear	Oblong-ovate or lanceolate	Oblong-ovate or lanceolate	Linear or lanceolate	Obovate	Ovate	Linear
Bracts	Pinnate and linear coexist	Pinnate	Pinnate	Pinnate	Pinnate and linear coexist	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate
Bracteoles	linear-lanceolate	Pinnate	Pinnate	Pinnate	Pinnate and linear coexist	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate and linear coexist
Mericarp surface	Hispid	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Mericarp shape	ovate or obovate to orbicular	Elliptic to ovate	Elliptic	Elliptic	Elliptic to ovate	Elliptic	Elliptic to oblong	Ovate	Elliptic to ovate	Elliptic
Calyx teeth	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous	Conspicuous
Dorsal compression	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong
Median rib shape	Filiform	Filiform	Filiform	Filiform	Filiform	Keeled	Filiform	Filiform	Filiform	Keeled
Vittae each furrow	2–3	1–3	1–3	1–3	1–3	2–3	3–4	1–3	1–4	1–3
Commissural vittae	6	6	6	6	6	6	8	6	4–6	6

leaf blade in outline (Fig. 3C, D) (vs. oblong-ovate or oblong-lanceolate), pinnate and linear coexist bracts (Fig. 3F) (vs. pinnate), linear-lanceolate bracteoles (Fig. 3F) (vs. pinnate) and hispid mericarp surface (Fig. 3G, H) (vs. smooth). *Peucedanum pubescens* can be distinguished from *L. wallichiana* (DC.) Pimenov et Kljuykov in having pinnate and linear coexist bracts (Fig. 3F) (vs. pinnate), linear-lanceolate bracteoles (Fig. 3F) (vs. pinnate and linear coexist), ovate or obovate to orbicular mericarp shape (Fig. 3G) (vs. elliptic), hispid mericarp surface (Fig. 3G, H) (vs. smooth), 2–3 vittae in each furrow (Fig. 3H) (vs. 1–3) and filiform median rib shape (Fig. 3G, H) (vs. keeled).

Plastome feature of *Peucedanum pubescens*

The plastome of *Peucedanum pubescens* is a typically quadripartite structure, including a large single copy region (LSC), a small single copy region (SSC), and a pair of inverted repeat regions (IR) (Fig. 1). The overall size of plastome is 148,260 bp, and that of the LSC, IR, and SSC are 91,819 bp, 19,411 bp, and 17,619 bp, respectively. GC content analysis shows that the overall GC content is 37.0%, and the IR regions (43.8%) are higher than LSC (35.9%) and SSC (30.9%). The whole plastid genome contains 129 genes including 36 tRNAs, 8 rRNAs, and 85 protein-coding genes.

Phylogenetic analyses

The phylogenetic trees based on plastome CDs and ITS were given in Fig. 4 and Fig. 5, respectively. Both tree topologies strongly supported that *Peucedanum pubescens* nested in the genus *Ligusticopsis* (PP = 1.00 & BS = 100%; PP = 0.99 & BS = 88%). Although the phylogenetic position of this species could not be resolved in ITS tree, phylogenetic tree constructed based on plastome CDs showed that *Peucedanum pubescens* was sister to the clade that included the species *L. rechingeriana* (type species of the genus *Ligusticopsis*) and *L. involucrata* with high support (PP = 1.00 & BS = 99%).

Discussion

Peucedanum sensu stricto and *Ligusticopsis* both belong to the Selineae tribe of Apiaceae, and members of these two genera are similar in the dorsally compressed fruits with filiform dorsal ribs, and winged marginal ribs (Spalik et al. 2004; Sheh and Watson 2005; Li et al. 2022), but the former genus can be distinguished significantly from the latter by having ternate leaves, linear-subulate, caducous or lacking bracts, one vitta in a furrow and two vittae in commissure in mericarp (Kadereit and Bittrich 2018), while the latter can also be distinguished from the former by possessing pinnate leaves, pinnate bracts, numerous vittae in each furrow and in commissure (Li et al. 2022). *Peucedanum pubescens* is more similar to the genus *Ligusticopsis* in having pinnate leaves, linear and pinnate coexisting bracts, numerous vittae in each furrow and in commissure (Table 2), rather than *Peucedanum* sensu stricto. This result was further

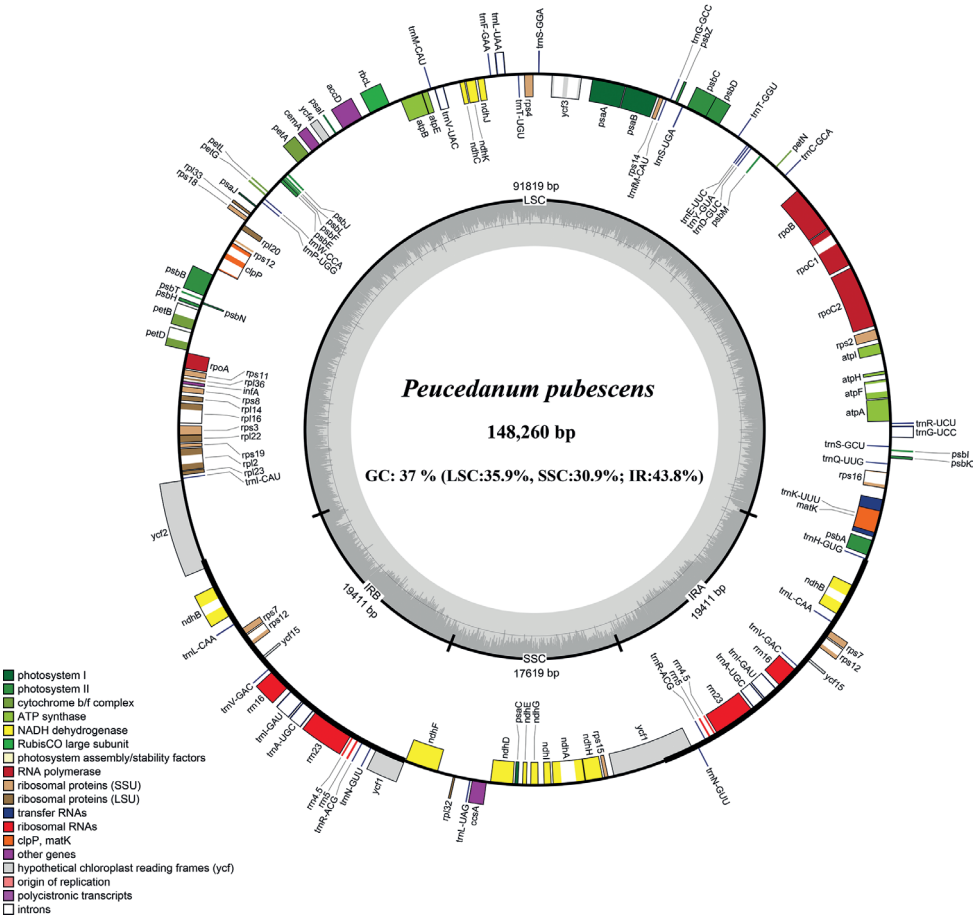

Figure 1. Plastome map of *Peucedanum pubescens*.

supported by the molecular phylogenetic analyses that *Peucedanum pubescens* nested in *Ligusticopsis*. As a result, *Peucedanum pubescens* is here transferred to *Ligusticopsis* as an independent species and a new combination in *Ligusticopsis* made, so that this genus now includes ten recognized species. The species is easily distinguished from other members of *Ligusticopsis* by the hispid fruit and linear-lanceolate bracteoles.

Taxonomic treatment

Ligusticopsis pubescens **(Hand.-Mazz.) J.J.Deng, C.K.Liu & X.J.He, comb. nov.**
urn:lsid:ipni.org:names:77307802-1
Figs 2, 3

Basionym. *Peucedanum pubescens* Hand.-Mazz. (1933: 728).

Type. China. Yunnan centralis: In regionis calide temperatae ad orientem fluminis Dsolin-ho, declivibus siccis inter vicos Mabou schan et Bölu, ad elevationem 1900–2000 m, 9 November 1916, Handel-Mazzetti 13043 (lectotype: WU! (WU0029560); isolectotypes: E (E00002620), W!).

Description. Perennials. Plants 30–70 cm, densely pubescent throughout. Stem solitary, hollow, prominent striated protrusions, branches few, short and stout, base densely clothed with fibrous leaf remains. Basal leaves few; petioles with broadly scarious-margined sheaths; leaf blade triangular-ovate in outline, 8–10 × 8–10 cm, 1–2-pinnate, pinnae sessile or subsessile; ultimate segments obovate, 1–4.5 × 0.8–2 cm, rather thick, both surfaces tomentose, more densely so on abaxial nerves, coarsely serrate or crenate, base cuneate or truncate. Leaves reduced upwards, uppermost very small, 3-lobed or toothed, petioles wholly sheathing. Synflorescence subcorymbosely branched; umbels 2.5–4 cm across; peduncles angled; bracts 6 to 8, pinnate and linear coexist, hispid throughout; rays 10 to 15, subequal, 1–2 cm; bracteoles 5 to 7, linear-lanceolate, longer than flowers; umbellules ca. 10-flowered. Calyx teeth conspicuous, subulate. Petals white, stylopodium conical, styles long, ca. 2 mm. Fruit ovate or obovate to orbicular, ca. 4 × 3 mm,

Figure 2. *Peucedanum pubescens* **A** isolectotype (E00002620) **B** lectotype (WU0029560).

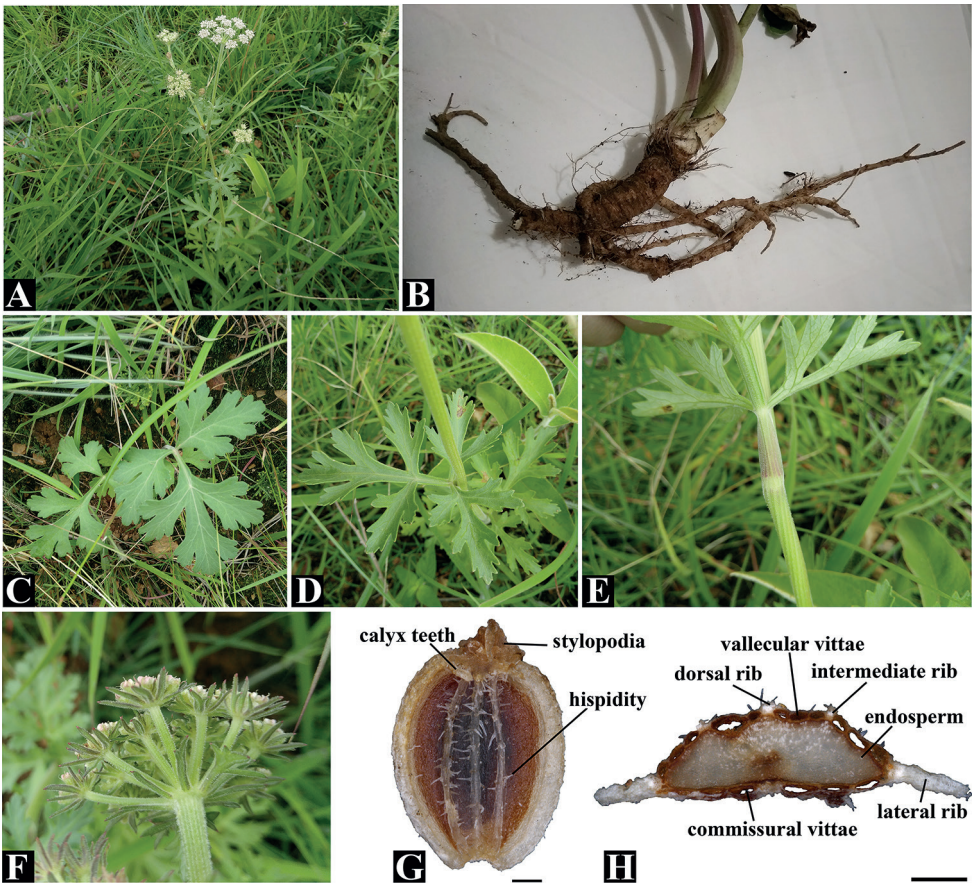

Figure 3. *Peucedanum pubescens* **A** habit **B** root **C** basal leaf **D** middle leaf **E** middle leaf with scarious-margined sheaths **F** bracts and bracteoles **G** dorsal view of mericarp **H** transverse section of mericarp. Scale bars: 0.5 mm (**G, H**).

hispid; strong dorsal compression, vittae large, 2–3 in each furrow, 6 on commissure. Seed face plane.

Phenology. Flowering and fruiting: August to October.

Vernacular name. Máo qián hú (Chinese pronunciation), 毛前胡 (Chinese name).

Distribution and habitat. This species is endemic to China and distributed in Yunnan (Lufeng, Luquan, Wuding) and Sichuan (Huili, Miyi) provinces. It grows in alpine meadows, elevation 1900–3000 m.

Diagnosis. *Ligusticopsis pubescens* significantly differs from other *Ligusticopsis* species by linear-lanceolate bracteoles and hispid fruit.

Additional specimen examined. China. Sichuan: Huili, Hongge, 2200 m, 12 October 1958, *Z. He*, *S.G. Tang* & *B.Q. Li 11593* (NAS); Panzhihua, Baishapo, 26°35'17"N, 101°59'1"E, 1854 m, 17 August 2021, *C.K. Liu LCK2020817001* (SZ).

Key to related species

1 Fruit hispid ..... ***L. pubescens***
– Fruit smooth **2**
2 Bracteoles pinnate and linear coexist **3**
– Bracteoles pinnate **4**
3 Blade shape broadly ovate; calyx teeth linear ..... ***L. wallichiana***
– Blade shape triangular-ovate; calyx teeth lanceolate ..... ***L. brachyloba***
4 Bracts pinnate and linear coexist; petals white obcordate..... ***L. integrifolia***
– Bracts pinnate; petals white purplish obcordate..... **5**
5 Commissural vittae 8 ..... ***L. modesta***
– Commissural vittae 4 to 6 or 6..... **6**
6 Plants hispid throughout **7**
– Plants sparsely pilose or glabrous..... **8**
7 Rays extremely elongated, elongate up to 24 cm; calyx teeth linear .... ***L. hispida***
– Rays subequal, (1–)3 cm; calyx teeth lanceolate ..... ***L. capillacea***
8 Stem unbranched; vittae per furrow 1 to 4, commissural vittae 4 to 6..... ..... ***L. scapiformis***
– Stem usually branched; vittae per furrow1 to 3, commissural vittae 6 ..... **9**
9 Ultimate leaf segments oblong-ovate; mericarp elliptic..... ***L. involucrate***
– Ultimate leaf segments ovate; mericarp elliptic to ovate ..... ***L. rechingeriana***

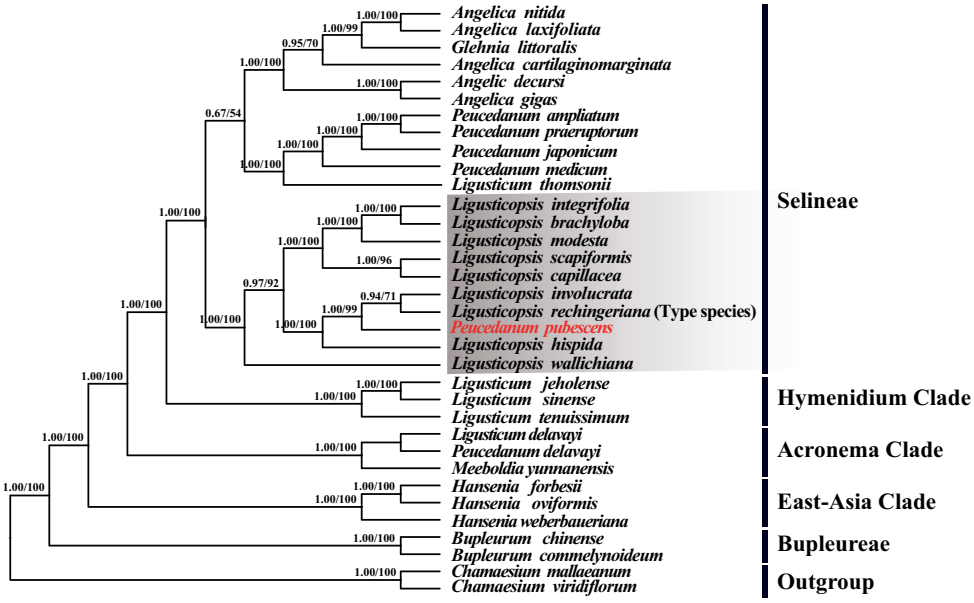

Figure 4. Phylogenetic tree inferred from Maximum-Likelihood (ML) and Bayesian Inference (BI) analyses based on 79 commonly shared CDs. PP/BS indicated posterior probabilities/bootstrap values.

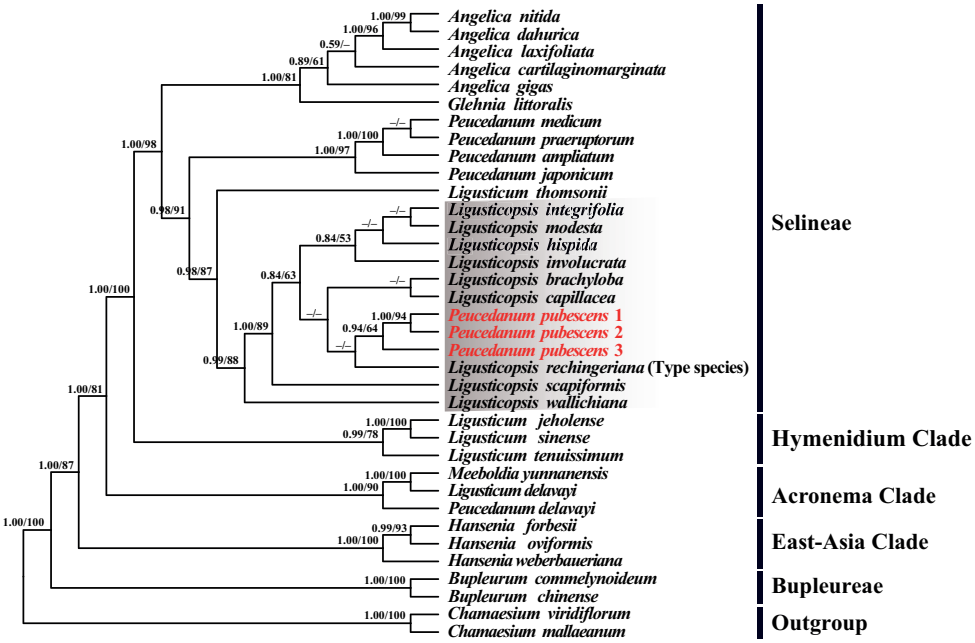

Figure 5. Phylogenetic tree of *Peucedanum pubescens* inferred from Maximum-Likelihood (ML) and Bayesian Inference (BI) analyses based on 36 ITS sequences. PP/BS indicated posterior probabilities/bootstrap values, respectively. Short line indicates values < 50%.

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Integrating morphological and genetic limits in the taxonomic delimitation of the Cuban taxa of *Magnolia* subsect. *Talauma* (Magnoliaceae)

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Abstract

An accurate taxa delimitation, based on a full understanding of evolutionary processes involved in taxa differentiation, can be gained from a combination of ecological, morphological, and molecular approaches. The taxonomy of *Magnolia* subsect. *Talauma* in Cuba has long been debated and exclusively based on traditional morphological study of a limited number of individuals. A more accurate description of leaf morphology variation using geometric morphometrics combined with genetic data could bring consistency to taxa delimitation in this group. Leaf samples for the morphological (243) and genetic (461) analyses were collected throughout the entire distribution range. The variability of each taxon was analyzed through multivariate and geometric morphometry, and 21 genetic markers (SSR). The observed leaf morphological variability was higher than previously described. Morphological and genetic classifications were highly congruent in two out of four taxa. Our data brought evidence that *Magnolia orbiculata* can be considered a true species with very clear genetic and morphological limits. The main taxonomic

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issues concern the north-eastern Cuban populations of *Magnolia* subsect. *Talauma*. The data supported the existence of two clear groups: corresponding mainly to *M. minor-M. oblongifolia* and *T. ophiticola*. However, these two groups cannot be considered fully delimited since genetic markers provided evidence of genetic admixture between them. Due to the likely absence of, at least strong, reproductive barriers between these three taxa, we propose therefore to consider them as a species complex.

Keywords

Gene flow, Genetic structure, integrative taxonomy, mclust, speciation, species delimitation

Introduction

Defining what a species is has been the subject of long debates in the history of biology, debates that have produced multiple species concepts (SC) over time (e.g. Genetic SC, Morphological SC, Phylogenetic SC, Ecological SC, Biological SC, among others (Mayr 1996; de Queiroz 2007). However, as pointed out by Hey (2006), this theoretical dilemma should not hinder the fact that biologists agree on simple and general ideas such as that species are fundamental units in biology, and that individuals belonging to the same species share a higher co-ancestry than with individuals from other species. The problem arises when one should define criteria for defining what a species is, and because criteria are linked to methodologies used to delineate species, therefore leading to different ways to define species (Hey 2006).

In the last two decades, there is an ever-growing shared idea that species can be defined as separately evolving metapopulation lineages (Unified SC) (de Queiroz 1998, 2007). Delimiting species boundaries, therefore, calls for accumulating evidence that the considered taxa are currently evolving independently. This task is especially challenging for taxa that have recently diverged, due to several evolutionary and genetic factors that have been described (see Naciri and Linder 2015, for a review). It is now largely recognized that an accurate taxa delimitation, based on a full understanding of evolutionary processes involved in taxon differentiation, can be gained from, and even should rely on, a combination of ecological, morphological, and molecular approaches to assess within-taxon diversity and among-taxa differentiation, across their whole geographical range (de Queiroz 2007; Padial et al. 2010).

The combination of several species concepts to broadly support species limits is known as integrative taxonomy (Padial et al. 2010). Dayrat (2005) and Will et al. (2005) recommended that species should only be named when their limits are supported by multiple lines of evidence. Integrative taxonomy does not replace traditional taxonomy but uses complementarity among disciplines to improve accuracy (Schlick-Steiner et al. 2010; Yeates et al. 2011). The potential for such integrative taxonomic approaches has not yet been fully embraced in botany, particularly in the tropics (Damasco et al. 2019). The works of Zheng et al. (2017), Alvarado-Sizzo et al. (2018), Damasco et al. (2019), Denham et al. (2019), Moein et al. (2019), Yang et al. (2019) are good examples of the use of an integrative approach carried out to solve the taxonomic problems in different plant families.

The genus *Magnolia* L. is a good model for applying an integrative taxonomic approach. It is the largest genus of the family *Magnoliaceae* Juss. It includes three subgenera, 13 sections, and an equal number of subsections (Figlar and Nooteboom 2004; Veltjen et al. 2022). The section *Talauma* Baill., with a Neotropical distribution, includes around 120 species distributed in four subsections: *Dugandiodendron* Lozano, *Chocotalauma* A. Vázquez, Á.J. Pérez and F. Arroyo, *Cubenses* Imkhan., and *Talauma* Juss. (Figlar and Nooteboom 2004; Vázquez-García et al. 2017). The 85 species of the subsection *Talauma*, the most species-rich of all *Magnolia* subsections, occur both in lowlands and mountainous areas (0 - 3 300 m.a.s.l.) of Central and South America, and the Caribbean Islands (Vázquez-García et al. 2017). The genus *Magnolia* includes species that could be perceived morphologically (Treseder 1978; Callaway 1994) and genetically (Lee and Chappell 2008; Li et al. 2013; Shen et al. 2018; Sun et al. 2020) conservative. Recent molecular research is challenging species delimitation based on morphology (Azuma et al. 2011; Rico and Gutierrez-Becerril 2019; Aldaba-Núñez et al. 2021). Most of these studies conclude that more evidence from ecology and morphology is needed, to understand the discrepancies with molecular data.

Cuba has the highest diversity of magnolias among the Caribbean islands, with seven endemic taxa (Veltjen et al. 2019). The Cuban taxa of *Magnolia* belong to two sections, *Magnolia* and *Talauma*. The section *Talauma*, the most diverse with six taxa, is represented by two subsections in Cuba: *Cubenses* and *Talauma*. The taxonomy of *Magnolia* subsect. *Talauma* in Cuba has long been debated (Fig. 1, Suppl. material 7), although based until now only on leaf morphology. The first taxon described was *Talauma minor* Urb. followed by *T. orbiculata* Britton and P. Wilson, in 1912 and 1923, respectively (Urban 1912; Britton 1923). Howard (1948) recognized two species of *Talauma*, *T. minor* Urb., and *T. truncata* (Moldenke) R.A. Howard, previously described by Moldenke (1946) as *Svenhedinia truncata* Moldenke. Two years later, León and Alain (1950) described a variety of *Talauma minor* with extremely oblong leaves that they named *T. minor* var. *oblongifolia* León. In the Flora of Cuba, León and Alain (1951) mentioned four taxa of *Talauma: T. minor* var. *minor*, *T. minor* var. *oblongifolia*, *T. orbiculata* and *T. truncata*. These authors distinguished *T. orbiculata* and *T. truncata* based on the largely truncate leaves of the latter, which inhabits only areas around Pico Turquino (León and Alain 1951). However, due to the large variation of leaf-base shape observed in these two taxa, they were not recognized as separated entities in the subsequent taxonomic reviews of the group (e.g., Bisse 1988; Imkhanitzkaja 1993; Palmarola et al. 2016).

In the Supplemental Material of the Flora of Cuba, Alain (1969) suggested that all previously described taxa of *Talauma* from Cuba could be considered as one single taxon. Later, Borhidi and Muñiz (1971) considered *T. minor* as the only species of *Talauma* in Cuba and defined two subspecies: *T. minor* subsp. *oblongifolia* (León) Borhidi and *T. minor* subsp. *orbiculata* (Britton and P. Wilson) Borhidi. Afterward, Bisse (1974) described *T. ophiticola* Bisse and recognized *T. oblongifolia* (León) Bisse. Years later, Bisse (1988) referred four species: *T. orbiculata*, *T. minor*, *T. oblongifolia* and *T. ophiticola*. The delimitation of each taxon was mainly supported, as in previous works, by leaf morphological characters.

Based on anatomical and morphological (vegetative and reproductive) traits described by Nooteboom (1993), Frodin and Govaerts (1996) made the combination of *Talauma minor* to *Magnolia minor* (Urb.) Govaerts and considered all the other names of the Cuban *Talauma* species as synonyms of *M. minor* (Acevedo-Rodríguez and Strong 2012; Rivers et al. 2016). The latest taxonomic review of *Magnolia* subsect. *Talauma* in Cuba recognized three species: *Magnolia orbiculata* (Britton and P. Wilson) Palmarola, *Magnolia minor*, and *Magnolia oblongifolia* (León) Palmarola (Palmarola et al. 2016). In the absence of additional evidence, Palmarola et al. (2016) considered *T. ophiticola* synonym of *M. oblongifolia* due to the existence of one specimen (Bisse and Kohler HFC 5358 HAJB) that has leaves with the characteristics used by Bisse (1974, 1988) to define both taxa. A recent work (Testé et al. in press) analyzed the ecological niche of the group, concluding that *M. orbiculata* is the only species that could be considered ecologically distinct from the others.

All abovementioned taxonomic revisions (e.g., León and Alain 1950, 1951; Bisse 1974, 1988; Imkhanitzkaja 1993; Palmarola et al. 2016) were exclusively based on traditional leaf morphological descriptors and only a few individuals, limiting their ability to elucidate taxon boundaries. A more accurate description of leaf morphology variation using geometric morphometric combined with genetic data could significantly bring consistency to taxa delimitation in this group. The present work focuses on the Cuban taxa of *Magnolia* sect. *Talauma* subsect. *Talauma* and aims to **(1)** assess their phenotypic variability of leaf morphological traits across their full geographic range **(2)** based on morphological data, evaluate the three main classification systems (called CS hereafter) of these taxa proposed up to date: the two taxa CS of León and Alain (1951), the four taxa CS of Bisse (1988), and the three taxa CS of Palmarola et al. (2016) (see Fig. 1); **(3)** infer the genetic structure of *Magnolia* subsect. *Talauma* in Cuba; **(4)** integrate morphological and genetic data to review taxon delimitation in *Magnolia* subsect. *Talauma* in Cuba.

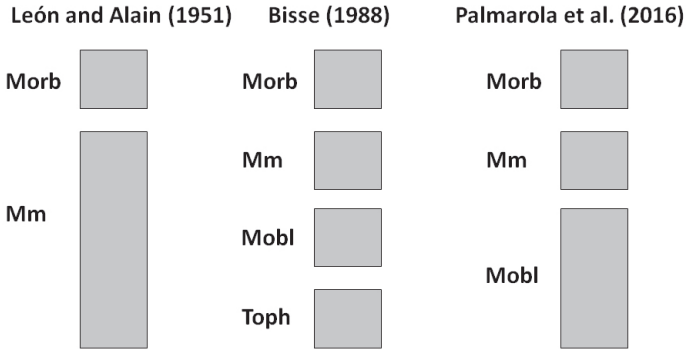


Figure 1. The three main classification systems (CS) of *Magnolia* subsect. *Talauma* in Cuba. Morb: *M. orbiculata* (Britton & P. Wilson) Palmarola; Mm: *M. minor* (Urb.) Govaerts; Mobl: *M. oblongifolia* (León) Palmarola; Toph: *Talauma ophiticola* Bisse.

Materials and methods

Sampling and taxon identification

The leaf samples for the morphological and genetic analyses were collected between 2015 and 2020 from individuals representing of *Magnolia* sect. *Talauma* subsect. *Talauma* throughout their entire distribution range in the mountains of Nipe-Sagua-Baracoa and Sierra Maestra in eastern Cuba (Fig. 2). In the field, individuals were identified based on tree and leaf shape according to the morphological criteria outlined by Bisse (1988), because this author defined the highest number of species units (Fig. 1). León and Alain (1951) considered *T. truncata* an independent species. However, in the present work, individuals that could have been considered as *T. truncata*, were considered as part of the variability of *M. orbiculata*, as has been recognized by Bisse (1988), Imkhanitzkaja (1993), and Palmarola et al. (2016). To confirm species identity, 43 herbarium vouchers were collected or reviewed (Table 1). All herbarium vouchers were deposited in the Herbarium Johannes Bisse (HAJB, herbarium acronyms follow Thiers 2022) at the National Botanic Garden (University of Havana). The number of samples per species and localities is shown in Table 1.

For the morphological analyses, 4–8 healthy leaves from 200 individuals were randomly collected, across the entire range of taxa within each locality. A leaf was considered healthy if the full outline of the leaf was undamaged. Leaves were photographed with a Nikon camera on a white background with a fixed ruler. The petiole of the leaf was removed before taking pictures, and the camera was mounted on a tripod to standardize the angle and distance of the photographs. To expand the geographic scope of our study, we also included leaf samples from 43 herbarium specimens (deposited in HAC, HAJB, and B). Hence, in total 243 individuals of *Magnolia* sect. *Talauma* subsect. *Talauma* in Cuba were morphologically analyzed.

For the genetic analyses, young leaf samples of a total of 461 individuals, belonging to 26 of 30 known localities, were stored in self-sealed bags with silica gel for DNA extraction. The resulting number of DNA samples represented 52% of the known individuals of *Magnolia* subsect. *Talauma* in Cuba (close to 900 individuals).

Multivariate and geometric morphometry

Analyses based on morphological variables were aimed at comparing the relevance of each of the three CS previously proposed: the two taxa CS, *Magnolia minor* and *M. orbiculata*, of León and Alain (1951); the four taxa CS, *M. minor*, *M. orbiculata*, *M. oblongifolia*, and *Talauma ophiticola*, of Bisse (1988); and the most recent, the three taxa CS, *M. minor*, *M. orbiculata*, and *M. oblongifolia*, of Palmarola et al. (2016) (Fig. 1). Two types of morphological analyses were carried out on three independent datasets: 1) multivariate morphometry analysis: a linear and angular measures dataset, 2) geometric morphometry analysis: an outline dataset and a landmarks coordinates dataset.

Table 1. Recorded localities, demographic information, DNA samples and herbarium voucher of the taxa of *Magnolia* subsect. *Talauma* in Cuba. HFC: "Serie Flora de Cuba". All the samples were deposited in HAJB (Herbarium Johannes Bisse of National Botanic Garden-University of Havana). **NP:** National Park; **ER:** Ecological Reserve; **NOE:** Natural Outstanding Element; **PAMR:** Protected Area of Management resources. * Extinct in the locality; ? No field data available; NV: no voucher.

Taxa	Localities (AP)	Abrev.	Indiv.	Leaf	DNA	Voucher
M. orbiculata	NP Pico La Bayamesa	BAY	6	0	1	Molina Y. HFC 89590
M. orbiculata	NP Turquino	TUR	43	26	20	Palmarola A. & González-Torres L.R. HFC 89394
M. orbiculata	ER El Gigante	GIG	4	4	1	Testé E. HFC 90667
M. orbiculata	ER Pico Caracas	CAR	26	1	14	Palmarola A. et al. HFC 89194
M. orbiculata	Loma del Gato	GAT	?	4	0	León Hno. 23366
M. minor	Calizas de Mucaral (NP Alejandro de Humboldt)	MUC	35	9	16	Bécquer E.R. et al. HFC 90656
M. minor	Camarones-Río Báez (PAMR Cuchillas del Toa)	CRB	16	5	15	Bécquer E.R. et al. HFC 89579
M. minor	Cañón del Río Yumurí (NOE Cañón del Río Yumurí)	CRY	5	5	4	Bécquer E.R. et al. HFC 89829
M. minor	Cayo Guam	CG	43	3	22	Palmarola A. et al. HFC 89243
M. minor	Cayo Mujeres	CM	2	0	1	Palmarola A. et al. HFC 89213
M. minor	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	34	4	19	Falcón B. et al. HFC 88955
M. minor	El Recreo (NP Alejandro de Humboldt)	REC	4	2	4	Bécquer E.R. et al. HFC 89467
M. minor	La Melba (NP Alejandro de Humboldt)	MEL	5	1	5	Palmarola A. et al. HFC 89584
M. minor	Mina la Hoya (NOE Cañón del Río Yumurí)	MH	29	9	12	NV
M. minor	Monte Fresco (NP Alejandro de Humboldt)	MF	18	0	12	García A. et al. HFC 90715
M. minor	Naranjo del Toa (NP Alejandro de Humboldt)	NT	15	7	13	NV
M. minor	Pico Cristal (NP Pico Cristal)	PC	16	13	15	Bécquer E.R. et al. HFC 89921
M. minor	Piedra La Vela (NP Alejandro de Humboldt)	PV	13	3	11	Bécquer E.R. et al. HFC 90519
M. minor	NOE Pinares de Montecristo	PM	33	8	16	Bécquer E.R. et al. HFC 90421
M. minor	Región del Toa (NP Alejandro de Humboldt)	RT	29	7	15	Bécquer E.R. et al. HFC 90660
M. minor	Río Yamanigüey (NP Alejandro de Humboldt)	YAM	72	5	28	Bécquer E.R. et al. HFC 89449
M. minor	Sur de las Delicias del Duaba	SDD	2	1	2	Díaz J. et al. HFC 89435
M. minor	Yumurí del Sur	YS	8	5	5	Bécquer E.R. et al. HFC 89510
M. minor	NOE Yunque de Baracoa	YUN	3	2	3	Bisse J. HFC 5321
M. minor	Siera de Imías	IMI	?	2	0	Alvarez A. et al. HFC 27534
M. minor	Presa de Cola de Moa	–	*	1	0	Wright 1100
M. minor	Presa de Mayarí	–	*	1	0	Shafer 8335
M. oblongifolia	Calizas de Mucaral (NP Alejandro de Humboldt)	MUC	1	1	1	Bécquer E.R. et al. HFC 90655
M. oblongifolia	Cayo Guam	CG	11	11	3	Palmarola A. et al. HFC 89249
M. oblongifolia	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	31	12	15	Falcón B. et al. HFC 88959
M. oblongifolia	La Melba (NP Alejandro de Humboldt)	MEL	5	0	2	Palmarola A. et al. HFC 89589
M. oblongifolia	Pico Cristal (NP Pico Cristal)	PC	5	4	3	Bécquer E.R. et al. HFC 89933
M. oblongifolia	Piedra La Vela (NP Alejandro de Humboldt)	PV	4	3	3	Bécquer E.R. et al. HFC 90543
M. oblongifolia	Río Yamanigüey (NP Alejandro de Humboldt)	YAM	6	6	6	Bécquer E.R. et al. HFC 89452
M. oblongifolia	Sur de las Delicias del Duaba	SDD	1	1	1	Díaz J. et al. HFC 89435
M. oblongifolia	Yunque de Baracoa	YUN	2	2	1	Bécquer E.R. et al. HFC 89531
T. ophiticola	Cayo Guam	CG	130	15	33	Bécquer E.R & Testé E. HFC 89439
T. ophiticola	Cupeyal del Norte (NP Alejandro de Humboldt)	CN	82	23	39	Falcón B. et al. HFC 88950
T. ophiticola	La Melba (NP Alejandro de Humboldt)	MEL	12	5	12	Palmarola A. et al. HFC 89587
T. ophiticola	Mina Iberia (NP Alejandro de Humboldt)	MI	77	16	45	Palmarola A. et al. HFC 89261

Taxa	Localities (AP)	Abrev.	Indiv.	Leaf	DNA	Voucher
T. ophiticola	Monte Fresco (NP Alejandro de Humboldt)	MF	11	0	8	NV
T. ophiticola	Pico Cristal (NP Pico Cristal)	PC	8	6	7	Bécquer E.R. et al. HFC 89917
T. ophiticola	Piedra La Vela (NP Alejandro de Humboldt)	PV	4	0	3	Bécquer E.R. et al. HFC 90531
T. ophiticola	Subida a la Melba (km 10)	SM	7	0	7	Alvarez A. et al. HFC 42531
T. ophiticola	Sur de las Delicias del Duaba	SDD	12	8	10	Bécquer E.R. et al. HFC 89556
T. ophiticola	NOE Yunque de Baracoa	YUN	19	2	8	Bécquer E.R. et al. HFC 89529

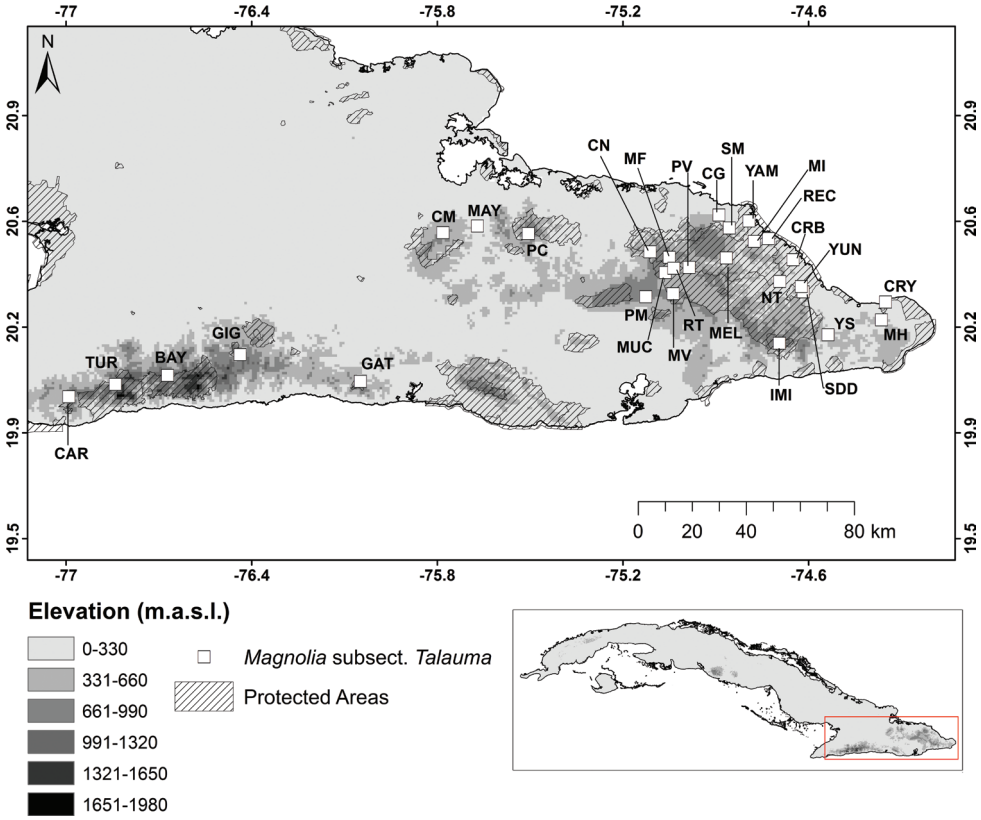


Figure 2. Geographic distribution of sampling locations of *Magnolia* subsect. *Talauma* in Cuba.

In the multivariate morphometry analysis, linear and angular measures of leaf characters were automatically taken from the digital photographs using the R v. 3.4.1 (R Core Team 2017) package FOLIOMETRIK v. 0.2.2 (Ramírez-Arrieta and Denis 2020). Eleven leaf variables were measured: central axis length (Length), maximum width, width at the three main quartiles (25, 50, and 75 quartiles), the perimeter of the contour (Perimeter), surface area (Area), and internal angles (v1 = angle of the base, v2 = angle of the apex; m1 and m2 = lateral angles at the maximum width) (Fig. 3A). Additional to the eleven measured variables, we calculated the maximum width/length ratio, named Calculated Index of Bisse (B_{ci}) for each leaf. The eleven variables were

recorded for each leaf. Subsequently, the twelve variables were averaged per individual for the 4–8 leaves available per individual. These averages of the twelve variables were used for all the subsequent statistical analyses.

In the geometric morphometry analysis, the outline dataset was obtained through a semi-automated shape analysis performed in FOLIOMETRIK v. 0.2.2 (Ramírez-Arrieta and Denis 2020). We set the program outputs to the Elliptic Fourier Descriptors (EFDs) (Jensen 2003), to obtain the first 25 harmonics (Chuanromanee et al. 2019). The harmonics were normalized to eliminate the differences in size, position, rotation, and starting point. This allowed removing the undesired experimental source of random variation and analyzing true differences of leaf shape between individual measurements (Jensen 2003). The landmarks coordinates dataset was obtained as follows. The positions of landmarks were determined by placing a quadratic grid with six lines on each leaf. In between the intersections of the grid and the border of the leaf we set 14 landmarks, two of them anatomical (type 1, i.e. apex and base) and the other 12 mathematically defined (type 2) (Fig. 3B). All analyses were carried out in FOLIOMETRIK v. 0.2.2. The landmarks X and Y coordinates were standardized using a Generalized Procrustes Analysis in PAST v. 2.14 (Hammer et al. 2001). Next, two variables: the Sum EDMA (Euclidean Distance Matrix Analysis) and centroid size were calculated.

The statistical significance of the differences among taxa for each measured variable (linear and angular variables, Sum EDMA, and centroid size) was assessed by a MonteCarlo analysis in PopTools v. 3.23 (Hood 2010) with 10 000 random permuta-

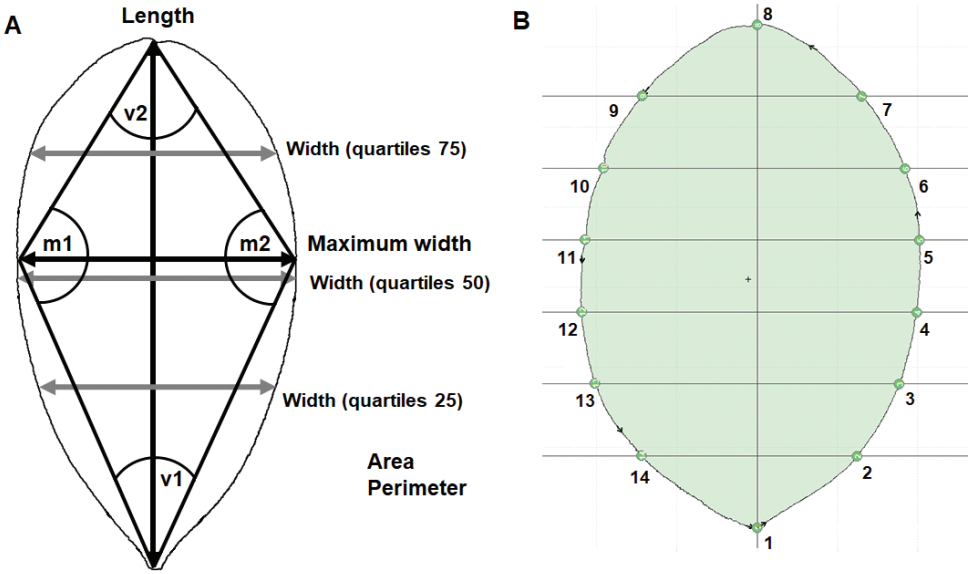


Figure 3. A the 11 morphological variables measured on leaves of *Magnolia* subsect. *Talauma* in Cuba; v1 = angle of the base, v2 = angle of the apex; m1 and m2 = lateral angles in the maximum wide **B** quadratic grid with six lines and the position of the 14 landmarks (type 1: points 1 and 8; type 2: the other 12 points), placed on the leaves of *Magnolia* subsect. *Talauma* in Cuba.

tions. The variability of the whole sample was described by using a normalized Principal Component Analysis (PCA). Differences among taxa were tested according to a one-way nonparametric MANOVA, using Euclidian distance, with 10 000 randomizations. Correction of p-values for multiple testing was done using the Bonferroni method. The multivariate comparisons were done independently for each dataset (linear and angular measures dataset, outline datasets, and landmarks coordinate dataset). All statistical analyses were conducted in R v. 3.4.1 (R Core Team 2017) and PAST v. 2.14 (Hammer et al. 2001), and the threshold used to decide for statistical significance was a p-value of 0.001.

Clustering analysis based on morphological variability

A Bayesian clustering approach based on Gaussian finite mixture models was carried out using each of the three datasets of morphological variables using the "mclust" R package (Scrucca et al. 2016). The method tests the number of clusters and different mixture models that best fit the data according to the number of clusters (G) chosen a priori. The method allows comparing the quality of the discrimination among clusters based on the Bayesian Information Criteria (BIC) allowing to choose the best value(s) of G, without any information about individual assignation to the different clusters. The default "mclust" setting was used to assess the 14 types of models which all differ in the covariance matrix landscape (see Scrucca et al. 2016; Zhang and Di 2020, for further details about the models). We varied G values between 1 and 9 (default option). Three independent analyses were performed using the three datasets; to compare the power of those three groups of variables to discriminate among taxa. Because the analyses sometimes provided clusters with only one individual, those clusters were considered "ghost" clusters and not considered as true clusters.

DNA extraction and PCR

DNA was extracted from dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1990) with MagAttract Suspension G solution mediated cleaning (Xin and Chen 2012). DNA quality was assessed using a spectrophotometer NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Individuals were genotyped using 21 microsatellite markers (simple sequence repeats, SSR) (Suppl. material 8) developed on four Neotropical *Magnolia* species: *M. lacandonica* A. Vázquez, Pérez-Farr. and Mart.-Camilo (MA39), *M. mayae* Vázquez and Pérez-Farrera (MA40), *M. dealbata* Zucc. (MA41) and *M. cubensis* subsp. *acunae* Imkhan. (MA42) (Veltjen et al. 2019), using four-primer PCR multiplex method (Vartia et al. 2014). PCR conditions and primer labeling followed Veltjen et al. (2019). The combination and parameters of the four multiplex reactions are given in Suppl. material 8. The lengths of the DNA fragments were detected using an ABI 3130XL fragment analyzer, quantified with a GeneScanTM 500 LIZ size standard (Thermo Fisher Scientific), and analyzed in Geneious v. 8.0.5 (Kearse et al. 2012) with the microsatellite plugin.

Genetic structure

Genetic diversity values were calculated for each taxa using GeneAlex v. 6.5 (Peakall and Smouse 2012) and Genepop v. 4.7.5 (Rousset 2008). Genetic differentiation between taxa was estimated through pairwise comparisons of F_{ST} (Weir and Cockerham 1984) and D_{JOST} values (Jost 2008) using the fastDivPart function of the R package diveRsity (Keenan et al. 2013). The identification of genetic clusters and the assignment of individuals was performed using STRUCTURE v. 2.3.4 (Pritchard et al. 2000), which uses a Bayesian clustering approach using MCMC for posterior distribution sampling. STRUCTURE analyses were conducted using a model that assumes admixture, correlated allele frequencies, and without prior population information. First, 10 replicates were run for each genetic clusters (K), with K varying between 1 to 20 and a burn-in period of 50 000 iterations followed by a run-length of 150 000 iterations of the Markov Chain. The most probable number of groups was determined according to the method of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER v. 0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester) (Earl and von Holdt 2012). Then, 100 new repetitions of the MCMC method were run for the best K value. CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) was used to estimate similarities between runs and to average the membership probabilities. Final bar plots displaying individual admixture coefficients were obtained thanks to Structure Plot v. 2.0 (Ramasamy et al. 2014). An individual was considered a member of a genetic group when its probability of belonging to that group was higher than or equal to 0.9. A second STRUCTURE analysis was executed (using the same configuration) without considering the individuals of *M. oblongifolia* (sensu Bisse 1988).

Because the MCMC method implemented in STRUCTURE is based on a population genetic model, the results of genetic clusters and assignment of individuals, may be affected by the potential low model fit to data. Thus, a non-model-based multivariate clustering analysis was also performed. A DAPC analysis (Discriminant Analysis of Principal Components) was executed in R v. 3.6.1 (R Core Team 2017) using the adegenet R package (Jombart et al. 2010). Firstly, a PCA was run on the whole dataset for which the first 200 Principal Components (PCs) were retained. Secondly, a discriminant analysis was executed using the number of genetic clusters defined in the previous step. Parallel to the STRUCTURE analysis, a second DAPC analysis was done without *M. oblongifolia* (sensu Bisse 1988). An individual was considered a member of a genetic group when its probability of belonging to that group was higher than or equal to 0.9.

For all analyses, graphical representations of outputs were built using the four taxa CS to have a representative overview of the correspondence between genetic clusters and each already defined taxon.

Integrating morphological and genetic limits

Because 138 individuals were analyzed both at the morphological and the genetic level, the correspondence between the groups inferred from both type of characters was assessed. The distributions of individual assignment to each morphological

(mclust) and genetic (STRUCTURE) clusters were compared with a Chi^2 test carried out on the Contingency Assignment Table using PAST v. 2.14 (Hammer et al. 2001). A heatmap was made, in R v. 3.4.1, to analyze the variation in the cluster assignation inside each taxon.

Results

Multivariate and geometric morphometry

The results of the multivariate morphometry analysis are summarized in Figs 4, 5, Suppl. materials 1–3, 9. The Calculated Index of Bisse (B_{ci}) showed significant differences between the defined species whatever the CS tested ($p < 0.0001$). *Magnolia orbiculata* and *Talauma ophiticola* displayed the highest and the lowest mean B_{ci}, respectively. Most of the eleven other variables showed significant differences between taxa, whatever the CS (Suppl. materials 1–3). There were three exceptions. The leaf perimeter did not show significant differences between *Magnolia minor* and *Talauma ophiticola* ($p = 0.211$), and between *M. oblongifolia* and *T. ophiticola* ($p = 0.132$) when the four taxa CS was considered. Likewise, the leaf area between *M. minor* and *M. oblongifolia* ($p = 0.115$) did not show differences when the three taxa CS was used. When following the two taxa CS (Fig. 4A, Suppl. material 1), eight variables (Maximum width, B_{ci}, Width-quartiles 50, Width-quartiles 75, Internal angles-v1, Internal angles-m1, Internal angles-v2, Internal angles-m1) showed an intra-taxon bimodal pattern within *M. minor*.

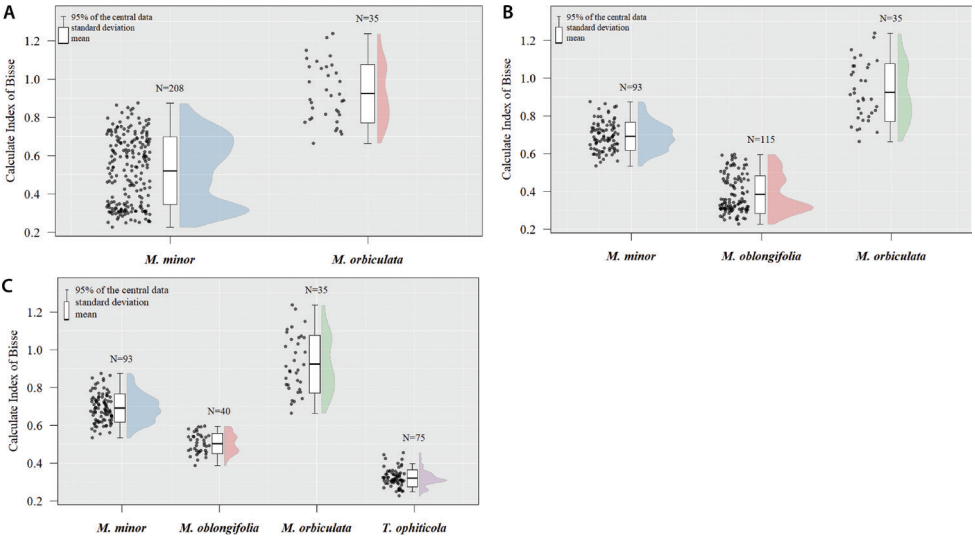


Figure 4. Graphic representation of Calculated Index of Bisse (B_{CI}) measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba. Two taxa CS (**A**), four taxa CS (**B**), three taxa CS (**C**).

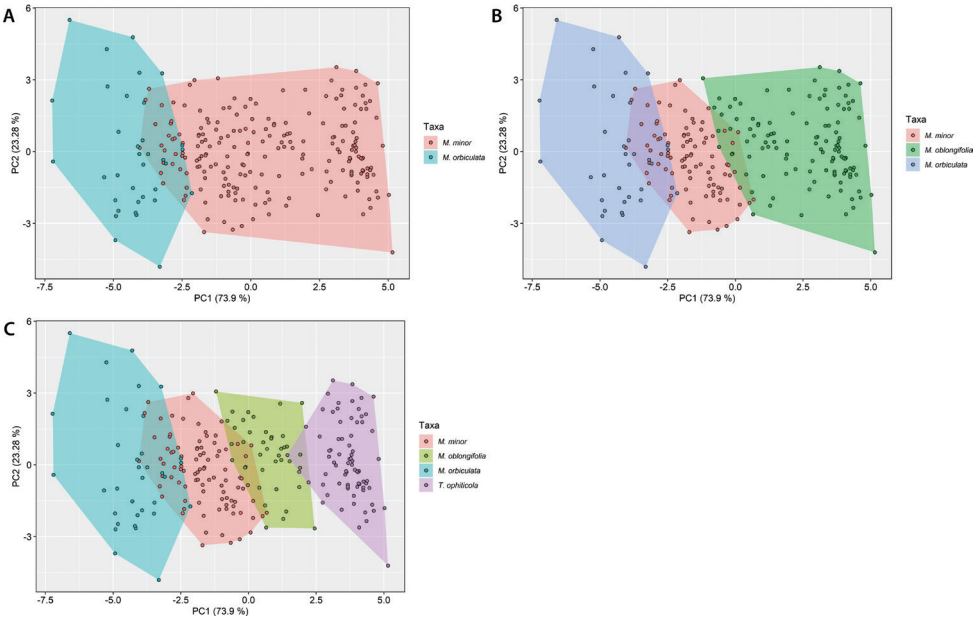


Figure 5. Principal Component Analysis for the multivariate morphometric variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba. Two taxa CS (**A**), three taxa CS (**B**), four taxa CS (**C**).

Despite the clear morphological differentiation between taxa, overlap in the multivariate distributions of leaf morphology variables was observed (Fig. 5). The internal angle of the base (-0.324) and the leaf perimeter (0.5627) displayed the highest weight in the first two principal components, respectively (Suppl. material 9). The NPMANOVA showed significant statistical differences ($p < 0.0001$) between taxa for each of the CS (Suppl. material 10). The comparison between groups, based on Sum EDMA and centroid size, showed significant differences for most comparisons (Suppl. material 4). The exceptions were: the Sum EDMA between *M. minor* and *M. oblongifolia* ($p = 0.316$) and between *M. orbiculata* and *T. ophiticola* ($p = 0.406$), when referring to the four taxa CS (Suppl. material 4). Fig. 6 illustrates PCAs on the outline dataset (Fig. 6A, C, E), and the landmark dataset (Fig. 6B, D, F) for the two (Fig. 6A, B), three (Fig. 6C, D) and four (Fig. 6E, F) taxa CS. Based on PCA for elliptic Fourier descriptors and Landmark, the different taxa had little overlap in the ordination space (Fig. 6). However, a clearer distinction among taxa was obtained with landmark positions than with other quantitative variables. This was especially obvious with *M. orbiculata*, which was strongly differentiated from other taxa when using landmark positions, no matter the CS considered.

The NPMANOVA showed significant statistical differences ($p < 0.001$) between taxa for each of the CS in the linear and angular measures dataset (Suppl. material 10). Similarly, the NPMANOVA showed significant statistical differences ($p < 0.001$) between the groups in the outline and landmark datasets (Suppl. material 10).

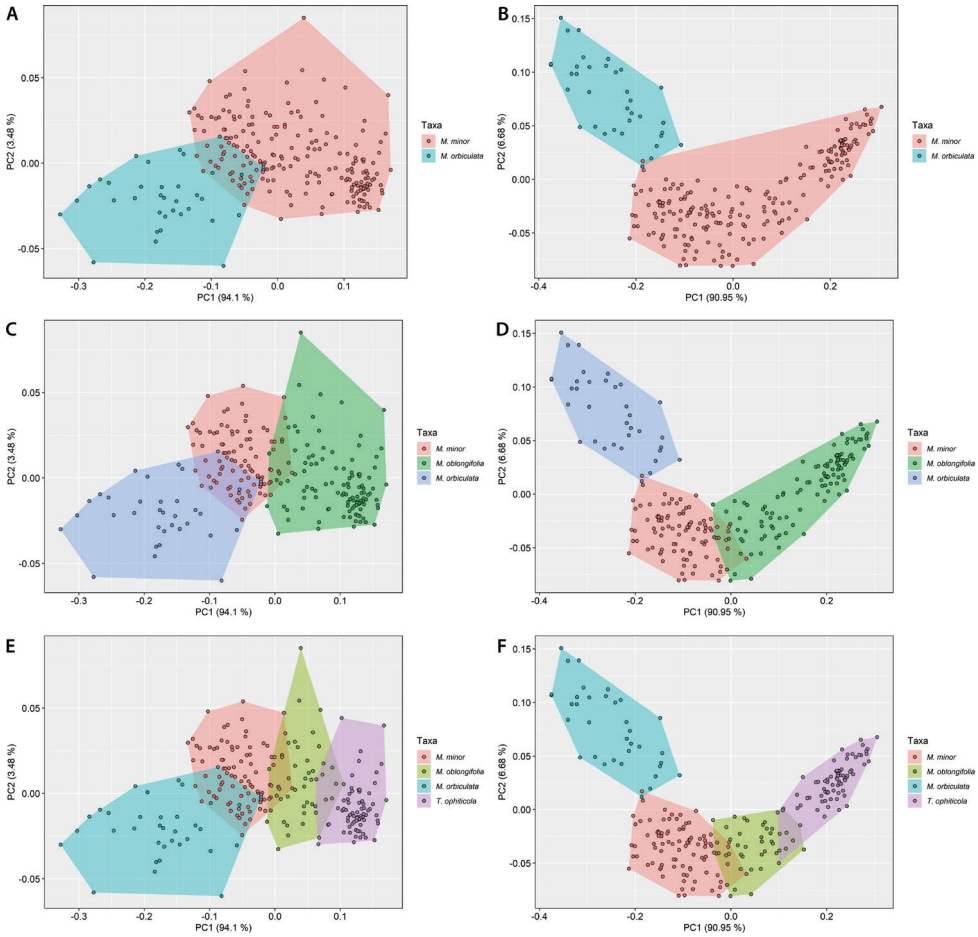


Figure 6. Principal Component Analysis for the Elliptic Fourier Descriptors (**A, C, E**) and Coordinates of the landmark (**B, D, F**) which characterized the leaves of *Magnolia* subsect. *Talauma* in Cuba. Two taxa CS (**A, B**), three taxa CS (**C, B**), four taxa CS (**E, F**).

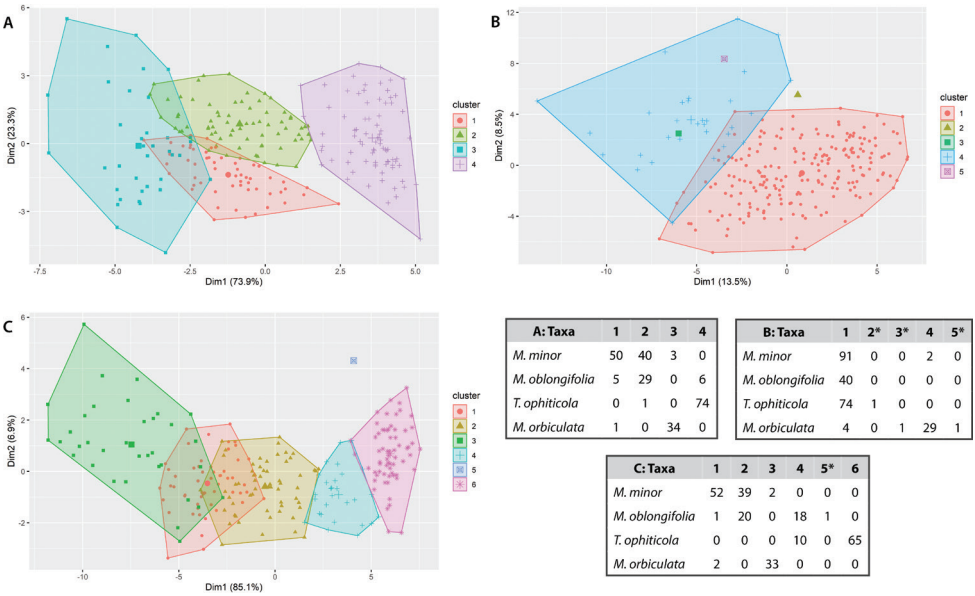
Clustering analysis based on morphological variability

The clustering analysis based on morphological variability showed differences in the number of groups inferred by the best models, according to the different datasets (Fig. 7; Suppl. material 11). The highest BIC scores were retrieved for G = 4 for linear and angular dataset, G = 2 for the Elliptic Fourier Descriptors dataset (with other 3 ghost clusters), and G = 6 for the Landmark dataset (with other ghost clusters) (Fig. 7; Suppl. material 11). It was noticeable that for each data set, the probabilities of assignment of each individual were higher than 0.9 in all cases based on the Elliptic Fourier Descriptors. In the case of the linear and angular variables and matrix of landmarks, only 5 and 22 individuals showed probabilities of an assignment less than 0.9, respec-

tively (data not shown). The linear and angular variables allowed a clear discrimination between *M. orbiculata*, *T. ophiticola* and *M. minor*, the latter taxa being split into two clusters. One of these two clusters was shared only with the majority of *M. oblongifolia* individuals.

The clustering analysis based on Elliptic Fourier Descriptors provided only two clusters (Fig. 7). The assignment of individuals was therefore different from that obtained with linear and angular variables. Indeed, all individuals of *M. oblongifolia*, and most individuals of *T. ophiticola* and *M. minor*, were assigned to the same cluster (cluster 1), while most individuals of *M. orbiculata* were assigned to a different cluster (cluster 4). Therefore, Elliptic Fourier descriptors were efficient to discriminate between *M. orbiculata* on the one hand and the 3 other taxa on the other hand. Finally, the analysis carried out on the matrix of landmarks showed a similar pattern to that obtained with the linear and angular variables for *M. minor*, *T. ophiticola* and *M. orbiculata*. The main difference between these two analyses (matrix of landmarks and linear and angular dataset) was that in the first one, *M. oblongifolia* was split into two clusters, one of which was shared with *M. minor* and the other one with *T. ophiticola* (Fig. 7).

Thus, despite a continuous variation of leaf morphology across taxa, a clear delimitation of *M. orbiculata* is shown by our analyses whichever data set was used. In cases where individuals of the same taxon were assigned to different clusters, no obvious correspondence between the assigned clusters and the geographic origin of those individuals was found. Indeed, many individuals of the same taxon/locality were assigned to different clusters (data not shown).



A: Taxa	1	2	3	4
M. minor	50	40	3	0
M. oblongifolia	5	29	0	6
T. ophiticola	0	1	0	74
M. orbiculata	1	0	34	0

B: Taxa	1	2*	3*	4	5*
M. minor	91	0	0	2	0
M. oblongifolia	40	0	0	0	0
T. ophiticola	74	1	0	0	0
M. orbiculata	4	0	1	29	1

C: Taxa	1	2	3	4	5*	6
M. minor	52	39	2	0	0	0
M. oblongifolia	1	20	0	18	1	0
T. ophiticola	0	0	0	10	0	65
M. orbiculata	2	0	33	0	0	0

Figure 7. Graphic representation and classification matrix obtained after the cluster analysis using the morphological data of *Magnolia* subsect. *Talauma* in Cuba. **A** Linear and angular variables **B** elliptic Fourier Descriptors **C** matrix of landmarks. * Ghost Cluster.

Genetic structure and taxon differentiation

The species with the greatest genetic diversity were *Magnolia minor* and *Talauma ophiticola*, while the lowest diversity was found in *Magnolia orbiculata*. The expected heterozygosity was similar in the four taxa (Table 2). The genetic differentiation among taxa was relatively high (global F_{ST} = 0.10, D_{JOST} = 0.23). *Magnolia orbiculata* contributed mainly to this result since it was highly differentiated from the three other taxa, while *M. minor* and *M. oblongifolia* were the less differentiated taxa (Table 3). The Bayesian clustering analysis clearly provided three genetic clusters as the unambiguously best solution in the two analyses (with and without *M. oblongifolia*) (Fig. 8A, B, Suppl. material 5: fig. S5A, B). In the following, an individual was considered to be correctly assigned to a unique genetic cluster if the ancestry coefficient of this individual to this cluster was higher than or equal to 0.9. One of those clusters corresponded obviously to *M. orbiculata* (red cluster in Fig. 8C). The 88.8% (32/36) of individuals from *M. orbiculata* were assigned to this cluster, while the 4/36 *M. orbiculata* individuals were considered unclear. The second cluster (green cluster in Fig. 8C) consisted mainly of the majority of *M. minor* (171) *and M. oblongifolia* (16) individuals, but also included some individuals (14) of *T. ophiticola*. (Fig. 8C). The third cluster (blue cluster on Fig. 8) was predominantly composed of *T. ophiticola* with only one individual of *M. minor*. We will therefore refer hereafter to the "orbiculata", "minor-oblongifolia" and "ophiticola" genetic clusters, keeping in mind that ancestry coefficients within each taxon of these genetic clusters still varied. Indeed, despite a clear delimitation between three genetic clusters, a significant proportion of individuals (130/461) displayed genetic admixture (on the basis of a 0.9 admixture coefficient value as a threshold). Based on these "admixed" individuals, the level of genetic admixture varied according

Table 2. Average values and standard deviation of the measures of genetic diversity by taxa of *Magnolia* subsect. *Talauma* in Cuba. N: sample size, N_P: number of private alleles, N_A: number of mean alleles, A_R: allele richness, N_E: number of effective alleles, He: expected heterozygosity.

Taxa	N	Np	Na	Ar	Ne	He
M. orbiculata	36	0.524±1.030	6.81±1.18	6.652±5.237	3.618±0.543	0.564±0.064
M. minor	218	2.286±2.217	13.619±2.043	9.091±6.472	5.732±1.169	0.588±0.072
M. oblongifolia	35	0.333±0.483	9.810±1.360	9.674±6.134	5.420±0.929	0.630±0.065
T. ophiticola	172	1.619±2.037	12.524±1.896	9.163±5.986	5.658±1.023	0.650±0.057

Table 3. Pairwise genetic differentiation measures: fixation indices (F_{ST}) (below diagonal) and allelic differentiation index (D_{JOST}) (above diagonal) calculated for the taxa of *Magnolia* subsect. *Talauma* in Cuba. In all case significant differences were found ($p < 0.001$).

Taxa	*M. orbiculata*	*M. minor*	*M. oblongifolia*	*T. ophiticola*
M. orbiculata	–	0.3127	0.2937	0.3921
M. minor	0.1721	–	0.0056	0.0999
M. oblongifolia	0.1613	0.0092	–	0.0705
T. ophiticola	0.1982	0.0859	0.045	–

to taxa. The mean value of probability to belong to their a priori cluster (defined from their taxonomic status) was 0.624 (±0.104) for *M. orbiculata*, 0.678 (±0.172) for *M. minor* (including *M. oblongifolia*), and only 0.477 (±0.262) for *T. ophiticola*.

Magnolia orbiculata was strongly homogeneous pertaining to ancestry coefficient values with only four individuals displaying genome admixture with the "minor-oblongifolia" cluster (Fig. 8C). This is strongly in agreement with what was observed for leaf characteristics. *Magnolia minor* and *M. oblongifolia* displayed a high level of genome admixture with the "ophiticola" cluster. 35 individuals (16.1%) of the individuals of *M. minor* showed genome admixture with the "ophiticola" cluster. In *M. oblongifolia*, 51% of the individuals exhibited an ancestry coefficient over 0.9 to the "minor-oblongifolia" cluster, the rest showed high admixture levels. Moreover, it is noticeable that one individual of *M. oblongifolia* displayed a very high ancestry to *M. orbiculata*. The localities of Cupeyal del Norte (CN), Monte Fresco (MF), Piedra la Vela (PV), and Cayo Guam (CG) show the highest levels of misclassification of *M. minor* and *M. oblongifolia* into the "ophiticola" cluster.

For *T. ophiticola*, 56.4% (97/172) of individuals could be assigned to the "ophiticola" genetic cluster while 8.14% (14/172) could be assigned to the "minor-oblongifolia" genetic cluster (referred to as "misclassified" individuals hereafter). Similar to *M. minor* and *M. oblongifolia*, many individuals of *T. ophiticola* (61/172) also displayed signals of genetic admixture, mainly with the "minor-oblongifolia" cluster, but also, for a few of them, with the "orbiculata" cluster. The localities of Subida a la Melba (SM), Mina Iberia (MI), and Sur de las Delicias del Duaba (SDD) showed the lowest levels of misclassification. Four individuals from Cupeyal del Norte (CN) were clustered with the group of *M. orbiculata.* Most individuals from La Melba (MEL), Pico Cristal

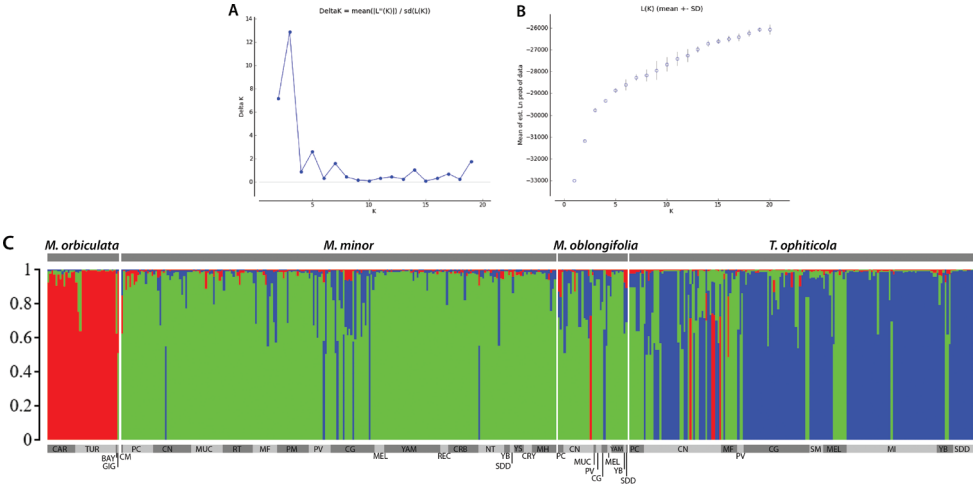


Figure 8. Structure results of *Magnolia* subsect. *Talauma* in Cuba for the complete dataset **A** Delta K plot **B** the mean Ln(K) plot **C** representative bar plot (out of 100 en replicates) for K = 3.

(PC), and Monte Fresco (MF) showed an ancestry coefficient similar to the "minor-oblongifolia cluster". The clustering analysis without individuals of *M. oblongifolia* also provided K = 3 as the best solution (Suppl. material 5: fig. S5A, B). Moreover, it was striking that this analysis provided an ancestry pattern very similar (Suppl. material 5: fig. S5C) to the analysis including this taxon (Fig. 8C). This demonstrated the very good stability of inferences on individuals' ancestry coefficients which could be explained by the strong genetic delimitation between the three identified genetic clusters.

The PCA analysis on the whole SSR data set showed that the 200 first principal components explained 99.3% of the variation, which were therefore kept for the discriminant analyses. Based on the number of taxa that have been defined across the history of Cuban *Talauma* taxonomy, but also on the STRUCTURE results, two solutions for the number of genetic clusters were considered in the following discriminant analysis (DAPC) K = 3 and K = 4. When K = 3, individual assignment displayed a pattern very similar to that found with the Bayesian clustering approach; with one cluster predominantly composed by *M. minor* and *M. oblongifolia*, the other cluster with *T. ophiticola*, and the third one with the individuals of *M. orbiculata*. In the three clusters, some level of misclassification was found. Many individuals "misclassified" in the DAPC analysis were the same that were "misclassified" based on the STRUCTURE analysis. The DAPC analysis confirmed the correspondence of *M. orbiculata* to a unique genetic cluster as expected because of its high genetic differentiation from the three other taxa (Suppl. material 6: fig. S6A). For K = 3 only one individual of *T. ophiticola* showed an assignment probability value less than 0.9.

K = 4 (Suppl. material 6: fig. S6B), seems to be a less meaningful solution. In this case, three clusters were predominantly composed of *M. minor*, *T. ophiticola* and *M. orbiculata* respectively, confirming the main pattern found with K = 3, with the difference that a higher proportion of *M. minor* and *T. ophiticola*, but also a majority of *M. oblongifolia* were not assigned to unique clusters. When K = 4 seven and three individuals of *M. minor* and *M. oblongifolia*, respectively, showed probabilities values under 0.9. As for structure, the analysis without considering *M. oblongifolia* with K = 3 displayed very similar results to the analysis including this taxon (Suppl. material 6: fig. S6C); in this case, only one individual of *T. ophiticola* showed probabilities values under 0.9.

Integrating morphological and genetic data

Overall, the morphological and genetic classifications were highly congruent (χ^2 = 173.69, $p < 0.0001$). The concordance between the two classifications (genetic and morphology) was especially high for *Magnolia orbiculata* and *M. minor*, and to a lesser extent for *M. oblongifolia* and *Talauma ophiticola* (Fig. 9). In this last taxon, the classification of several individuals based on genetic markers on one side and leaf traits on the other side were not congruent. Only a few genetic and morphological inconsistencies were also observed in *M. minor* and *M. oblongifolia*.

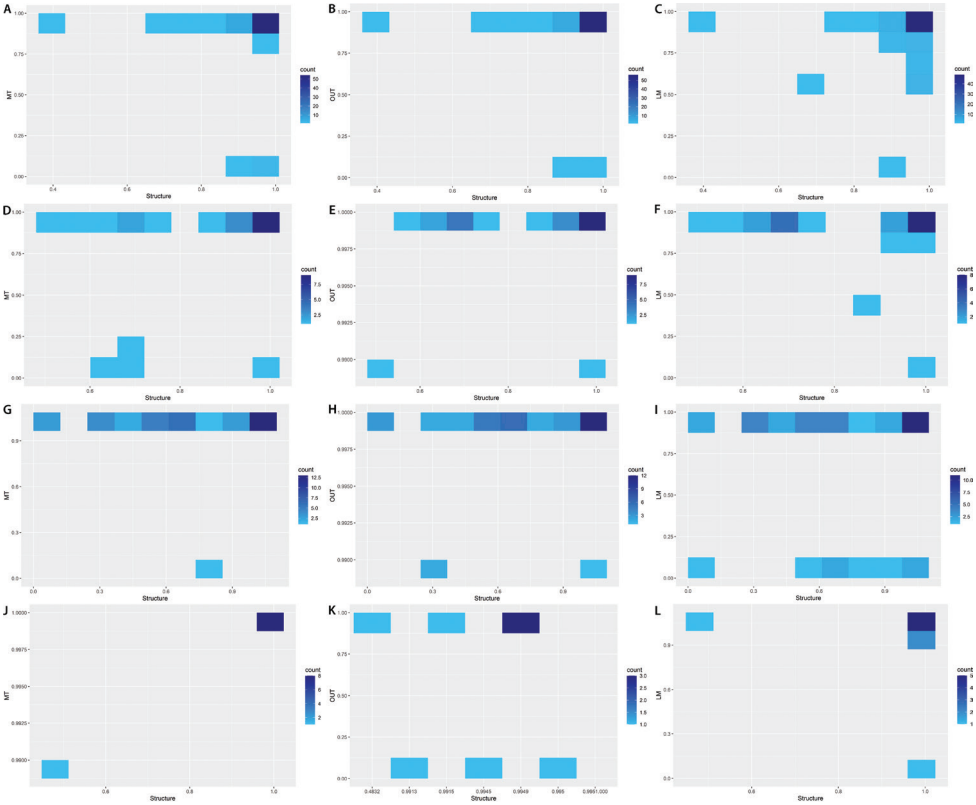


Figure 9. Heatmap with the congruence between morphological (MT: Multivariate, OUT: Elliptic Fourier Descriptors, LM: Matrix of Landmark) and genetic (Structure) cluster probabilities, inside each taxon of *Magnolia* subsect. *Talauma* in Cuba (**A–C**) *Magnolia minor* (**D–F**) *M. oblongifolia* (**G–I**) *Talauma ophiticola* (**J–L**) *M. orbiculata*. The blue color represents the number of individuals (less individuals: light blue; more individuals: dark blue).

Discussion

Morphological variability

The observed leaf morphological variability for Cuban magnolias was higher than that described by previous studies. According to the two taxa CS, the values of leaf length and width were higher than those reported by León and Alain (1950, 1951) for *Magnolia minor* and *M. orbiculata*. Likewise, in the four taxa CS, these values were higher than what was previously reported by Bisse (1974, 1988), except for *M. oblongifolia*. This difference with previous studies is due to the larger sample size used in the present work and its wider geographic representativeness. In the three taxa CS, Palmarola et al. (2016) reported similar values of length and width for *M. minor* and lower values for *M. oblongifolia* and *M. orbiculata*. The average values of Bisse

Index (B_{CI}) were similar to those reported by Bisse (1974, 1988) for *M. orbiculata* and *M. oblongifolia*. For *M. minor* and *T. ophiticola*, the average values of B_{CI} are slightly lower and slightly higher, respectively, than those reported by Bisse (1974, 1988). The high level of morphological differentiation between taxa observed in this study reinforces the value of leaf characteristics in taxonomic studies of Cuban magnolias (León and Alain 1950, 1951; Alain 1969; Bisse 1974, 1988; Imkhanitzkaja 1991, 1993; Hernández-Rodríguez 2014; Palmarola et al. 2016). Leaf morphological data are key traits for species delimitation (Jensen et al. 2002; Jensen 2003). This study confirmed they are highly relevant in groups like *Magnolia*, where very little variation is observed in flower and fruit characters (Treseder 1978).

An integrative classification of *Magnolia* subsect. *Talauma* in Cuba

In our study, *Magnolia orbiculata* was clearly distinguished from the other taxa of *Magnolia* subsect. *Talauma* in Cuba based both on morphology and genetic markers. The previously observed large variation of leaf morphology across subsection *Talauma* in Cuba, although based on the observation of only a few specimens, has been the basis for several authors to consider a unique species in this subsection, therefore including *M. orbiculata* within *M. minor* (Howard 1948; Alain 1969; Borhidi and Muñiz 1971; Lozano-Contreras 1994). In contrast, the present study, as well as lines of evidence already brought by molecular phylogeny of the subsection *Talauma* (Veltjen et al. 2022) and by studies on the ecological niches of Cuban *Talauma* (Testé et al. in press), strongly supported that *Magnolia orbiculata* should be considered as a well-delineated species.

However, in our study, a few cases of confusion with *M. minor* (sensu Bisse 1988 and Palmarola et al. 2016) on the basis of leaf morphology traits were observed. This confusion may be explained by the similar rounded shape and relation width-length present in both taxa. Different specialists have erroneously identified some herbarium specimens of *M. orbiculata* as *M. minor* in the past (personal observation in herbarium records). Moreover, our data showed that very few *M. orbiculata* individuals displayed genetic admixture with *M. minor.* Similarly, a few *M. oblongifolia* and *T. ophiticola* individuals displayed genome admixture with *M. orbiculata.* The levels of genetic differentiation among species are influenced by the time of separation and the amount of gene exchange (Hey and Pinho 2012). Genetic variation shared between closely related species may be due to the retention of ancestral polymorphisms because of incomplete lineage sorting (ILS) and/or introgression following secondary contact (Zhou et al. 2017).

Distinguishing between those two causes from observed patterns is challenging, although coalescence modeling can help (e.g. Zhou et al. 2017; Meleshko et al. 2021). However, in the case of *M. orbiculata* relative to other taxa, regular gene flow seems to be unlikely. The very clear morphological and genetic differentiation of *M. orbiculata* with other taxa in Cuba strongly suggested that the lowland between the Sierra Maestra (habitat of *Magnolia orbiculata*) and Nipe-Sagua-Baracoa (habitat of the other species) may have acted and still acts as a barrier to gene flow by strongly limiting pollination and seed dispersal. Hernández-Rodríguez (2022) reported high levels

of genetic differentiation between *Magnolia cubensis* Urb. subsp. *cubensis* (from the Sierra Maestra) and *Magnolia cristalensis* Bisse (from Nipe-Sagua-Baracoa), both from subsection *Cubenses*. Vázquez-García et al. (2016) stated that allopatric speciation seems to be a major driver of *Magnolia* diversification in the Neotropics. Therefore, it seems more likely that the admixture signal between *M. orbiculata* and the other taxa could rather be explained by shared ancestral polymorphism with other Cuban talaumas due to the likely recent diversification of the subsection in Cuba, that is less than 5 mya according to Veltjen et al. (2022), and the recent separation of *M. orbiculata* from the other taxa. However, the possibility of rare events of inter-taxa hybridization involving *M. orbiculata* as one parent cannot be totally ruled out, especially because individuals displaying admixed genome involving *M. orbiculata* have intermediate ancestry coefficients, which is compatible with a hypothetical first- or early-generation hybrid status. Testé et al. (in press) have also shown that the ecological niche of *M. orbiculata* is differentiated from that of the other taxa considered in this study. This may suggest that selection against first- or early-generation hybrids due to local adaptation could also contribute to preventing genetic exchanges between that taxon and the other taxa of *Magnolia* subsect. *Talauma* in Cuba.

Undoubtedly, our data confirmed that the main taxonomic issues concern the north-eastern Cuban populations of *Magnolia* subsect. *Talauma*. León and Alain (1950) have stated that individuals of *M. minor* with more oblong leaves, considered by them as *Talauma minor* var. *oblongifolia*, may belong to a different species. However, the authors did not assign the species rank to this group because of the absence of reproductive structures in the available specimens. On the other hand, Bisse (1974, 1988) proposed to divide *Magnolia minor* (sensu León and Alain 1950, 1951) into three separate species (*M. minor*, *M. oblongifolia* and *T. ophiticola*). Our morphological and genetic data did not support those two proposals. Indeed, concerning *M. oblongifolia* (sensu Bisse 1988), the foliar phenotype observed in this taxon appears to be intermediate between *M. minor* and *T. ophiticola*. A recent diversification process or natural hybridization might explain the intermediate characteristics of *M. oblongifolia*, as has been observed for *Quercus* species (Burgarella et al. 2009; An et al. 2017) and the genus *Rhizophora* (Francisco et al. 2018). Rather, considering *M. minor* and *M. oblongifolia* as separate taxa is supported neither by morphological (see Figs 4–7) data nor by genetic data (Fig. 8) of the present study. On the other hand, the existence of a single species, including those three taxa, (*Magnolia minor* sensu León and Alain 1951) was supported neither by our morphological results, nor by genetic markers, which both showed a clear differentiation between *M. minor* and *T. ophiticola*. Yet, our results did not support either the combination of *Talauma ophiticola* and *Magnolia oblongifolia* (sensu Bisse 1974, 1988) in a unique taxon, as recently proposed by Palmarola et al. (2016) on the basis of the specimen HFC 5358 from the coast of Moa, which shows both oblong and elliptical leaves. Nevertheless, the delimitation of *T. ophiticola* is still challenging. In the present study, a significant proportion of individuals that were assigned to this taxon based on leaf morphology was unambiguously assigned to the "minor-oblongifolia" genetic cluster, while only one individual of *M. minor* was assigned to

the "*ophiticola*" genetic cluster. Also, for each taxon, a significant proportion displayed high genetic admixture between the two genetic clusters identified (and as discussed above rare cases of admixture with *M. orbiculata*). This could be explained by a recent diversification of the three taxa that led to numerous genetic loci with incomplete lineage sorting and to overlaps in the distribution of morphological traits. In trees, factors such as long generation time, and large effective population sizes, increase the opportunity of sharing ancestral polymorphisms through incomplete lineage sorting which makes species identification based on neutral markers even more problematic (Zhou et al. 2017).

The taxa from the north-eastern part of Cuba live in the same habitats and in similar ecological conditions (Testé et al. in press), a situation that is not favorable for the emergence of reproductive barriers. Moreover, those three taxa are also found in sympatry in several locations. The phylogenetic closeness between those three taxa has recently been reported by Veltjen et al. (2022). Therefore, the high admixture level observed in these taxa with SSR markers, as well as the few cases of reciprocal "miss-assignment", suggest gene flow between the taxa of northeastern Cuba has occurred recently and may still be occurring, producing recombinant and therefore intermediate genotypes and phenotypes. This hypothesis is reinforced by the observation that reciprocal genetic admixture between the two genetic clusters, corresponding mainly to *M. minor* and *T. ophiticola*, is more frequent in the localities where both taxa occur. According to Callaway (1994), hybridization is a common process in magnolias and is more common when the distribution ranges of two or more highly related taxa overlap (Soltis and Soltis 2009).

Conclusions

The Cuban taxa of *Magnolia* subsect. *Talauma* showed a high intra-specific leaf morphological variability, which reinforces the value of leaf characteristics in taxonomic studies of Cuban magnolias. As it has been shown in other groups of plants, the integrative approach was efficient to build an accurate classification in *Magnolia* subsect. *Talauma*. Indeed, according to this study, *Magnolia orbiculata* appears to be an evolutionary lineage separated from other Cuban magnolias of the subsection, with very clear genetic, morphological delimitations, which is consistent with its ecological delimitation already shown (Testé et al. in press). This taxon can thus be considered a true species. Concerning the group of northeastern Cuba taxa, the data supported the existence of two clear groups: corresponding mainly to *M. minor-M. oblongifolia* on the one hand and *T. ophiticola* (sensu Bisse 1988) on the other hand. However, the integrative approach also showed that these two groups cannot be considered as fully delimitated lineages since hybridization between them seems to have occurred recently, or is still ongoing. Because of the likely absence of, at least strong, reproductive barriers between these taxa, we propose therefore to consider them as a species complex.

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Supplementary material 1

Graphic representation of the leaf´s morphological variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba following the two taxa CS

Authors: Ernesto Testé

Data type: morphological

Explanation note: The P-values were less than 0.001 in all cases.

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl1

Supplementary material 2

Graphic representation of the leaf´s morphological variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba following the three taxa CS

Authors: Ernesto Testé

Data type: morphological

Explanation note: The P-values were less than 0.001 in all cases; except for the area for the comparison between *M. minor-M. oblongifolia* (p = 0.115)

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl2

Supplementary material 3

Graphic representation of the leaf´s morphological variables measured in the individuals of *Magnolia* subsect. *Talauma* in Cuba following the four taxa CS

Authors: Ernesto Testé

Data type: morphological

Explanation note: The P-values were less than 0.001 in all cases; except for the perimeter for the comparison between *Magnolia minor-Talauma ophiticola* (p = 0.211) and for the comparison between *M. oblongifolia-T. ophiticola* (p = 0.132).

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl3

Supplementary material 4

Graphic representation of the Sum of EDMA and Centroid Size calculated in the individuals of *Magnolia* subsect. *Talauma* in Cuba following the different CS

Authors: Ernesto Testé

Data type: morphological

Explanation note: The pair *M. minor-M. oblongifolia* (p = 0.316) and *M. orbiculata-T. ophiticola* (p = 0.406), of the four taxa CS, were the only comparisons with MonteCarlo probabilities over 0.001.

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl4

Supplementary material 5

Structure results of *Magnolia* subsect. *Talauma* in Cuba without *M. oblongifolia*

Authors: Ernesto Testé

Data type: Genetic

Explanation note: Delta K plot (**A**); The mean Ln(K) plot (**B**); Representative bar plot (out of 100 en replicates) for K = 3 (**C**).

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl5

Supplementary material 6

Discriminant Analysis of Principal Components (DAPC) of *Magnolia* subsect. *Talauma* in Cuba

Authors: Ernesto Testé

Data type: Genetic

Explanation note: The axes represent the first two linear discriminants. The upper left graph (principal component analysis (PCA) eigenvalues) inset displays the variance explained by the principal component axes used for DAPC and the bottom-right inset (DA eigenvalues) displays in relative magnitude the variance explained by the two discriminant axes plotted. DAPC graph of the all the taxa (**A–B**), and without *M. oblongifolia* (**C**), 200 principal components (PCs) retained. The individuals with probabilities less than 0.9 were not considered in the contingency tables.

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl6

Supplementary material 7

Historical classification of the Cuban taxa of *Magnolia* subsect. *Talauma*

Authors: Ernesto Testé

Data type: Taxonomy

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl7

Supplementary material 8

Multiplex designed with the 21 microsatellites marker used for the genetic characterization of *Magnolia* subsect. *Talauma* in Cuba

Authors: Ernesto Testé

Data type: Genetic

Explanation note: Conc.: Concentration; Tm: Primer Melting Temperature (temperature at which one-half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability); Unp: unpublished.

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Supplementary material 9

The Eigenvalue and percent of the total variance of the three first principal components (PC); and the relative weight of each variable to the Principal Component Analysis for the Cuban taxa of *Magnolia* subsect. *Talauma*

Authors: Ernesto Testé

Data type: Morphology

Explanation note: * The variable with the highest weight per component.

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Supplementary material 10

F-values (F) of the NPMANOVA (one-way) based on Euclidian distance, 10 000 random permutations and Bonferroni-corrected p values (p); calculated on the individuals of *Magnolia* subsect. *Talauma* in Cuba

Authors: Ernesto Testé

Data type: Morphology

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Supplementary material 11

Number of most probable (highest BIC score) groups resulting for the morphological data of the taxa of *Magnolia* subsect. *Talauma* in Cuba

Authors: Ernesto Testé

Data type: Morphology

Explanation note: Abbreviations: VEV (ellipsoidal, equal shape), EEI (diagonal, equal volume and shape), VVV (ellipsoidal, varying volume, shape, and orientation), EEE (ellipsoidal, equal volume, shape and orientation), BIC (Bayesian information criterion), ICL (integrated complete-data likelihood criterion)

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Link: https://doi.org/10.3897/phytokeys.213.82627.suppl11

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RESEARCH ARTICLE

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Wikstroemia fragrans (Thymelaeaceae, Daphneae), a new species from Mount Danxia, China based on morphological and molecular evidence

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Abstract

A new species, *Wikstroemia fragrans* (Thymelaeaceae, Daphneae), from Danxiashan National Park, Shaoguan, Guangdong of China is described and illustrated. It is similar to the sympatric *W. trichotoma*, but can be differentiated easily from the latter by its shorter racemose inflorescences, yellowish green calyx tube, and smaller leaves. It also resembles the allopatric *W. fargesii*, but differs from it by its strigose-pubescent ovary and disk scale that is 2- or 3-dentate apically. Phylogenetic analysis using the nuclear DNA internal transcribed spacer (ITS) region revealed that *W. fragrans* falls within the *Wikstroemia* clade; based on current sampling, *W. fragrans* is closely-related to *W. capitata*. It is also the first species of *Wikstroemia* known to be endemic to the Danxia landform and is classified provisionally as Critically Endangered according to the IUCN Red List Categories and Criteria.

Keywords

Danxia landform, flora, internal transcribed spacer (ITS), IUCN Redlist, phylogenetics, taxonomy, *Wikstroemia*

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Introduction

Thymelaeaceae comprise 50 genera and about 900 species widely distributed in both temperate and tropical regions (Herber 2002, 2003). It is circumscribed to include three subfamilies: Thymelaeaoideae (ca. 42 genera), Octolepidoideae (8 genera), and a yet to be validly published "Tepuianthoideae" (1 genus). *Wikstroemia* Engl. is a member of the Daphne group (Daphneae) of the Thymelaeaoideae, comprising approximately 70 species. *Wikstroemia* is widely distributed in Asian and Oceanic regions, with some populations scattered around the Hawaiian Islands. Among these species, 54 were reportedly naturally distributed in China (Rogers 2009-onwards).

Since 2004, we have conducted a series of biological surveys on Mount Danxia to elucidate biodiversity patterns in the Danxia landform. Mount Danxia in Guangdong, China, from which the name Danxia landform is derived, is characterized by steep slopes derived from sandstones and conglomerates (Peng et al. 2018). Owing to its unique geological and geomorphic structure, the ecological succession of biota is strongly differentiated and a variety of ecosystems appear in a small scale. Mount Danxia harbors a handful of endemic plant species, of which more than ten have been discovered in the last decade, including the recently published *Selaginella orientali-chinensis* Ching & C.F. Zhang ex H.W. Wang & W.B. Liao (Selaginellaceae) (Wang et al. 2022) and *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang (Fabaceae) (Zhao et al. 2021). The discovery of these species serves to reveal the biodiversity richness of Mount Danxia.

During one of our floristic inventories in Danxiashan National Park in 2022, a plant species that most closely resembled *Wikstroemia* was found. It was previously overlooked and misidentified as *W. nutans* Champion ex Benth, which is a common species widely distributed in Guangdong. The 5-lobed calyx obviously differed from the 4-lobed calyx of *W. nutans*. After careful morphological comparison using herbarium specimens, digital images, and relevant literature of other similar species, we confirmed that our specimens represented an undescribed species, which we here describe as *W. fragrans*. Taxonomic information, including the distribution, habitat, phenology, etymology, and the International Union for Conservation of Natures (IUCN) preliminary conservation status also are provided. The epithet, fragrans, refers to the attractive scent of the flowers, which is like a mixed aroma of orchid and jasmine. A molecular phylogenetic analysis based on the nuclear DNA internal transcribed spacer (ITS) was conducted to evaluate the phylogenetic position and relationship of *W. fragrans* within *Wikstroemia*.

Materials and methods

Morphological study

The morphological characters of *Wikstroemia fragrans* were compared with similar species, using living plants, relevant literature, and herbarium specimens, including the

Chinese Virtual Herbarium (https://www.cvh.ac.cn/) and the China Field Herbarium (https://www.cfh.ac.cn/). Morphological examination was conducted in the Herbarium of Sun Yat-Sen University (SYS). Herbarium acronyms are based on those reported by Thiers (2022).

Taxon sampling and molecular analyses

Three individuals of *Wikstroemia fragrans* were collected in Danxiashan National Park, Guangdong, China, during the March to April flowering season in 2022. Voucher specimens were deposited in SYS. The ITS region was used for phylogenetic reconstruction of *Wikstroemia*. Despite insufficiency in delimiting plants at the species level, the ITS regions of most *Wikstroemia* species are publicly available and partly provide an insight into phylogenetic relationships between closely related species (He et al. 2021; Lee et al. 2022). We downloaded all species of Daphneae (Thymelaeaceae) with ITS gene sequences available in NCBI GenBank for analysis. In total, 24 taxa were selected, including 17 species of *Wikstroemia*. Two closely-related species, *Aquilaria sinensis* (Lour.) Spreng. (Thymelaeaceae, Aquilarieae) and *Edgeworthia chrysantha* Lindl. (Thymelaeaceae, Daphneae) were included as outgroups. The GenBank accession numbers for each species used in this study are listed in Suppl. material 1.

Total genomic DNA was extracted from silica-gel-dried leaves using the modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). The quality and quantity of the DNA extract were determined using Nanodrop spectrophotometer (Thermo Fisher, USA). Polymerase chain reaction was carried out based on the program setting as proposed by Lee et al. (2022) using the universal primers, ITS1 and ITS4 (White et al. 1990). The amplicon was verified under UV and the PCR product was Sanger sequenced both forward and reverse directions. The ITS sequence was assembled and multiple sequence alignment was performed using ClustalW which is embedded in MEGA-X (Kumar et al. 2018). The sequences in the alignment were manually trimmed, in which the nucleotides that correspond to the primer regions were removed from the sequences to obtain a clean sequence read. Phylogenetic trees were reconstructed using the maximum likelihood (ML) and Bayesian inference (BI) methods via MEGA-X (Kumar et al. 2018) and MrBayes v3.2.7a (Ronquist et al. 2012), respectively. For ML, the optimum DNA substitution model calculated using the "Find best DNA/Protein Models (ML) function embedded in MEGA-X was Kimura two-parameter model (K2P) with gamma incorporated (+G) (=K2P+G). All branch nodes were calculated with 1000 bootstrap replicates. For BI, a mixed substitution type and a four by four nucleotide substitution model were selected for the likelihood model, and the Markov chain Monte Carlo simulations were run twice independently for 2000000 generations. Four chains were selected and sampling of data was conducted every 100 generations.

Results

Morphological comparison

Wikstroemia fragrans is similar to the sympatric *W. trichotoma* (Thunb.) Makino. The two species share identical features such as papery leaves, a glabrous calyx tube, 5-lobed calyx, and a lobed disk scale. However, *W. fragrans* differs from the latter by its smaller leaves (1.2–1.6×0.5–0.9 vs. 1.2–3.5(-8) × (0.5-)1–2.2(-4) cm), densely racemose to capitate inflorescence (vs. loose panicle), yellowish green calyx (vs. white), and strigose-pubescent ovary (vs. apically strigose ovary) (Table 1).

Among the *Wikstroemia* species known from China, *W. fragrans* also resembles the allopatric and stenochoric *W. fargesii* (Lecomte) Domke: both species have capitate inflorescences, yellowish green calyces, glabrous tubes, 5-lobed calyces, and a linear disk scale. However, *W. fragrans* differs from *W. fargesii* by its strigose-pubescent ovary (vs. apically strigose ovary) and disk scale 2- or 3-dentate apically (vs. entire or slightly retuse apically) (Table 1).

Molecular analysis

The final sequence alignment based on the ITS dataset was 687 bp. Both the ML and BI trees revealed identical topologies; thus, the trees were merged and only the ML tree is displayed (Fig. 1). The backbone of the phylogenetic tree was not well-supported

Table 1. Comparison of morphological features and distribution between *Wikstroemia fragrans*, *W. capitata, W. fargesii*, and *W. trichotoma*.

Characters	*W. fragrans*	*W. capitata**	*W. fargesii**	*W. trichotoma**
Leaf shape	ovate to ovate-lanceolate	elliptic or obovate-elliptic, rarely obovate-oblong	elliptic, suborbicular, or oblong-lanceolate	ovate to ovate-lanceolate
Leaf size (cm)	1.2–1.6 × 0.5–0.9	1–2 × 0.4–1.0	1–2.2 × 0.8–2.0	1.2–3.5(-8) × (0.5–)1–2.2(-4.0)
Inflorescences	densely racemose to nearly capitate, 4–8-flowered	capitate, 3–7-flowered	capitate, 7–10-flowered	a panicle of spikes, few to 10(-26)-flowered
Indumentum of tube abaxially	Glabrous	sericeous-strigose	glabrous	glabrous
Number of calyx lobes	Five	four	five	five
Color of calyx	yellowish green	yellowish green	yellowish green	white, rarely yellowish green
Indumentum of ovary	strigose-pubescent	strigose-pubescent	apically strigose	apically strigose
Shape of subgynoecial disk scale	linear or linear-oblong, apex 2- or 3-dentate	linear, apex 2- or 3-dentate	linear, apex entire or slightly retuse	linear or linear-oblong, membranous, lobed or truncate
Distribution (Province)	Guangdong	Guizhou, Hubei, Shanxi, Sichuan	Chongqing, Hunan**	Anhui, Guangdong, Guangxi, Hunan, Jiangxi, Zhejiang

*Characters of *Wikstroemia capitata, W. fargesii*, and *W. trichotoma* are from the original descriptions in *Flora of China* (2007). **We checked the herbarium specimens of *Wikstroemia fargesii* collected from Mount Tianmen, Hunan (CSFI026054, CSFI034280) and confirmed their identifications.

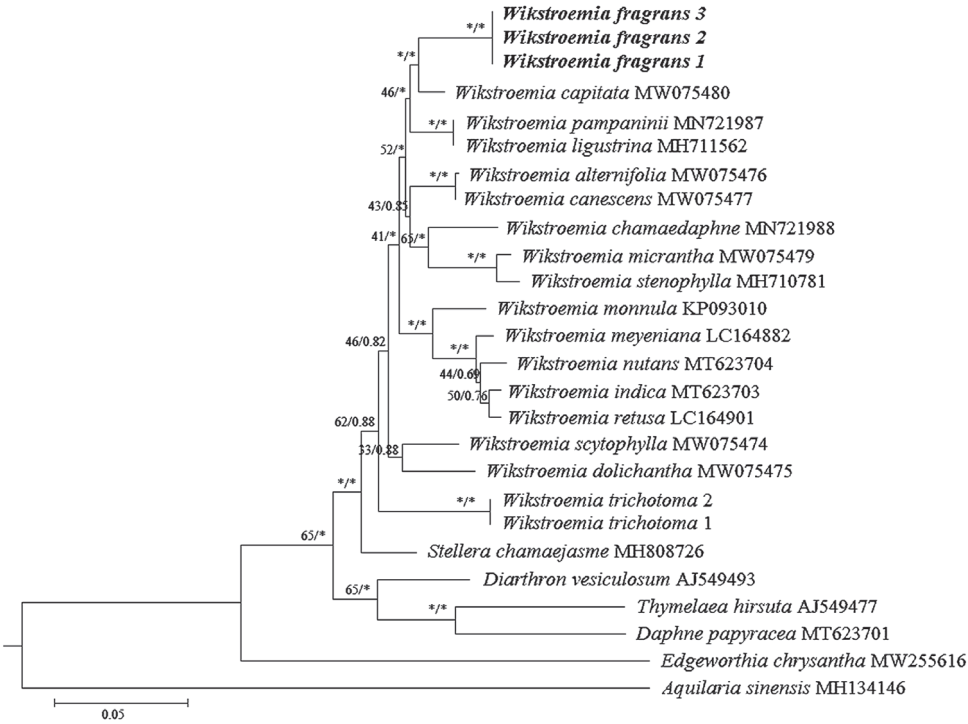


Figure 1. Phylogenetic inference of *Wikstroemia fragrans* and related species based on ITS sequences. Values of Bootstrap support (BS) and posterior probability (PP) are labeled at each branch node, in which BS ≥ 75% and PP ≥ 0.90 are indicated with an asterisk (*). *Wikstroemia fragrans*, described in this study, is shown in bold.

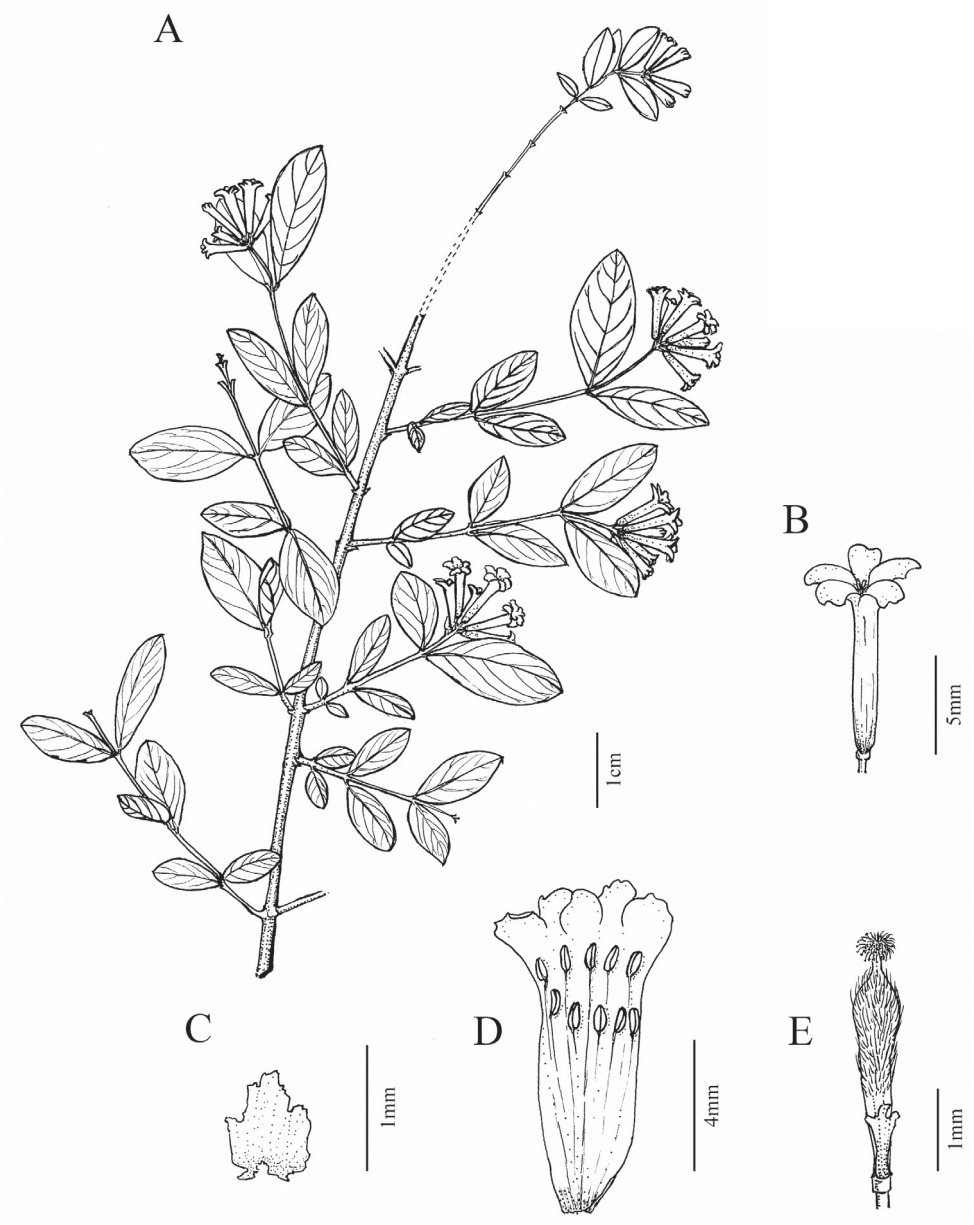
when using both the ML and BI methods, in which the bootstrap support (BS) value was less than 75% and the posterior probability was less than 0.90. Based on current sampling, species of *Wikstroemia* are monophyletic; *W. fragrans* was placed close to *W. capitata* and the divergence between the two species was strongly supported (BS = 75%, PP = 0.98). *Wikstroemia trichotoma* was placed distant from *W. fragrans* (Fig. 1).

Taxonomic treatment

Wikstroemia fragrans **W.B.Liao, Q.Fan & J.R.Chen, sp. nov.**
urn:lsid:ipni.org:names:77307946-1
Figs 2–4

Type. China. Guangdong Province, Danxia National Park, 25.0°N, 113.7°E, 74 m alt., 16 March 2022 [fl.], *Qiang Fan*, *DNPC 1597* (Holotype SYS! Barcode SYS00236854, Isotypes SYS! Barcode SYS00236855, CSFI!).

Diagnosis. *Wikstroemia fragrans* differs from *W. trichotoma*, by its smaller leaves (1.2–1.6×0.5–0.9 vs. 1.2–3.5(-8) × (0.5-)1–2.2(-4) cm), densely racemose to near-



Figures 2. *Wikstroemia fragrans* W.B.Liao, Q.Fan & J.R.Chen, sp. nov. **A** flowering branch **B** flower **C** subgynoecial disk scale opened out **D** dissected flower showing the normal condition of 10 stamens **E** strigose ovary and glabrous disk scale **A–E** *DNPC 1597* deposited in SYS. Drawn by Rong-En Wu.

ly capitate inflorescence (vs. loose panicle), yellowish green calyx (vs. white), and strigose-pubescent ovary (vs. apically strigose ovary). *Wikstroemia fragrans* differs from *W. fargesii* by its strigose-pubescent ovary (vs. apically strigose) and a disk scale 2- or 3-dentate apically (vs. entire or slightly retuse apically).

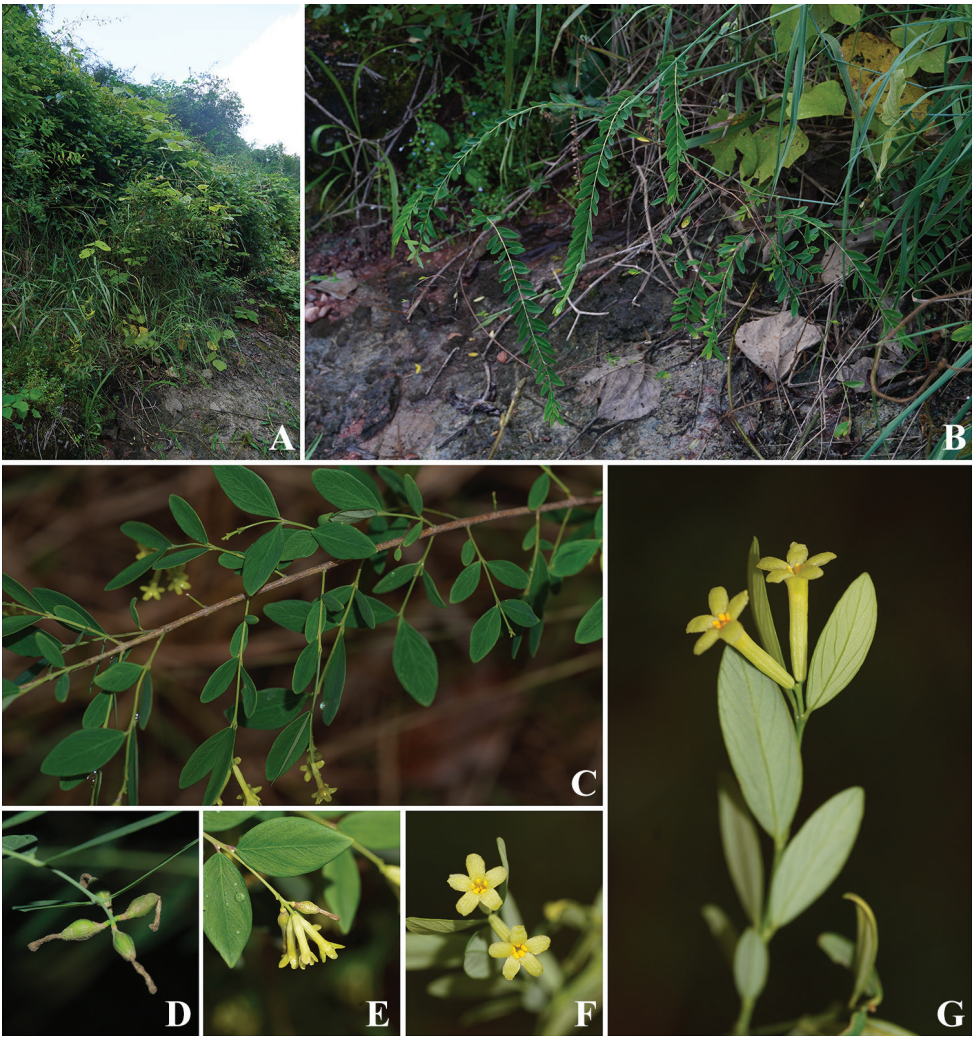


Figure 3. Habitat and morphological features of *Wikstroemia fragrans* W.B.Liao, Q.Fan & J.R.Chen, sp. nov. **A, B** habitat, hillsides thickets of Danxiashan National Park **C** flowering branches **D** immature fruits **E** densely racemose inflorescence **F, G** flowers, showing 5-lobed calyx. (**A–C, E–G** photographs by Jing-Rui Chen of unvouchered plants in the original habitat in April 2022 **D** photograph by Jian-Qiang Guo taken in April 2021).

Description. Shrub, 0.6–1.6 m tall; perennial branches reddish brown, rugose, annual branches yellowish green, glabrous. ***Leaves*** opposite or subopposite, ovate to ovate-lanceolate, 1.2 – 1.6×0.5 – 0.9 cm, thinly papery, grayish green adaxially, light yellowish green abaxially, glabrous on both surfaces, apex acuminate or obtuse, margin entire, base cuneate or subrounded, midrib flat adaxially, prominent abaxially, secondary veins 4–8 pairs per side, slightly prominent abaxially; petiole ca. 1 mm long. ***Inflorescence*** 4–8-flowered, densely racemose to capitate; peduncle 5 – 15 mm long, glabrous; pedicels absent or ca 0.4–0.7 mm long, glabrous. ***Calyx*** tube yellowish green;

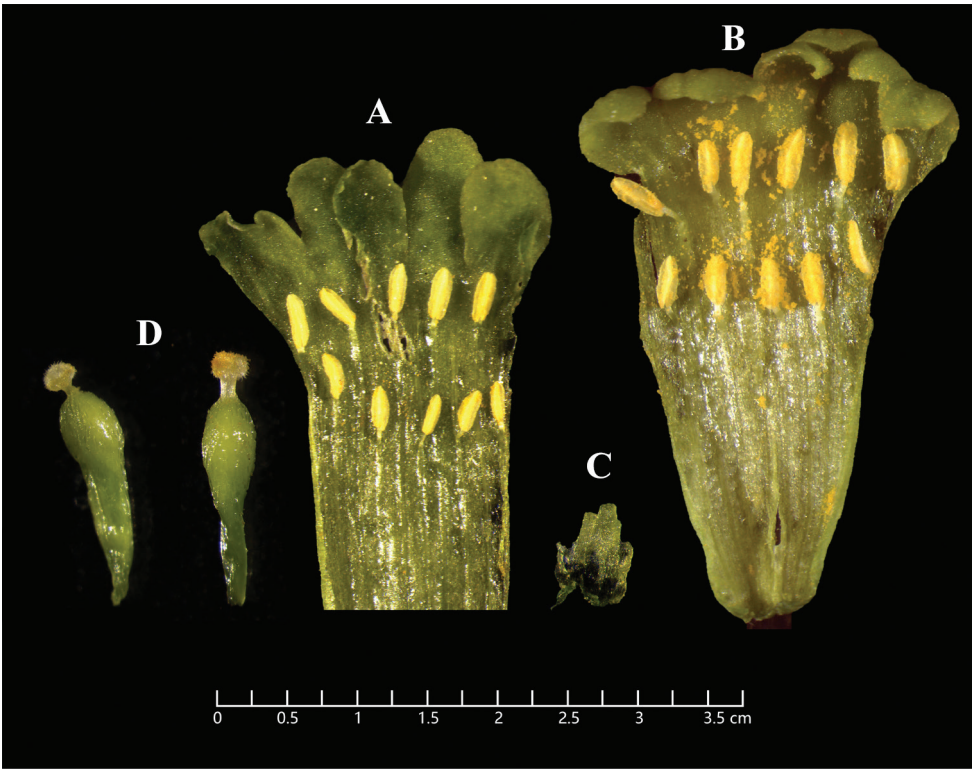


Figure 4. *Wikstroemia fragrans* W.B.Liao, Q.Fan & J.R.Chen, sp. nov. **A** hypanthium dissected showing 10 stamens **B** hypanthium dissected showing abnormal 11 stamens **C** subgynoecial scale disk, linear-oblong, apex 2–3 dentate **D** pistil with strigose-pubescent ovary, glabrous style, and light green stigma. **A–D** *DNPC 1597* deposited in SYS.

9–11 mm long, exterior glabrous, lobes 5 (sometimes abnormally 6), elliptic, 2.5–3.3×1.2–1.7 mm, margin undulate, glabrous on both surfaces. ***Stamens*** 10 (sometimes abnormally 11), lower whorls of 5 anthers inserted 2–4 mm above middle of hypanthium, upper whorl of 5 anthers at throat; free portion of filaments ca 0.3 mm long; anther linear-oblong, ca 0.8 mm long; subgynoecial disk scale 1, linear or linear-oblong, apex 2- or 3-dentate, 0.8–1.0×0.3–0.7 mm, membranous, glabrous. ***Ovary*** obovoid, 3–4 mm long, ca 0.6 mm in diam., subsessile, strigose pubescent; style ca 0.2 mm long, glabrous; stigma yellow, globose, ca 0.5 mm in diam., surface papillate. ***Drupe*** ca. 6 mm long, yellowish green, ovoid-globose, glabrous, 1-seeded, enclosed by persistent calyx; fruiting pedicel ca 8 mm long. ***Seed*** ovoid, ca. 4 mm long, black, glabrous.

Distribution and habitat. *Wikstroemia fragrans* is currently known only from the type locality, Danxiashan National Nature Reserve (Ba Zhai, Mount Shaoshi, Shuang He Zhai, Yu Nv Lan Jiang), Guangdong, China. It occurs in xerophytic hillside thickets on sandstone and conglomerate based soil at 100–300 m elevation. In this habitat, the most common shrubby and herbaceous species are *Lagerstroemia indica* Linn.

(Lythraceae), *Symplocos tanakana* Nakai (Symplocaceae), *Decaspermum gracilentum* (Hance) Merr. et Perry (Myrtaceae), *Viola hybanthoides* W. B. Liao & Q. Fan (Violaceae) and *Salvia scapiformis* Hance (Lamiaceae).

Phenology. *Wikstroemia fragrans* was observed flowering from March to April, fruiting from April to June.

Etymology. Latin *fragrans*, smell or odor, alluding to sweet-scented flowers. The Chinese name is given as 香花荛花 (xiāng huā ráo huā).

Conservation status. During our intensive floristic inventories in Danxiashan National Nature Reserve from September 2021 to May 2022, only 5 populations of *Wikstroemia fragrans* comprising 5–20 individuals each were found. Due to the limited extent of occurrence (ca. 40 km^2) and area of occupancy (ca. 5 km^2) and small population sizes (<100 individuals totally), *W. fragrans* is proposed to be classified as Critically Endangered (CR B1ac(i)+2ac(i)) according to the IUCN Categories (IUCN 2012).

Additional specimens examined (paratypes). China, Guangdong Province, Danxia National Park, 25.0°N, 113.7°E, 384 m alt., 14 August 2022, *Wan-yi Zhao & Jing-rui Chen*, *DNPC 2966* (SYS); China, Guangdong Province, Danxia National Park, 24.9°N, 113.7°E, 162 m alt., 17 August 2022, *Wan-yi Zhao & Jing-rui Chen*, *DNPC 3029* (SYS).

Discussion

The taxonomic status of *Wikstroemia* has long been debated; species exhibit continuous morphological variation, which has complicated efforts to distinguish between species and has created problems in the classification of the genus and its sister genera (Skottsberg 1972; Halda 2001; Wang et al. 2007; Zhang et al. 2007). However, it is generally accepted that *Wikstroemia* can be distinguished from related genera through its inflorescence and scale type (distinct or annular) (Domke 1934; Herber 2002; 2003; Wang et al. 2007).

Wikstroemia fragrans was previously confused with *W. nutans*, but can be distinguished easily by the length of its inflorescence axis and number of calyx lobes. After checking all species of *Wikstroemia* distributed in Guangdong, we turned our eyes to the sympatric *W. trichotoma*. These two species share identical features such as papery leaves, a glabrous calyx tube, five calyx lobes, and a lobed disk scale, but differ in leaf size, inflorescence, and calyx color. To support these morphological findings, molecular methods to distinguish closely similar species were also utilized. However, DNA studies of *W. trichotoma* are limited; there is no publicly available record of the ITS sequence of *W. trichotoma*. Thus, we sequenced two individuals of *W. trichotoma* collected from Mount Babao, which is adjacent to Mount Danxia, to be included in our phylogenetic analysis. The phylogenetic tree placed *W. trichotoma* distant from *W. fragrans*, indicating that *W. trichotoma* is at least not considered to be the most immediately genetically affiliated species to *W. fragrans*. Additionally, we noticed that among the Chinese mainland species the allopatric *W. fargesii* is also morphologically similar to *W. fragrans*. We

faced enormous difficulties in collecting samples of *W. fargesii*, which had not been collected since the 1890s until it was allegedly rediscovered on limestone in Mount Tianmen, Hunan, but with the help of CSFI, we acquired pieces of flowers and leaves from herbarium specimens of *W. fargesii* from Mount Tianmen. This enabled us to make further comparisons. Dissection of these herbarium materials confirmed the identification of *W. fargesii* and showed differences from *W. fragrans* in the ovary indumentum and shape of the disk scale (Table 1). As the two species grow on different types of substrates (*W. fargesii* on limestone and *W. fragrans* on sandstone and conglomerate from red beds), we are convinced that they represent two independent species.

It is worth mentioning the fragrant flowers of *Wikstroemia fragrans*, which are quite rare in *Wikstroemia*. In general, members of Thymelaeaceae with fragrant flowers are usually found in the sister genus *Daphne*. Based on its fragrance, *W. fragrans* has the potential to be domesticated and cultivated for horticulture purposes. Based on the morphological and molecular evidence obtained through this study, we confirmed that the newly described *W. fragrans* is a distinct species.

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Supplementary material 1

List of the GenBank accession numbers of the ITS sequences of sampled species in this study

Authors: Jing-Rui Chen, Shiou Yih Lee, Jian-Qiang Guo, Jie-Hao Jin, Qiang Fan, Wen-Bo Liao

Data type: Phylogenetic.

Explanation note: The appendix shows the GenBank accession numbers of the ITS sequences of sampled species in this study.

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RESEARCH ARTICLE

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Hansenia trifoliolata, a new species (Apiaceae) from Shaanxi, China

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Abstract

Hansenia trifoliolata Q.P.Jiang & X.J.He (Apiaceae), is described as new from Shaanxi Province, northwest China. The mericarp features of *H. trifoliolata* resemble *H. himalayensis* and *H. phaea* and molecular phylogenetic analyses (combining ITS and plastid genomes data) suggest that *H. trifoliolata* is closely related to the group formed by *H. oviformis* and *H. forbesii*. The new species *H. trifoliolata* has unique 3-foliolate leaves and differ from other *Hansenia* species in its leaves, umbel numbers and size. A comprehensive description of *H. trifoliolata* is provided, including habitat environment and detailed morphological traits.

Keywords

Apiaceae, *Hansenia*, new species, phylogenetic analyses

Introduction

The Apiaceae is a large family with high morphological diversity, the generic and tribal delimitations within it being notoriously difficult (Shan and Sheh 1992; Plunkett and Downie 1999). Fruit characteristics have long been regarded as one of the most important sources of evidence for generic and tribal delimitation within the family (Drude 1898; Liu et al. 2003, 2007, 2009; Winter et al. 2008; Magee et al. 2010, 2011).

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Notopterygium H. Boissieu (Apiaceae) was first established by Boissieu in 1903 with two species, *N. forbesii* H. Boissieu and *N. franchetii* H. Boissieu, which later appeared to be identical (Boissieu 1903). In some later studies, the genus *Notopterygium* contained six species: *N. forbesii*, *N. forrestii* H. Wolff, *N. oviforme* Shan, *N. incisum* Ting ex H. T. Chang, *N. pinnatiivolucellatum* Pu et Y. P. Wang and *N. tenuifolium* Sheh et Pu (Wolff 1930; Shan 1943; Chang 1975; Pu and Wang 1994; She and Pu 1997; Pu et al. 2000; She and Watson 2005a). The roots of *N. incisum* and *N. forbesii* are used in traditional Chinese medicine and named "Qiang Huo" (Wang et al. 1996; She and Watson 2005a; Wei et al. 2019). Although *Notopterygium* has long been treated as an endemic genus in China, Pimenov et al. (2008) merged it into the genus *Hansenia*.

Hansenia Turcz. belongs to the East Asia Clade of Apiaceae and it was first established by Turczaninow in 1844, with *H. mongolica* Turcz. as the type species (Turczaninow 1844; She and Watson 2005a; Pimenov et al. 2008; Downie et al. 2010; Pimenov 2017; Gou et al. 2020). *Hansenia* used to be treated as a monotypic genus and then some species had been transferred into this genus. Pimenov et al. (2008) transferred all the species of *Notopterygium*, except *N. tenuifolium*, to *Hansenia* through comparative morphological and molecular phylogenetic analyses and proposed five new combinations: *H. forbesii* (H. Boissieu) Pimenov & Kljuykov, *H. forrestii* (H. Wolff) Pimenov & Kljuykov, *H. oviformis* (R. H. Shan) Pimenov & Kljuykov, *H. weberbaueriana* (Fedde ex H. Wolff) Pimenov & Kljuykov and *H. pinnatiinvolucellata* (F. T. Pu & Y. P. Wang) Pimenov & Kljuykov (Pimenov et al. 2008; Pimenov 2017). Due to the lack of relevant material, *N. tenuifolium* was still retained in the genus *Notopterygium*. Subsequently, based on morphological and molecular data, Jia et al. (2019) considered that *H. pinnatiinvolucellata* was a synonym of *H. weberbaueriana*. Additionally, Tan et al. (2020), based on morphological characters and molecular data, transferred the two species of the genus *Haplosphaera* Handel-Mazzetti (Apiaceae) (She and Watson 2005b) into the genus *Hansenia* and proposed two new combinations: *H. himalayensis* (Ludlow) J.B. Tan & X.G. Ma and *H. phaea* (Handel-Mazzetti) J.B. Tan & X.G. Ma (Tan et al. 2020). Therefore, there are six species in *Hansenia* and one species in *Notopterygium* to date.

During a botanical expedition to Feng County in western Shaanxi Province in 2019, a umbelliferous species with thin stem and unusual 3-foliate leaves was collected. Species with 3-foliolate leaves are rare in Apioideae and only *Trachydium trifoliatum* H. Wolff is known in China (Shan and Sheh 1992). 3-foliolate leaves are commonly found in the genus *Sanicula* L. (Apiaceae) (She and Phillippe 2005), but the fruits were significantly different from *Sanicula*. After consulting relevant floras and literature, we identified that the fruit of the new species resembles *H. himalayensis* and *H. phaea* and further molecular phylogenetic analyses supported our conclusion. Based on careful morphological and molecular analyses, we identified it as a new species of *Hansenia*.

Materials and methods

DNA extraction and sequencing

Fresh leaves of *Hansenia trifoliolata* were collected from wild plants, desiccated and stored in silica gel. The herbarium specimens were stored in the Herbarium, College of Life Sciences, Sichuan University (SZ). Specimen voucher details were provided in Table 1 and Suppl. material 1: Fig. S2. Total genomic DNA was extracted from the stored dry leaves, using a CWBIO plant genomic DNA extraction kit (CWBIO, Beijing, China), following the manufacturer's protocols. PCR-amplification of the complete ITS region used the primers of ITS4 (5'-TCC TCCGCT TAT TGA TAT GC- 3') and ITS5 (5'-GGA AGTAAA AGT CGT AAC AAG G-3'; White et al. 1990). PCR amplification was undertaken in a 30 µl volume reaction, containing 3 µl plant total DNA, 1.5 µl of each forward primer and reverse primer, 10 µl ddH_2O and 15 µl 2×Taq MasterMix (CWBIO, Beijing, China). The PCR amplification of the nrITS region had an initial denaturation for 4 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 45 s at 53 °C and 60 s at 72 °C, then a final extension of 10 min at 72 °C. All PCR products were sent to Sangon (Shanghai, China) for sequencing after being examined using a 1.5% (w/v) agarose TAE gel. The DNA sequences of nrITS were applied for phylogenetic analyses and detailed information as outlined in Table 1.

Plastid genome sequencing, assembly and annotation

We sequenced, assembled and annotated the plastid genome of *Hansenia trifoliolata*, then compared it with other species of *Hansenia*. The processes of plastid genome sequencing, assembly and annotation were performed as follows.

The Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA) at Novogene (Beijing, China) was used to sequence the resultant DNA with Novaseq 150 sequencing strategy. The remaining clean data were assembled using NOVOPlasty 2.7.1 (Dierckxsens et al. 2017) with the default K-mer value 39 and rbcL of *H. oviformis* (GenBank accession No.: MF787597.1) being used as seed input. Preliminary genome annotation was conducted using PGA (Qu et al. 2019), with manual modifications for uncertain genes and uncertain start and stop codons, based on comparison with other related plastid genomes, using Geneious R11 soft (Kearse et al. 2012). Protein-coding sequence (CDS) was extracted from the plastid genome using the PhyloSuite programme (Zhang et al. 2020). The plastid genome of *H. trifoliolata* was submitted to GenBank and the accession number was listed in Table 1.

Table 1. Voucher details and GenBank accession number of *Hansenia trifoliolata*.

Taxa (Species number)	Voucher	Locality	Genbank number	
			Plastid genome	ITS
*Hansenia trifoliol*ata	JQP19082004	Feng County, Shannxi Province	OM281945	OM800961 OM800962

Phylogenetic analyses

We used MEGA7 (Kumar et al. 2016) to align DNA sequences with manual adjustment to improve the accuracy of sequence alignment. Phylogenetic analyses were undertaken applying Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Based on the Akaike Information Criterion (AIC) implemented in MrModelTest version 2.2 (Nylander 2004), the best-fit nucleotide substitution models for the ITS sequences (GTR+G) and protein-coding sequences (GTR+G+I) were determined, respectively. ML analyses were undertaken using RAxML v.8.2.4 (Stamatakis 2014) with the best-fit model and 1000 bootstrap replicates. BI analyses were conducted with MrBayes version 3.2 (Ronquist et al. 2012). Four simultaneous runs were performed using Markov Chain Monte Carlo (MCMC) simulations for 10 million generations, starting from a random tree and sampling one tree every 1000 generations. The first 20% of obtained trees were discarded as burn-in and the remaining were used to calculate a majority-rule consensus topology and posterior probability (PP) values.

Results

Morphological study

We collected several specimens of *H. trifoliolata* from Feng County, Shannxi Province and the type locality at an elevation of 2300–2500 m (Fig. 1). After field observation, we investigated the fruit morphological characteristics of *H. trifoliolata* (Fig. 2), including fruit shape and size, ribs, vittae and endosperm which are highly similar to *H. himalayensis* and *H. phaea*. We compared the morphological characteristics of *H.trifoliolata* with the related species (*H. himalayensis*, *H. phaea*, *H. oviformis*, *H. forbesii* and *H. forestii*), including life form, leaves, umbel rays and fruits (Table 2).

Phylogenetic analyses

The phylogenetic analysis result, based on ITS data, is shown in Fig. 3. The details of the ITS dataset that we sequenced for phylogenetic analysis are listed in Table 1. The phylogenetic trees derived from BI and ML analyses were topologically consistent. Thus, only the BI tree is shown in Fig. 3, with bootstrap support values obtained from ML analyses. The phylogenetic tree showed that *H. trifoliolata* was sister to *H. oviformis*, with strong support (Bayesian inference posterior probability, BI = 1.00; maximum parsimony bootstrap, ML = 96%). Additionally, *H. trifoliolata* and other *Hansenia* species formed a monophyletic group with the support very close to maximum (BI = 1.00; ML = 99%).

The result of the phylogenetic analysis, based on the plastid genome data, is shown in Fig. 4. The plastid genome GenBank number of *H. trifoliolata* is listed in Table 1.

The phylogenetic trees derived from BI and ML analyses were topologically consistent. Therefore, only the BI tree is shown in Fig. 4, with bootstrap support values obtained from ML analyses. The phylogenetic tree showed that *H. trifoliolata* clustered with the communities of *H. oviformis* and *H. forbesii* (BI = 1.00; ML = 68%). This is the same as the ITS tree, with *H. trifoliolata* and other *Hansenia* species forming a monophyletic group with maximum support (BI = 1.00; ML = 100%).

Table 2. Diagnostic morphological characters of *Hansenia trifoliolata* and related species.

Character	Taxon					
	H. trifoliolata	***H. himalayensis***	***H. phaea***	***H. oviformis***	***H. forbesii***	***H. forestii***
Live form	monocarpic	polycarpic	polycarpic	monocarpic	polycarpic	monocarpic
Plant height (cm)	60–90	80–120	55–90	40–60	80–180	50–100
Leaf in outline (basal)	blade broad-triangular, 3-foliolate	blade ovate-triangular, 3-pinnate	blade broad-triangular or triangular-ovate, ternate-1–2-pinnate	broadly triangular 2-pinnate	oviform 3-pinnate	broadly triangular, 2-pinnate
Median leaflets (pinnae) (basal)	cuneate-obovate or rhombic, base cuneate, with irregularly doubly serrate, apex obtuse	pinnatifid, pinnae 3–6 pairs, triangular or narrowly ovate-triangular, ultimate segments, mucronate, acute-dentate	ovate or obovate, 3-parted, base cuneate; with irregularly doubly serrate or serrate, apex obtuse	(broadly) obovate to almost round, base cuneate, margins serrulate, apex obtuse	broadly lanceolate to oviform-lanceolate, base obtuse or cuneate, margins serrate	oviform to lanceolate, base cuneate, margins irregular or sharply serrate
Lateral leaflets (pinnae) (basal)	oblique-ovate, base oblique, often shallowly or deeply uneven 2-parted or not divided; irregularly doubly serrate, apex obtuse	pinnatifid, pinnules 3–4 pairs, ultimate segments mucronate, acute-dentate	ovate to ovate-lanceolate, base oblique; with irregularly doubly serrate or serrate, apex obtuse	ovate or elliptic, base truncate; margins serrulate, apex obtuse	broadly lanceolate to oviform-lanceolate, base obtuse or cuneate; margins serrate	oviform to lanceolate, base cuneate, base oblique; margins irregular or sharply serrate
Umbels	compound umbel, 3–7-rayed, unequal	compound umbel, 2–6-rayed, subglobose, unequal	Subglobose	compound umbel, 5–9-rayed, rays very unequal	compound umbel, 11–20-rayed, rays ± equal	compound umbel, 6–9-rayed, unequal
Calyx teeth	ovate-triangular, 0.3–0.5 mm	inconspicuous, triangular, ca. 0.1 mm	ovate-triangular, 0.4 × 0.5 mm	short, triangular, ca. 0.4 mm	short, lanceolate, ca.0.5 mm	ovate-lanceolate, 0.3–0.6 mm
Fruit	obovoid-oblong or long-ellipsoid, 4–6 mm × 1.4–2.1 mm; constricted at the commissure	obovoid-oblong or long-ellipsoid, 6–7 mm × 1.5–2 mm, slightly constricted at the commissure	obovoid-oblong, 4–5 mm × 2–2.5 mm; no constricted at the commissure	globose, 4–5 × 2–3 mm; no constricted at the commissure	oblong-ellipsoid, ca. 5 × 4 mm; no constricted at the commissure	subglobose, ca. 3–3.5 × 2.5–3 mm; no constricted at the commissure
Stylopodium	conic	low-conic	depressed	flat	conic	depressed
Mericarp ribs	± equal, prominent to narrow-winged	± equal, conspicuous, narrowly winged	± equal, narrow-winged	± equal, broadly winged	± equal, winged	± equal, winged
Endosperm (at commissural side)	concave	deeply concave	concave	slightly concave	broadly and not deeply concave	concave
Vittae in dorsal furrows	3 (4)	3	3	1–2	2–4	3
Vittae in commissure	2–5	6	4–6	4	4–5	4–6

Discussion

The fruits of *H. trifoliolata* were similar to *H. himalayensis* and *H. phaea* in fruit shape and size, mericarp ribs and both vittae in dorsal furrows and in the commissure. Additionally, the endosperm (at the commissural side), slightly or deeply concave, was common in *Hansenia* (Pimenov et al. 2008; Tan et al. 2020). The fruit shape of *Hansenia* can be divided into two groups by the shape and ribs: fruits oblong-ellipsoid, subglobose or globose, all ribs winged or broadly winged ribs (including *H. forrestii*, *H. forbesii*, *H. mongolica*, *H. oviformis* and *H. weberbaueriana*); fruits obovoid-oblong or long-ellipsoid, ribs prominent to narrowly winged (including *H.trifoliolata*, *H. himalayensis* and *H. phaea*). All species' fruit ribs are 5 and ± equal, except for *H. weberbaueriana* where the ribs are 3–5, equal or a little unequal (Jia et al. 2019). Moreover, there is a constriction at the commissure in *H. trifoliolata*, with a similar phenomenon being found in *H. himalayensis* that has a slight constriction at the commissure (She and Watson 2005a; Tan et al. 2020).

The life form of *H. trifoliolata* is monocarpic, which is uncommon in *Hansenia*, except for *H. forrestii* which seems to be similar (Pimenov et al. 2008). Through observation of the specimens of *H. oviformis*, we believed that *H. oviformis* is monocarpic. The leaves of *Hansenia* species are often 1–3-pinnate, leaflets pinnatifid (including *H. mongolica*, *H. himalayensis* and *H. weberbaueriana*) or leaflets not pinnatifid (including *H. forrestii*, *H. forbesii*, *H. oviformis*, *H. phaea* and *H.trifoliolata*). The stylopodium shape in the genus is continuous, from depressed to flat, to low-conic and conic. All species of *Hansenia* display compound umbels, except for *H. phaea* and the number of rays are either below ten (including *H. trifoliolata*, *H. himalayensis*, *H. oviformis* and *H. forrestii*) or ten to twenty (including *H. forbesii*, *H. mongolica* and *H. weberbaueriana*) (Pimenov et al. 2008; Tan et al. 2020).

In our phylogenetic analyses, *H. trifoliolata* and other *Hansenia* species formed a monophyletic group in both ITS and plastid trees with very strong support (ITS trees: BI = 1.00, ML = 99%; plastid trees: BI = 1.00, ML = 100%). Though the position of *H. trifoliolata* within *Hansenia* had a slight difference between ITS trees and plastid trees (ITS trees: *H. trifoliolata* was sister to *H. oviformis*, then clusters with *H. forbesii*; plastid trees: *H. trifoliolata* clustered with the communities of *H. oviformis* and *H. forbesii*), there is no doubt that *H. trifoliolata* is a member of the genus *Hansenia*.

H. trifoliolata overlaps in its distribution with *H. forbesii* and *H. weberbaueriana* in the western Shaanxi Province and south-eastern Gansu Province.

The molecular data and morphological evidence strongly support the circumscription of *H. trifoliolata* as a new species belonging to *Hansenia*.

Key to the species of *Hansenia*

1a Fruit oblong-ellipsoid, subglobose or globose or elliptic, all ribs winged or broadly winged, wings equal or unequal **2**

2a Rays below ten, unequal **3**

3a Ultimate leaf segments ovate-lanceolate, 2.5–8 cm; bracteoles linear, shorter than flowers ..... ***H. forrestii***
3b Ultimate leaf segments ovate, 1.5–3.5 cm; bracteoles filiform, longer than flowers ..... ***H. oviformis***
2b Rays ten to twenty, ± equal..... **4**
4a Leaves pinnatisect, leaflets pinnatifid..... **5**
5a Bracteoles linear or pinnatifid, fruit ribs 3–5, ultimate leaf segments oblong, margin pinnatifid or variously laciniate-dentate ..... ***H. weberbaueriana***
5b Bracteoles linear, ribs 5, ultimate leaf segments broadly ovate to oblong, at the margin toothed, teeth obtuse ..... ***H. mongolica***
4b Leaves pinnate, leaflets not pinnatifid, ultimate leaf segments ovate to oblong-ovate, margin entire or coarsely toothed..... ***H. forbesii***
1b Fruit obovoid-oblong or long-ellipsoid, ribs prominent to narrowly winged.... **6**
6a Basal leaves and cauline leaves 3-foliolate, umbels 2–5 cm across, rays unequal..... ***H. trifoliolata***
6b Basal leaves ternate-1–3-pinnate, flowers densely crowded into a compact, globose heads..... **7**
7a Basal leaves ternate-1–2-pinnate; petals obovate, apex narrowly inflexed..... ..... ***H. phaea***
7b Basal leaves 3-pinnate; petals broad-ovate, spoon-shaped apex acute..... ..... ***H. himalayensis***

Description of the new species

Hansenia trifoliolata **Q.P.Jiang & X.J.He, sp. nov.**
urn:lsid:ipni.org:names:77307988-1
Figs 1, 5; Suppl. material 1: Fig. S2

Diagnostic characters. Monocarpic. Root cylindrical, branched or partial rhizomes. Leaves 3-foliolate. Umbels 2–5 cm across, rays 3–7, unequal. Stylopodium conical. Fruits are obovoid-oblong or long-ellipsoid, have 5 ribs, ribs prominent to narrow-winged and endosperm (at commissural side) concave. It is clearly distinguished from *H. phaea* and *H. himalayensis* in leaves (ternate-1–2-pinnate and 3-pinnate vs. 3-foliolate). Compared to other *Hansenia* species (i.e. *H. forrestii*, *H. oviformis* and *H. forbesii*), *H. trifoliolata* also shows distinctive morphological characters, especially in fruits characters (shape and ribs) and leaves (3-foliolate is unique in *Hansenia*).

Type. China, Shaanxi Province: Tongtianhe National Forest Park, Feng County, elevation 2430 m a.s.l., 34°14'N, 106°33'E, 28 Sep 2021, Q. P. Jiang, JQP21092801, fruiting (Holotype: SZ).

Description. Biennial, herb, 60–90 cm high. Root cylindrical, branched or partial rhizomes. Stem purplish-green, thinly ribbed, glabrous, thin. Leaves 3-foliolate, green, blade broad-triangular, irregularly doubly serrate, teeth mucronate; central leaflets cuneate-obovate or rhombic, 4–6 × 2–3.5 cm, with irregularly doubly serrate, base

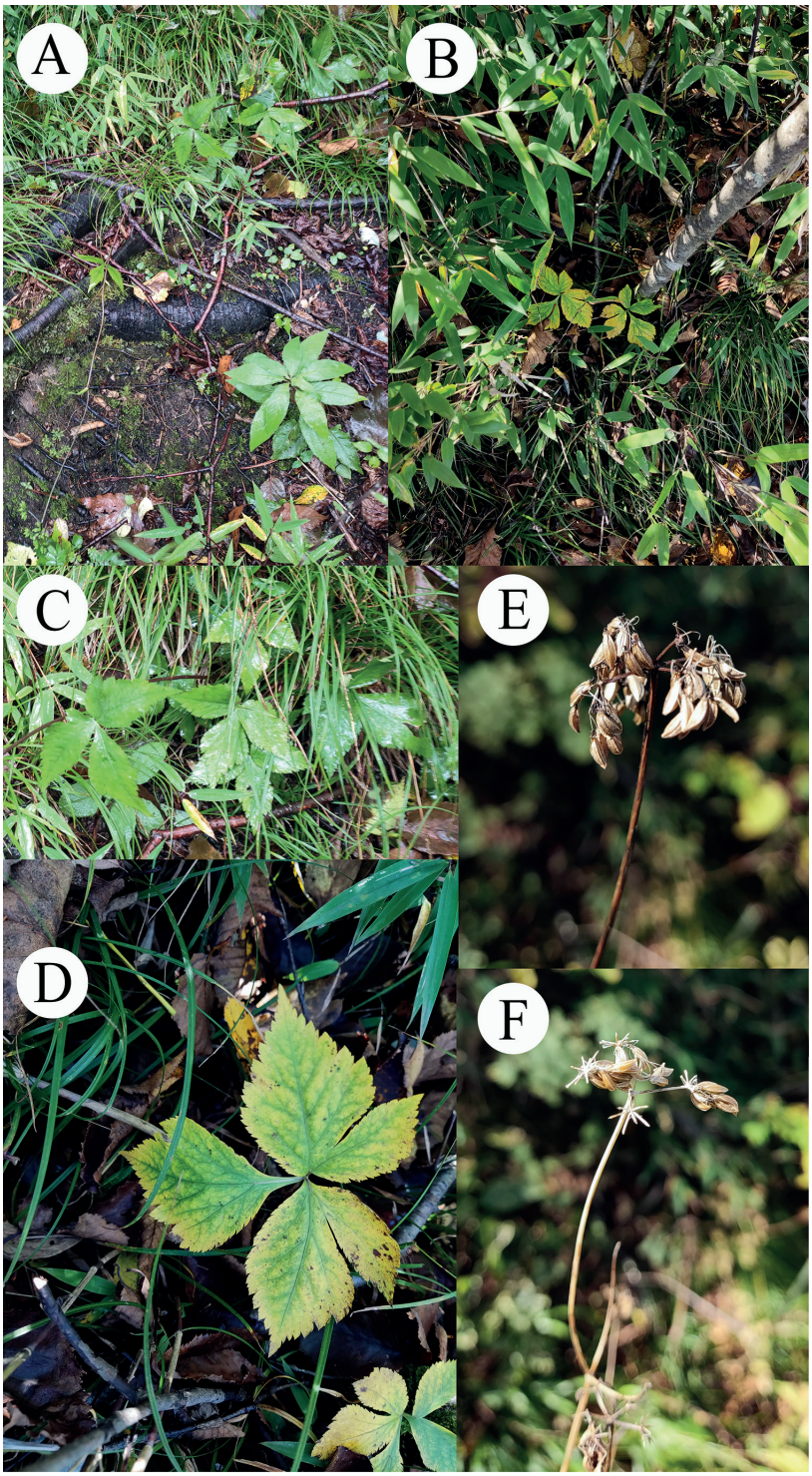

Figure 1. *Hansenia trifoliolata* in the field **A, B** habitat **C** cauline leaves **D** basal leaves **E, F** umbels and fruits.

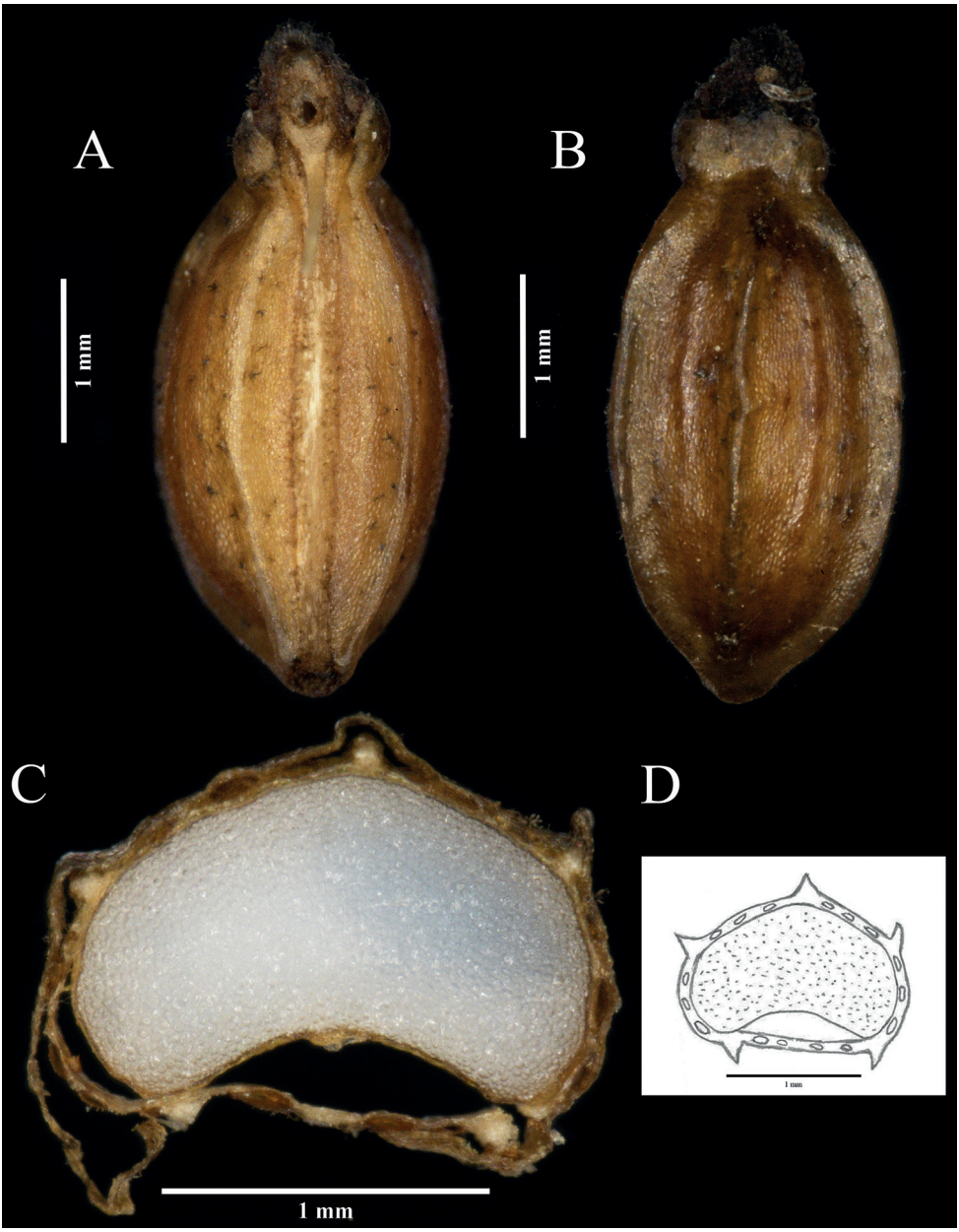

Figure 2. Fruit characters of *Hansenia trifoliolata* **A** commissural side of fruit **B** dorsal view of fruit **C** cross-section of fruit **D** the illustration of the fruit in transverse section. Voucher: JQP21092801.

cuneate; lateral leaflets oblique-ovate, base oblique, often shallowly or deeply uneven 2-parted or not divided, 2–5 × 3.5–6.5 cm. Basal petioles 15–20 cm, petioles shorten upwards; sheaths narrow-oblong, glabrous, with margin irregularly coarse-cuspidate-

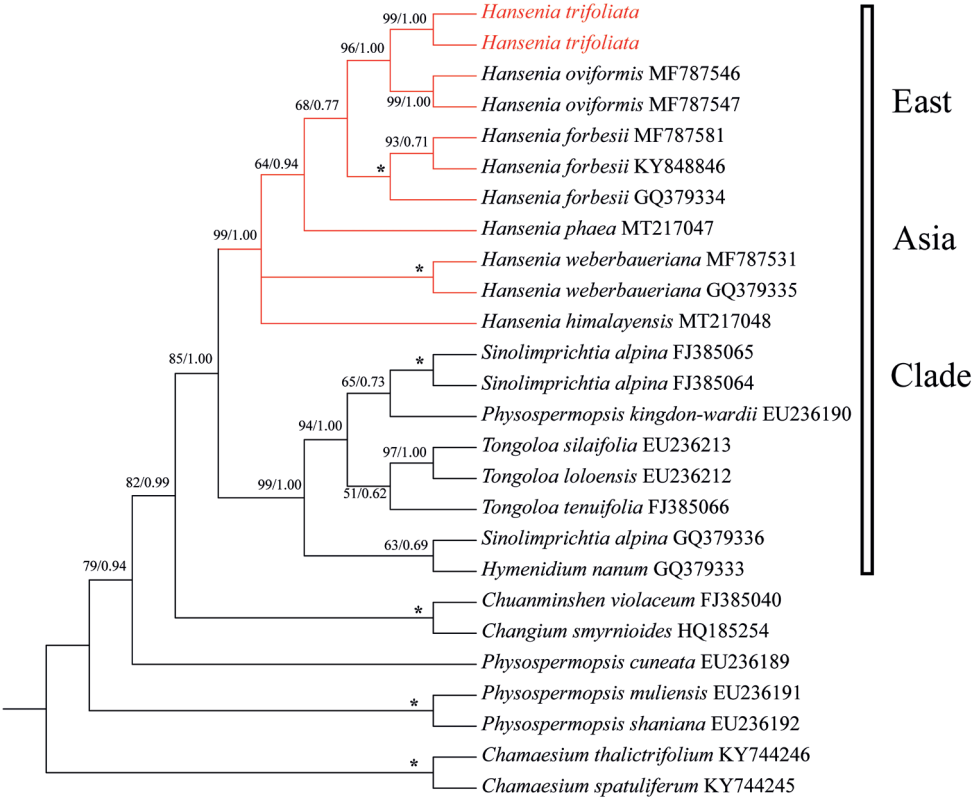

Figure 3. Bayesian 50% majority-rule consensus tree of *Hansenia trifoliolata*, other species of *Hansenia* and related species inferred from ITS sequences using a GTR+G nucleotide substitution model. The tree is rooted with two species of *Chamaesium*. Maximum Likelihood bootstrap support (ML BS) and Bayesian posterior probabilities (BI PP) are presented at the nodes, * representing the best support (100%). The ITS sequences obtained from NCBI exhibited the GenBank number adjacent to the species names.

serrate. Umbels 20–50 mm across; peduncles 5–20 mm long, glabrous; bracts 0 to 2, linear; rays 3 to 7, 5–25 mm long, glabrous; bracteoles 2 to 7, linear, 3–8 mm long; raylets 5 to 11, 1–3 mm long. Flowers unknown; calyx teeth ovate-triangular, 0.3–0.5 mm; petals unknown; stylopodium conical. Fruit obovoid-oblong or long-ellipsoid, 1.4–2.1 × 4–6 mm; mericarps 5-ribbed, ribs prominent to narrow-winged; vittae 3 (4) in each furrow, 2–5 on commissure; endosperm (at commissural side) concave, commissure width 0.8–1.35 mm.

Etymology. The specific epithet refers to the distinctive 3-foliolate leaves.

Phenology. Flowering from July to August, and fruiting from August to September.

Distribution, habitat and ecology. At present, this new species has only been found in the type locality in Tongtianhe National Forest Park, Feng County, Shaanxi

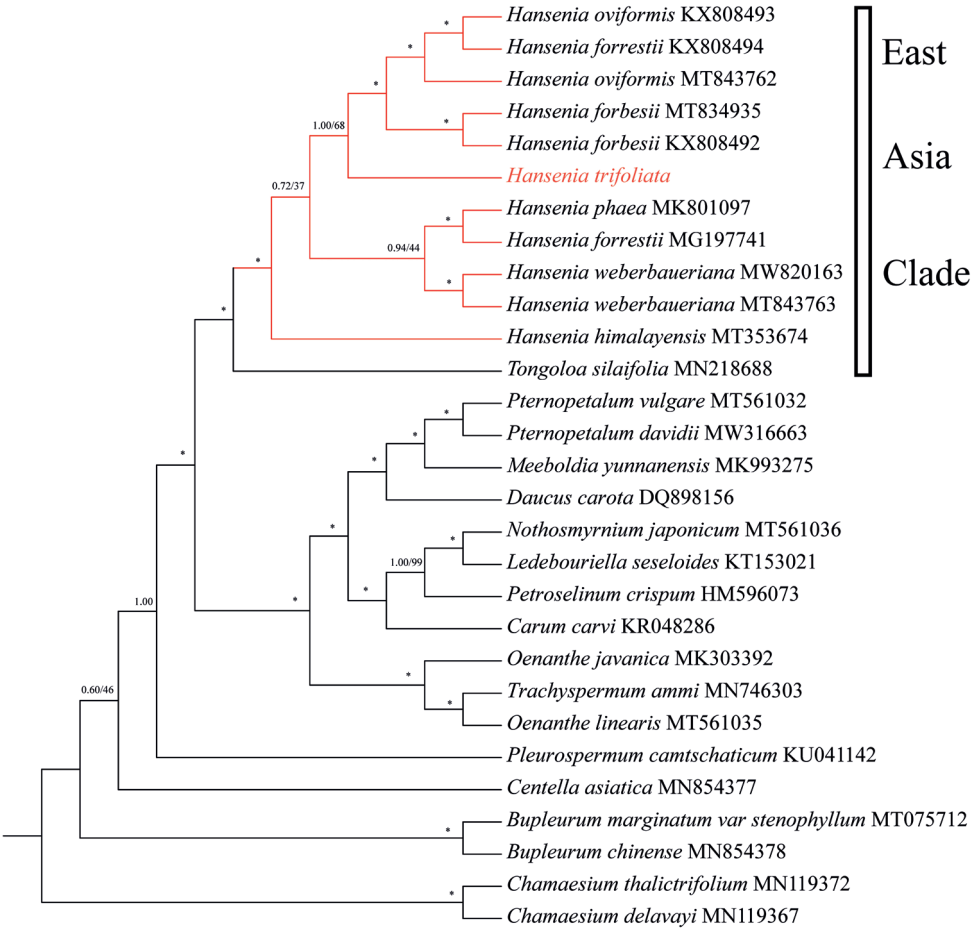

Figure 4. Bayesian 50% majority-rule consensus tree of *Hansenia trifoliolata*, other species of *Hansenia* and related species inferred from protein-coding genes of plastid genomes using a GTR+G+I nucleotide substitution model. The tree is rooted with two species of *Chamaesium*. Maximum Likelihood bootstrap support (ML BS) and Bayesian posterior probabilities (BI PP) are presented at the nodes,* representing the best support (100%). The plastid genome sequences obtained from NCBI exhibited the GenBank number adjacent to the species names.

Province, China. According to the growing environment, we speculate it may inhabit forests at an elevation of 2300 m to 2500 m in western Shaanxi Province and south-eastern Gansu Province. This new species grows in humid environments under the forests.

Additional specimens examined (paratypes). China: Shaanxi Province, Baoji City, Feng County, Tongtianhe National Forest Park, elevation 2430 m a.s.l., 34°14'N, 106°33'E, 20 Aug 2019, Q. P. Jiang and X. Y. Zhang, JQP19082004 (photo SZ !).

Figure 5. Holotype of *Hansenia trifoliolata*, fruiting. Vouchers: JQP21092801.

Acknowledgements

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Supplementary material 1

Figure S1, S2

Authors: Qiu-Ping Jiang, Megan Price, Xiang-Yi Zhang, Xing-Jin He

Data type: Docx file.

Explanation note: **Figure S1.** Fruit of *Hansenia trifoliolata*. **Figure S2.** Isotype of *Hansenia trifoliolata* and paratype of *H. trifoliolata*.

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RESEARCH ARTICLE

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Three new species of *Polycarpaea* (Caryophyllaceae) from Kerala, South India

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Abstract

Three new species of *Polycarpaea*, *Polycarpaea barbellata*, *P. ebracteata* and *P. psammophila*, are described from the Palakkad district of Kerala, India. The new species are allied to *P. corymbosa* and *P. aurea* but can be visibly distinguished by unique character combinations, *viz.* shape of sepal, petal, bract and bracteole and seed morphology. Detailed descriptions along with illustrations and photographs are provided.

Keywords

Caryophyllales, Palakkad gap, *Polycarpaea*, Western Ghats

Introduction

The genus *Polycarpaea* Lamarck (1792: 3) (Caryophyllaceae Juss.) comprises approximately 50 species which are mostly distributed in the tropics and subtropics of the old world and a few occur in the New World tropics (Dequan and Gilbert 2001; Mabberley 2008). The genus is represented in India by seven species (Arya et al. 2021).

During the field exploration carried out as part of the floristic studies of the southern Western Ghats in the Kerala region, several specimens of morphologically unique *Polycarpaea* were collected from the hillocks of Palakkad district (Northern

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Kerala, India). On the basis of critical evaluation of collected specimens, comparison with various herbaria and through literature review, we found that these specimens are distinct from all other known species. Hence, we propose them as novel species.

Materials and methods

Forest exploration trips were carried out during the period of June–January of 2020–21. Herbarium specimens of collected plants were deposited in the Herbarium UCBD. Additional herbarium specimens were examined from the Herbaria E, MH, K, TBGT, UCBD (acronyms according to Thiers 2022 [continuously updated]). Relevant literatures were analyzed (Wight 1843, 1850; Edgeworth and Hooker 1874; Dunn 1915; Majumdar 1993; Daniel et al. 2000; Venu et al. 2001; Daniel 2005; Mastakar et al. 2015; Geethakumary et al. 2019). A total of more than 50 flowers from each species were assessed to confirm the consistency of traits in the collected specimens and to validate the character occurrence.

Results and discussion

Polycarpaea ebracteata **S. Arya, V.S.A. Kumar, V. Suresh & Alen Alex, sp. nov.**
urn:lsid:ipni.org:names:77307989-1
Figs 1, 2

Type. India. Kerala, Palakkad district, Kollengode forest range, Nenmeni, Vengappara 10°34'33.6"N, 76°42'47.1"E, 160 m a.s.l., 20 September 2021, Suresh V., V.S.A. Kumar & Arya S., 2077 (holotype UCBD! isotype UCBD!).

Diagnosis. *Polycarpaea ebracteata* is morphologically similar to *Polycarpaea corymbosa* in terms of having prominent villous stem nodes and shape of petals but differs with respect to stipules (short, ovate less than 1mm, *vs.* lanceolate, long, 5 mm) bracts (absent *vs.* present), bracteoles (absent *vs.* present), sepals (ovate, acute at apex, not membranous *vs.* lanceolate, acuminate apex, hyaline, membranous), petals (dark brown, ovate-elliptical *vs.* whitish-pink, broadly ovate), stamens (filament reduced, 0.1 mm *vs.* filaments equal to the length of anther 1 mm), capsule (style not persistent, tips curved *vs.* style persistent, tips not curved) and seeds (3–4 or rarely 2, yellow, ovate *vs.* 5–13, brown, reniform).

Description. Annual herbs, erect or sub-erect, branched at base, 2.5–8 cm high. Stem terete, densely villous, nodes green, swollen, internodes ca. 5 mm long. Leaves whorled or verticillate, sessile, linear, green, 0.7–0.9 cm long, base cordate, margins smooth, apex acute or acuminate, surface glabrous, blade 1-veined, prominent on abaxial side; stipules scarious, ovate-obovate, (0.2–0.8 × ca. 0.6 mm), margins entire, acute, not nerved, yellowish or greenish at the base, white above. Inflorescence terminal, branched cyme, ca. 1.0 cm long; Flowers 2.2–2.6 mm long; Bracts absent. bracteoles absent; pedicels 1.0–1.3 mm long, green, villous. Sepals 5, free, ovate (1.3–1.4 × ca. 0.7 mm), entire at the margin, acute or obtuse at apex, white, non-membranous

Figure 1. *Polycarpaea ebracteata* **A** habit **B** flower **C** flower bud **D** stipule **E, F** sepal **G** petal **H** gynoecium **I** capsule **J** seed. Photos by Arya Sindhu.

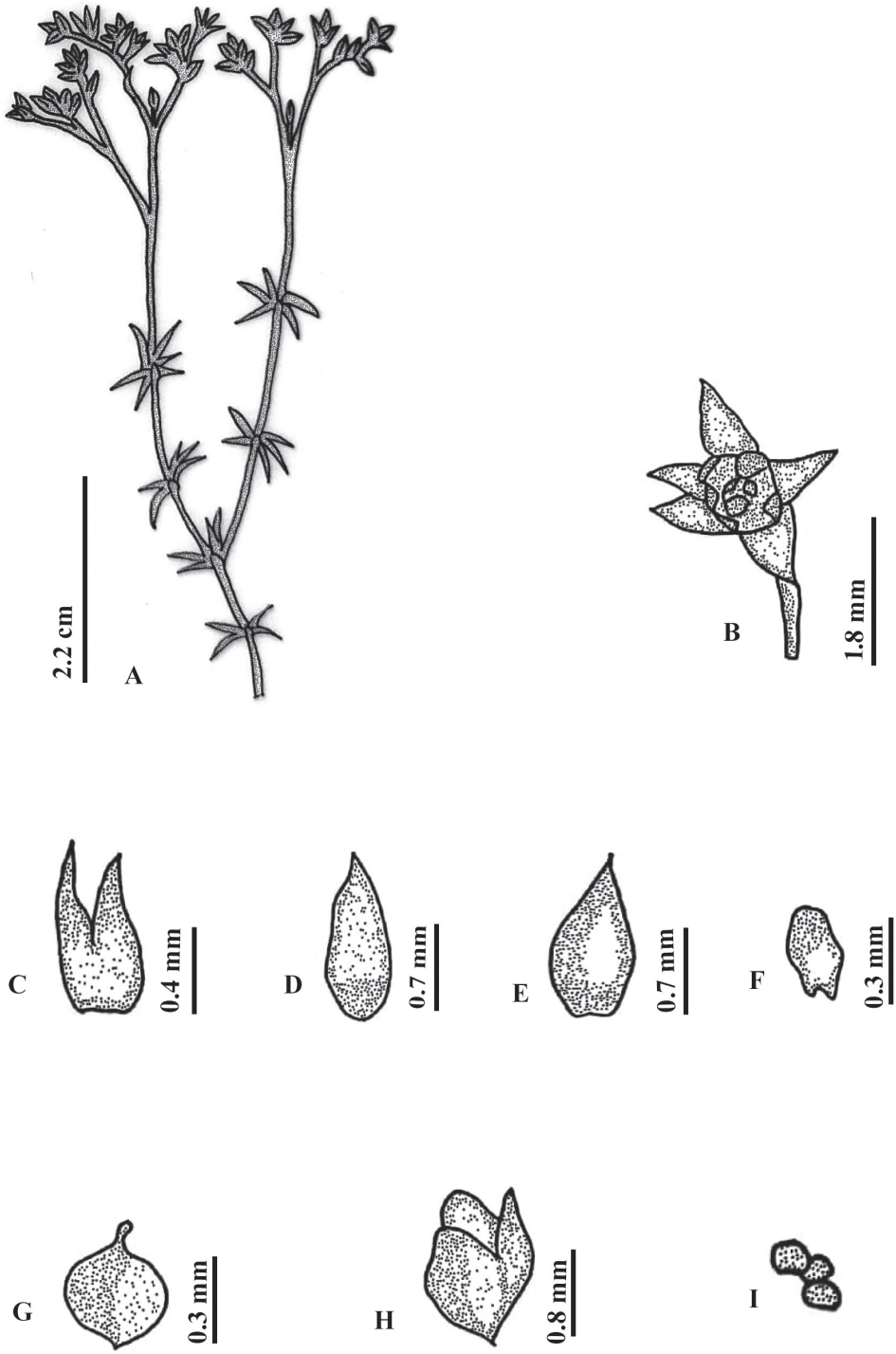

Figure 2. *Polycarpaea ebracteata* **A** habit **B** flower **C** stipule **D, E** sepal **F** petal **G** gynoecium **H** capsule **I** seed. Illustration by Ambika Viswanathanpillai.

base round, midrib not prominent. Petals 5, ovate (0.1–0.3 × 0.1–0.3 mm), margins entire, oblong to round at apex, partially enclosing the ovary, 1/4 as short as sepals, dark red-brown. Stamens 5, forming a ring with petals and encircling the ovary, ca. 0.2 mm long; anthers yellow, oblong, basifixed. Ovary 1-loculed, shortly stipitate, spheroidal, 0.3–0.5 × 0.1–0.2 mm, glabrous, placentation free central; style 0.08–0.1 mm, shorter than the ovary, slender; stigma capitate. Capsule ovoid (1.4–1.6 × ca. 0.6 mm), shortly stipitate, 3-valved, breaks along the suture, brownish, scarious along margin. Seeds 3–4 (rarely 2), ovate (0.2–0.3 × 0.1–0.2 mm), yellow with no striations.

Micromorphology of the seed shows that it is round-oblong with a winged margin. The surface has sub parallel striations which are prominent. The striations do not cross each other and the encircling surface of the striations are punctate. Along the margins, the surface has parallel striations (Fig. 7E, F).

Etymology. Latin prefix e-, without, bractea, bract, and suffix -ata, possession, alluding to absence of bracts, a diagnostic character.

Phenology. Flowering and fruiting during August – December

Distribution and habitat. The primary habitat of *Polycarpaea ebracteata* is the hillock terrains in Palakkad district (Granite outcrop in the southern side of Palakkad gap, the largest break in the Western Ghats having an arid climate with seasonal fires, in the state of Kerala). One of the common species that emerges after the initial rain are members of the Genus *Polycarpaea*, especially *Polycarpaea aurea* (Wight 1850: 44) Dunn (1915: 65). *Polycarpaea ebracteata* is seen associated with *Allmania nodiflora* (L.) R. Br. ex Wight, *Indigofera aspalathoides* DC. and *Fimbristylis cymosa* R. Br. (Fig. 8).

Conservation status. The present study could report only three populations each with 15–20 individuals. Since *Polycarpaea ebracteata* could occur in further sites in SW-India (and India as a whole), we think that further data is required to ascertain the conservation status of the new taxon. As a consequence, the new species is here assessed as DD (Data Deficient) according to the IUCN criteria (IUCN 2021).

Additional specimens examined. *Polycarpaea ebracteata* India. Kerala, Palakkad district, Kollengode, Cheerani. 12 September 2021, Suresh V. & Alen Alex Philip, 2061 (UCBD!); 20 September 2021, Sojan Jose & Suresh V, 2078 (UCBD!).

Polycarpaea psammophila **V. Suresh, V.S.A. Kumar, S. Arya, & Alen Alex, sp. nov.**
urn:lsid:ipni.org:names:77307990-1
Figs 3, 4

Type. India. Kerala, Palakkad district, Nenmara, Ayinampadam, 10°35'29.4"N, 76°34'48.2"E, 140 m a.s.l., 21 September 2021, Suresh V. & Arya S., 2081 (holotype UCBD! isotype UCBD!).

Diagnosis. *Polycarpaea psammophila* is morphologically similar to *Polycarpaea corymbosa* with respect to the whorled arrangement of leaves and pilose nature of stem but differs with respect to stipules (linear to lanceolate with acuminate apex *vs*. lanceolate-ovate, with acute apex), bract (lanceolate-oblanceolate white, exceeding the length of

sepal *vs.* lanceolate-ovate, shorter than the sepal), bracteoles (linear with acicular apex *vs*. lanceolate with acute apex), petals (ovate – oblate, keeled, dark brown, apex pointed upwards *vs*. broadly ovate, not keeled, whitish-pink, round at apex), gynoecium (oblate spheroidal, reddish yellow *vs*. ovate short, green), capsule (four valved *vs.* three valved) and seeds (20–25 yellowish brown, ovate *vs*. 5–13, brown, reniform).

Description. Annual herbs, erect or sub-erect, 18–25 cm high. Stems terete, densely villous, nodes green, swollen, internodes ca. 1.5–2 cm long. Leaves whorled, sessile, linear-lanceolate, green, 2.3–3.1cm long, base round, margin smooth, daggered in young leaf, apex acute or obtuse abaxial surface glabrous, adaxial surface pubescent along the mid vein; blade 1–2 veined, prominent on abaxial side; stipules prominent, linear to lanceolate, fused at the base (5–8 × ca. 2 mm), base golden yellow with unicellular setae; setae hyaline; margins entire, often bifurcated into two, branches acicular at apex, not nerved, milky white. Inflorescence terminal, irregular, branched lax cyme, ca. 10 cm long; Bracts lanceolate-oblanceolate, exceeding the length of the sepal (2.0 – 2.3 × ca. 0.3 mm); base smooth, margin entire, apex acuminate. Bracteole 1.3mm linear with acicular apex, holding the bracts in position. Flowers 8–10 per cyme, 4–5.5 mm long; pedicels 1.0–1.3 mm long, green villous. Sepals 5, free, obovate-oblanceolate (2.3–2.6 × ca. 0.7 mm), entire at the margin, acute or obtuse at apex, white, non-membranous base round, midrib faint. Petals 5, ovate-oblate (1–1.3 × 1.1–1.3 mm), margin entire, keeled, pointed upward at apex, partially or completely enclosing the ovary, 1/2 as short as sepals, dark red-brown. Stamens 5, forming a ring with petals and encircling the ovary, ca. 0.3 mm long; anthers yellow, oblong, basifixed. Ovary 1-loculed, shortly stipitate, spheroidal, reddish-yellow 1.2–1.3 × 1–2 mm, glabrous, placentation free central; style 0.2–0.3 mm, shorter than the ovary, often very reduced and slender; stigma capitate. Capsule oblate-prolate (1.4–1.6 × ca. 0.6 mm), style persistent, shortly stipitate, 4-valved, breaks along the suture, brownish, scarious along margin. Seeds (20–25) ovate (0.2–0.3 × 0.1–0.2 mm), yellowish brown with striations.

Micromorphology of the seed exhibits a sub-orbicular shape with striations that are not parallel and cross each other towards the margin. The epidermal cell pattern is angular to spheroidal. Seed margin is entire and along the margin the cells are rectangular shaped (Fig. 7G, H).

Phenology. Flowering and fruiting during August– December.

Etymology. Greek psammos, sand, and philios, loving, alluding to exclusive habitat of sandy marginal zones of granite hills.

Habitat and distribution: The primary habitat of *Polycarpaea psammophila* is the hillock terrains in Palakkad district along the sandy margins. It is seen associated with *Tephrosia purpurea* (L.) Pers., *Parasopubia delphiniifolia* (L.) H.-P. Hofm. & Eb. Fisch. and *Glinus oppositifolius* (L.) A. DC. (Fig. 8).

Conservation status. The current study is based on two different populations ranging from 50–80 individuals. We believe that further data is needed to determine the conservation status of *Polycarpaea psammophila* because it could be found in other locations in SW-India (or India as a whole). As a result, according to IUCN criteria, the new species is classified as DD (Data Deficient) (IUCN 2021).

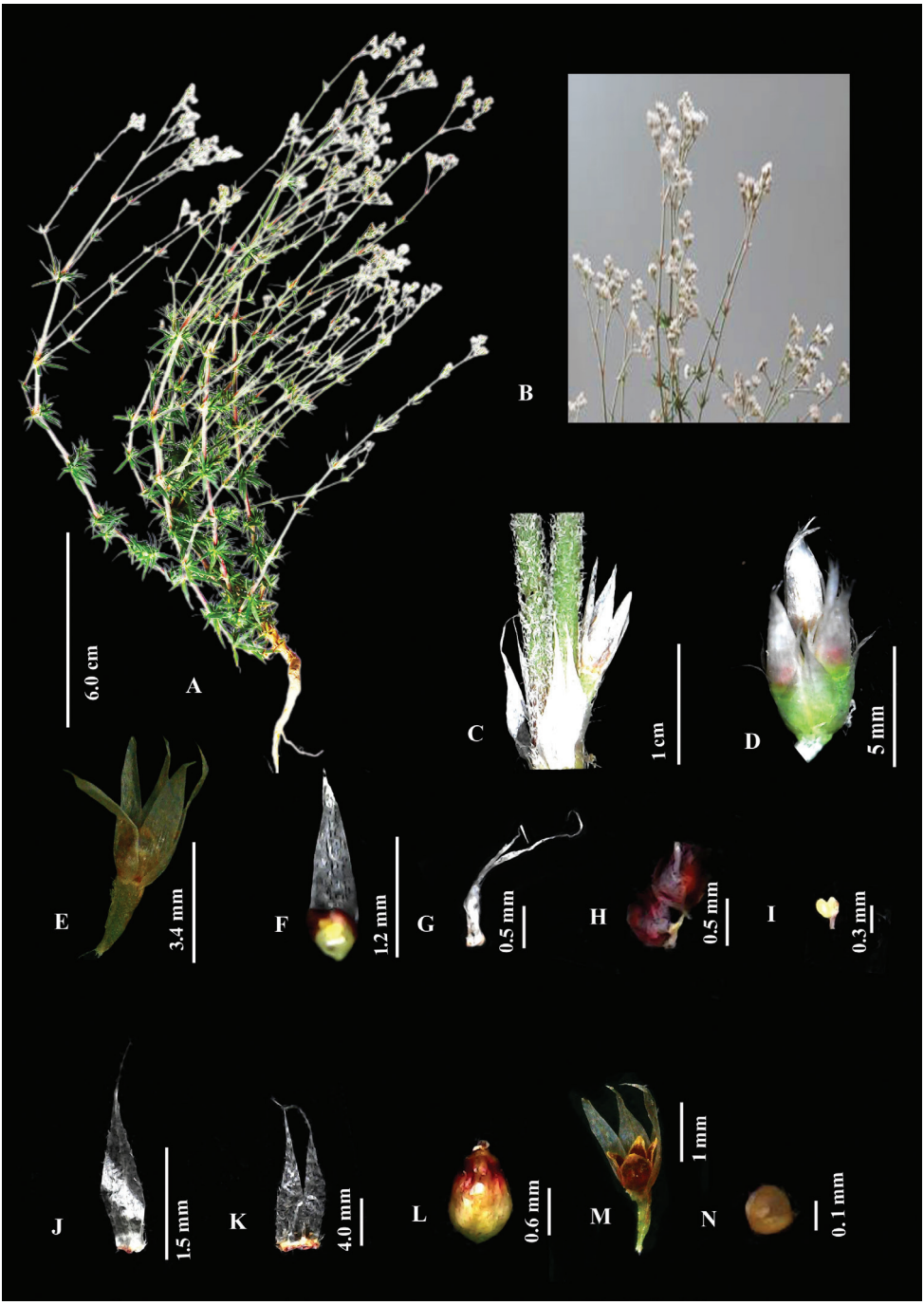

Figure 3. *Polycarpaea psammophila* **A** habit **B** inflorescence **C** internode **D** flower cluster **E** flower **F** bract **G** bracteole **H** petal **I** stamen **J** sepal **K** stipule **L** gynoecium **M** capsule **N** seed. Photos by Suresh V.

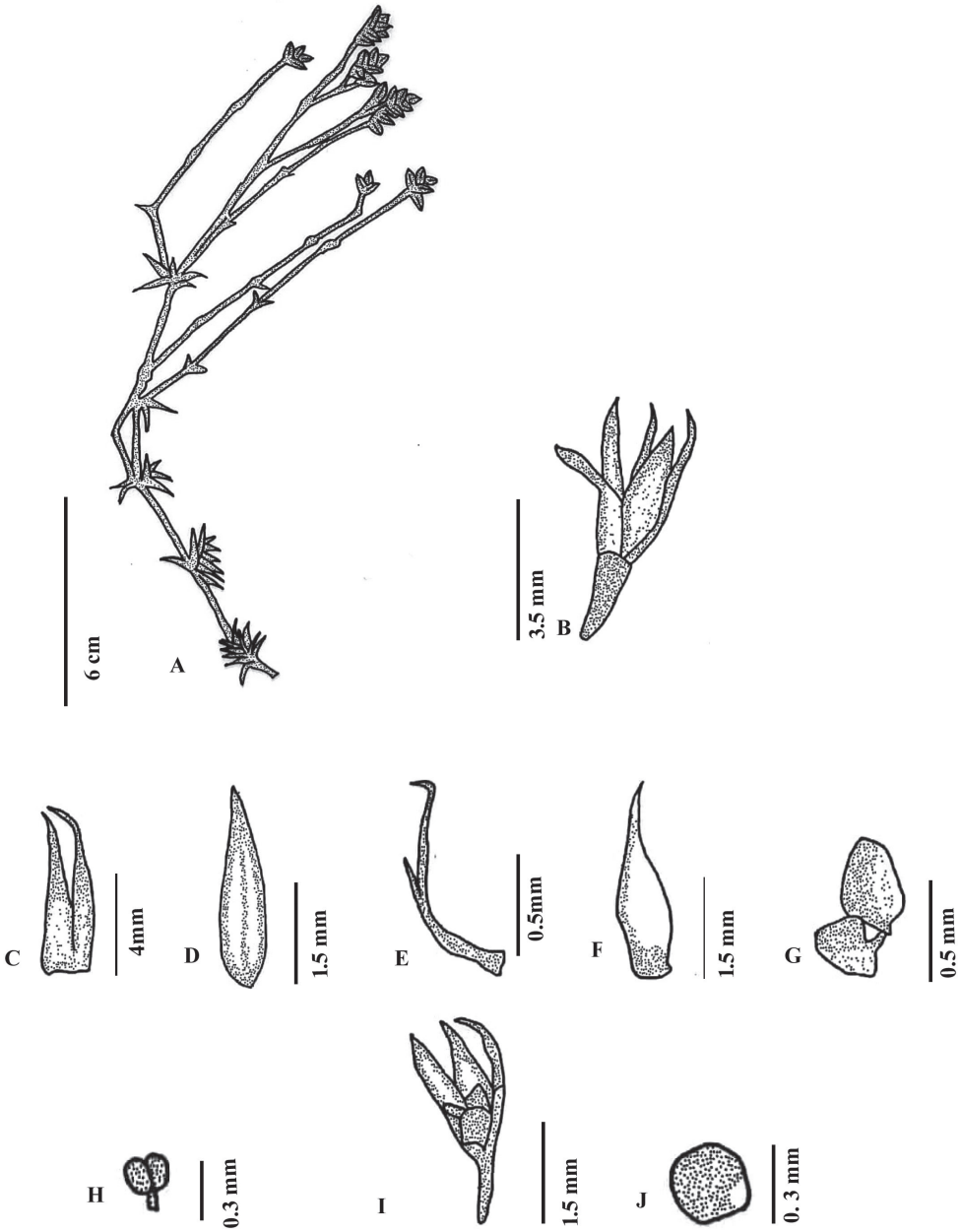

Figure 4. *Polycarpaea psammophila* **A** habit **B** flower **C** stipule **D** bract **E** bracteole **F** sepal **G** petal **H** stamen **I** capsule **J** seed. Illustration by Ambika Viswanathanpillai.

Additional specimens examined. India. Kerala, Palakkad district, Nenmara, Vallangi, 12 September 2021, Suresh V. & Alen Alex Philip, 2065 (UCBD!); 21 September 2021, Sojan Jose & Kumar V.S.A., 2083 (UCBD!).

Polycarpaea barbellata **V.S.A. Kumar, S. Arya, V. Suresh & Alen Alex, sp. nov.**
urn:lsid:ipni.org:names:77307991-1
Figs 5, 6

Type. India. Kerala, Palakkad district, Kuthanur, Chedukamala 10°41'42.6"N, 76°31'06.3"E, 150 m a.s.l., 20 October 2021, V.S.A Kumar, Suresh V & Arya S., 3010 (holotype UCBD! isotype UCBD!).

Diagnosis. *Polycarpaea barbellata* is morphologically similar to *Polycarpaea aurea* with respect to yellow-orange color of sepals but differs with respect to stipules (oblong, parted into 3 with a long acicular structure in the center and other two parts barbellate *vs.* lanceolate parted into 2, free, with no central structure), Inflorescence (dense cyme *vs.* lax cyme), bract (linear, white, acicular apex *vs.* lanceolate-ovate, greyish-brown, acuminate apex), bracteoles (Capillaceous with acicular apex *vs.* ovate lanceolate with acute apex), petals (wedge shaped, whitish-lilac *vs.* broadly ovate-oblong, yellowish-brown), Gynoecium (spheroidal, yellowish green reduced *vs.* conical, short yellow), capsule (style not persistent, tip not recurved, 2–3 seeded *vs.* style persistent, tip recurved, 5–many seeded).

Description. Annual herbs, erect or sub-erect, branched from the base 10–15 cm high. Stem terete, sparsely villous, nodes red swollen, internodes 1.5–2 cm long. Leaves whorled, sessile, lanceolate-oblanceolate, green, 1.3–2.1cm long, base round, margin smooth or wavy, apex acute or acuminate, abaxial surface glabrous, adaxial surface pubescent; lamina 1–2 veined, prominent on abaxial side; stipules prominent, oblong, parted into 3 (2 equal barbellate parts), central part has a long acicular structure ca. 1 mm long, fused at the base (1–1.2 × ca. 0.4 mm), base golden yellow, smooth; margin entire, apex acicular, milky white. Inflorescence terminal, branched regular dense cyme, ca. 4.5 cm long; Bracts linear – lanceolate, 0.8 – 1.0 mm, white, equal or sub-equal to the length of the sepal; base smooth, margin entire, apex acicular. Bracteole capillaceous with acicular apex, holding the bracts in position. Flowers 4–6 per cyme, 3.5–3.8 mm long; pedicels 1.5–2.3 mm long, green villous. Sepals 5, fused at base, ovate (1–1.2 × ca. 0.8 mm), entire at the margin, acute to obtuse at apex, white, non-membranous base round, midrib faint. Petals 5, broadly wedge shaped (0.5–1 × 0.5–0.6 mm), margin entire, completely enclosing the ovary, 1/3 as short as sepals, whitish-lilac. Stamens 5, forming a ring with petals and encircling the ovary, ca. 0.9 mm long; anthers yellow, ovate, basifixed. Ovary 1-loculed, shortly stipitate, spheroidal, yellowish green, 1.2–1.3 × 1–2 mm, glabrous, placentation free central; style 0.01–0.03 mm, shorter than the ovary; stigma capitate. Capsule oblate-prolate (1.4–1.6 × ca. 0.6 mm), shortly stipitate, style not persistent, tip not recurved, 3-valved, breaks along the suture, brownish, scarious along margin. Seeds (2–3) ovate (0.1–0.15 × 0.1–0.2 mm), yellow with striations.

Micromorphology of the seed is ovate-sub-orbicular in its outline with depressions all over the seed surface. The margin is entire and the epidermal cell pattern is faintly angular. Striations are also faint (Fig. 7I, J).

Phenology. Flowering and fruiting during August-December.

Etymology. Latin barba, stiff hairs, suffix ella, diminutive, and -ata, possession, alluding to barbellate nature of stipules, a diagnostic character.

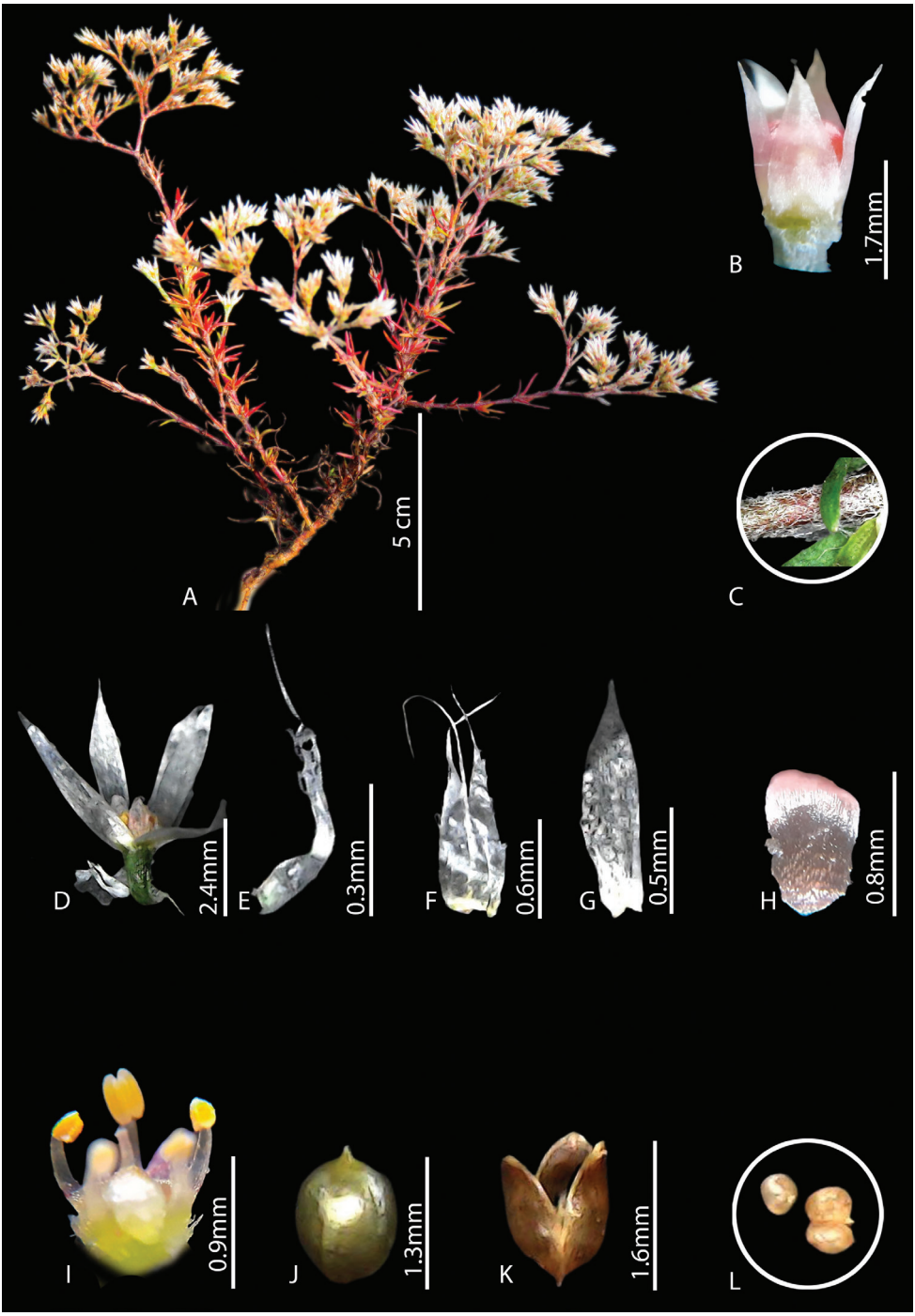

Figure 5. A habit **B** flower bud **C** internode **D** flower **E** bract **F** stipule **G** sepal **H** petal **I** stamen **J** gynoecium **K** capsule **L** seed. Photos by V.S.A. Kumar.

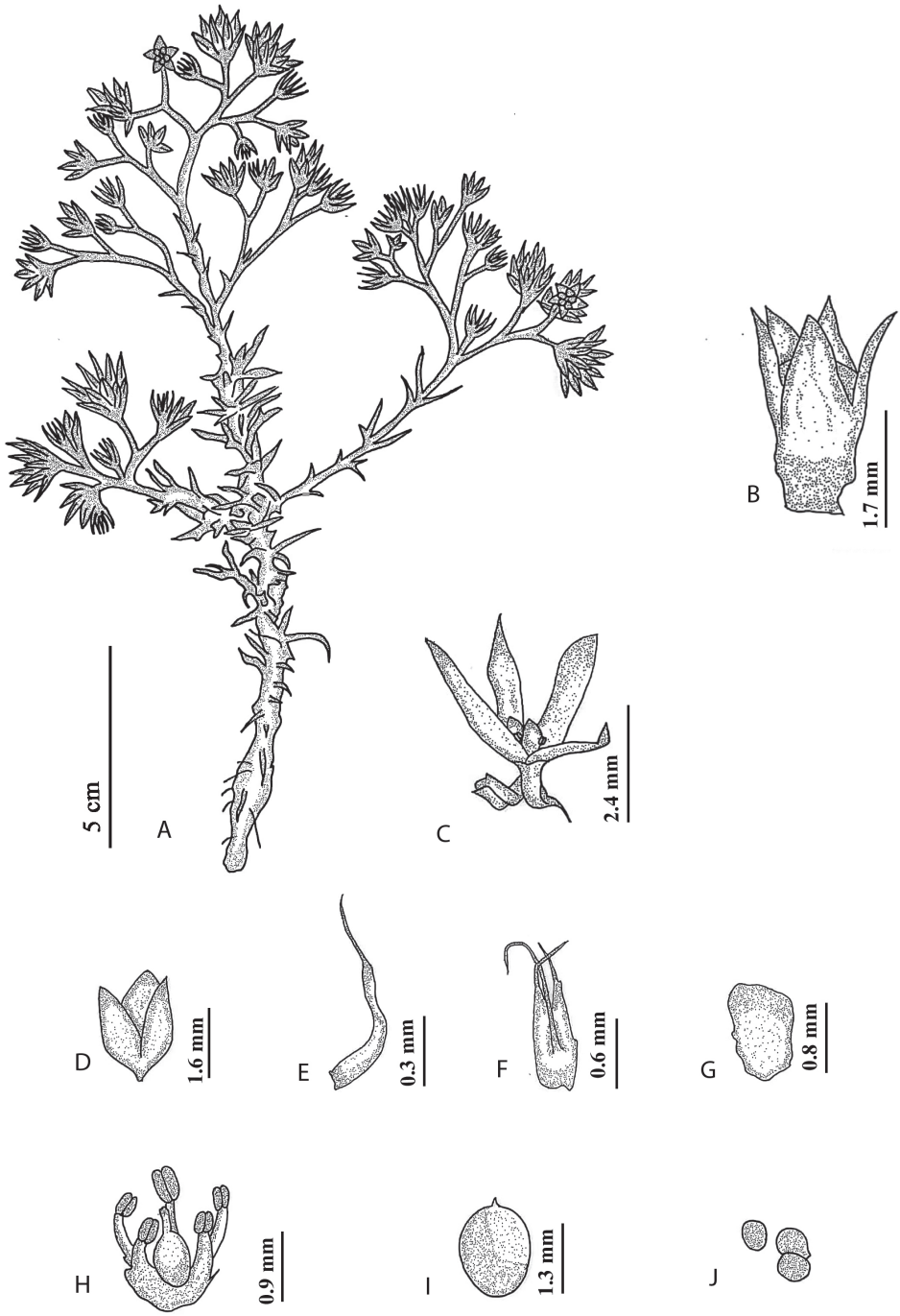

Figure 6. *Polycarpaea barbellata* **A** habit **B** flower bud **C** flower **D** capsule **E** bract **F** stipule **G** petal **H** stamen **I** gynoecium **J** seed. Illustration by Ambika Viswanathanpillai.

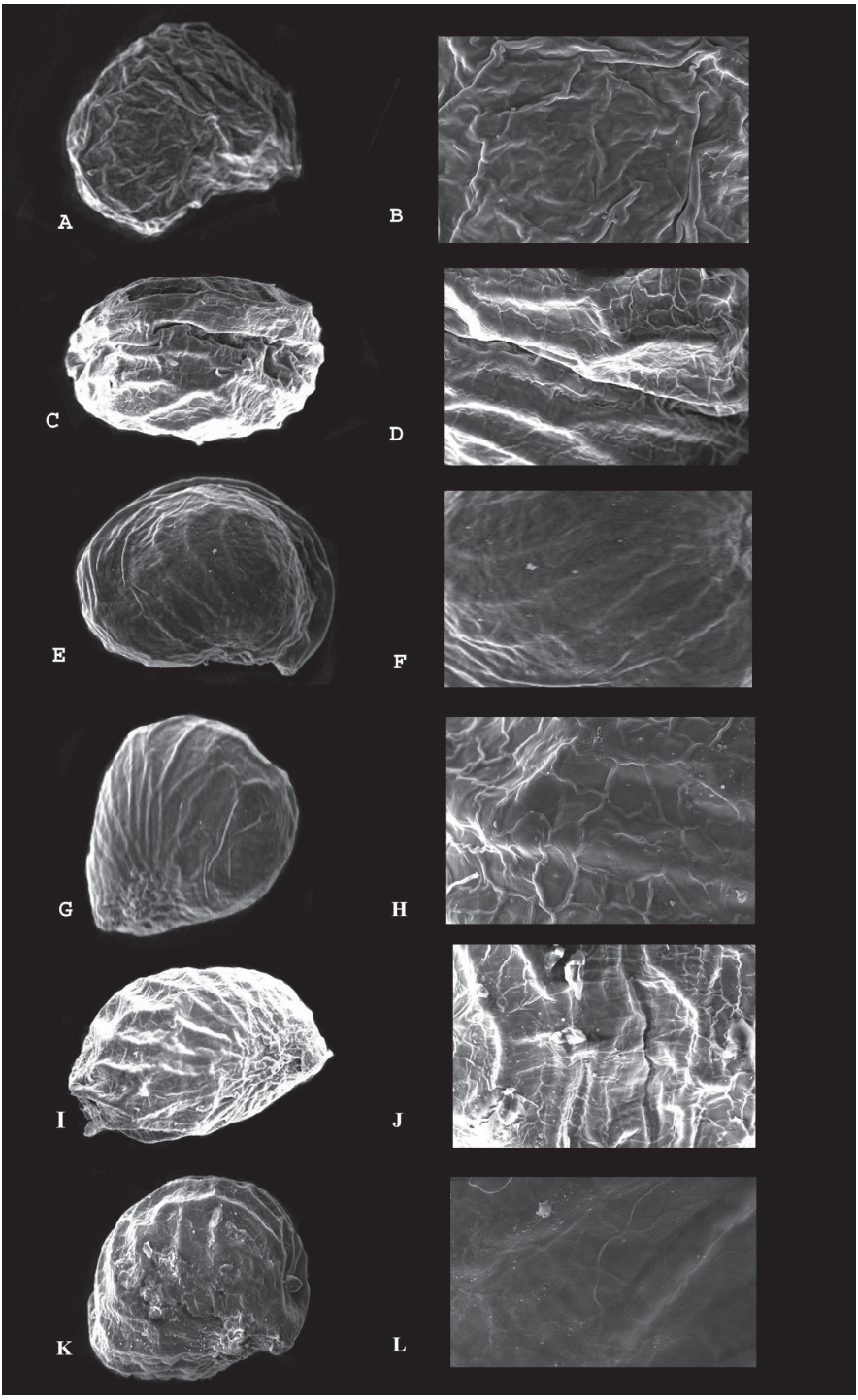

Figure 7. SEM of seeds (*P.corymbosa*) **A** seed **B** seed surface **C, D** *P. palakkadensis* **E, F** *P. ebracteata* **G, H** *P. psammophila* **I, J** *P. barbellata* **K, L** *P. aurea*.

Habitat and distribution. The primary habitat of *Polycarpaea barbellata* is the hillock terrains in Palakkad district (Kerala granite outcrop in the northern side of Palakkad gap, the largest break in the Western Ghats having an arid climate with seasonal fires). *Polycarpaea barbellata* is also seen associated with *Polycarpaea corymbosa*, *Fimbristylis* sp. and *Indigofera enneaphylla* (Fig. 8).

Conservation status. The present study could report two populations with 20–35 individuals each. We consider that further evidence is needed to determine the new taxon's conservation status because *Polycarpaea barbellata* could be found in other regions in Southwest (and India as a whole). As a consequence, the new species is now categorized as DD (Data Deficient) by the IUCN (IUCN 2021).

Additional specimens examined. India. Kerala, Palakkad district, Kuzhalmannam, Kariyanchirachola, 2 November 2021, Suresh V. & Sojan Jose, 3077 (UCBD!); 10 November 2021 Alen Alex Philip & Suresh V., 3084 (UCBD!).

Taxonomic notes. In India, the genus *Polycarpaea* is represented by seven species (Arya et al. 2021). The proposed three new species are closely allied to *Polycarpaea corymbosa* (Linnaeus 1753: 205) Lamarck (1792: 129) and *Polycarpaea aurea* that has wide distribution along with *P. palakkadensis*. The new species also resembles *Polycarpaea palakkadensis* superficially but differs distinctly with respect to characters summarized in Table 1.

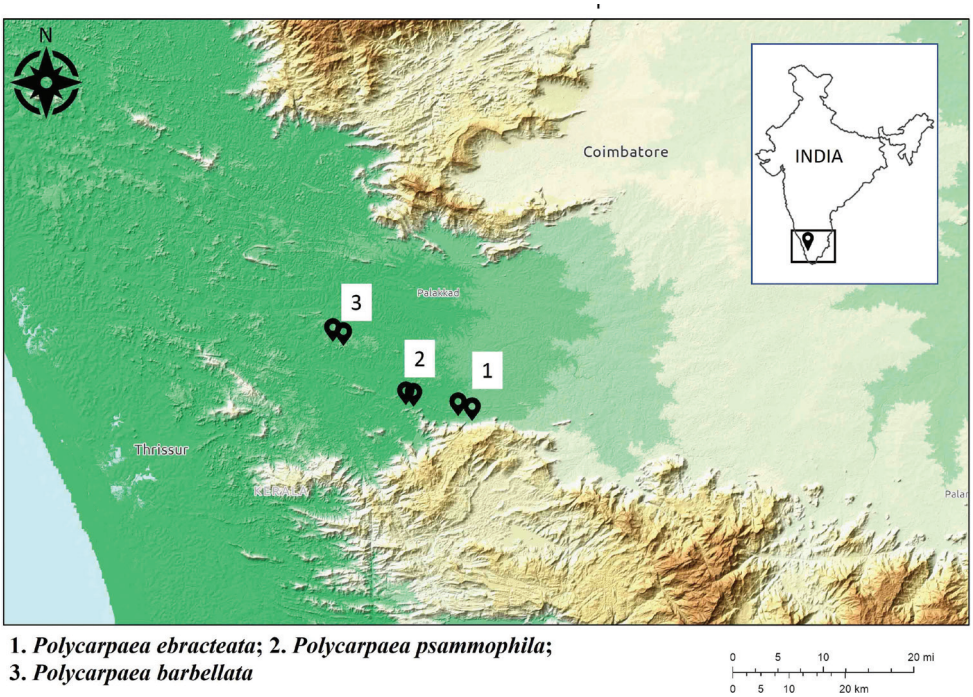

Figure 8. Distribution map of *Polycarpaea ebracteata*, *P. psammophila* and *P. barbellata*.

Table 1. Morphological comparison between *Polycarpaea ebracteata*, *P. psammophila*, *P. barbellata* with *P. corymbosa*, *P. aurea* and *P. palakkadensis*.

Characters	*Polycarpaea ebracteata*	*Polycarpaea psammophila*	*Polycarpaea barbellata*	*Polycarpaea corymbosa*	*Polycarpaea palakkadensis*	*Polycarpaea aurea*
Leaves	Verticillate	Whorled	Whorled	Opposite or apparently whorled	Verticillate (erroneously given as Pseudoverticillate in Protologue)	Opposite decussate
Stipules	Ovate-obovate, 0.2–0.8 mm, apex acute base yellowish or greenish, setae absent, white	Linear to Lanceolate, 5–8 mm, base golden yellow with unicellular setae, apex often bifurcated, branches acicular at apex, milky white	Oblong, parted into 3 (2 equal parts), central part has a long acicular structure ca 1 mm long, fused at the base 1–1.2 × ca. 0.4 mm, base golden yellow, , apex acicular, milky white	Lanceolate, long, 5 mm, base without setae, hyaline	Linear-lanceolate, 1.2 – 2 mm, base without setae, apex acute, creamy white	Lanceolate, acuminate at apex, ca. 3 mm long, base without setae, colourless or yellowish-brown, slightly silvery
Inflorescence	Regular branched lax cyme	Irregular branched lax cyme	Regular branched dense cyme	Irregular branched dense cyme	Irregular, dense cyme	Regular lax cymes
Bract	Absent	Lanceolate-oblanceolate, exceeding the length of the sepal; base smooth, margin entire, apex acuminate.	Lanceolate, equal or sub-equal to the length of the sepal; base smooth, not fused.	Lanceolate-ovate, shorter than the sepal.	Ovate-oblong (erroneously given as linear-lanceolate in protologue), fused at the base, creamy white.	Ovate-lanceolate, not fused at base, grey with a faint brownish tinge.
Bracteole	Absent	Linear with acicular apex	Capillaceous with acicular apex	Lanceolate with acute apex	Capillaceous, not prominent	Ovate-lanceolate
Sepal	Ovate 1.3–1.4mm, entire at the margin, acute or obtuse at apex, white, non-membranous base round, midrib faint.	Obovate-oblanceolate 2.3 –2.6 mm, acute or obtuse at apex, white.	Ovate 1–1.2 mm, acute to obtuse at apex, white, non-membranous base round, midrib faint.	Lanceolate, acuminate apex, hyaline, membranous.	Ovate-oblong, entire margin, acute or blunt apex, white.	Ovate-lanceolate, acute-acuminate at apex, scarious, bright orange-reddish.
Petal	Ovate 0.1–0.3 mm, oblong to round at apex, partially enclosing the ovary, 1/4 as short as sepals, dark red-brown.	Ovate-oblate 1–1.3 mm, keeled, pointed upward at apex, partially or completely enclosing the ovary.	Broadly wedge shaped 0.5–1 mm completely enclosing the ovary, 1/3 as short as sepals, whitish-lilac.	Broadly ovate round at apex; silvery white to pink or purplish red.	Ovate-cordate, fimbriate margin, round to mucronate at apex, dark red – brown.	Oblong-obovate, margin entire, obtuse at apex, yellowish-brown.
Stamens	0.2 mm long, filament inconspicuous	0.3 mm long; filament longer than anther.	0.9 mm, filament same length as anther.	2 mm, Filament equals the length of anther.	0.1 mm, filament very short	1 mm, filament as long as anther
Gynoecium	Spheroidal	Spheroidal	Spheroidal	Ovoid	Oblate spheroid	Conical
Capsule	Style not persistent ovoid 3-valved, breaks along the suture, brownish,	Style persistent, Oblate-prolate, tip not curved after dehiscence 4 valved.	Style not persistent, 3 valved, tips straight after dehiscence	Style persistent, tips not curved, 3 valved	Style not persistent, 4 valved, smooth, tips not recurved after dehiscence	Style persistent smooth, shining, glabrous, tips recurved after dehiscence
Seed	3–4 seeds, yellow to brown no striation	20–25 seeds yellowish brown, smooth	2–3 seed, ovate yellow with striations	5–13, brown, reniform	1–2 Ovoid-elliptical creamy white	5-many seeded, reniform brown

A key to demarcate the new three species from the other seven species found in India

1 Habitat in rocky terrains, reaching a height of 2–15cm, petal ovate – oblong, apex obtuse or round, not keeled **2**

– Habitat in sand, reaching a height of 18–25cm, petal ovate-oblate, apex shortly acicular, keeled ..... ***P. psammophila***

2 Leaves radical and cauline; flowers in spike; capsule thin walled..... ***P. spicata***

– Leaves cauline; flowers in dense or lax cyme; capsule thick walled..... **3**

3 Bract present..... **4**

– Bract absent ..... ***P. ebracteata***

4 Petal pinkish-purple; plant glabrous..... ***P. diffusa***

– Petals pinkish-yellow or whitish-lilac or yellowish-brown; plant densely tomentose..... **5**

5 Stem with greyish hairs; petal lightly coloured or hyaline; leaves set with green slender node ..... ***P. corymbosa***

– Stem with white hairs; petal brightly colored; leaf set with reddish swollen nodes..... **6**

6 Plants not stunted; sepal bright white or red or orange; petals yellow-brown or whitish lilac **7**

– Plants stunted; sepal colorless; petals violet ..... ***P. majumdariana***

7 Leaves opposite-decussate; anthers white-cream ..... ***P. aurea***

– Leaves pseudo whorled, whorled or verticillate, nodes red villous, anthers bright yellow..... **8**

8 Stipule barbellate parted into three halves with central part acicular..... ..... ***P. barbellata***

– Stipules smooth parted into two halves with no central structure..... **9**

9 Sepals ovate-oblong; petals ovate-cordate, apex round; gynoecium oblate spheroidal, capsule 1–2 seeded, seed ovoid..... ***P. palakkadensis***

– Sepals lanceolate, petals ovate-lanceolate, apex acute; gynoecium prolate; capsule 3–10 seeded, seed sub-reniform ..... ***P. rangaiahiana***

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RESEARCH ARTICLE

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Pimpinella saxifraga subsp. *rupestris* (Apiaceae) – taxonomy and nomenclature of stenoendemic taxon from Karkonosze Mountains (Sudetes, Poland)

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Abstract

Pimpinella saxifraga subsp. *rupestris* (Apiaceae) grows in a glacial cirque (Karkonosze Mountains, Sudetes, Poland) on a basalt substrate. Specimens of this species were first collected and described at the end of the 19th century, and their taxonomic distinctiveness and endemic status were determined by Weide in 1962. The typification of the name *Pimpinella saxifraga* subsp. *rupestris* is discussed. The protologue of the name and the diagnostic phrase are evaluated based on herbarium specimen. The lectotype is designated. The paper also presents diagnostic morphological features of this and a closely related species *Pimpinella saxifraga* subsp. *saxifraga*.

Keywords

endemic taxon, lectotypification, morphology, Poland, Sudetes, tribe Pimpinelleae

Introduction

The genus *Pimpinella* L. is one of the most numerous genus in the family Apiaceae, subfamily Apioideae, tribe Pimpinelleae, and consists of ca. 180 species. Its wide geographic scope covers Europe, Asia and Africa, including Madagascar. However, nearly 70% of the species grow in Asia. It is also introduced to North and South America and

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southern Australia (Bentham 1867; Tutin 1968; Pimenov and Leonov 2004; Pu and Watson 2005; Plunkett et al. 2018).

Pimpinella saxifraga, the type species of the genus (Downie et al. 2010; Fernandez Prieto et al. 2018), is a polymorphic taxon with high morphological plasticity, being variable in stem size, leaf shape, dentation of leaflet margins, and structure of umbels. Several infraspecific taxa at the ranks of subspecies, varieties and forms (Sprengel 1820; Wolf 1927) have been proposed to accommodate the observed morphological diversity, although not all of these are currently accepted. Included among these intra-specific taxa are two related mountain subspecies *Pimpinella saxifraga* subsp. *alpestris* (Sprengel) Vollmann, confined to alpine and subalpine belts of the Alps, Carpathians, Dinaric and Balkans Mountains (Reduron 2008; Pignatti 2018); and *Pimpinella saxifraga* subsp. *rupestris* Weide, a highly ecologically specialized stenoendemic taxon restricted to single locality on Sudetes, on the Polish side of the Karkonosze Mts. (Weide 1962; Šourek 1967). Here, we provide morphological characteristic and diagnostic features of this endemic taxon, list its herbarium specimens and, because the holotype (Weide 1962) is missing, we designate a lectotype from among the extant isotypes.

Materials and methods

We have studied specimens held by the herbaria G, JE, KRA, KRAM, PR, WRSL (acronyms according to Thiers 2022).

The typification process follows Article 9.12 of the ICN Schenzen Code (Turland et al. 2018). The results are based on the analysis of relevant literature, examination of herbarium specimens and original field research. We attempted to locate all original material in the herbaria G, JE, PR, Museum Coburg as well as all other available specimens of the subspecies. As the holotype is missing from herbarium of Naturhistorisches Museum Coburg (Heimo Rainer, pers. comm.), we designate as lectotype its best duplicate housed in herbarium PR.

Taxonomic treatment

***Pimpinella saxifraga* L. subsp. *rupestris* Weide, 1962 (Weide, Fedd. Repert. 64: 259. 1962; Šourek, Preslia 39: 70)**

Holotype.–Poland. Europe, Sudetes: Kleine Schneegrube des Riesengebirges (Hirte, Naturwissenschaftlichen Museum Coburg) [missing, Heimo Rainer, pers. comm.]. – Lectotype (designated here): Poland. Flora des Westsudeten. Basalt in der Kleinen Schneegrube [Karkonosze Mountains–Mały Śnieżny Kocioł Cirque, basaltic rocks, ca 1300 m a.s.l.], 14 August 1891, leg. *G. Hirte*, (PR 162605! – Fig. 1; isolectotypes: G00379179, G00379180, G00848072, JE00028396, JE00028397, JE00028398, PR162596).

All specimens (isolectotypes) come from the same collection by G. Hirte (August 14, 1891) and were sent as duplicates to various herbaria (Geneva, Jena, Pruhonice).

Figure 1. Lectotype of *Pimpinella saxifraga* L. subsp. *rupestris* Weide, 1962: National Museum in Prague, Czech Republic, PR162205.

Figure 2. Living plants of *Pimpinella saxifraga* L. subsp. *rupestris* Weide 1962 **A** general view of type locality - *locus classicus*, Mały Snieżny Kocioł glacial cirque, Karkonosze Mountains, Sudetes, Poland, Europe **B** stem **C** pinnate leaf **D** leaf rosette **E** inflorescence **F** infrutescens (**A** photo by P.Kwiatkowski; **B–F** photo by L.Przewoźnik).

Description. Plants (5-)10–35 cm tall. Rosette leaves with ± long petioles, 2-pinnate with 3–6(-8) pairs of sessile leaflets; leaflets rounded to ovate, evenly dentate or serrate. Stems cylindrical, sometimes slightly striate, rarely branched in the upper part,

leafless or with 1–3 cauline leaves. Lower cauline leaves 1-pinnate, dentate with obtuse teeth, light green; middle cauline leaves 2-pinnate, sessile with short sheaths; leaflets of upper cauline leaves reduced, linear or lanceolate. Umbels small, with 7–14 rays of uneven length; rays smooth or ± ciliate; involucres and involucels usually absent. Petals whitish, yellowish, sporadically pink, up to 0.7–1.0 mm long, cordate, incurved at tips. Fruits 1.0–2.0(–2.5) × 0.5–1.5(–2.0) mm, ovoid, slightly compressed, smooth with ribs distinct only at maturity (Fig. 2). The most important differences in the morphological structure between the nominative taxa of *Pimpinella saxifraga* subsp. *saxifraga* and the discussed *P. saxifraga* subsp. *rupestris* are given in Table 1.

Poland. Sudetes: Riesengebirge [Karkonosze Mountains]: Kleine Schneegrube [Mały Śnieżny Kocioł Glacial Cirque], 14 August 1891, *Hirte* (Flora silesica exsiccata No 375, 1891), ut *Pimpinella saxifraga* L. var. *alpestris* Spreng. (G00379179, G00848072, JE00028397); Am Basalt in der Kleinen Schneegrube [basalt outcrop in the Mały Śnieżny Kocioł Glacial Cirque], 14 August 1891, *Hirte*, ut *Pimpinella saxifraga* L. var. *alpestris* Spreng. (G00379180, JE00028396, JE00028398, PR162605); M. Sněžná jáma [Mały Śnieżny Kocioł Glacial Cirque], August 1921, *Pilát*, ut *Pimpinella saxifraga* var. *petraea* (PR162596).

Table 1. Morphological differences between the subspecies of *Pimpinella saxifraga* in Karkonosze Mountains.

Characters	*P. saxifraga* subsp. *saxifraga*	*P. saxifraga* subsp. *rupestris*
Stems	angular to slightly striate; (20)50–100(150) cm high; usually branched; only lower parts hairy	cylindrical (oval), slightly striate; (5)10–35 cm high; mostly single stems, very rarely branched in upper part
Leaves	± shiny, from light to dark green	± dull, from light to dark green
Rosette leaves	2-pinnate with (2)3–5(8) pairs of ovate or oval, evenly dentate leaflets	on ± long petioles, 2- pinnate, with 3–6(8) pairs of oval, unevenly dentate or serrate leaflets
Umbells	with (7)9–16(26) peduncles which reach a length of 2.0–4.0 cm	with (7)8–11(14) smooth or ± ciliated peduncles of unequal length
Flowers	with white, yellow or red petals up to 1.0–1.5 mm long	with whitish, white-yellowish or sporadically pink petals up to 0.7–1.0 mm long
Fruits	spherical-ovate, slightly laterally compressed, dimensions 1.5–2.5 × 1.0–2.0 mm	ovate, slightly laterally compressed, dimensions 1.0–2.0 (2.5) × 0.5–1.5 (2.0) mm

Nomenclature

This particular population of *Pimpinella saxifraga* from Mały Śnieżny Kocioł in the Polish side of Karkonosze Mountains (Sudetes) was formally recognized for the first time under the name *Pimpinella saxifraga* var. *alpestris* Sprengel by Rudolf von Uechtritz in the late 19th century, and reported in publications by Fiek (1881), Čelakovský (1881) and Winkler (1881). In turn, the oldest herbarium specimens of *Pimpinella saxifraga* var. *alpestris* Sprengel from the Karkonosze Mountains were collected by G. Hirte in year 1891. Similarly, in older literature the specimens from the Karkonosze Mountains were included in subsp., var. or f. *alpestris* (Spreng.) Vollmann (Callier 1892; Schube 1903; Kruber 1913; Schustler 1918; Thellung 1927; Wolf 1927; Limpricht 1930).

In the mid-twentieth century, Weide (1962) conducted critical taxonomic studies of the *Pimpinella saxifraga* complex in Europe. He distinguished five subspecies within the complex that differ in the morphology of leaves, stems and umbels, as well as the preference to specific habitat conditions and type of geographical distribution. Among those, he also described plants occurring in Mały Śnieżny Kocioł Glacial Cirque as a stenoendemic taxon *Pimpinella saxifraga* subsp. *rupestris* Weide. The protologue of the name of this taxon consists of the following diagnostic phrase (nomen specificum legitimum): *Planta foliolis foliorum axillarium primorum subrotundis*, *obtuse dentatis vel subovatis*, *serratis*; *foliorum axillorum secundorum subovatis*, *serratis vel subovatis*, *inciso-serratis*, *glaberrimis. Caule humili*, *sulcato*, *subter*, *pubescente*. Weide (1962) noted that some morphological features place specimens from the Karkonosze Mountains closer to plants found in the Alps, i.e. *Pimpinella saxifraga* subsp. *alpestris.* In particular, the number of umbel rays is similar (*Pimpinella saxifraga* subsp. *rupestris* 7–14, *P. saxifraga* subsp. *alpestris* 8–12). However, in alpine plants the stem is always angular, surrounded at the base by a cluster of dead leaves, while the leaflets have pointed and spreading teeth.

Further research was conducted by Josef Šourek (1967). This excellent Czech botanist devoted special attention to the study of rare species of vascular plants of the Karkonosze Mountains, including taxa with extremely limited geographical range. For herbarium specimens of the genus *Pimpinella* from the Karkonosze Mountains, Alps, Dinaric Mountains and Carpathians, he compared this population in respect of size of leaflets, the number of teeth on a single leaflet, and the number of umbel rays with other material from Central European mountains. He found distinct differences between specimens from Mały Śnieżny Kocioł and other mountain ranges, and adopted the classification proposed by Weide (1962). Since then this taxonomic separateness has been generally accepted (Meusel et al. 1978; Kwiatkowski 1997, 2008; Štěpánek 1997; Fabiszewski and Kwiatkowski 2002; Krahulec 2006; Mozolová 2007). *Pimpinella saxifraga* subsp. *rupestris* has been included among the endemic taxa of the Karkonosze Mountains vascular flora. *Pimpinella saxifraga* subsp. *rupestris* is restricted to very specific stand, basalt rocky outcrop in steep northern slope of Mały Śnieżny Kocioł Glacial Cirque in altitude 1265–1385 m a.s.l., which is known by occurrence of several relic and/or endemic taxa, e.g. *Alchemilla corcontica* Plocek, *Euphrasia minima* Jacq., *Festuca versicolor* Tausch, *Galium sudeticum* Tausch, *Myosotis alpestris* F.W.Schmidt, *Rhodiola rosea* L., *Saxifraga bryoides* L., *S. moschata* Wulfen subsp. *basaltica* Braun-Blanq., *S. nivalis* L., *Woodsia alpina* (Bolton) Gray.

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RESEARCH ARTICLE

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Oreocharis qianyuensis, a new species of Gesneriaceae from Southwest, China based on morphological and molecular evidence

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Abstract

Oreocharis qianyuensis, a new species of Gesneriaceae from Southwest, China, is described and illustrated based on morphological comparisons and molecular phylogenetic analyses. Phylotranscriptomic analyses of the new species in the context of a comprehensive phylogeny with dense sampling of 88% (111/126) of all species of the genus indicated that the new species was most closely-related to *O. fargesii*. The new species is morphologically similar to *O. fargesii* and *O. nanchuanica* in the shape, color and structure of flowers and the number of stamens, but differs in the leaf blade shape, margin and the indumentum characters of the inflorescence. Its morphological relationship with similar species is discussed, the detailed descriptions, colour photographs, distribution, as well as the IUCN threatened status based on the IUCN Red List Categories and Criteria are also provided.

Keywords

Flora of China, Morphology, *Oreocharis*, Phylotranscriptomics

* These authors contributed equally to this paper.

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Introduction

Möller et al. (2011) redefined *Oreocharis* s.l. (Gesneriaceae) and recognized 102 species. In the following decade of research, some taxa (e.g., *Ancylostemon dimorphosepalus* W.H. Chen & Y.M. Shui, *Beccarinda baolianis* Q.W. Lin, *Boeica guileana* B.L. Burtt, *Briggsia acutiloba* K.Y. Pan, *B. muscicola* (Diels) Craib, *Tremacron hongheense* W.H. Chen & Y.M. Shui) were incorporated into the genus (Burtt 1977; Pan 1988; Chen et al. 2012, 2014; Middleton et al. 2013; Möller et al. 2014; Cai et al. 2015; Möller 2015; Lin 2016; Yang et al. 2021; *Bournea sinensis* Oliv. and *B. leiophylla* (W.T. Wang) W.T. Wang & K.Y. Pan were removed from the genus based on molecular and palynological evidence (Chen et al. 2020), together with the publication of some dozens of new species (e.g., Wei et al. 2016; Cai et al. 2017, 2020; Guo et al. 2018; Pan et al. 2019; Yang and Shi 2021; Le et al. 2022), *Oreocharis* s.l. hitherto comprises ca. 160 species, mainly distributed in South and Southwest China (150 species), with several species occurring in North Vietnam (ten species with eight endemic), Myanmar (two species), Bhutan (one species), India (one species), Japan (one endemic species) and Thailand (one endemic species) (Wang et al. 1990, 1998; Li and Wang 2005; Cai et al. 2020; Wen et al. 2021). *Oreocharis* shows extremely diverse floral charateristics, particularly regarding the corolla shape ranging from narrowly or widely tubular, campanulate, urceolate, to flat; symmetry from zygomorphic to actinomorphic; color from white, red, yellow, pink to purple (Jin et al. 2021). A recent extensive study based on transcriptomic data of 88% (111/126) of all species of the genus revealed the spatiotemporal diversification and the possible driving forces (Kong et al. 2022). This study provided a robust phylogenetic hypothesis of the relationships of most species and acts as firm basis for further studies such as species identification and delimitation.

In 2019, during a field investigation in Kaili City, Guizhou, China, an anomalous plant of Gesneriaceae with few flowers caught the authors' attention, and in July 2020, it was recollected at flowering time. Coincidentally, similar specimens were collected by Si-Rong Yi from Pengshui County, Chongqing, China in 2021. We identified it as a member of the previously recognised genus of *Isometrum* Craib (Pan 1986; Wang et al. 1990; Li and Wang 2005), which now belongs to the genus *Oreocharis* s.l. based on the flower and fruit characteristics, such as: four stamens coherent in pairs, anther thecae not confluent, capsule dehiscent on one side (Wang et al. 1990, 1998; Li and Wang 2005). After examination of the specimens stored in the related herbaria (E, HITBC, IBK, HN, K, KUN, P, PE and VMN) including digital specimens online, such as Chinese Virtual Herbarium (http://www.cvh.ac.cn/) in China and Global Plants on JSTOR (https://plants.jstor.org/), and consulting the related taxonomic publications of *Oreocharis* from the adjacent regions (Wang et al. 1990, 1998; Li and Wang 2005; Li and Li 2015; Guo et al. 2018; Cai et al. 2019; Fu et al. 2019a, b), we could not match the species to any previously published one of this genus. Then we carried out transcriptome sequencing using the leaves from two individuals of Guizhou Population and two individuals of Chongqing Population respectively, and added them to the recently published large data set of orthologous nuclear genes screened from the transcriptomic data of 111 *Oreocharis* species to reconstruct the phylogeny of the genus including the sus-

pected new species (Kong et al. 2022). The results suggested that the plants were nested within *Oreocharis* and the analysed material was phylogenetically distinct from other species. Here, *Oreocharis qianyuensis* Lei Cai, J.W.Yang & Q.Zhang is described and illustrated based on the morphological comparisons and molecular phylogenetic analyses.

Materials and methods

We measured and recorded the morphological characters at least from more than ten mature individuals at flowering and fruiting from Guizhou and Chongqing populations. In addition, four relatively young leaves from each of the four individuals (two from Guizhou population and two from Chongqing population) were collected and sent to Novogene Technology Co., Ltd. for transcriptome sequencing. After filtering the low-quality reads, the remaining clean reads were used for denovo assembling with the package Trinity v2.11.0 (Grabherr et al. 2011). Referring to the published data consisting of 574 orthologous genes and including 111 *Oreocharis* species (Kong et al. 2022), we extracted the corresponding orthologous genes and added them to the data set for phylogenetic reconstruction. Phylogenetic tree was inferred based on the data set of the concatenated genes using maximum likelihood (ML) in RAxML v8.0.X (Stamatakis 2014) with parallel computation employing 100 threads on a server (ThinkSystem SR860). The parameters were set as GTR substitution model and a random starting tree with all others left as default. 100 bootstrap replicates were used to assess the robustness of the branches in the ML tree.

Data availability statement

The transcriptome data of four individuals in this study are openly available from NCBI: https://www.ncbi.nlm.nih.gov/sra/PRJNA813939 (the two individuals from Chongqing population) and https://www.ncbi.nlm.nih.gov/sra/PRJNA861104 (the two individuals from Guizhou population).

Results

For the 574 target nuclear orthologous genes, 566, 566, 561, 567 nuclear orthologous genes were screened out from each of the four transcriptomes (*Oreocharis qianyuensis*_CQ1, *O. qianyuensis*_CQ2, *O. qianyuensis*_GZ1, *O. qianyuensis*_GZ2), respectively, and one gene failed to be obtained from any of the four individuals. Hence 573 genes were included and the concatenated matrix had a length of 839193 bp. The matrix contained 376988 variable sites and 203260 parsimony informative sites, with an overall average GC content of 44.39%. The phylogenetic analyses using ML showed that the four individuals of the new species were clustered together and they in turn were grouped with *O. fargesii* (Franch.) Mich. Möller & A. Weber with full support (BS=100%), followed by *O. rubrostriata* F. Wen & L.E. Yang (BS=100%) in a lineage in *Oreocharis* (Fig. 1).

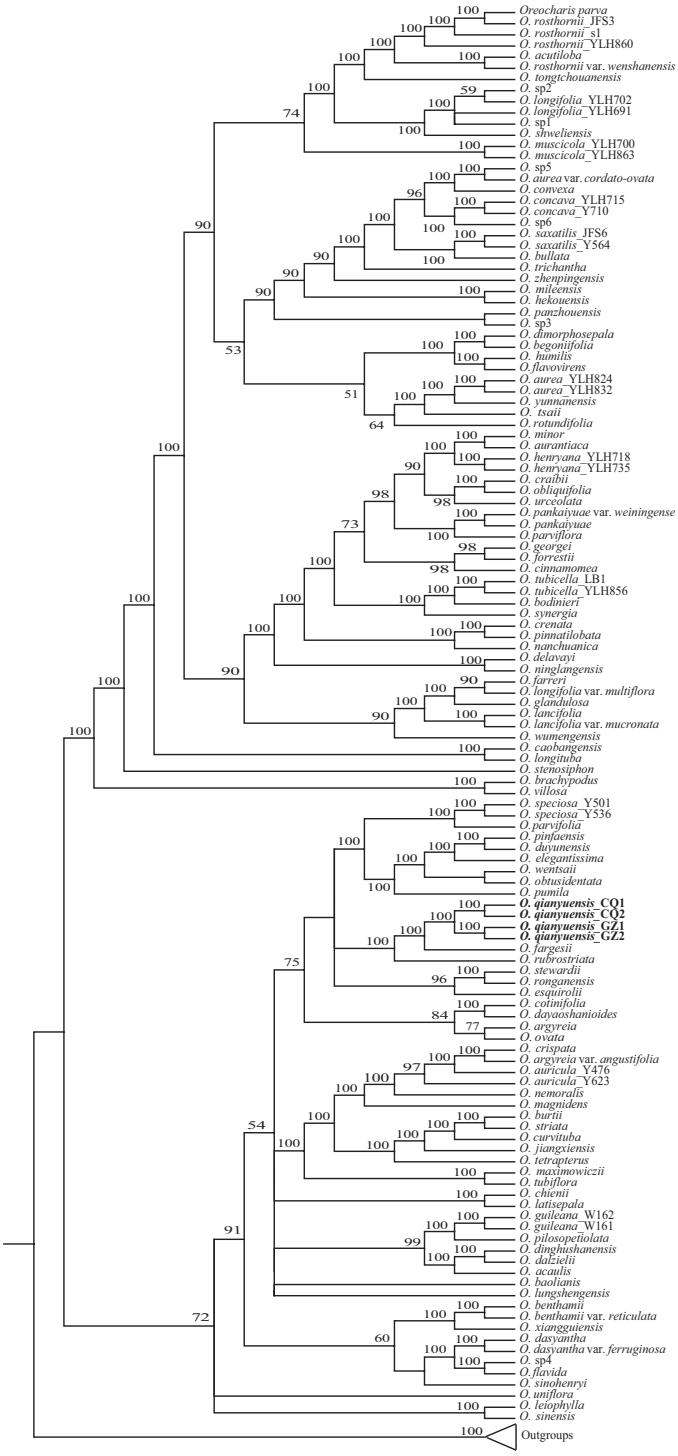

Figure 1. A maximum likelihood (ML) phylogeny of *Oreocharis* based on the concatenated data set of 573 loci with bootstrap support values (> 50%) shown below or above the branches around the corresponding nodes.

Taxonomic treatment

Oreocharis qianyuensis **Lei Cai, J.W.Yang & Q.Zhang, sp. nov.**
urn:lsid:ipni.org:names:77308124-1
Figs 2–5

Diagnosis. The new species is morphologically most similar to *Oreocharis fargesii* (Franch.) Mich. Möller & A. Weber and *O. nanchuanica* (K.Y. Pan & Z.Y. Liu) Mich. Möller & A. Weber in the color, shape and structure of flowers, but differs from the latter two in its inconspicuously petiolate, obovate to flabellate leaf blade with adaxially surface sparsely rust-brown pubescent to glabrescent, abaxially densely rust-brown villous, apex rounded, base extending downward into a wing shape, margin crenate, and the peduncle and pedicel densely glandular pubescent. A comparison of morphological differences between the related species is provided in Table 1.

Type. China, Guizhou Province: Kaili City, Dafengdong Town, Shuangjiangkou Village, Taiyanghe, 26°42'30"N, 107°49'32"E, elev. ca. 845 m, on the surfaces of rocks under the thicket, in flowering, 15 July 2020, Jia-Wen Yang et al. CL2020247 (Holotype: KUN!; Isotypes: KUN!, P!).

Description. Perennial herb, rhizome short. Leaves 4–7, basal; without petiole or extremely inconspicuous petiole, leaf blade obovate to flabellate, 3.0–12 × 2.0–8.5 cm, adaxially sparsely rust-brown pubescent to glabrescent, abaxially rust-brown villous, densely along veins, lateral veins 3–6 on each side of midrib, apex rounded, obtuse to nearly truncate, base extends downward into wing shape, basally enclosed with dense and long rust-brown villous tuft, margin crenate. Cymes axillary 2–5, 2–12-flowered per inflorescence; peduncle 4.5–14 cm long, cover with rust-brown villous and densely glandular pubescent, basally enclosed with dense and long rust-brown villous tuft; bracts 2, linear triangle to lanceolate, margin entire, 3.0–4.5 × 1.6–2.0 mm, outside rust-brown villous; pedicel 2.0–5.0 cm long, densely glandular pubescent. Calyx 5-parted to base, lobes equal, lanceolate triangle, 3.0–5.0 × 1.0–1.4 mm, margin denticulate, outside rust-brown villous, inside glabrous. Corolla brownish red to dark purple, 7–12 mm long, outside and inside glabrous, tube campanulate, 5–8 mm long, 4.5–5.5 mm in diameter at the widest position; limb 2-lipped; adaxial lip 2-lobed from the middle, lobes semiorbicular, 3.5–4.5 × 2.5–3 mm, abaxial lip 3-lobed to base, lobes semiorbicular, 4–4.5 × 3–3.5 mm. Stamens 4, 4.5–6 mm long, adnate to corolla 2–2.5 mm from base; filaments linear, glabrous; anthers broadly ovate, 2-loculed, coherent in pairs, connective glabrous; staminode 1,ca. 0.5 mm long, inserted ca. 1 mm from base. Disc 1–1.5 mm high, yellow, margin undulate. Pistil 5–8 mm long; ovary long cylindrical, glabrous, 3.0–4.5 mm long; style ca. 2.0–3.5 mm long, glabrous; stigma orbicular, emarginate in the middle, slight bilobed when dry. Capsule linear, glabrous, 2.0–2.8 cm long, 2–3 mm in diameter, dehiscing predominantly on one side.

Phenology. Flowering from July to August in Guizhou and from September to October in Chongqing; time of fruiting unknown.

Etymology. The specific epithet '*qianyuensis*' refers to the known distribution at the time of publication in Guizhou and Chongqing in China. Qian is an alternative name for Guizhou and Yu is an alternative name for Chongqing.

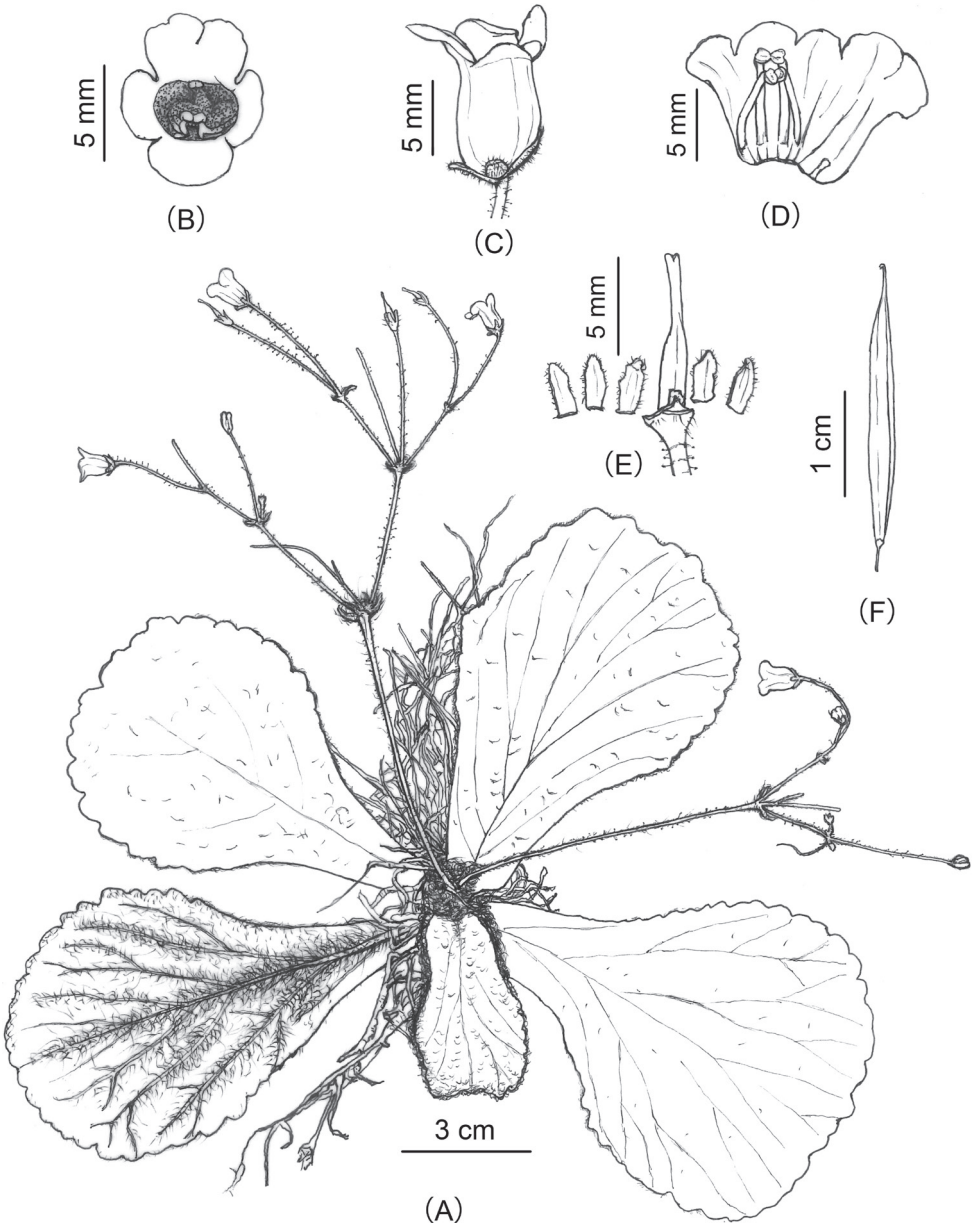

Figure 2. *Oreocharis qianyuensis* sp. nov. **A** habit **B** front view of a flower **C** side view of a flower **D** opened corolla showing stamens and staminode **E** pistil with disc and calyx **F** old fruit. Drawn by Xuan-Lin Zhu.

Vernacular name. The Chinese name of the new species is "Qian Yu Ma Ling Ju Tai" (黔渝马铃苣苔). The first two characters mean this species is distributed in Guizhou and Chongqing, and the last four characters represent the Chinese name of the genus *Oreocharis*.

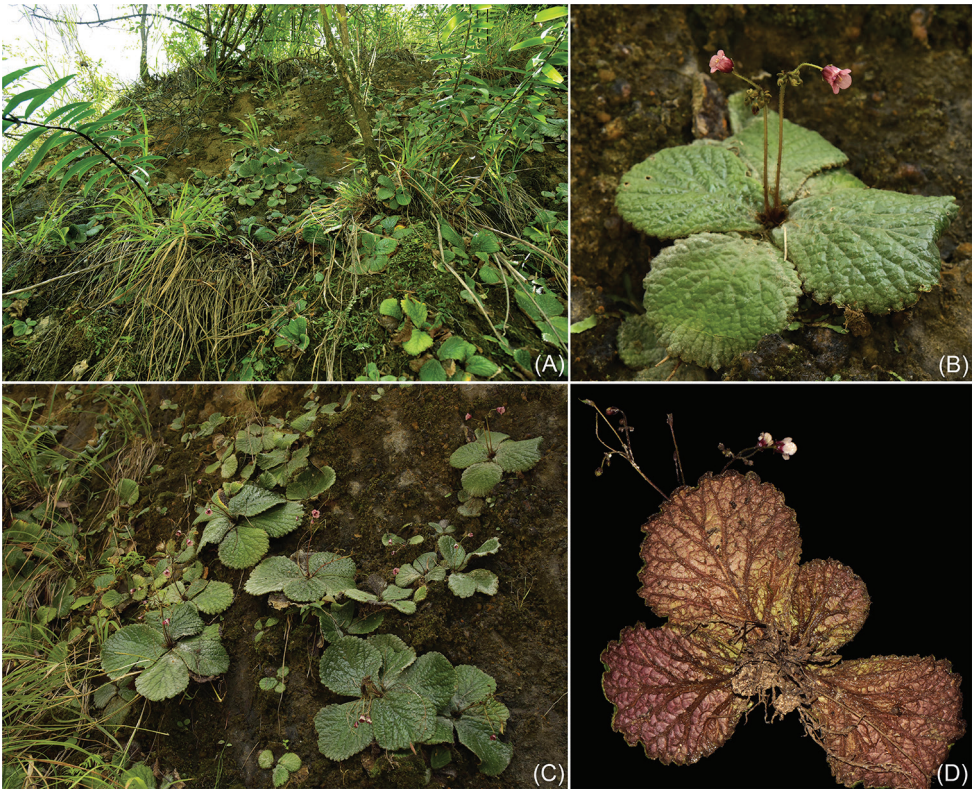

Figure 3. *Oreocharis qianyuensis* sp. nov. (Population in Guizhou) **A, C** habitat **B, D** plants with flowers.

Distribution and conservation status. *Oreocharis qianyuensis* was observed to grow on the surfaces of rocks under forest in karst region in Kaili City, Guizhou, and on limestone rock surface or crevices under deciduous forests in Pengshui County, Chongqing. The species is currently known from one population of ca. 2000 individuals within 5500 m^2 (AOO) in Guizhou and one population of ca. 300 individuals within 1000 m^2 (AOO) in Chongqing. Since no special surveys were carried out for its distribution, and the threat is that the population is close to roadside in Guizhou and possible continuous drought in Chongqing, so it is very likely to be damaged or excavated, so this species was provisionally considered to be Endangered [EN B2ab(iii)] in terms of IUCN Red List categories and criteria (IUCN 2022).

Specimens examined. China. Chongqing: Pengshui County, Hanjia Town, on rock walls, 29°1'90.94"N, 108°13'23.16"E, elev. 290 m, 4 October 2021, Si-Rong Yi YSR9297 (Paratypes: IBK!).

Taxonomic affinities

The molecular evidence (phylogenetic tree) clearly supports that this new species belongs to the genus *Oreocharis* s.l. (Fig. 1). Our phylogenetic results presented here

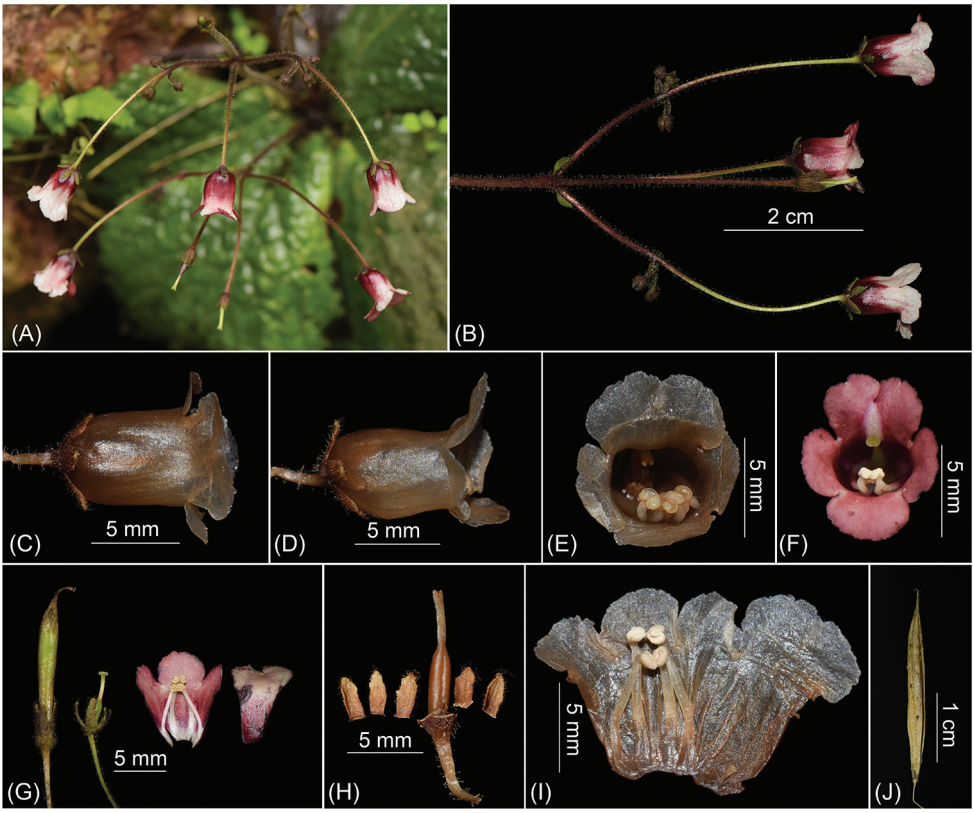

Figure 4. *Oreocharis qianyuensis* sp. nov. (Population in Guizhou) **A, B** inflorescence **C** top view of flower **D** side view of flower **E, F** front view of flowers **G** young fruit, pistil with disc and calyx, opened corolla showing stamens and staminode **H** pistil with disc and calyx **I** opened corolla showing stamens and staminode **J** old fruit.

are congruent with the results presented by Chen et al. (2020), and here, we choose to support the decision of removing the genus *Bournea* from the enlarged *Oreocharis*. Although the latest conclusions from the phylogenetic study of *Oreocharis* s.l. by Lv et al. (2022) are different, which may be related to the data these authors used providing a different view of early and rapid evolutionary radiation of the *Oreocharis*. Further work is needed to clarify these incongruent results. Another important aspect for us is to classify this species into this genus based on some characteristics of its flowers and capsules, such as: four stamens coherent in pairs, anther thecae not confluent, capsule dehiscent on one side (Wang et al. 1990, 1998; Li and Wang 2005). *O. qianyuensis*, *O. fargesii* and *O. nanchuanica* have characters shared with the previously recognized genus *Isometrum* Craib based on the anthers attached in pairs, corolla purple, tube campanulate and not swollen (Pan 1986; Wang et al. 1990, 1998; Pan and Liu 1995; Li and Wang 2005). *O. qianyuensis*, *O. fargesii* and *O. nanchuanica* were listed

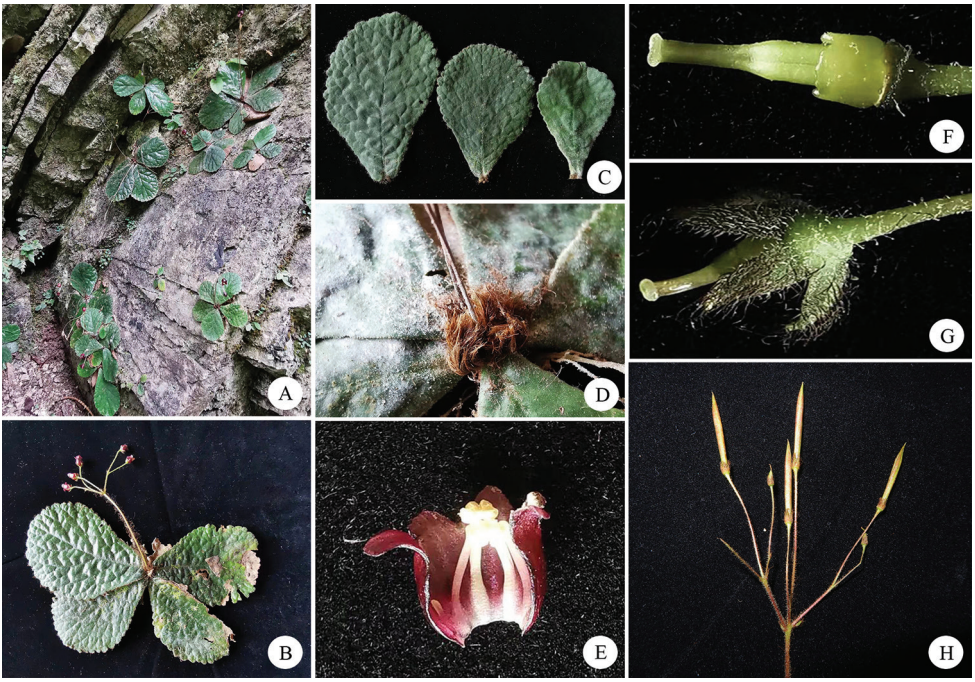

Figure 5. *Oreocharis qianyuensis* sp. nov. (Population in Chongqing) **A** habit **B** plant with flowers **C** leaves **D** pilose tuft **E** opened corolla with stamens and staminode **F** pistil and disc **G** calyx with pistil **H** infructescence.

Table 1. Morphological comparison among *Oreocharis qianyuensis* sp. nov., *O. fargesii* and *O. nanchuanica*.

Characters	*O. qianyuensis*	*O. fargesii*	*O. nanchuanica*
Leaf blade	obovate to flabellate, adaxially sparsely rust-brown pubescent to glabrescent	obovate to narrowly oblong, sometimes oblique, adaxially appressed puberulent-strigillose	ovate, adaxially appressed gray puberulent
apex	rounded, obtuse to nearly truncate	rounded to obtuse	acute
margin	crenate	serrate near apex	serrate
base	extends downward forming a wing	cuneate	cordate
Peduncle indumentum	rust-brown villous and densely glandular pubescent	rust-brown villous, glabrescent	brown puberulent and glandular puberulent
Petiole and indumentum	almost invisible	to 1.5 cm long, rust-brown villous	to 8.3 cm long, brown pubescent
Bract indumentum	rust-brown villous	rust-brown villous	brown puberulent
Pedicel indumentum	rust-brown villous and glandular pubescent	rust-brown villous, glabrescent	brown puberulent and glandular puberulent
Calyx lobes	lanceolate triangle, margin denticulate	lanceolate to triangular, margin entire	lanceolate, margin entire
Corolla tube	campanulate, not constricted at throat	campanulate, not constricted at throat	urceolate, constricted at throat
Staminode	ca. 0.5 mm long	ca. 2 mm long	ca. 0.7 mm long

as members of Sect. *Pachysiphon* K.Y. Pan and can be distinguished from the other *Isometrum* species by their corolla tubes short and thick (e.g., 6–12 mm long, 5–9 mm in the diameter, the length is 1.2–1.5 times the width), outside glabrous (Pan 1986; Wang et al. 1990, 1998; Li and Wang 2005). *O. qianyuensis* morphologically resembles *O. fargesii* and *O. nanchuanica* in the purple flowers, 5-parted to the base of calyx, campanulate corolla and coherent in pairs of anthers, however, *O. qianyuensis* can be easily differentiated from them by the shape, margin, apex and base of leaf blade shape, indumentum characters of the inflorescence. Detailed diagnostic characters of the new species are listed and compared with other morphologically similar species in Table 1.

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RESEARCH ARTICLE

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Coptis huanjiangensis, a new species of Ranunculaceae from Guangxi, China

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Abstract

Coptis huanjiangensis, a new species of Ranunculaceae distributed in the valleys of Jiuwanshan National Natural Reserve in Huanjiang county (Guangxi, China), is described and illustrated for the first time based on morphological and plastome sequences data. It differs from *C. chinensis*, *C. deltoidei* and *C. omeiensis* mainly by having notably longer petiole, scape, bigger leaf blade with lobes obviously remote and robust rhizomes without stolons. Phylogenetic analyses support that *C. huanjiangensis* is sister to *C. omeiensis* and *C. deltoidei*.

Keywords

China, *Coptis*, Guangxi, new taxa, taxonomy

Introduction

The genus *Coptis* Salisb. (Ranunculaceae), containing 15 recognized species, is one of the most medicinally important genera in China and demonstrates a classical eastern Asian and North American disjunct distributional pattern. According to the morphology, especially flower and leaf, genus representatives distributed in China had been

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classified into six species and one variant, i. e., *C. chinensis* Franch. (endemic to SW China), *C. chinensis* var. *brevisepala* W. T. Wang & P. K. Hsiao (endemic to SE China), *C. deltoidei* C. Y. Cheng & P. K. Hsiao (endemic to Sichuan, China), *C. omeiensis* (Chen) C. Y. Cheng (endemic to Sichuan, China), *C. quinquefolia* Miq. (distributed in Taiwan province and Japan), *C. quinquesecta* W. T. Wang (endemic to Yunnan, China) and *C. teeta* Wall. (endemic to SW China) (Tamura 1995; Dezhi and Robinson 2001).

All these species have branched rhizomes, basal and long petioled leaves splitting into three–five segments, small and actinomorphic flowers. Most of these species are less than 30 cm in height and grow in shady places in forest valleys at an altitude of 600–2500 meters. Due to the richness in benzylisoquinoline alkaloids, various *Coptis* species have been used in China ethnomedicine, and three of them, *C. chinensis*, *C. deltoidea*, and *C. teeta*, are used as official Huanglian 'Weilian', 'Yalian' and 'Yunlian' in the Chinese Pharmacopeia respectively (Liu et al. 2021; Wang et al. 2022).

The south-western limestone area is one of the biodiversity centers in China, especially in Guangxi (López-Pujol et al. 2011; Huang et al. 2019). During the Fourth National Survey of Chinese Materia Medica Resources in Guangxi (August, 2018), we found an unusual species of *Coptis* with an outstanding plant size and robust rhizomes that are distinctive from other species in Jiuwanshan National Natural Reserve, Huanjiang county. Subsequently, an in-depth field investigation, detailed morphological observations and phylogenetic reconstruction by plastomes were carried out. The comprehensive morphology and molecular results suggested that it is a new species, which is described as follows.

Materials and methods

Taxon sampling and DNA extraction

Samples of the new species were collected in the field and 12 related species of *Coptis* (a total of nineteen accessions) were obtained from the herbarium of PE (Herbarium, Institute of Botany, CAS, Beijing, China) and CMMI (Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China). *Asteropyrum peltatum* and *A. cavaleriei* were taken as outgroups, and the plastome sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov) with accession numbers MG734862.1 and MG734861.1, respectively. Sample information is listed in Suppl. material 1.

Total genomic DNA was extracted from specimens using a modified cetyl trimethyl ammonium bromide (CTAB) method and purified with the Genebetter DNA clean-up kit (GeneBetter Biotech Corporation, Beijing, China) (Li et al. 2013). All the DNA and molecular material were deposited in the herbarium of the Institute of Chinese Materia Medica (CMMI).

Plastome sequencing and assembly

PE150 sequencing was conducted on an Illumina HiSeq XTen platform at Novogene (Tianjin, China). The raw data of the PE150 sequencing were filtered using the Trim-

momatic 0.39 software to obtain high-quality reads (Bolger et al. 2014). The de novo assembly of the high-quality reads was performed by GetOrganelle v1.7.5 with the following settings: -F embplant_pt, -R 15 and -K 105 (Jin et al. 2020). Ambiguous regions and four junctions between IRs and SCs in the plastid were confirmed manually in Geneious v8.1 (Wang et al. 2021; Dong et al. 2022).

Phylogenetic reconstruction

A total of 23 plastid sequences were aligned using the MAFFT online service and manually adjusted using MEGA X (Kumar et al. 2018; Katoh et al. 2019). And ambiguous regions were trimmed by the Gblocks 0.91b program (Castresana 2000). Phylogenetic reconstruction was carried out using the maximum likelihood (ML) and Bayesian inference (BI) methods in PhyloSuite (Zhang et al. 2020). The program ModelFinder was used to select the best-fit model according to the Bayesian information criterion (Kalyaanamoorthy et al. 2017). The ML tree was inferred using IQ-TREE with the TVM+F+R2 model and 5,000 ultrafast bootstraps (Nguyen et al. 2015). The BI tree was implemented with the GTR+F+I+G4 model and the Markov chain Monte Carlo (MCMC) chains were run for 1,000,000 generations. The trees were sampled every 1000 generations and the initial 25% were discarded as burn-in. Trees were visualized in FigTree v1.3.1.

Results

Phylogenetic analysis

To better clarify the evolutionary position of the new species within *Coptis*, phylogenetic analyses were constructed based on the 23 complete plastid sequences with *Asteropyrum* as outgroups. The aligned sequences were 154,249 bp in length for analysis. The topologies of the ML and BI trees were identical with all the branches strongly-supported (ML BS = 100 and BI PP = 1) (Fig. 1). All the accessions of *Coptis* formed a monophyletic group with 100% support. The two samples of the new species (*C. huanjiangensis* sp. nov.) were clustered into one clade and sister to the clade consisting of *C. omeiensis* and *C. deltoidei*.

Taxonomic treatment

Coptis huanjiangensis **L.Q.Huang, Q.J.Yuan & Y.H.Wang, sp. nov.**
urn:lsid:ipni.org:names:77308125-1
Figs 2, 3

Diagnosis. *Coptis huanjiangensis* is morphologically similar to *C. chinensis*, *C. deltoidei* and *C. omeiensis*, but it differs from these species by having notably longer petioles (15–40 cm), scapes (20–32 cm), and bigger leaf blades with lobes remote obviously.

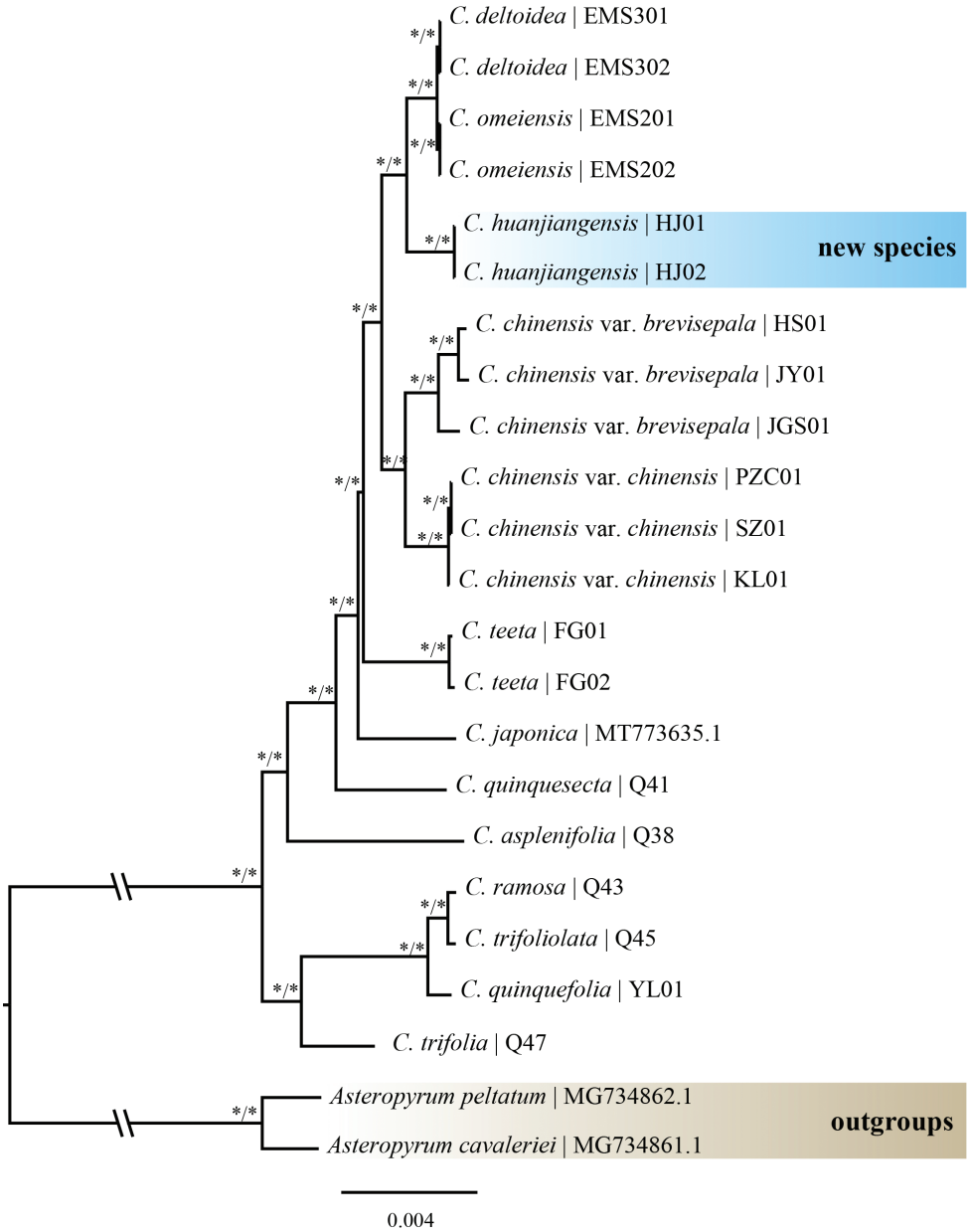

Figure 1. The Maximum likelihood and Bayesian inference tree of *Coptis huanjiangensis* and related species. Numbers on branches correspond to Maximum likelihood bootstrap support (BS) and Bayesian posterior probability (PP), respectively. An asterisk (*) indicates BS = 100% or PP = 1.0.

Type. China. Guangxi: Huanjiang County, Jiuwanshan National Natural Reserve, 1082 m, 25°12'1.07"N, 108°38'28.32"E, valleys, 24 January 2022, Yiheng Wang HJ220124I02 (holotype CMMI!, isotype CMMI!) (Suppl. materials 2, 3).

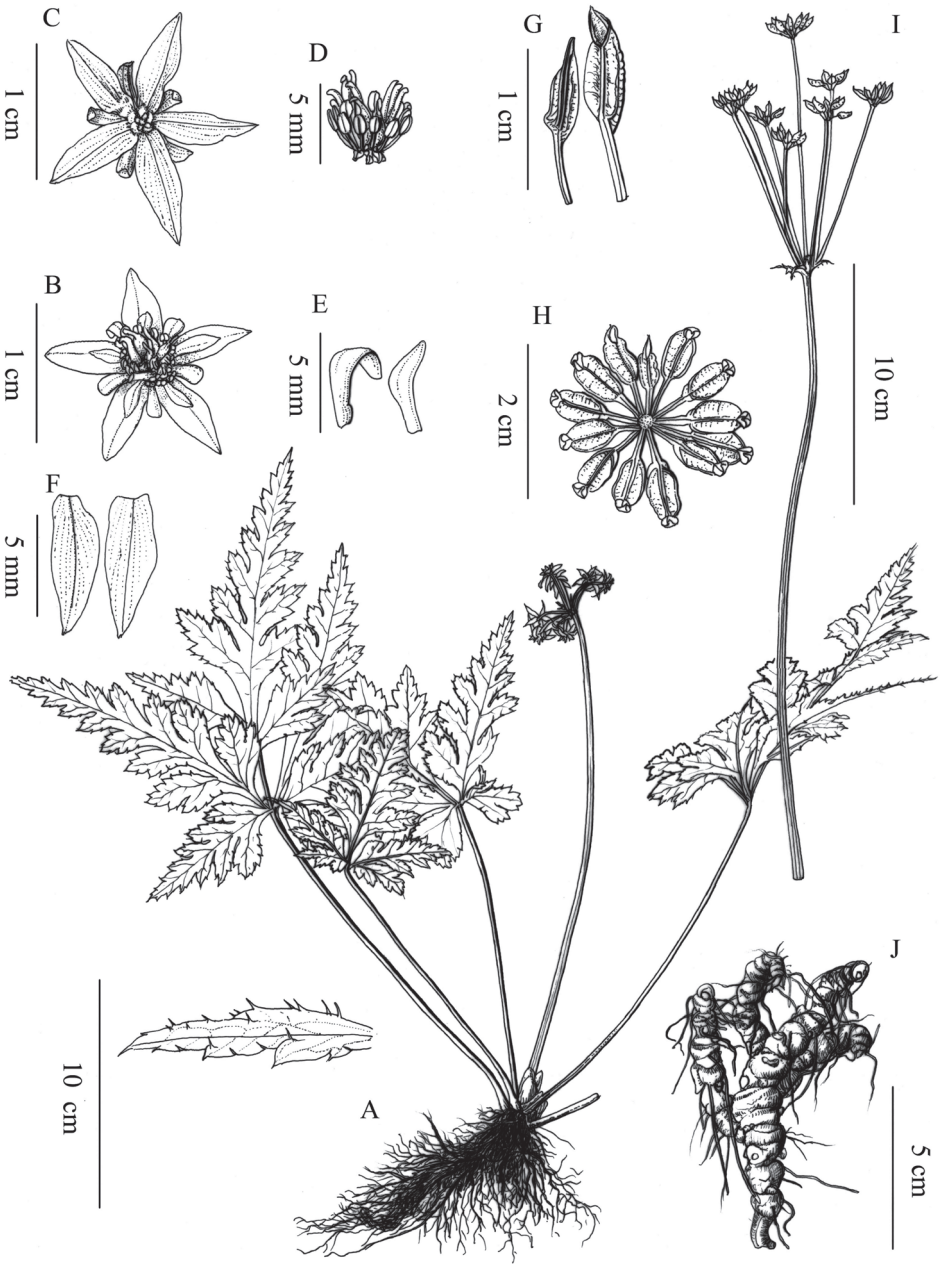

Figure 2. *Coptis huanjiangensis* L.Q.Huang, Q.J.Yuan & Y.H.Wang, sp. nov. **A** habit **B** flower, frontal view **C** flower, back view **D** opened corolla **E** petals **F** sepals **G, H** follicles **I** inflorescence **J** root. Drawn by Yingbao Sun.

Description. Herbs perennial, rhizomes branched, without stolons. Leaves basal, petiole 15–40 cm, glabrous. Leaf blade ovate-triangular, 12–22 × 9–22 cm, three-sect, papery to subleathery, abaxially glabrous, adaxially nearly glabrous on veins, base cor-

Figure 3. *Coptis huanjiangensis* L.Q.Huang, Q.J.Yuan & Y.H.Wang, sp. nov. **A** species habitat (Jiuwanshan National Natural Reserve, Huanjiang County, Guangxi, China) **B** plant in florescence stage **C** plant in fruiting stage **D** leaf, frontal and back view **E** margin with sparsely upturned spiny hairs **F–H** inflorescence and flowers **I–K** follicles and seeds **L** root. Photos by Yiheng Wang, Jingyi Wang & Qiang Mao.

date, margin with sparsely upturned spiny hairs; central segment petiolulate (petiole 2.5–4 cm), ovate-rhombic, 11–18 × 7–14 cm, deeply four-ten-lobed, lobes remote, ultimate lobes margin acute serrate, apex acute or obtuse; lateral segments similar to or slightly shorter than the central one, obliquely ovate, unequally two-parted. Scapes one to several, erect, longer or shorter than the leaves, 20–32 cm tall, glabrous, sulcate. Inflorescences terminal, often monochasial, five-ten-flowered; flowers small, actinomorphic, bisexual; bracts lanceolate, palmately divided. Sepals five or six, greenish or redish yellow, long ellipsoid or lanceolate, 5.5–9.0 × 1.8–3.5 mm, sparsely puberulous. Petals spatulate, 2–5 mm long, glabrous, apex rounded to obtuse, 1/3–1/2 as long as sepals. Stamens numerous, glabrous, 2–4 mm-long, outer ones slightly shorter than petals. Pistils 8–14, 3–5 mm long; follicles 4.5–9.0 mm long, stipitate; seeds ellipsoid, ca. 1–2 mm long, brown.

Distribution and habitat. This species has only been found in the valleys of Jiuwanshan National Natural Reserve, Huanjiang County up until now. It grows in shaded places in valleys at 800–1200 m. a. s. l.

Etymology. The specific epithet is derived from the type locality, Huanjiang County, Guangxi.

Phenology. The species was observed flowering in February – March and fruiting in April–June.

Note. There are seven species and one variant of *Coptis* distributed in China. An identification key is presented below.

Key to the species of *Coptis* in China

1 Leaves five-sect **2**
– Leaves three-sect **3**
2 Rhizome robust; leaf blade 5.5–14 cm wide, central segment pinnately divided, apex Attenuate ..... ***C. quinquesecta***
– Rhizome slender; leaf blade 2–6 cm wide, central segment three-lobed, apex acute ..... ***C. quinquefolia***
3 Leaf blade lanceolate to narrowly ovate; lateral segments 3–3.5 × shorter than central segment; sepals linear-lanceolate ..... ***C. omeiensis***
– Leaf blade ovate to ovate-triangular; lateral segments slightly shorter than central segment; sepals lanceolate, elliptic, or narrowly ovate **4**
4 Petals spatulate **5**
– Petals lanceolate to linear-lanceolate **6**
5 Inflorescences three–five-flowered ..... ***C. teeta***
– Inflorescences more than five-flowered ..... ***C. huanjiangensis***
6 Leaf segment lobes ± contiguous to each other; stamens ca. 1/2 as long as petals ..... ***C. deltoidei***
– Leaf segment lobes remote; outer stamens slightly shorter than petals **7**
7 Sepals 9–13 mm, ca. 2 × as long as petals ..... ***C. chinensis* var. *chinensis***
– Sepals ca. 6.5 mm, slightly longer than petals.. ***C. chinensis* var. *brevisepala***

Discussion

Plastoms have been extensively used in phylogeny reconstruction and species delimitation studies because of their moderate evolution rate and abundant phylogenetic information (Dong et al. 2021; Wang et al. 2021; Wang et al. 2022). The relationships of *Coptis* species were clearly resolved by phylogenetic studies. *Coptis huanjiangensis* possesses an independent phylogenetic position and is located in the clade formed by *C. chinensis*, *C. deltoidei*, and *C. omeiensis*. And the phylogenetic relationship of these four species is also supported by the morphological characters of these species in having a similar leaf blade shape (leaves three-sects), leaf blade texture (papery to subleathery), and a long and erect scape with five to ten small actinomorphic flowers. However, *C. huanjiangensis* can be distinguished from the latter three species by having notably longer petioles (15–40 cm) (vs other species having petioles shorter than 18 cm), spatulate petals (vs lanceolate or linear in other species), bigger leaf blades with lobes obviously remote and robust rhizomes without stolons. The detailed comparison between *C. huanjiangensis* and close species is represented in Table 1. Herein, both morphological and molecular studies indicated that *C. huanjiangensis* is an independent species.

Table 1. Distinguishing features of *C. huanjiangensis* in comparison with other related species.

Characters	*C. huanjiangensis*	*C. deltoidei*	*C. omeiensis*	*C. chinensis* var. *chinensis*	*C. chinensis* var. *brevisepala*
Leaf blade	ovate-triangular, 12–22 × 9–22 cm, papery to subleathery	ovate, 4–16 × 5–15 cm, papery to subleathery	lanceolate to narrowly ovate, 6–16 × 3.5–6.3 cm, subleathery	ovate-triangular, 4–10 × 4–10 cm, papery to subleathery	ovate-triangular, 4–10 × 4–10 cm, papery to subleathery
Leaf margin	deeply 4–10 lobed, lobes remote	4–6 lobed, lobes ± contiguous to each other	7–14 lobed, lobes remote	deeply 3–5 lobed	deeply 3–5 lobed
Relationship of lateral segment and central segment in length	lateral segments similar to or slightly shorter than central one	lateral segments slightly shorter than central one	lateral segments 3–3.5 × shorter than central one	lateral segments slightly shorter than central one	lateral segments slightly shorter than central one
Petiole length	15–40 cm	6–18 cm	5–14 cm	5–12 cm	5–12 cm
Scape length	20–32 cm	slightly longer than leaves	15–27 cm	12–25 cm	12–25 cm
Sepal number	5 or 6	5	5	5	5
Sepal shape	long ellipsoid or lanceolate	narrowly ovate	linear-lanceolate	lanceolate	lanceolate
Petal shape	Spatulate	lanceolate	linear-lanceolate	linear-lanceolate	linear-lanceolate
The length ratio of sepal vs petal	ca. 2–3 times	ca. 2–3 times	ca. 2 times	ca. 2 times	sepal slightly longer than petals
Are there any stolons	No	Yes	Yes	No	No

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Supplementary material 1

Accession number of 23 sequenced or downloaded chloroplast genome in this study

Authors: Yiheng Wang

Data type: table (excel file)

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Link: https://doi.org/10.3897/phytokeys.213.96546.suppl1

Supplementary material 2

The photo of holotype stored at the CMMI with accession number HJ220124I02
Authors: Yiheng Wang, Jiahui Sun, Jingyi Wang, Qiang Mao, Wenpan Dong4, Qingjun Yuan, Lanping Guo, Luqi Huang
Data type: figure (JPG image)
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Link: https://doi.org/10.3897/phytokeys.213.96546.suppl2

Supplementary material 3

The photo of isotype stored at the CMMI with accession number HJ220124I01
Authors: Yiheng Wang, Jiahui Sun, Jingyi Wang, Qiang Mao, Wenpan Dong4, Qingjun Yuan, Lanping Guo, Luqi Huang
Data type: figure (JPG image)
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RESEARCH ARTICLE

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The floral morphology of four Chinese *Bambusa* species (Poaceae, Bambusoideae) previously described only from vegetative material

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Abstract

Due to their specialised flowering biology where frequent or even annual flowering is uncommon, reproductive materials of bamboos are not always available, so hampering taxonomic interpretation and research into other aspects. *Bambusa contracta*, *B. corniculata*, *B. cornigera* and *B. subtruncata* were established only based on vegetative materials and flowering or fruiting material has been hitherto unknown. The floral morphology of these four species is described for the first time and, correspondingly, epitypes are designated to support a more complete interpretation of the species.

Keywords

Bambuseae, epitype, pseudospikelet, taxonomy

Introduction

The subfamily Bambusoideae (Poaceae) includes forest grasses that number more than 1680 species in 127 genera classified into three tribes, viz., Olyreae, Bambuseae and Arundinarieae (Sungkaew et al. 2009; Triplett and Clark 2010; Zhang et al. 2012; Vorontsova et al. 2016; Clark and de Oliveira 2018). *Bambusa* Schreb. is a genus of

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Bambuseae including more than 100 species and is distributed in tropical and subtropical Asia (Xia et al. 2006). In China, there are about 80 *Bambusa* species of high practical and economic value (Jia et al. 1996; Xia et al. 2006).

Phylogenetic studies have shown that *Bambusa* is not monophyletic and it has complicated genetic relationships with *Dendrocalamus* Nees and *Gigantochloa* Kurz ex. Munro (Goh et al. 2010, 2011, 2013; Guo 2010; Zeng 2014; Qin 2019). Therefore, the reticulate alliance amongst these three genera has been called the *Bambusa-Dendrocalamus-Gigantochloa* complex (or 'BDG' complex) (Goh et al. 2010, 2011, 2013). The vegetative morphology of *Bambusa* and its allies can sometimes be rather confusing. The main characters distinguishing these three genera are found in their floral morphology. *Bambusa* (except subgenus *Dendrocalamopsis* L.C. Chia & H.L. Fung) has elongate and disarticulating rachilla segments which in *Dendrocalamus*, *Gigantochloa*, as well as *Bambusa* subgenus *Dendrocalamopsis*, are absent (Wong 1995; Xia et al. 2006). In addition, *Gigantochloa* consistently features a firm filament tube while the other two typically have separate filaments (Wong 1995). Lack of detailed knowledge on the flowering characters, combined with the long flowering interval and frequent clump death after reproduction has resulted in floral material of a number of these bamboo species being unavailable. Thus, it is valuable to document the reproductive characteristics for every bamboo species, which benefits identification and the clarification of relationships amongst various taxa.

Bambusa contracta L.C. Chia & H.L. Fung, *B. corniculata* L.C. Chia & H.L. Fung, *B. cornigera* McClure and *B. subtruncata* L.C. Chia & H.L. Fung were published, based only on vegetative materials (McClure 1940; Chia and Fung 1981). The type of *B. cornigera* was collected from Wuzhou, Guangxi in 1928 (McClure 1940). The types of *B. subtruncata* (introduced from Xinyi, Guangdong), *B. contracta* and *B. corniculata* (introduced from Dongxing, Guangxi) were collected from cultivated material in the South China Botanical Garden (Chia and Fung 1981). Up to now, studies about *B. contracta*, *B. corniculata*, *B. cornigera* and *B. subtruncata* mainly focused on resource collection, protection, utilisation and evaluation of growth characteristics (Qiu et al. 2006; Wu 2008; Huang et al. 2013; Huang et al. 2014; Wu 2014; Huang et al. 2017), introduction and reproduction (Jin and Wang 1990; Zhang 2008), leaf epidermis micromorphology (Wang et al. 2002; Tao 2021), chromosome characteristics (Li et al. 2001; Jia et al. 2016) and vascular bundle morphology (Wen and Chou 1984; Fang et al. 1998). Studies on their floral morphology are unknown.

In this study, the pseudospikelet and floral morphology of these four *Bambusa* species are compared with those of closely-related species. Epitypes are designated here to support a more complete representation of the species.

Materials and methods

All flowering materials were collected from plants cultivated in the Bambusetum of South China Botanical Garden, Chinese Academy of Sciences. They are deposited in

the IBSC Herbarium of the South China Botanical Garden, as *Qin & Ni QQM 16* (*B. subtruncata*), *QQM 39* (*B. contracta*), *QQM 40* (*B. corniculata*) and *QQM 41* (*B. cornigera*). Dissection was carried out using a stereomicroscope (Olympus SZX16). Morphological comparisons were based on characters recorded in the relevant literature including protologues, as well as the study of type specimens. The specimens and photographic images were used for making descriptions.

New epitypes and descriptions including flowering material

***Bambusa contracta* L.C. Chia & H.L. Fung (1981:376)**
Figs 1, 2

Holotype. China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences (plants originally from Guangxi, Dongxing), 15 August 1978, *Nan-Zhu 2061* (IBSC!).

Epitype (designated here). China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences, 31 March 2016, *Qin & Ni QQM 39* (IBSC!).

Description including flowering material. Culms 5–6 m tall, 2–3 cm diameter, erect, apically drooping; internodes 34–57 cm long, plain green, initially slightly white waxy, with sparse long white hairs; wall ca. 3 mm thick; nodes flat, glabrous. Branch complement at mid-culm with a slightly dominant central branch and many subequal branches, those at culm base without thorny branchlets. Culm leaf sheath slightly white waxy, usually glabrous or basally dark brown hispid, apically arched with asymmetric sides; auricles unequal, oblong to lanceolate, undulate, wrinkled, larger auricle slightly slanted downwards, ca. 3 cm long, 0.7–1 cm wide, ca. 2 times larger than smaller one, bristles on the margin undulate; ligule ca. 2 mm high, margin sparsely dentate; blade erect, narrowly ovate, ca. 2/5 as long as sheath, base rounded, slightly overlapping with auricles for 2–3 mm, ca. 1/4 as wide as sheath apex, apex involute and acuminate. Foliage leaf sheath glabrous; auricles subovate, margin with long bristles; ligule very low, margin sparsely dentate; blade linear to linear-lanceolate, 10–15 cm long, 1.3–1.5 cm wide, abaxial surface densely pubescent, adaxial surface glabrous. Pseudospikelets fasciculate at each node of flowering branches, linear, sessile, basally subtended by several bud-bearing bracts, 2.5–3.5 cm long; florets 4–6, middle 2–4 florets fully developed; prophylls 1–2 mm long, 2-keeled, keels sparsely ciliolate; bracts 2–4, ovate to oblong, 3–5 mm long, glabrous, apically ciliolate, adaxial surface apex puberulent, obscurely 0–5-veined, apex obtuse to acute, mucronate or not; rachilla disarticulating between florets, segments compressed, 1.5–3 mm long, glabrous, lower segments distally inflated, upper segments distally only slightly inflated; glumes 1–3, oblong, 6–7 mm long, glabrous, sometimes adaxial surface apex puberulent, obscurely 11–13-veined, apex obtuse to acute, mucronate or not; lemma oblong, 7–10 mm long, glabrous, abaxial surface purple-spotted, 19–21-veined, apex

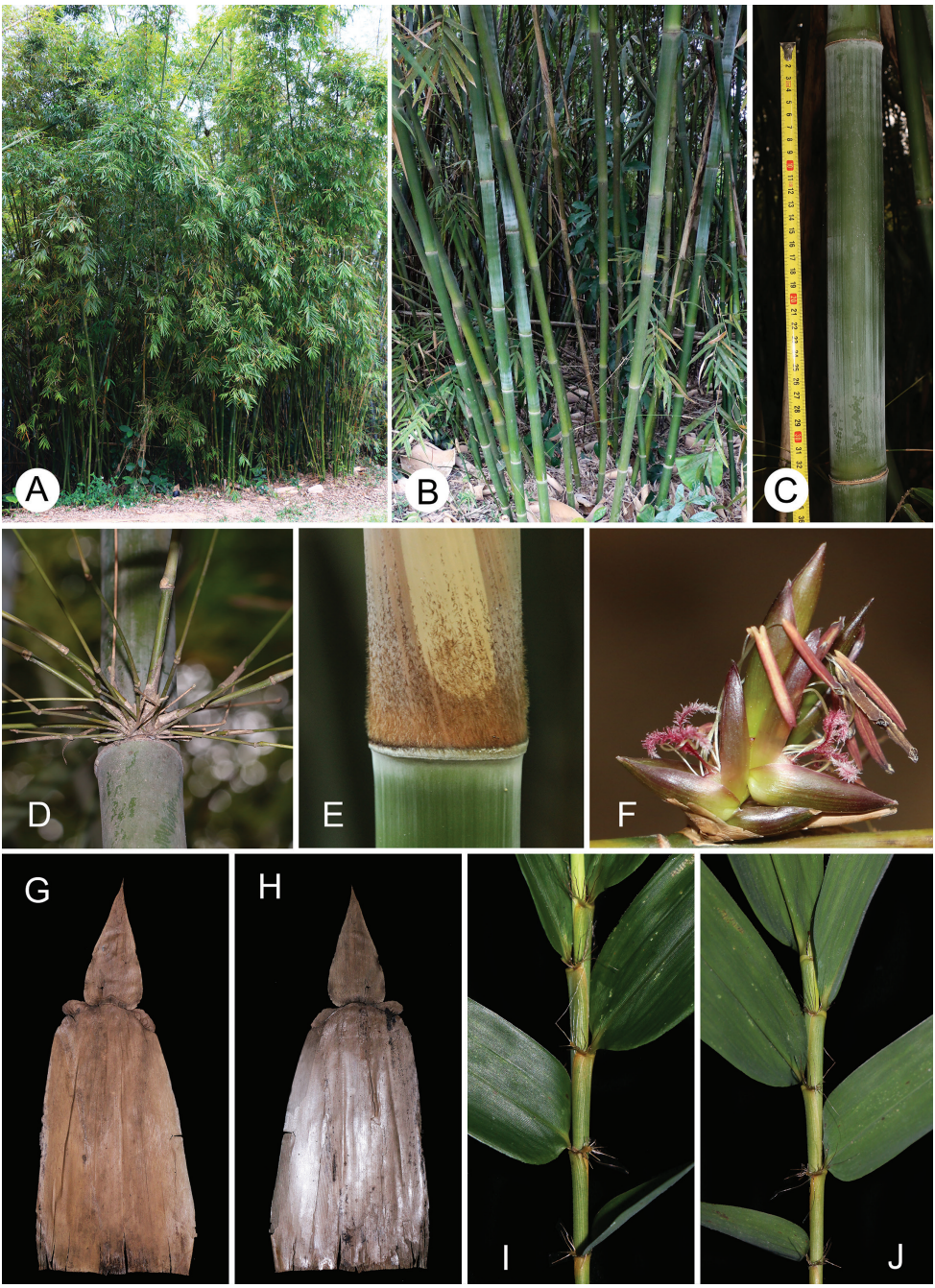

Figure 1. Vegetative morphology and pseudospikelets of *B. contracta* **A** clumps **B** clump base **C** culm internode **D** branch complement **E** culm node with velvety hairs **F** pseudospikelets **G** culm leaf (abaxial view) **H** culm leaf (adaxial view) **I** the distal part of a leafy branch (upper side) **J** the distal part of a leafy branch (lower side).

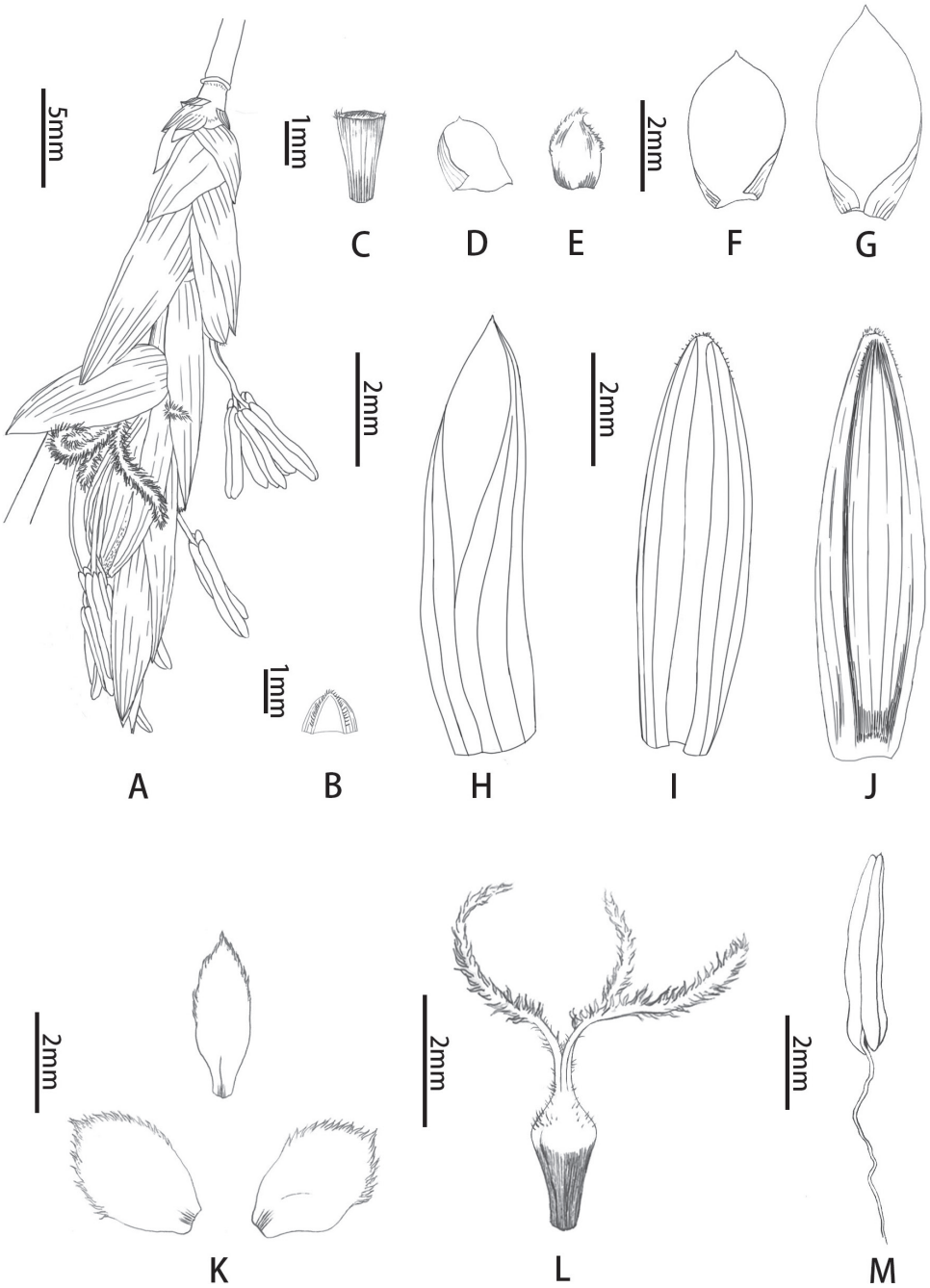

Figure 2. Floral morphology of *B. contracta* **A** pseudospikelet **B** prophyll (abaxial view) **C** rachilla segment **D** bud-bearing bract (adaxial view) **E** axillary bud subtended by bract in (**D**) **F, G** glumes (adaxial view) **H** lemma showing margins **I** palea showing margins **J** back of palea **K** lodicules **L** pistil **M** stamen. Drawn by Qiao-Mei Qin.

acute mucronate, calluses no more than 5 mm long, glabrous; palea 9–11 mm long, 2-keeled, keels apically ciliolate, 4–5-veined between keels, each side 2-veined, apex truncate; lodicules 3, apex ciliate, anterior two broadly oblong, 2–3 mm long, posterior one narrowly oblong, 2–3 mm long; stamens 6, filaments filiform, anthers brown to yellowish, 5.5–7 mm long, apex retuse; ovary obovoid, 1.8–2 mm long, apex hispidulous, styles 3, 0.5–0.7 mm long, stigmas 3, plumose, 4–5 mm long.

Note. We made a comparison between this species and its relative, *Bambusa textilis* McClure. The result shows that they share similar floral characters. *B. contracta* differs from *B. textilis* in having 4–5 veins between palea keels (versus 10), 2 veins on each wing of the palea (versus 4) and 11–13 veins on the glumes (versus 21).

Bambusa corniculata L.C. Chia & H.L. Fung (1981:368)

Figs 3, 4

Holotype. China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences (plants originally from Guangxi, Dongxing), 15 August 1978, *Nan-Zhu 2599* (IBSC!).

Epitype (designated here). China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences, 31 March 2016, *Qin & Ni QQM 40* (IBSC!).

Description including flowering material. Culms ca. 8 m tall, 4–7 cm diameter, basally slightly zig-zag, apically drooping; internodes 20–32 cm long, basal ones conspicuously shorter, usually flat and shallowly grooved above branches, plain green, initially white waxy and with caducous sparse hispid hairs; wall ca. 8 mm thick; basal nodes with grey-white sericeous ring-like zones below and above sheath insertion, with short aerial roots. Primary branch bud horizontally elliptic, prophyll margins apically ciliate. Branch complement at lower culm nodes typically with only one branch bearing short, curved, weak, thorny branchlets; at mid-culm with 3 to several branches, central branch dominant. Culm leaf sheath glabrous, apex subtruncate, with a triangular protuberance on one shoulder; auricles unequal, lager auricle oblong or elliptic, ca. 8 mm wide, ca. 3 times larger than smaller one, margin with undulate bristles ca. 1 cm long; ligule ca. 3 mm high, short-fimbriate; blade erect, triangular or narrowly ovate, base 4/5 as wide as sheath apex. Foliage leaf sheath glabrous; auricles absent or tiny, semicircular to ovate, margin with undulate bristles; ligule very low, fimbriate; blade linear to lanceolate, 13–20 cm long, 1–2 cm wide, abaxial surface pubescent, adaxial surface glabrous. Pseudospikelets fasciculate at each node of flowering branches, linear, sessile, basally subtended by several bud-bearing bracts, 2–4 cm long; florets 7–9, middle 2–5 florets fully developed; prophylls ca. 4 mm long, 2-keeled, keels apically sparsely ciliolate; bracts 2–3, lanceolate, 4–6 mm long, glabrous, 1–9-veined, apex acute, mucronate or not; rachilla disarticulating between florets, segments compressed,

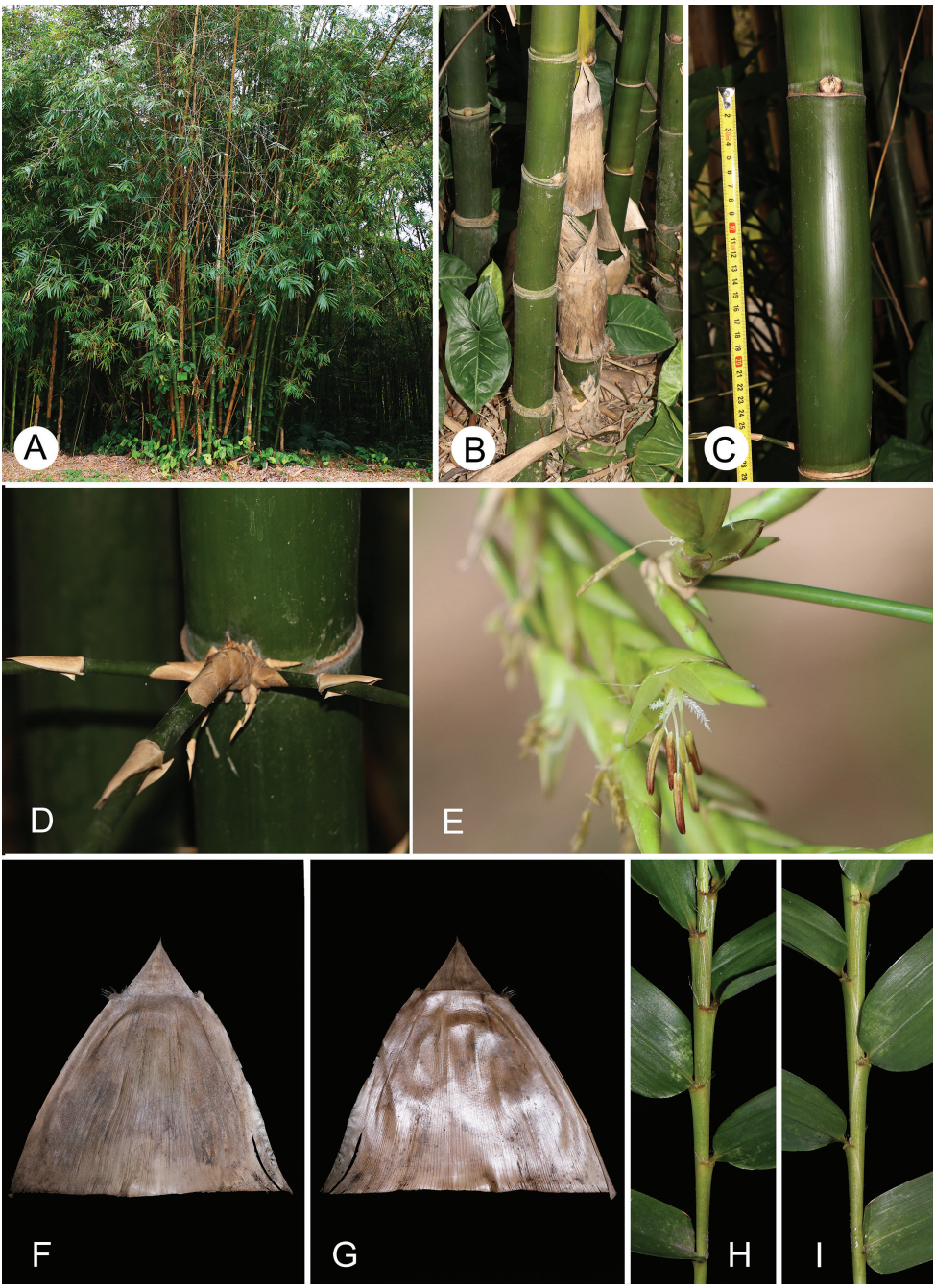

Figure 3. Vegetative morphology and pseudospikelets of *B. corniculata* **A** clump **B** clump base **C** culm internode **D** thorny branches at culm base **E** pseudospikelets **F** culm leaf (abaxial view) **G** culm leaf (adaxial view) **H** the distal part of a leafy branch (upper side) **I** the distal part of a leafy branch (lower side).

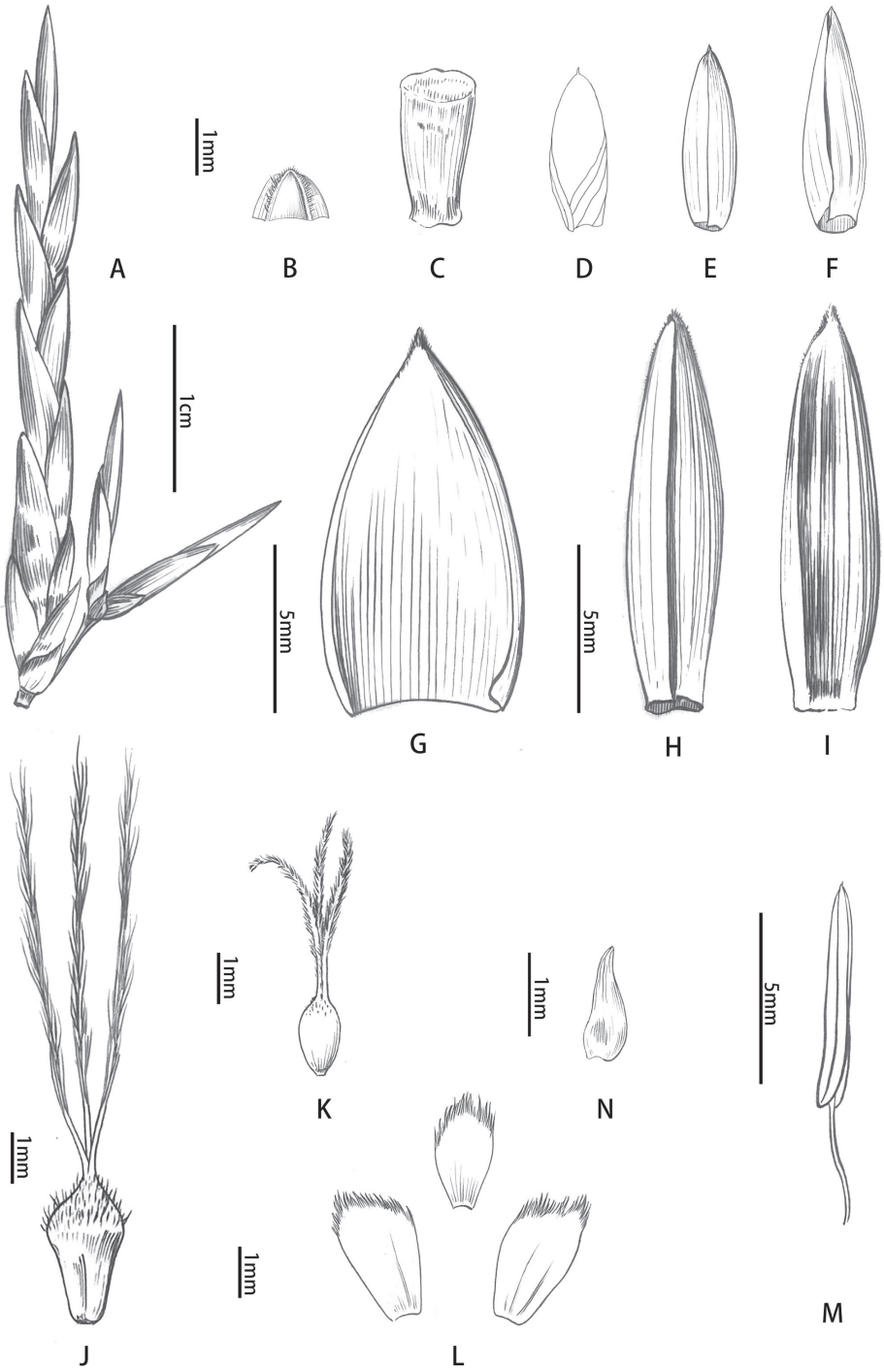

Figure 4. Floral morphology of *B. corniculata* **A** pseudospikelets **B** prophyll (abaxial view) **C** rachilla segment **D** empty bract (adaxial view) **E, F** glumes (abaxial view) **G** lemma (adaxial view) **H** palea showing glabrous margins **I** back of palea **J** pistil **K** young pistil **L** lodicules **M** stamen **N** terminal floret. **D** drawn by Qiao-Mei Qin, **A–C** and **E–N** drawn by Ding-Han Cui.

2–4 mm long, glabrous, apex ciliolate, lower segments distally inflated, upper segments distally only slightly inflated; glumes 1–3, ovate, ca. 7 mm long, glabrous, adaxial surface puberulent at the upper half, 15-veined, apex acute mucronate; lemma oblong, 8–12 mm long, glabrous, abaxial surface purple-spotted, 18–21-veined, apex acute mucronate, calluses ca. 0.5 mm long, glabrous; palea 8–13 mm long, 2-keeled, keels apically sparsely ciliolate, 4-veined between keels, each side 2-veined; lodicules 3, apex ciliate, anterior 2 obliquely oblong, 2.5–3 mm long, posterior one obovate, ca. 2.5 mm long; stamens 6, filaments filiform, anthers yellow to brownish, 5.5–6 mm long, apex retuse; ovary obovoid, 1.5–2 mm long, apex sparsely hispidulous, styles 3, 0.8–1 mm long, stigmas 3, plumose, 2.5–8 mm long.

Note. This species is closely related to *Bambusa gibba* McClure in vegetative morphology. However, *B. corniculata* can differ from *B. gibba* by apically acute (versus obtuse) bracts, glabrous (versus puberulent) rachilla segments, apically sparsely ciliolate (versus glabrous) keels of the palea, more veins on the palea and unstalked (versus stalked) ovary.

***Bambusa cornigera* McClure (1940:7)**
Figs 5, 6

Holotype. China, Guangdong Province: Guangzhou City, cultivated in Lingnan University Bamboo Garden under BG 1833 (living type, originally from Guangxi, Wuzhou, Cangwu, Changzhou Island, West River, above Wuzhou), 10 September 1933, *H. Fung 20712* (US!).

Epitype (designated here). China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences, 31 March 2016, *Qin & Ni QQM 41* (IBSC!).

Description including flowering material. Culms 8–13 m tall, 6–8 cm diameter, basally straight or zig-zag, apically pendulous; internodes 24–28 cm long, slightly curved, basally somewhat swollen, glabrous, plain green, initially slightly white waxy; basal nodes with grey-white sericeous ring-like zones below and above sheath insertion. Branch complement at lower culm nodes with branchlets sometimes specialised into curved, weak thorns; at mid-culm with several branches, central 3 branches dominant. Culm leaf sheath ribbed-striate, with caducous, stiff, appressed, pale hairs above the middle, apex somewhat truncate; auricles equal, oblong, small, margins with fine bristles; ligule up to 3 mm high, entire, margin ciliate; blade erect, triangular to narrowly triangular. Foliage leaf sheath glabrous; auricles absent, without or with only a few bristles; ligule very low; blade lanceolate to oblong-lanceolate, 12–20 cm long, 2–3 cm wide, abaxial surface pubescent, adaxial surface glabrous. Pseudospikelets fasciculate at each node of flowering branches, linear, sessile, basally subtended by several bud-bearing bracts at base, 2.5–4.5 cm long; florets 10–12, middle 5–7 florets fully developed; prophylls 2–4 mm long, 2-keeled, keels apically sparsely ciliolate; bracts 2–3, lanceolate, 3–4 mm long, glabrous, apically ciliolate, sometimes adaxial surface apex puberulent, apex acute, mucronate or not; rachilla not disarticulating between florets, segments

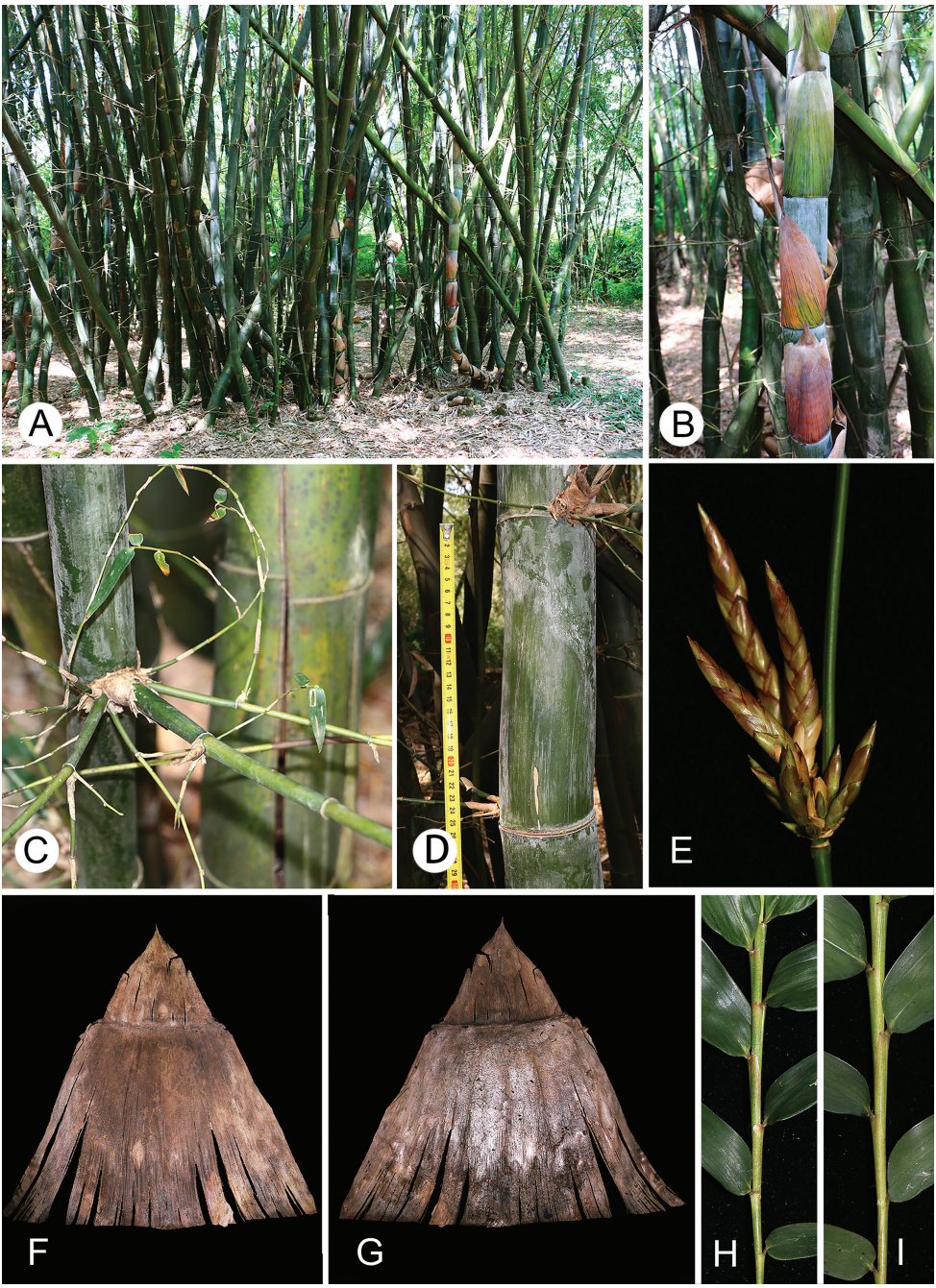

Figure 5. Vegetative morphology and pseudospikelets of *B. cornigera* **A** clumps **B** young culm **C** thorny branches at culm base **D** culm internode **E** pseudospikelets **F** culm leaf (abaxial view) **G** culm leaf (adaxial view) **H** the distal part of a leafy branch (upper side) **I** the distal part of a leafy branch (lower side).

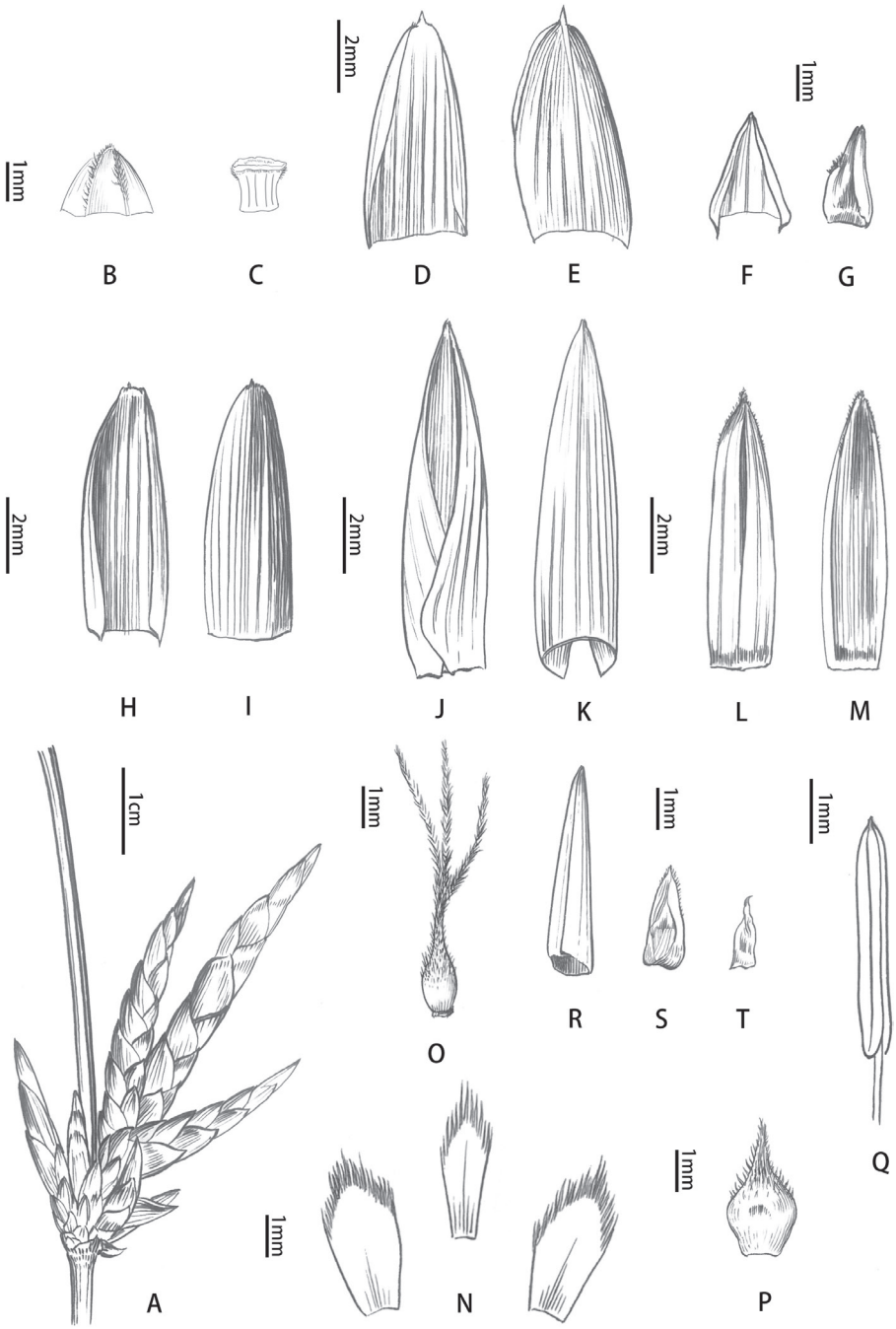

Figure 6. Floral morphology of *B. cornigera* **A** pseudospikelets **B** prophyll (abaxial view) **C** rachilla segment **D** empty bract (adaxial view) **E** empty bract (abaxial view) **F** bud-bearing bract (adaxial view) **G** axillary bud subtended by bract in (**F**) **H** glume (adaxial view) **I** glume (abaxial view) **J** lemma showing margins **K** lemma (abaxial view) **L** palea showing margins **M** back of palea **N** lodicules **O** pistil **P** ovary **Q** stamen **R** lemma of subterminal floret **S** palea of subterminal floret **T** terminal floret. Drawn by Ding-Han Cui.

compressed, 2–3 mm long, glabrous, apex slightly ciliolate and flat; glumes 1–2, lanceolate to oblong, 5–7 mm long, glabrous, both surface apex puberulent, obscurely 11–13-veined, apex acute or obtuse, mucronate; lemma broadly elliptic, 10–12 mm long, glabrous, 19–23-veined, apex acute mucronate, calluses no more than 0.5 mm long, glabrous; palea 8–11 mm long, 2-keeled, keels apically ciliolate, 4–6-veined between keels, each side 2–3-veined; lodicules 3, apex ciliate, anterior 2 obliquely oblong, 3–3.5 mm long, posterior one narrower, ca. 3.5 mm long; stamens 6, filaments filiform, anthers yellow, 5–7 mm long, apex apiculate; ovary broadly ovoid, 1–2 mm long, apex hispidulous, style 1, 0.7–1 mm long, stigmas 3, plumose, 4–5 mm long.

Bambusa subtruncata L.C. Chia & H.L. Fung (1981:378)

Figs 7, 8

Holotype. China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences (plants originally from Guangdong, Xinyi, Qingshui mountain), 5 August 1976, *Nan-Zhu 2312* (IBSC!).

Epitype (designated here). China, Guangdong Province: Guangzhou City, cultivated in South China Botanical Garden, Chinese Academy of Sciences, 27 November 2015, *Qin & Ni QQM 16* (IBSC!).

Description including flowering material. Culms 4–5 m tall, 2–2.5 cm diameter, basally nearly straight, apically slightly drooping; internodes 25–33 cm long, glabrous, initially slightly white waxy, green, basal ones typically with yellow stripes; wall ca. 7 mm thick; basal nodes with grey-white sericeous ring-like zones below and above sheath insertion. Primary branch bud broadly ovate, prophyll margins apically ciliate. Branch complement at mid-culm with many branches, central 3 branches dominant, those at culm base without thorny branchlets. Culm leaf sheath initially green with yellow stripes, glabrous or brown hispid especially near margins, apex subtruncate; auricles unequal, larger one about 2.5 times as large as smaller one, moderately to broadly elliptic, ca. 2 cm long, ca. 1.3 cm wide, wrinkled, margins with undulate bristles; ligule 1.5–2 mm high, margin ciliate; blade caducous, erect, triangular to narrowly triangular, base slightly rounded, overlapping with auricles for 6–7 mm, about 3/5 as wide as sheath apex. Foliage leaf sheath glabrous; auricles subovate or tiny, margin with undulate bristles; ligule low, entire; blade linear-lanceolate, 8–15 cm long, 0.9–1.3 cm wide, abaxial surface densely pubescent, adaxial surface glabrous. Pseudospikelets fasciculate at each node of flowering branches, linear, sessile, basally subtended by several bud-bearing bracts at base, 2.5–3 cm long; florets 9–10, middle 4–7 florets fully developed; prophylls ca. 3 mm long, 2-keeled, keels apically sparsely ciliolate; bracts 4–5, lanceolate, 3.5–10.5 mm long, glabrous, adaxial surface puberulent at the upper half, 5–15-veined, apex acute mucronate; rachilla disarticulating between florets, segments compressed, 4–5 mm long, apex ciliolate and flat; no glume; lemma

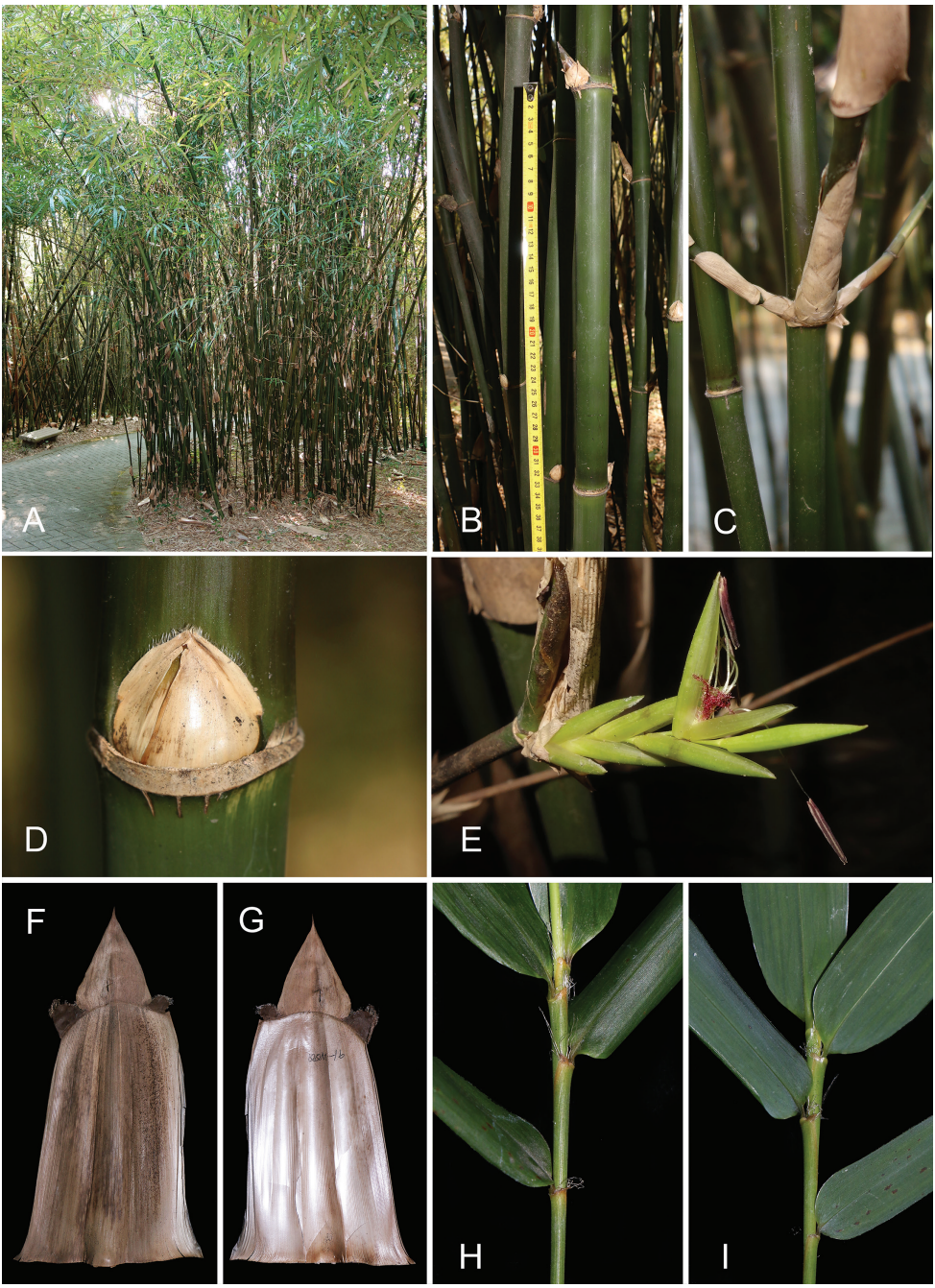

Figure 7. Vegetative morphology and pseudospikelets of *B. subtruncata* **A** clump **B** culms internode **C** branch complement **D** primary branch bud **E** pseudospikelet **F** culm leaf (abaxial view) **G** culm leaf (adaxial view) **H** the distal part of a leafy branch (upper side) **I** the distal part of a leafy branch (lower side).

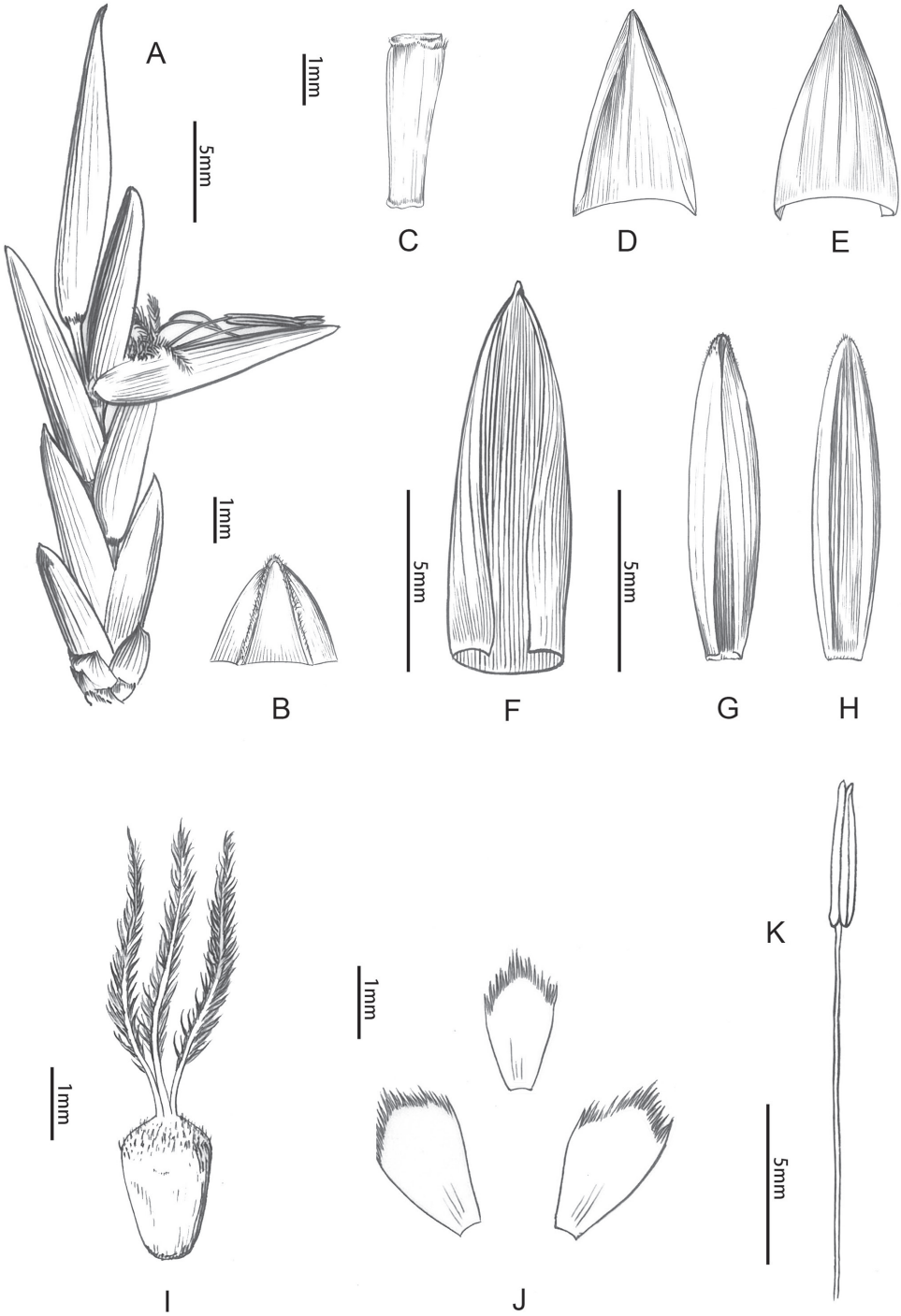

Figure 8. Floral morphology of *B. subtruncata* **A** pseudospikelet **B** prophyll (abaxial view) **C** rachilla segment **D** empty bract (adaxial view) **E** empty bract (abaxial view) **F** lemma (adaxial view) **G** palea showing margins **H** back of palea **I** pistil **J** lodicules **K** stamen. Drawn by Ding-Han Cui.

oblong-lanceolate, 12–14 mm long, glabrous, 17–19-veined, apex acute mucronate, calluses ca. 1 mm long, glabrous; palea 12–13 mm long, 2-keeled, keels apically sparsely ciliolate, 4-veined between keels, each side 2-veined, apex slightly puberulent; lodicules 3, apex ciliate, anterior 2 obliquely oblong, 2.5–3.5 mm long, posterior one narrowly obovate, 2.5 mm long; stamens 6, filaments filiform, anthers brown to yellowish, 5–6.5 mm long, apex retuse; ovary obovoid, ca. 1.5 mm long, apex hispidulous, styles 3, 0.5–0.6 mm long, stigmas 3, rarely 4, plumose, 2.5–3 mm long.

Note. After the comparison of floral characters between this species and a very similar congener, *Bambusa tuldoides* Munro, the following differences were found: *B. subtruncata* has a flat rachilla segment apex (versus inflated), 4–5 bracts (versus 2), no glumes (versus usually just 1), an acute lemma apex (versus obtuse) and 2 veins on each side of the palea (versus 4 veins).

Acknowledgements

We are grateful to Mr. Ding-Han Cui for preparing the illustrations. This work was supported by the National Natural Science Foundation of China (Grants 31670196), the Science and Technology Program of Guangzhou, China (grant no. 202201011461), the Young Innovative Talents Project (Natural Science) in Colleges and Universities of Guangdong Province (grant no. 2021KQNCX189) and the Natural Science Project of the Guangdong Eco-engineering Polytechnic (grant no. 2020kykt-xj-zk05).

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RESEARCH ARTICLE

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Artemisia calcicola (Asteraceae, Anthemideae), a new species from karst region in Guizhou, southwestern China

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Abstract

Artemisia calcicola (Asteraceae, Anthemideae), a new species from karst region in Shibing county, Guizhou province, southwestern China, is described and illustrated. The species can be readily assigned to *A.* subg. *Artemisia* in having fertile disk florets and glabrous receptacles. Within this subgenus, *A. calcicola* is distinguished by having (2- or) 3-pinnatipartite leaves and narrowly ellipsoid involucres 0.9–1.3 mm in diameter. It resembles *A. annua* to some extent, but differs immediately by the plant duration, stem and leaf indumentum, and involucre shape and size. A detailed description and distribution map of this species are also provided herein.

Keywords

Compositae, limestone flora, morphology, taxonomy

Introduction

Artemisia L. (Asteraceae), the largest genus of the tribe Anthemideae, comprises 300–500 species mainly distributed in the northern hemisphere (Ling 1991; Shulz 2006; Ling et al. 2011; Pellicer et al. 2014, 2018; Malik et al. 2017). China is considered one of the most important species centers of this genus, with ca. 190 species and 40

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varieties recorded (Ling 1988, 1991; Ling et al. 2011; Shultz and Boufford 2012; Guo et al. 2020, 2021, 2022). This genus is well known for containing various remarkable bioactive compounds, especially the efficient antimalarial agent artemisinin extracted from the leaves of *A. annua* L. (Tu 2011, 2017; Pellicer et al. 2018).

During a botanical trip to Guizhou in southwestern China in 2021, we discovered an unusual population of *Artemisia* in a karst region in Shibing (Fig. 1). At first glance, the plants were easily referred to *A.* subg. *Artemisia* due to their fertile disk florets and glabrous receptacles. Further critical observations revealed that they are rather distinct within this subgenus by having (2- or) 3-pinnatipartite leaves and narrowly ellipsoid involucres 0.9–1.3 mm in diameter. Morphologically, they are superficially similar to *A. annua*, a species in the same subgenus and widely distributed in the northern hemisphere, in having (2- or) 3-pinnatipartite stem leaves, ovate-acuminate or ovate, entire or (1- or) 2-toothed leaf lobules, and a narrow to broad panicle-like synflorescence (Fig. 1), but differ markedly by being perennial (vs. annual) and by having arachnoid-tomentose (vs. glabrous or sparsely pubescent) stems and leaves, narrowly ellipsoid (vs. globose or hemispheric) involucres 0.9–1.3 mm (vs. 1.5–2.5 mm) in diameter (Table 1). We therefore determined that the population in question represents a hitherto undescribed species, which we name *A. calcicola* and describe below.

Materials and methods

For morphological comparison, we critically examined physical or digitalized herbarium specimens of the genus *Artemisia* deposited at several major herbaria in China including CDBI, HNWP, IBSC, KUN, NAS, PE, SZ, and WUK (acronyms follow Thiers (2022)). Plants of *A. calcicola* were collected and photographed during our 2021 field investigation to Guizhou province. Morphological observations and measurements were based on fresh materials as well as herbarium specimens deposited at IBSC.

Taxonomic treatment

***Artemisia calcicola* X.Q.Guo & L.Wang, sp. nov.**
urn:lsid:ipni.org:names:77308334-1
Figs 1, 2

Diagnosis. *Artemisia calcicola* is distinguished within the *A.* subg. *Artemisia* in having (2- or) 3-pinnatipartite leaves and narrowly ellipsoid involucres 0.9–1.3 mm in diameter. Within this subgenus, it is merely superficially similar to *A. annua* in having (2- or) 3-pinnatipartite stem leaves, ovate-acuminate or ovate, entire or (1- or) 2-toothed leaf lobules, and a narrow to broad panicle-like synflorescence, but differs by being

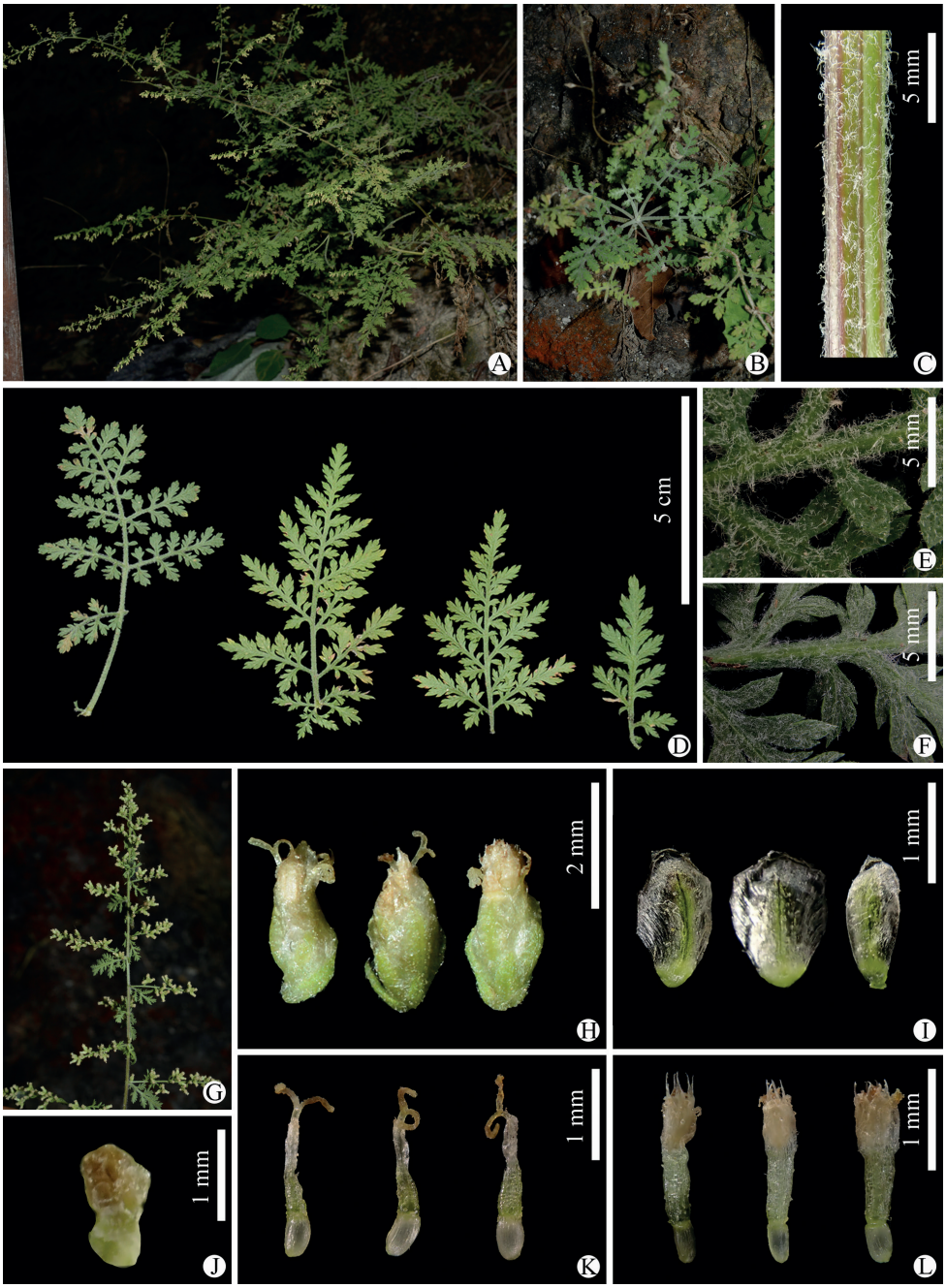

Figure 1. *Artemisia calcicola* sp. nov. **A** habitat and habit **B** leaf rosette of a vegetative branch **C** portion of stem **D** leaves **E** adaxial side of leaf **F** abaxial side of leaf **G** portion of synflorescence **H** capitula **I** phyllaries (abaxial side) **J** receptacle **K** marginal female florets **L** disk florets. All photographs by Long Wang.

Table 1. Morphological comparison between *Artemisia annua* and *A. calcicola* sp. nov.

	A. annua	*A. calcicola*
Duration	Annual	perennial
Stem	glabrous, sparsely pubescent	arachnoid-tomentose
Leaf	glabrous, sparsely pubescent; middle stem leaves 3 (or 4)-pinnatipartite; segments 5–8 (–10) pairs	arachnoid-tomentose; middle stem leaves (2- or) 3-pinnatipartite; segments 3–6 pairs
Capitulum	shortly pedunculate	sessile or subsessile
Involucre	globose to hemispheric; 1.5–2.5 mm in diameter	narrowly ellipsoid; 0.9–1.3 mm in diameter
Marginal female floret	10–20	4–6
Disk floret	10–30; corolla yellow or dark yellow	7–9; corolla creamy yellow

perennial and by having arachnoid-tomentose stems and leaves and narrowly ellipsoid involucres 0.9–1.3 mm in diameter (a detailed morphological comparison between the two species is given in Table 1).

Type. China. Guizhou: Shibing, Yuntai Shan, 27°06'N, 108°06'E, calcareous cliffs, 873 m a.s.l., 12 October 2021 (fl.), *Long Wang & Cheng-Sheng Li 4521* (holotype: IBSC; isotypes: IBSC, PE). Fig. 2.

Description. Herbs, perennial, 40–80 (–100) cm tall. Rhizome woody, up to 0.7 cm in diameter at base. Stems arachnoid-tomentose, more or less branched, erect or ascending. Basal stem leaves usually withered at anthesis, petiolate; (2- or) 3-pinnatipartite. Middle stem leaves petiolate; petiole 1–3 cm long; leaf blade ovate or ovate-oblong, 3–7 cm long, 3–5 cm broad, light green adaxially, grayish green abaxially, arachnoid-tomentose on both surfaces, (2- or) 3-pinnatipartite; segments 3–6 pairs, elliptic or ovate-elliptic, 1–2.5 cm long, 0.5–1 cm broad; lobes 3–6 pairs on each segment, elliptic or ovate, 5–8 mm long, 3–5 mm broad, with lobules ovate-acuminate or ovate, entire or (1- or) 2-toothed. Upper stem leaves subsessile or sessile; leaf blade ovate or ovate-elliptic, 3–5 cm long, 3–4 cm broad, (2- or) 3-pinnatipartite, arachnoid-tomentose on both sides; segments 3–7 pairs, elliptic or ovate, 1–2 cm long, 0.5–1.5 cm broad; lobes 3–5 pairs on each segment, elliptic or ovate, 0.5–1 cm long, 3–5 mm broad, with lobules ovate-acuminate or ovate, entire or (1- or) 2-toothed. Uppermost stem leaves subsessile; leaf blade ovate or ovate-elliptic, 1.5–2 cm long, 1.5–2 cm broad, (2- or) 3-pinnatipartite, arachnoid-tomentose on both sides; segments 3–6 pairs, elliptic or ovate, 0.5–1 cm long, 0.5–1 cm broad; lobes 2–4 pairs on each segment, ovate, with lobules ovate-acuminate or ovate, apex mucronate, entire or (1- or) 2-toothed. Synflorescence a narrow or broad panicle. Capitula sessile or subsessile, usually 3–7 clustered together. Involucres narrowly ellipsoid, 1.8–2.2 mm high, 0.9–1.3 mm in diameter. Phyllaries 3–4 rows, abaxially sparsely arachnoid-pubescent (outermost row) to glabrous (inner rows), obovate, ovate-oblong to elliptic, green when fresh, margin membranous. Receptacle glabrous. Marginal female florets 4–6, ca. 2 mm long, fertile; corolla tubular, 0.7–1 mm long, apex 2-toothed; style exserted. Disk florets 7–9, ca. 2 mm long, bisexual, fertile; corolla creamy yellow, 0.8–1 mm long, apex 5-toothed. Achenes cylindrical. Pappus absent.

Distribution and habitat. *Artemisia calcicola* is currently known only from the type locality, i.e. Yuntai Shan in Shibing, Guizhou, southwestern China (Fig. 3). It grows on calcareous cliffs at an altitude of ca. 900 m above sea level.

Figure 2. Holotype sheet of *Artemisia calcicola* sp. nov.

Etymology. Latin *calcis*, genitive singular of *calx*, limestone, and *cola*, *dweller*, alluding to habitat on calcareous cliffs.

Phenology. Flowering from October to November; fruiting from November to December.

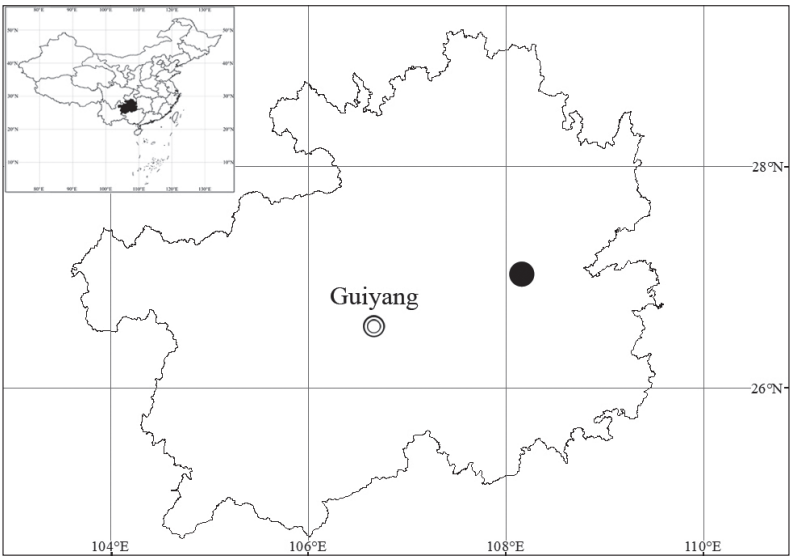

Figure 3. Distribution of *Artemisia calcicola* sp. nov. (black circle).

Vernacular name. 灰岩蒿 (Chinese pinyin: huī yán hāo).

Conservation status. *Artemisia calcicola* is currently known only from its type locality, i.e. Yuntai Shan in Shibing, Guizhou, southwestern China. The single population we discovered consists of no more than 20 individuals. Before acquiring adequate information to make a conclusive assessment of its risk of endangerment, the conservation status of *A. calcicola* is here recommended as "Data Deficient (DD)" (IUCN 2019).

Notes. In *Artemisia* subg. *Artemisia*, *A. calcicola* is also somewhat similar to *A. lancea* Vaniot, a species widely distributed in China, India, Japan, Korea, and Russia, particularly in the narrowly ellipsoid involucres. However, *A. calcicola* differs remarkably from *A. lancea* by an array of characters, including the arachnoid-tomentose (vs. glabrescent or sparsely arachnoid) stems, (2- or) 3-pinnatipartite (vs. 1-pinnatisect to 3-partite, or undivided), arachnoid-tomentose (vs. adaxially sparsely arachnoid, abaxially densely tomentose) stem leaves, and elliptic or ovate (linear-lanceolate or linear if divided) leaf segments.

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