RESEARCH ARTICLE



Lectotypification, epitypification and history of the name *Plagiothecium neglectum* Mönk. (Plagiotheciaceae)

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Academic editor: Peter de Lange | Received 9 November 2021 | Accepted 21 December 2021 | Published 24 January 2022

Citation: Wolski GJ, Proćków J (2022) Lectotypification, epitypification and history of the name Plagiothecium neglectum Mönk. (Plagiotheciaceae). PhytoKeys 189: 1–8. https://doi.org/10.3897/phytokeys.189.77839

Abstract

In the protologue of *Plagiothecium neglectum*, Mönkemeyer (1927) does not indicate any herbarium specimen as a type. The author only gave a short description and attached a figure illustrating selected features of this taxon. The original materials from the Mönkemeyer collection were deposited in the HBG herbarium; however, it is not currently possible to determine their location. Furthermore, one specimen of *P. neglectum*, currently known from the original Mönkemeyer collections, was found in the Herbarium B (B 30 0105646). The features given in the diagnosis of this taxon are consistent with those of the lectotype of *Stereodon nemoralis* Mitt.; only the leaf apex from Figure 207c (Mönkemeyer 1927) is different and thus suggests mixed material. According to Art. 9.1 of the *Shenzhen Code*, Figure 207c represents a holotype of *P. neglectum*. However, due to differences in the leaf apex and according to Art. 9.3 of the *Shenzhen Code*, the part representing the apex should be excluded from the holotype, and the remainder of Figure 207c is consequently designated as a lectotype of the name *P. neglectum*. However, because the lectotype does not include a complete set of significant distinguishing features, an epitype (B 30 0105646) was designated.

Keywords

Herbarium B, Herbarium HBG, iconotypus, Mönkemeyer collection, *Orthophyllum* section, synonym, typification

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Introduction

Plagiothecium neglectum Mönk. was described by Mönkemeyer in *Die Laubmoose Europas* (Mönkemeyer 1927). The name was created for specimens previously known as *P. sylvaticum auct. non* (Brid.) Bruch & Schimp. (= *P. nemorale* (Mitt.) A.Jaeger), wherein the author also retains and uses the name *P. silvaticum* (*nom. illeg. orthogr. pro P. sylvaticum* (Brid.) Bruch & Schimp.) which is now understood as *P. denticulatum* var. *obtusifolium* (Turner) Moore (Persson 1952; Iwatsuki 1970).

In the diagnosis, Mönkemeyer (1927) did not indicate any herbarium specimen as a type; the only original element is Figure 207c. The taxon is characterized, e.g., by shrunken turf in a dry condition, dense foliage, strong costae, and a very loose areolation of cells. In addition, Mönkemeyer (1927) compared this species with *P. silvaticum* and *P. succulentum* (Wilson) Lindb., also describing that the cell areolation in *P. neglectum* is as wide as that of the former, and the appearance of the turf and the leaves and habitat are similar to the latter.

For decades, *P. neglectum* was treated as a separate species (Jedlička 1948, 1950, 1961; Podpěra 1954; Iwatsuki 1963), as a synonym of *P. silvaticum* (*P. sylvaticum*) (Jensen 1939; Nyholm 1965; Ireland 1969), or a variety of this species – *P. silvaticum* var. *neglectum* (Mönk.) F.Koppe (Koppe 1949; Barkman 1957). Iwatsuki (1970), while examining the lectotype of *Hypnum sylvaticum* Brid., pointed out that this specimen is identical to *P. denticulatum* (Hedw.) Schimp. This researcher also suggested that the plants previously named *P. sylvaticum* and *P. neglectum* should instead be assigned the earlier available epithet, *P. nemorale* (Mitt.) A.Jaeger.

Nyholm (1965) and Ireland (1969), who proposed a synonymization of *P. ne-glectum* with *P. silvaticum* (*P. sylvaticum*), did not analyze the original materials of this taxon. Also, while Iwatsuki (1970) did not cite herbarium specimens (original material) of *P. neglectum*, his proposed synonymization of this taxon with *P. nemorale* has nevertheless been widely accepted (e.g., Lewinsky 1974; Koponen et al. 1977; Gangulee 1980; Corley et al. 1981; Iwatsuki 1991, 2004; Suzuki 2016; Wolski 2020), and although this proposal was based only on Figure 207c (Mönkemeyer 1927), it was made correctly as this figure is listed in the protologue only.

Considering the above facts, efforts have been made to revise the original herbarium materials of *P. neglectum* from the collection of Wilhelm Mönkemeyer to confirm the correctness of the previous synonymization, which was made in the absence of herbarium specimens.

Materials and methods

Before starting the research, efforts were made to find all herbaria in which W. Mönkemeyer collections are deposited. Sayre (1977) pointed out that his materials are deposited in the HBG, H-BR, and S herbaria. Additionally, an analysis of the Index of Botanists (https://kiki.huh.harvard.edu, accessed 15th of May 2021) found some of his herbarium specimens to be stored in the B, H, M and MANCH herbaria. To find the original materials of *P. neglectum*, contact was made with the staff of all the above-mentioned institutions. All curators indicated that the original materials of the analyzed taxon had been deposited at the Herbarium HBG. This was also confirmed in a paper by Walter and Martienssen (1976) describing the bryological collection of this herbarium.

Results

In the diagnosis, Mönkemeyer (1927) did not designate any specimen or collection as a type, or identify any original material or even geographical locations of the collection sites of the analyzed specimens. He added only a figure (207c) to the description of the taxon (Fig. 1A). An analysis of the bryological collection of the HBG herbarium (Walter and Martienssen 1976) revealed the presence of 13 "syntypes" of *P. neglectum* (Fig. 2); however, these specimens are incorrectly defined as syntypes because none is cited in the protologue *P. neglectum* (Mönkemeyer 1927). Nevertheless, based on contact with the curator of the bryological collection in the HBG herbarium, it was found that the original material of this taxon has been lost and their location is currently unknown and cannot be determined (Herbarium staff, pers. comm.).

Nevertheless, our research yielded only one currently known original specimen of *P. neglectum* collected by Mönkemeyer (Wesergebirge: in Erlenbrüchen bei Eschershausen, Juli 1900, *Mönkemeyer s.n.*, B 30 0105646). However, like the other ones, this is also not mentioned in the protologue (Fig. 1B).

The characteristics given in the diagnosis (e.g., shrunken turf; strong costae; loose cell areolation) and figures (e.g., rather flat, symmetrical, ovate leaves; straight apex; short, wide, hexagonal, or narrowly hexagonal cells) are consistent with those of the *Stereodon nemoralis* Mitt. lectotype (Wolski et al. 2020). Only the leaf apex from the Mönkemeyer (1927) figure is not serrate, as should be in this case. Nevertheless, based

A 18. Plagiothecium neglectum Moenkem. (Fig. 207 c). Habituell wie *P. succulentum*. Rasen gewöhnlich braungrün, trocken stark schrumpfend. Stengel niederliegend mit aufstrebenden Trieben. Diese unten klein beblättert, dann dichtgelagert, allseits abstehend. Blattform wie bei *succulentum*, aus verengtem Grunde oval-lanzettlich, allmählich gespitzt. Rippe kräftig. Zellnetz sehr locker wie bei *P. silvaticum*, reichlich mit Chlorophyllkörnern. Das herablaufende Band schmal, aus verlängerten Zellen bestehend. Kapsel auf roter Seta länglich-zylindrisch, aufrecht, glatt. Deckel geschnäbelt. Sporen hellgrün, glatt. Zweihäusig. Reift im Sommer.

An gleichen Stellen wie *P. succulentum*, nicht selten im gleichen Rasen. Zerstreut. Durch die angegebenen Merkmale von *succulentum* und *silvaticum* leicht zu unterscheiden.



Figure 1. A Diagnosis and specimen from the original Mönkemeyer collection. Diagnosis of *P. neglectum* (Mönkemeyer 1927; changed) (accessed from http://bjbdigital.com; 18 October 2021) **B** sheet with the specimen (B 30 0105646) stored in the Berlin herbarium B. neglectum Moenk.1927 : 866. - Thüringen: Eisenach, Annatal, 26. 7.1898, u. Wartburg, 2.5.1915 (J.Bornmüller); Wesergebirge: Eschershausen, Juli 1900, u. Bodenwerder, Königszinne, Juli 1901 (W.Mönkemeyer); Hessen, Rhön: Gr.Nallen, Juli 1906 (W.Mönkemeyer); Vogtland: Plauen, Triebtal, 25.7.1904 (E.Stolle); Bayern: Allgäu, Hinterstein, Sauwald, Aug.1906, u. Regensburg, U-Lichtenwald, Schindelmacherhänge, Nov.1906 (I.Familler), Prien/Chiemsee, 550 m, Juni 1911 (T.Linder); Mähren: Oppafall, Juli 1904 (J.Podpera); Ostpreußen: Labiau, Juli 1864 (H.v. Klinggräff); Kurland: Usmaitensee, Moritzholm, Mengwald, 3.8. 1913 (K.R.Kupffer); sine loc.et dat. (Wüstnei 380). - SYNTYFEN.

Figure 2. Fragment of the specimen list of the bryological collection of HBG concerning *P. neglectum* (Walter and Martienssen 1976).

on the other features given above, the proposal by Nyholm (1965), Ireland (1969), and Iwatsuki (1970) that *P. neglectum* is the plant currently understood as *P. nemorale* (syn. *P. sylvaticum*) appears correct.

Due to the existence of Figure 207c of this taxon, to which Mönkemeyer (1927) only refers to in the protologue, and according to Art. 9.1 of the *Shenzhen Code* (Turland et al. 2018), it should be assumed that Figure 207c is the holotype of that name. However, because the leaf apex of the holotype is not serrate, this suggests that the material used to make Figure 207c was derived from two different taxa (mixed material).

According to Art. 9.3 of the *Shenzhen Code* (Turland et al. 2018), "A lectotype is one specimen or illustration designated from the original material (Art. 9.4) as the nomenclatural type, in conformity with Art. 9.11 and 9.12, (...) if a type is found to belong to more than one taxon (see also Art. 9.14)". Therefore, the part of the figure representing the leaf apex, which is not serrate, should be excluded from the holotype, this being Fig. 207c (Mönkemeyer 1927: 862) (Fig. 3), and the rest of Figure 207c should be designated as the lectotype of *P. neglectum*.

However, due to the fact that the newly designated lectotype does not include the figure of the leaf apex, which is an important taxonomic feature, a specimen recently found in Herbarium B (B 30 0105646) should be designated as the epitype; it is the original material (as stated in Article 9.4 of the *Shenzhen Code*), collected by Mönkemeyer and signed by him as "*P. neglectum*". The plants of specimen B 30 0105646 also show a serrate leaf apex (Fig. 4), which indisputably indicates an association with *P. nemorale*.

This is an excellent choice because this specimen comes from the original Mönkemeyer collection; this way, it is practically impossible to change the understanding of the name *P. neglectum*. In addition, it is not possible to find the remaining original materials, which, however, are not quoted in the protologue of *P. neglectum* but listed in the Walter and Martienssen (1976) catalog.



Figure 3. Lectotype of *Plagiothecium neglectum* – outlined with a red line; the part of the figure showing the leaf apex is crossed out and excluded from the lectotype (figure 207c; Mönkemeyer 1927; changed).



Figure 4. Epitype of *P. neglectum* and some of its most important taxonomic characteristics. **A** specimen from herbarium B (B 30 0105646) **B–C** stem leaves **D** serrate leaf apex **E** cells of the middle part of the leaf.

Taxonomic treatment

Stereodon nemoralis Mitt., J. Linn. Soc. Bot. Suppl. 1: 104 (1859) \equiv Plagiothecium nemorale (Mitt.) A.Jaeger, Ber. St. Gall. Naturw. Ges. 1876–1877: 451 (1878) \equiv P. silvaticum var. nemorale (Mitt.) Paris, Index Bryol.: 967 (1898). **Type citation**: Hab. In Himalayae orient. reg. temp., Sikkim, in monte Tonglo (ad radicem filicis cujus dam), J. D. Hooker ! Lectotype: "Herb. Ind Or Hook. Fil. & Thomson Stereodon nemorale m. Hab. Sikkim, Tonglo Regio temp. Alt. – J. D. H." – BM 1030713!: isolectotype: NY 913349! = P. neglectum Mönk., Laubmoose Europas 866 (1927). Lectotype (designated here): Figure 207c without a part of the figure with the top of the leaf (Mönkemeyer 1927: 862), see Figure 3. Epitype (designated here): Wesergebirge: in Erlenbrüchen bei Eschershausen, Juli 1900, Mönkemeyer s.n., B 30 0105646 (Figure 4).

The remaining original material according to Walter and Martienssen (1976) was confirmed to have been lost at HBG: Thüringen: Eisenach, Annatal, 26.7.1898, u. Wartburg, 2.5.1915 (*J. Bornmüller s.n.*); Wesergebirge: Bodenwerder, Königszinne, Juli 1901 (*W. Mönkemeyer s.n.*); Hessen, Rhön: Gr. Nallen, Juli 1906 (*W. Mönkemeyer s.n.*). Vogtland: Plauen, Triebtal, 25.07.1904 (*E. Stolle s.n.*); Bayern: Allgäu, Hinterstein, Sauwald, Aug. 1906, u. Regensburg, U-Lichtenwald, Schindelmacherhänge, Nov. 1906 (*I. Familler s.n.*); Prien/Chiemsee: 500 m, Juni 1911 (*T. Linder s.n.*); Mähren: Oppafall, Juli 1904 (*J. Podpěra s.n.*); Ostpreuβen: Labiau, Juli 1864 (*H. v. Klinggräff s.n.*); Kurland: Usmaitensee, Moritzholm, Mengwald, 3.8.1913 (*K. R. Kupffer s.n.*); sine loc. et dat. (*Wüstnei s.n. 380*).

Acknowledgements

We thank all the curators and employees of the herbaria mentioned in the paper for their help and contact regarding the original materials of *P. neglectum* and the collection of Wilhelm Mönkemeyer.

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RESEARCH ARTICLE



Pulvinatusia (Brassicaceae), a new cushion genus from China and its systematic position

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Academic editor: Karol Marhold | Received 12 November 2021 | Accepted 3 January 2022 | Published 24 January 2022

Citation: Chen H-L, Al-Shehbaz IA, Qian L-S, Zhang J-W, Xu B, Zhang T-C, Yue J-P, Sun H (2022) *Pulvinatusia* (Brassicaceae), a new cushion genus from China and its systematic position. PhytoKeys 189: 9–28. https://doi.org/10.3897/phytokeys.189.77926

Abstract

The new genus and species *Pulvinatusia xuegulaensis* (Brassicaceae) are described and illustrated. The species is a cushion plant collected from Xuegu La, Xizang, China. Its vegetative parts are most similar to those of *Arenaria bryophylla* (Caryophyllaceae) co-occurring in the same region, while its leaves and fruits closely resemble those of *Xerodraba patagonica* (Brassicaceae) from Patagonian Argentina and Chile. Family-level phylogenetic analyses based on both nuclear ITS and plastome revealed that it is a member of the tribe Crucihimalayeae, but the infra-/intergeneric relationships within the tribe are yet to be resolved.

Keywords

Crucihimalayeae, cushion plants, molecular phylogenetics, new species, Xizang

Introduction

Cushion plants represent a special life form which usually has character combinations such as short-node intervals, compact branches, solitary flowers or few-flowered racemes, and dome- or mat-shaped cushions. They are common among perennial herbs growing on high-altitude mountains and are thought to be associated with dry and cold environments, such as the high Andes and Patagonia, Himalayas, and New Zealand Alps (Aubert et al. 2014; Boucher et al. 2016). Hauri and Schröter (1914) compiled the first worldwide list of cushion plants which included 338 species of 34 families and 78 genera. A century later, Aubert et al. (2014) updated the cushion plants catalogue in which they recognized 1,309 species of 63 families and 273 genera. An online database was also created for easy access and timely update (http://www.cushionplants.eu/).

The mustard family (Brassicaceae) is distributed primarily in temperate areas, and many of its species grow on high mountains. Aubert et al. (2014) reported 100 species from 25 genera of cushion plants in Brassicaceae, within which six species within five genera occurred in China, i.e., *Alyssum klimesii* Al-Shehbaz (now *Ladakiella klimesii* (Al-Shehbaz) D.A. German & Al-Shehbaz), *Ptilotrichum canescens* (DC.) C.A. Mey (now *Stevenia canescens* (DC.) D.A. German), *Solms-laubachia eurycarpa* (Maxim.) Botsch., *Baimashania pulvinata* Al-Shehbaz, *B. wangii* Al-Shehbaz, and *Shangrilaia nana* Al-Shehbaz, J.P. Yue & H. Sun. Although many other Brassicaceae species were described as cushion plants and found to be occurring in China (Zhou et al. 2001; Al-Shehbaz 2015), they were not included in Aubert et al.'s catalogue (2014).

From 2000 to 2019, 58 new species of Brassicaceae from China were described (Du et al. 2020), the number of Chinese Brassicaceae species has grown to ca. 500 (Chen et al. 2019). During an expedition in August 2015 to Mt. Xuegu La, Damxung County, Xizang (Fig. 1), China, we collected a mustard plant with typical cushion characters and whitish pink flowers (Fig. 2G-H). We went back to the above-mentioned locality in August 2019 and collected fruiting material of this plant (Fig. 2A-F). Morphological studies family-wide revealed that it represents a new genus and species, hereafter recognized as *Pulvinatusia xuegulaensis*. We also carried out molecular studies to verify its systematic position within the family.

Material and methods

Taxon sampling and data collection

To assess the identity and systematic position of the new taxon, a family-level sampling strategy was adopted. Two datasets, the nuclear ITS and plastomes, were utilized to reconstruct the phylogeny of Brassicaceae. The ITS dataset included 125 species representing 98 genera, of which two accessions of the novelty were newly sequenced. The plastome dataset included 74 species representing 70 genera, of which 16 accessions representing 16 species were newly sequenced. The plastome of *Bivonaea lutea* (Biv.) DC. was extracted from raw sequencing data SRR8528386 deposited under NCBI BioProject PRJNA518905. *Cleome lutea* Hook. was chosen as outgroup for ITS and plastome datasets. Both ITS and plastome datasets comprised all 52 currently recognized tribes and nine genera which were not assigned to tribes within Brassicaceae.



Figure 1. Habitat and geographic distribution of *Pulvinatusia xuegulaensis* **A–C** alpine meadow habitat, white arrow in **A** points to the location, white arrows in **B** and **C** point to *P. xuegulaensis* **D** geographic distribution of *P. xuegulaensis*, marked with green circle. – Photos: **A** by Jianwen Zhang **B** and **C** by Lishen Qian.



Figure 2. Images of *Pulvinatusia xuegulaensis* **A** and **B** fruiting plants **C** fruits **D** septum and replum **E** and **F** seeds **G** and **H** flowering plants **I** and **J** stems **K** leaves. Scales bars: 1 mm. – Photos: **A–F** & **I–K** by Lishen Qian **G** and **H** by Jianwen Zhang.

Data downloaded from GenBank and newly generated for this study are listed in Appendices 1 and 2, respectively.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel-dried fresh leaves using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. The ITS region of one sample of *Pulvinatusia xuegulaensis* (voucher specimens ZBFC-510) was amplified with the primers ITS-18F as modified by Mummenhoff et al. (1997) and ITS4 (White et al. 1990). A 25-ml polymerase chain reaction (PCR) included 1–2µL sample DNA (approx. 1–10 ng), 12.5µL Premix Taq TM (Takara Biomedical Technology, Beijing, China), 1µL of 10 µM stock of each primer, adjusted to 25 µL with ddH₂O. The PCR program included a hot start with 4 min at 94 °C, and 30–32 cycles of amplification (1 min denaturing at 94 °C, 45–60 s annealing at 52–53 °C, 60–80 s extension at 72 °C), and a final elongation step for 10 min at 72 °C. The sequencing primers are the same as amplified primers. While the ITS region and plastome sequences of another sample of *P. xuegulaensis* (voucher specimens ZJW3454), together with the plastome data of 15 species listed in Appendix 2 were generated by genome skimming. Libraries for pair-end 150-bp sequencing was conducted using the Illumina HiSeq 2000 platform at Novogene Co. (Beijing, China).

Data assembly and annotation

For the genome skimming data, low-quality reads were filtered, and the clean data were assembled using the GetOrganelle pipeline (Jin et al. 2020). The nuclear ITS and plastomes were also annotated using Geneious 8.2.4 (Kearse et al. 2012) with the published ITS of *C. himalaica* (AY662283) and plastome of *Rudolf-kamelinia korolkowii* (Regel & Schmalh.) Al-Shehbaz & D.A. German (KX886350) as the reference, respectively. Positions of start and stop codons of plastome sequences were checked manually.

Sequence alignment and phylogenetic analyses

Two datasets, i.e., ITS and 75 plastid protein-coding genes (CDS) extracted from the annotated plastome sequences, were aligned using MAFFT v.7.311 (Katoh and Standley 2013) and manually adjusted with MEGA 7.0.14 (Kumar et al. 2016), ambiguous alignment regions within ITS dataset were trimmed by trimAl 1.2 (Capella-Gutiérrez et al. 2009). The 75 CDS were aligned one by one and then concatenated together, and substitutional saturation was assessed using DAMBE v.7.0.68 (Xia 2018).

Maximum parsimony (MP) and Bayesian Inference (BI) analyses were performed for the ITS dataset, while for the 75 CDS dataset, Maximum Likelihood (ML) method was utilized. No substitutional saturation was detected in 75 CDS dataset, as the index of substitution saturation (*Iss*) values were both significantly smaller than the critical *Iss* (*Iss.c*) values as defined by Xia et al. (2003). MP analysis was performed with heuristic searches of 1000 replicates with random stepwise addition using tree bisection reconnection (TBR) branch swapping as implemented in PAUP* 4.0a168 (Swofford 2020). All characters were weighted equally, and gaps were treated as missing data. BI and ML analyses were carried out with MrBayes v.3.2.6 (Ronquist et al. 2012) and RAxML 8.2.12 (Stamatakis 2014) implemented in the CIPRES Science Gateway v.3.3 (Miller et al. 2010), respectively. The best-fit model for nucleotide sequences was evaluated using jModeltest 2.1.6 (Darriba et al. 2012). Corrected Akaike Information Criterion (AICc) method was used to select the best-fit models. The SYM+I+G model were selected for ITS dataset in the BI analyses. Two independent runs each with four Monte Carlo Markov chains (MCMCs) were run for five million generations, and one tree sampled every 1000 generations. The first 1250 trees (25% of total trees) were discarded as burn-in. The remaining trees were summarized in a 50% majority-rule consensus tree, and the posterior probabilities (PP) were calculated. The ML analyses were conducted using the GTR+G model for 75 CDS dataset, with the option of rapid bootstrap of 1000 replicates.

Results

Morphological evaluation

With a single pivotal root, very short internode and compact branches, *Pulvinatusia xuegulaensis* forms a hemispherical (dome) shape (Fig. 1B-C and Fig. 2A). Its leaves are linear-lanceolate and imbricate (Fig. 2I-K), and its fruits are ovoid silicles with stout fruit pedicles (Fig. 2C-F). These character combinations were not seen in any other Brassicaceae taxa occurring in China, suggesting it might represent a new species.

Nuclear ITS and plastome assemblies

The ITS sequences for two accessions of the novelty were 628 bp long. Most of the 16 newly sequenced plastomes were assembled into complete circular genome, except one or two gaps remained in the noncoding regions of three accessions. Gaps information, voucher records, and GenBank accession numbers are provided in Appendix 2.

Phylogenetic analysis

The aligned ITS matrix was 496 bp long with 261 (52.6%) parsimony-informative sites. The aligned plastome CDS matrix was 61,713 bp long with 7,730 (12.5%) parsimony-informative sites. The resolution of MP analyses was relatively weaker than the outcome of BI analyses, thus only the topologies of Bayesian phylogenetic analysis were shown for ITS dataset. As our aim was to assess the systematic position of *Pulvinatusia xuegulaensis*, only clades containing this taxon were concerned. In the ITS phylogeny, two accessions of *P. xuegulaensis* clustered together and embedded in a clade consisting of *Crucihimalaya* species. This *P. xuegulaensis/Crucihimalaya* clade is sister to *Ladakiella klimesii* (Fig. 3). In the plastome phylogeny (Fig. 4), only three *Crucihimalaya* species

and one accession for each of *P. xuegulaensis* and *L. klimesii* were sampled. The sequence of *P. xuegulaensis* formed a clade with *L. klimesii*, and then sistered to a clade composed of three *Crucihimalaya* species. Therefore, both nuclear and chloroplast phylogenies indicated that *P. xuegulaensis* should be assigned to the tribe Crucihimalayeae.

Taxonomic treatment

Pulvinatusia J.P. Yue, H.L. Chen, Al-Shehbaz & H. Sun, gen. nov. urn:lsid:ipni.org:names:77249032-1 Fig. 2

Type. Pulvinatusia xuegulaensis J.P. Yue, H.L. Chen, Al-Shehbaz & H. Sun.

Diagnosis. As indicated above, the monospecific *Pulvinatusia xuegulaensis* and *Ladakiella klimesii* are the only members of the tribe Crucihimalayeae with pulvinate and scapose habit and pink to whitish pink petals. The former differs by having simple and fewer forked trichomes, thin papery leaves, solitary flowers, caducous sepals, and glabrous, somewhat flattened fruits. By contrast, *L. klimesii* has subdendritic trichomes with finely branched rays, thick and fleshy leaves, 2–4-flowered racemes, persistent sepals, and pubescent and terete fruits.

Description. Herbs perennial, cespitose, scapose, pulvinate, with well-developed caudex covered with petioles of previous years. Trichomes simple, mixed with fewer forked stalked ones. Leaves densely imbricate, sessile, thin, papery, densely long ciliate, midvein obscure, adaxially concave to nearly flat, base attenuate, apex subacute. Flowers solitary on short pedicels originating from axils of basal leaves. Fruiting pedicels stout, erect or ascending, often hidden among basal leaves. Sepals oblong, abaxially with trichomes similar to those on leaves. Petals whitish pink to pink; blade obovate to suborbicular, apex obtuse, rounded or rarely acute, claw subequaling or slightly shorter than sepals. Stamens 6, slightly tetradynamous; filaments unappendaged, free; anthers ovate or oblong, obtuse at apex. Ovules 2 or 3 per ovary, placentation parietal. Fruits dehiscent, latiseptate, ovoid to ellipsoid, inflated; valves thick leathery, carinate; replum rounded, visible; septum complete; style obsolete or short and to 0.4 mm long, stout; stigma capitate, entire, unappendaged. Seeds aseriate, wingless, oblong, seed coat smooth, not mucilaginous when wetted; cotyledons accumbent.

Name derivation. The generic name is derived from the pulvinate habit of the plant, and the species epithet from the Xuegu La (Xizang, China), where the type collection was made.

Pulvinatusia xuegulaensis J. P. Yue, H. L. Chen, Al-Shehbaz & H. Sun, sp. nov. urn:lsid:ipni.org:names:77249034-1 Fig. 2

Description. Herbs 0.9–1.6 cm tall; caudex many branched, to 4 mm in diam. Trichomes simple, to 0.6 mm long, mixed with fewer forked stalked ones. Leaves dense-



Figure 3. Bayesian Inference topology of the Brassicaceae relationships based on the nuclear ITS dataset. Bayesian inference posterior probability (PP) and maximum parsimony bootstrap (BS) are noted.



Figure 4. Maximum Likelihood cladogram of the Brassicaceae based on the plastome dataset. Maximum likelihood bootstraps (BS) are noted above the branch. Three Lineages of Brassicaceae (Beilstein et al. 2006; Walden et al. 2020) were marked.

ly imbricate, (3.5-) 4.0–4.2 (–4.7) × 0.7–1 mm, thin, papery, long ciliate on both sides and margin. Flowers solitary on short pedicels originating from axils of basal leaves. Fruiting pedicels stout, 4.5–6 mm long, often hidden among basal leaves.

Sepals oblong, $1-2 \times ca$. 1 mm. Petals whitish pink or pink; obovate to suborbicular, $3-3.5 \times 2.5-3$ mm, claw 2.5-3.4 mm long. Filaments 1.5-2 mm long; anthers 0.3-0.5 mm long. Ovules 2 or 3 per ovary. Fruit ovoid to ellipsoid, $1.6-1.9 \times 0.8-1$ mm; valves thick leathery, inflated, style 0.2-0.4 mm long. Seeds $1-1.5 \times 0.7-0.9$ mm, seed coat smooth, not mucilaginous when wetted; cotyledons accumbent.

Type. CHINA. Xizang: Xuegu La, alpine meadow, sandy area, 29°55' N, 90°7' E, 5300 m, 4 Aug. 2019, ZBFC-510 (holotype, KUN!; isotype, KUN!). *Paratype*. CHI-NA. Xizang: Xuegu La, alpine gravel slopes, sandy area, 29°54' N, 90°7' E, 5407 m, 28 Aug. 2015, ZJW3454 (KUN).

Discussion

Pulvinatusia xuegulaensis displays typical cushion-plants morphology, which belongs to the dome type of Aubert et al.'s category (2014). Many ball-shaped individuals grow together along alpine slopes and form a community with spectacular landscape (Fig. 1B-C). Without flowers and fruits, one can easily misidentify P. xuegulaensis as Arenaria bryophylla Fernald, a member of Caryophyllaceae family and one of the most typical cushion plants in the Sino-Himalayas. This might partially explain why this new taxon remained unrecognized until now; even the type locality is nearby a county road (Fig. 1A). Only with its conspicuous cruciform pink flowers and ovoid silicles, one can easily recognize it as Brassicaceae. To date, only one population of P. xuegulaensis has been found, within the family and the six cushion taxa (as mentioned in the Introduction) listed by Aubert et al. (2014) occurring in China, P. xuegulaensis is most similar to Ladakiella klimesii in gross morphology. Whereas it differs from the latter by more (vs. less) compact branches; imbricate (vs. rosulate) leaves; solitary flowers (vs. 2–4-flowered raceme) and stout (vs. slender) fruiting pedicel. By contrast, these distinct characters of P. xuegulaensis are also shown in Xerodraba patagonica (Speg.) Skottsb. (Eudemeae, Brassicaceae) (Table 1), a South American species endemic to southern Argentina and Chile at an altitude of 20 - 1050 m (Salariato et al. 2015a), demonstrating morphological homoplasy between unrelated taxa of different continents.

In both nuclear and chloroplast phylogenies, *Pulvinatusia xuegulaensis* fell in a clade consisting of *Ladakiella* and *Crucihimalaya* species, indicating that the new taxon is phylogenetically close to these two genera, which had been assigned to the tribe Crucihimalayeae by German and Al-Shehbaz (2010). This study therefore supported *Pulvinatusia* to be the third genus within Crucihimalayeae. However, the intergeneric relationship within this tribe was not resolved. In the nuclear rDNA (ITS) phylogeny, two accessions of *P. xuegulaensis* were embedded in a clade consisting of nine *Crucihimalaya* species and then sister to *L. klimesii* (Fig. 3). This indicates that the genus *Crucihimalaya* as currently delimited (German 2005; Al-Shehbaz et al. 2011) is not monophyletic. In fact, generic delimitation and systematic position of *Crucihimalaya* have been in dispute for a long time. This genus was first established by Al-Shehbaz et al. (1999) to accommodate nine species excluded from

	Pulvinatusia xuegulaensis	Ladakiella klimesii	Xerodraba patagonica	
Tribal assignments	Crucihimalayeae	Crucihimalayeae	Eudemeae	
Habit	perennial, pulvinate	perennial, pulvinate	perennial, pulvinate	
Type of cushions	hemispherical (dome shaped) cushion	hemispherical (dome shaped) cushion	low cushion	
Compactness	compact, hard	intermediate	compact, hard	
Leaf arrangement	imbricate	rosulate	imbricate	
Leaf shape	linear-lanceolate	obovate to spatulate	oblong-ovate	
Leaf texture	thin, papery	thickened, fleshy	thickened, fleshy	
Flower	solitary	raceme 2-4-flowered	solitary	
Petal color	whitish pink, pink	pink throughout or white with pink claws	white to pale yellow	
Fruit	ovoid to ellipsoid silicle	ovoid silicle	ellipsoid silicle	
Fruiting pedicels	stout	slender	slender	
Fruit valves	thick leathery, carinate, glabrous	papery, not veined, densely tomentose	leathery, carinate, glabrous	

Table 1. Tribal assignments and comparisons of morphological characters of *Pulvinatusia xuegulaensis*, *Ladakiella klimesii* and *Xerodraba patagonica*.

Arabidopsis based on morphological and molecular evidences (Price et al. 1994; O'Kane et al. 1995). This delimitation was followed by Zhou et al. (2001) and Appel and Al-Shehbaz (2003), and the genus had been assigned to the tribe Camelineae by Al-Shehbaz et al. (2006) in their first scheme of tribal classification. However, subsequent molecular studies revealed that Crucihimalaya is phylogenetically distant to taxa from Camelineae but formed a clade with species Arabis tibetica Hook.f. & Thomson, A. tenuisiliqua Rech.f. & Köie, A. rupicola Krylov, Transberingia bursifolia (DC.) Al-Shehbaz & O'Kane and Alyssum klimesii (O'kane and Al-Shehbaz 2003; Koch et al. 2007; Warwick et al. 2008; German et al. 2009). These species then had been transferred to *Crucihimalaya* and resulted in a heterogeneous genus including 13 species (German and Ebel 2005; German 2005; Al-Shehbaz et al. 2011), whereas a new genus Ladakiella was created to accommodate L. klimesii excluded from Alyssum (German and Al-Shehbaz 2010). Both Ladakiella and Crucihimalaya s.l. were assigned to the newly proposed tribe Crucihimalayeae (German and Al-Shehbaz 2010). The ITS phylogeny constructed in this study suggested either to combine *P. xuegu*laensis with Crucihimalaya s.l. or split the latter genus into several segregates. Pulvinatusia xuegulaensis is very similar to L. klimesii in gross morphology as they both share pulvinate habit and inflated ovoid silicles. These morphological similarities corresponded to their phylogenetic relationships revealed in the plastome phylogeny, within which these two species formed a clade sister to three Crucihimalaya species (Fig. 4). The discrepancy between nuclear and chloroplast phylogenies revealed in this study might be attributed to two main reasons: 1) sampling difference, i.e., there are nine species from Crucihimalaya s.l. sampled in the ITS phylogeny, but only three species sampled in the plastome phylogeny, especially lack of C. bursifolia and C. rupicola. 2) reticulate evolution caused by hybridization and/or introgression, of which evolutionary processes have been proposed for numerous taxa in the mustard family (Mummenhoff et al. 2004; Lihová et al. 2006; Dierschke et al. 2009; German and Friesen 2014; Mandáková et al. 2017; Hohmann and Koch 2017; Chen et al. 2020). To clarify inter- and infrageneric relationships within Crucihimalayeae, studies with comprehensive sampling and more molecular markers are needed.

The discovery of Pulvinatusia xuegulaensis added one new genus and species to the cushion plant list compiled by Aubert et al. (2014). The cushion habit had long been considered a good example of evolutionary convergence among various plants in alpine and arctic regions (Aubert et al. 2014). It had been suggested to evolve independently four times in South American Brassicaceae (Salariato et al. 2015b) and happened at least 115 times in whole angiosperms (Boucher et al. 2016). Characterized by dense branches and compact structure, cushion plants usually form hemispheric or mat shapes, which enables them to adapt to cold and/or dry harsh environments and also facilitate other alpine plant species by nurse trait effects (Körner 2003; Yang et al. 2010; Chen et al. 2015; Chen et al. 2017; Yang et al. 2017). However, nothing is known about the underlying genetic basis of adaptation to alpine environments of cushion plants. All the three genera of Crucihimalayeae coexist in Qinghai-Tibet Plateau, and all species of Crucihimalaya are not pulvinate, while both L. klimesii and P. xuegulaensis are cushion species, thus provide an excellent system to decode the genetic basis of the formation of cushion structure and study the adaptive evolution of cushion plants, and the available genome of *C. himalaica* (Zhang et al. 2019) can facilitate this process.

Acknowledgements

This study was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK0502), the Key Projects of the Joint Fund of the National Natural Science Foundation of China (U1802232), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA20050203), the National Natural Science Foundation of China (32000160, 32060237) and the Yunnan Ten-thousand Talents Plan Young & Elite Talent Project (YNWR-QNBJ-2019-154).

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Appendix I

Taxon and GenBank accession numbers for the ITS and plastid genome sequences downloaded from GenBank and used in this study.

ITS:Outgroup: Cleome lutea Hook. (AF137588); Ingroups: Aethionemeae: Aethionema saxatile (L.) W.T. Aiton (GQ284853), Alysseae: Clypeola lappacea Boiss. (EF514645), Odontarrhena chalcidica (Janka) Spaniel & al. (GQ284877), Alyssopsideae: Alyssopsis mollis (Jacq.) O. E. Schulz (GQ424523), Olimarabidopsis pumila (Stephan) Al-Shehbaz, O'Kane & R. A. Price (AY662277), Anastaticeae: Anastatica hierochuntica L. (GQ424524), Cithareloma lehmannii Bunge (DQ357528), Anchonieae: Sterigmostemum billardieri (DC.) D.A. German (DQ357512), Sterigmostemum sulphureum (Banks & Sol.) Bornm. (KJ663764), Aphragmeae: Aphragmus nepalensis (H. Hara) Al-Shehbaz (DQ165335), Aphragmus oxycarpus (Hook. f. & Thomson) Jafri (DQ165337), Arabideae: Arabis alpina L. (DQ060111), Baimashania pulvinata Al-Shehbaz (FJ187969), Baimashania wangii Al-Shehbaz (JQ919842), Asteae: Asta schaffneri subsp. pringlei (O.E. Schulz) Al-Shehbaz (HQ541169), Asta schaffneri subsp. stricta (Rollins) Al-Shehbaz (HQ541171), Biscutelleae: Biscutella laevigata L. (KF022694), Megadenia pygmaea Maxim. (KX943555), Bivonaeeae: Bivonaea lutea (Biv.) DC. (HQ327490), Boechereae: Boechera fendleri (S. Watson) W. A. Weber (JX146958), Borodinia macrophylla (Turcz.) O. E. Schulz (EU274865), Brassiceae: Brassica balearica Pers. (AF263402),

Raphanus sativus L. (FJ980407), Buniadeae: Bunias erucago L. (GQ497885), Calepineae: Calepina irregularis (Asso) Thell. (DO249822), Goldbachia laevigata (M. Bieb.) DC. (DQ357546), Camelineae: Camelina sativa (L.) Crantz (KJ623504), Capsella thracica Velen. (HE575243), Cardamineae: Cardamine occulta Hornem. (KX244391), Nasturtium officinale W. T. Aiton (AY254531), Chorisporeae: Chorispora tenella (Pall.) DC. (DQ249866), Diptychocarpus strictus (Fisch. ex M. Bieb.) Trautv. (DQ357534), Cochlearieae: Cochlearia officinalis L. (HQ268642), Ionopsidium abulense (Pau) Rothm. (HQ268661), Coluteocarpeae: Noccaea thlaspidioides (Pall.) F. K. Mey. (DQ249838), Noccaea vesicaria (L.) Al-Shehbaz (GQ497857), Conringieae: Conringia clavata Boiss. (AY722505), Zuvanda exacoides (DC.) Askerova (DQ357607), Cremolobeae: Cremolobus chilensis (Lag. ex DC.) DC. (GQ424530), Cremolobus rhomboideus Hook. (KF662762), Crucihimalayeae: Crucihimalaya bursifolia (DC.) D. A. German & A. L. Ebel (AF137557), Crucihimalaya himalaica (Edgew.) Al-Shehbaz, O'Kane & R. A. Price (AY662283), Crucihimalaya kneuckeri (Bornm.) Al-Shehbaz, O'Kane & R. A. Price (AF137550), Crucihimalaya mollissima (C.A. Mey.) Al-Shehbaz, O'Kane & R. A. Price (DO249845), Crucihimalaya lasiocarpa (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R. A. Price (AF137556), Crucihimalaya rupicola (Krylov) A. L. Ebel & D. A. German (FJ187923), Crucihimalaya stricta (Cambess.) Al-Shehbaz, O'Kane & R. A. Price (AF137554), Crucibimalaya tenuisiligua (Rech.f. & Köie) Al-Shehbaz, D. A. German & M. A. Koch (KF547304), Crucihimalaya wallichii (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R. A. Price (AY662282), Ladakiella klimesii (Al-Shehbaz) D. A. German & Al-Shehbaz (EF514608), Descurainieae: Descurainia sophia (L.) Webb ex Prantl (AF205587), Ianhedgea minutiflora (Hook.f. & Thomson) Al-Shehbaz & O'Kane (HQ896625), Dontostemoneae: Clausia trichosepala (Turcz.) F. Dvořák (LK021263), Dontostemon senilis Maxim. (LK021244), Erysimeae: Erysimum majellense Polatschek (KJ418042); Erysimum serpentinicum Polatschek (KJ418068), Euclidieae: Brava scharnhorstii Regel & Schmalh. (MH23787), Leiospora pamirica (Botsch. & Vved.) Botsch. & Pachom. (MH237698), Pycnoplinthus uniflora (Hook. f. & Thomson) O. E. Schulz (MH237701), Solms-laubachia pulcherrima Muschl. (MH237723), Strigosella africana (L.) Botsch. (MH237728), Eudemeae: Dactylocardamum imbricatifolium Al-Shehbaz (KM376257), Eudema nubigena Humb. & Bonpl. (KC174370), Onuris hatcheriana (Gilg ex Macloskie) Gilg & Muschl. (KM376239), Xerodraba lycopodioides (Speg.) Skottsb. (KM376221), Eutremeae: Eutrema japonicum (Miq.) Koidz. (JN387782), Eutrema scapiflorum (Hook. f. & Thomson) Al-Shehbaz, G. Q. Hao & J. Q. Liu (DQ518398), Halimolobeae: Halimolobos jaegeri (Munz) Rollins (AF137567), Pennellia longifolia (Benth.) Rollins (AF307627), Heliophileae: Heliophila coronopifolia L. (DQ249846), Heliophila pusilla L. f. (LN589686), Hesperideae: Hesperis sibirica L. (DQ357549), Hesperis isatidea (Boiss.) D.A. German & Al-Shehbaz (GQ497882), Hillielleae: Hilliella rivulorum (Dunn) Y. H. Zhang & H. W. Li (KX244376), Hilliella sinuata (K.C. Kuan) Y.

H. Zhang & H. W. Li (KX244377), Iberideae: Iberis saxatilis L. (LN589689), Iberis umbellata L. (AY237921), Isatideae: Isatis tinctoria L. (GQ131323), Myagrum perfoliatum L. (GQ424547), Kernereae: Kernera saxatilis subsp. boissieri (Reut.) Nyman (AJ440314), Rhizobotrya alpina Tausch (AJ440315), Lepidieae: Delpinophytum patagonicum (Speg.) Speg. (KM376225), Lepidium aretioides (Hedge) Al-Shehbaz (GQ497859), Lepidium papilliferum (L.F. Hend.) A. Nelson & J.F. Macbr. (JF541495), Lepidium mendocinensis (Hauman) Al-Shehbaz (GQ497890), Malcolmieae: Malcolmia maritima (L.) W. T. Aiton (AM905723), Malcolmia orsiniana (Ten.) Ten. (DQ357560), Megacarpaeeae: Megacarpaea delavayi Franch. (KX244385), Pugionium dolabratum Maxim. (JF978171), Microlepidieae: Blennodia pterosperma (J.M. Black) J. M. Black (DQ357519), Pachycladon radicata (Hook. f.) Heenan & A. D. Mitch. (EF015693), Scambopus curvipes (F. Muell.) O. E. Schulz (JX630167), Notothlaspideae: Notothlaspi australe Hook. f. (AF100689), Notothlaspi rosulatum Hook. f. (AF100690), Oreophytoneae: Murbeckiella huetii (Boiss.) Rothm. (GQ424546), Oreophyton falcatum (E. Fourn.) O. E. Schulz (GQ424549), Physarieae: Nerisyrenia linearifolia (S. Watson) Greene (AF055200), Physaria occidentalis (S. Watson) O'Kane & Al-Shehbaz (KU975797), Schizopetaleae: Mathewsia foliosa Hook, & Arn. (KC174387), Schizopetalon brachycarpum Al-Shehbaz (KC174407), Scoliaxoneae: Scoliaxon mexicanus (S. Watson) Payson (HQ541175), Shehbazieae: Shehbazia tibetica (Maxim.) D. A. German (LN713855), Sisymbrieae: Sisymbrium officinale (L.) Scop. (AF531565), Sisymbrium orientale L. (AF531592), Smelowskieae: Smelowskia borealis (Greene) W. H. Drury & Rollins (AY230571), Smelowskia calycina (Stephan ex Willd.) C. A. Mey. (AY230604), Stevenieae: Macropodium pterospermum F. Schmidt (GU182055), Stevenia cheiranthoides DC. (GU182059), Thelypodieae: Chaunanthus acuminatus (Rollins) R. A. Price & Al-Shehbaz (GQ497855), Thelypodium laciniatum (Hook.) Endl. (KJ953749), Thlaspideae: Didymophysa fedtschenkoana Regel (EF514647), Thlaspi arvense L. (KT220620), Turritideae: Turritis glabra L. (DQ518389), Turritis laxa (Sm.) Hayek (KF547126), Yinshanieae: Yinshania henryi (Oliv.) Y. H. Zhang (KX244390), Yinshania zayuensis Y. H. Zhang (KX244395), Unassigned genera: Asperuginoides axillaris (Boiss. & Hohen.) Rauschert (EF514626), Atacama nivea (Phil.) Toro, Mort & Al-Shehbaz (KC174381), Chamira circaeoides (L.f.) Zahlbr. (AJ862719, AJ862720), Dipoma iberideum Franch. (GQ497861), Fourraea alpina (L.) Greuter & Burdet (DQ518395), Hemilophia sessilifolia Al-Shehbaz, Arai & H. Ohba (KT762595), Idahoa scapigera (Hook.) A. Nelson & J. F. Macbr. (MF964066), Schrenkiella parvula (Schrenk) D. A. German & Al-Shehbaz (AF137579), Subularia aquatica L. (MF963829).

Plastid genome: Outgroup: *Cleome lutea* Hook. (MK637687), Ingroups: Aethionemeae: *Aethionema saxatile* (L.) W. T. Aiton (MK637661), Alysseae: *Alyssum turkestanicum* Regel & Schmalh. (KY498535), Alyssopsideae: *Alyssopsis mollis* (Jacq.) O. E. Schulz (MK637657), Anastaticeae: *Anastatica hierochuntica* L. (KY912021), Anchonieae: Sterigmostemum violaceum (Botsch.) H. L. Yang (MK637808), Asteae: Asta schaffneri (S. Watson) O. E. Schulz (MK637662), Biscutelleae: Biscutella lyrata L. (MH359179), Bivonaeeae: Bivonaea lutea (Biv.) DC. (SRR8528386), Boechereae: Boechera angustifolia (Nutt.) Dorn (MK637673), Brassiceae: Brassica napus L. (GQ861354), Buniadeae: Bunias erucago L. (LN877377), Calepineae: Calepina irregularis (Asso) Thell. (MK637682), Camelineae: Camelina sativa (L.) Crantz (LN877386), Cardamineae: Cardamine resedifolia L. (KJ136822), Chorisporeae: Litwinowia tenuissima (Pall.) Woronow ex Pavlov (MK637744), Cochlearieae: Cochlearia borzaeana (Coman & Nyár.) Pobed. (LN866844), Conringieae: Conringia orientalis (L.) C. Presl (MK637689), Cremolobeae: Cremolobus peruvianus (Lam.) DC. (MK637692), Crucihimalayeae: Crucihimalaya lasiocarpa (Hook. f. & Thomson) Al-Shehbaz, O'Kane & R. A. Price (MK637686), Crucihimalaya wallichii (Hook. f. & Thomson) Al-Shehbaz, O'Kane & R. A. Price (AP009372), Ladakiella klimesii (Al-Shehbaz) D. A. German & Al-Shehbaz (MK637741), Dontostemoneae: Dontostemon micranthus C. A. Mey. (KY912023), Euclidieae: Braya scharnhorstii Regel & Schmalh. (MT845129), Leiospora pamirica (Botsch. & Vved.) Botsch. & Pachom. (MT845148), Pycnoplinthus uniflora (Hook. f. & Thomson) O. E. Schulz (MT845156), Strigosella africana (L.) Botsch. (MT845193), Eudemeae: Brayopsis alpaminae Gilg & Muschl. (MK637666), Solms-laubachia pulcherrima Muschl. (MT845182), Eutremeae: Eutrema heterophyllum (W. W. Sm.) H. Hara (KT270358), Halimolobeae: Halimolobos jaegeri (Munz) Rollins (MK637824), Heliophileae: Heliophila amplexicaulis L. f. (MK637720), Hesperideae: Hesperis sylvestris Crantz (KY912027), Iberideae: Iberis amara L. (MK637733), Isatideae: Isatis tinctoria L. (KT591187), Kernereae: Kernera saxatilis (L.) Sweet (MK637737), Lepidieae: Delpinophytum patagonicum (Speg.) Speg. (K637706), Lepidium virginicum L. (AP009374), Malcolmieae: Malcolmia maritima (L.) W.T. Aiton (MK637751), Megacarpaeeae: Megacarpaea delavayi Franch. (KX886349), Microlepidieae: Arabidella trisecta (F. Muell.) O. E. Schulz (MK637664), Pachycladon cheesmanii Heenan & A. D. Mitch. (JQ806762), Stenopetalum lineare R. Br. ex DC. (MK637800), Notothlaspideae: Notothlaspi australe Hook. f. (MK637761), Oreophytoneae: Oreophyton falcatum (E. Fourn.) O. E. Schulz (MK637767), Physarieae: *Physaria pinetorum* (Wooton & Standl.) O'Kane & Al-Shehbaz (MK637778), Schizopetaleae: Schizopetalon walkeri Sims (MK637809), Scoliaxoneae: Scoliaxon mexicanus (S. Watson) Payson (MK637801), Shehbazieae: Shehbazia tibetica (Maxim.) D. A. German (MK637829), Thelypodieae: Thelypodium laciniatum (Hook.) Endl. (MK637813), Thlaspideae: Thlaspi arvense L. (KX886351), Unassigned genera: Atacama nivea (Phil.) Toro, Mort & Al-Shehbaz (MK637821), Chamira circaeoides (L. f.) Zahlbr. (MK637678), Dipoma iberideum Franch. (MK637702), Fourraea alpina (L.) Greuter & Burdet (MK637717), Hemilophia sessilifolia Al-Shehbaz, Arai & H. Ohba (MK637730), Idahoa scapigera (Hook.) A. Nelson & J. F. Macbr. (MK637735), Schrenkiella parvula (Schrenk) D. A. German & Al-Shehbaz (KT222186), Subularia aquatica L. (MK637792).

Appendix 2

Taxon	Voucher Specimens	Locations	ITS GenBank	Plastome Gen-	Plastome	Number of
	-		numbers	Bank numbers	length (bp)	plastome
						gap
Aphragmus sp.	YZC250 (KUN)	Daocheng, China		OL800589	153022	
Asperuginoides axillaris	SunHang17434 (KUN)	Uzbekistan		OL800590	153193	
Crucihimalaya himalaica	No specimen	Batang, China		OL800599	155112	
Dendroarabis fruticulosa	YC-XZ019 (KUN)	Altay, China		OL800587	152755	
Descurainia sophia	YC-XZ070 (KUN)	Fukang, China		OL800591	153829	
Erysimum wardii	YZC202 (KUN)	Lhasa, China		OL800596	154466	
Hilliella fumarioides	ZJW4302 (KUN)	Jinhua, China		OL800598	154988	
Hilliella yixianensis	ZJW4330 (KUN)	Yixian, China		OL800594	154334	2 gaps
Noccaea yunnanensis	YZC223 (KUN)	Shangrila, China		OL800588	152801	
Pulvinatusia xuegulaensis	ZBFC-510 (KUN)	Damxung, China	OL828562			
P. xuegulaensis	ZJW3454 (KUN)	Damxung, China	OL828563	OL800600	155134	
Sisymbrium brassiciforme	YC-XZ025 (KUN)	Altay, China		OL800593	154238	
Smelowskia tibetica	YC-XZ132 (KUN)	Rutog, China		OL800595	154433	
Stevenia canescens	YC-XZ140 (KUN)	Gar, China		OL800597	154667	
Turritis glabra	YC-XZ035 (KUN)	Burqin, China		OL800592	154196	1 gap
Yinshania henryi	ZJW4523 (KUN)	Nanchuan, China		OL800602	155553	1 gap
Yinshania zayuensis	ZJW4430 (KUN)	Zhangjiajie, China		OL800601	155401	

Species and data description of ITS and plastomes used in this study.

RESEARCH ARTICLE



Ultrastructure of three Species of Entomoneis (Bacillariophyta) from Lake Qinghai of China, with reference to the external areola occlusions

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Academic editor: Kalina Manoylov | Received 18 November 2021 | Accepted 6 January 2022 | Published 25 January 2022

Citation: Long J-Y, Williams DM, Liu B, Mo W-H, Quan S-J (2022) Ultrastructure of three Species of *Entomoneis* (Bacillariophyta) from Lake Qinghai of China, with reference to the external areola occlusions. PhytoKeys 189: 29–50. https://doi.org/10.3897/phytokeys.189.78149

Abstract

Three sympatric *Entomoneis* species, found at the same specific locality in Lake Qinghai, China, are studied by using light and scanning electron microscope. Two species are proposed as new to science and named as *E. sinensis* sp. nov. and *E. qinghainensis* sp. nov. The third species is identified as *E. paludosa* (W. Smith) Reimer. *Entomoneis sinensis* has a linear-lanceolate valve outline and Z-shaped keel, bears two distinct 8-shaped loops formed by the valvocopula pars media in each cell and each of its stria is composed of either a long hymen strip or a long hymen strip plus one separated areola close to the raphe. Its hymen strip belongs to Type Two, which is a siliceous membrane strip perforated by two rows of linear pores next to transapical costae and two rows of rounded pores between these two rows of linear pores. *Entomoneis qinghainensis* has large cells, very high keel and evident hymen strip regions like a U-shaped neck pillow at the middle of valve face. Its hymen strip belongs to Type One, which is a siliceous membrane strip perforated by irregularly distributed round pores. *Entomoneis paludosa* also has the hymen strip regions that are worm-like and close to the raphe canal. Its hymen strip is same as that of *E. qinghainensis*. The two kinds of the outside areola occlusions in *Entomoneis* are compared, summarised and discussed.

Keywords

brackish water, hymen strip, hymen strip region, junction line, valvocopula

Introduction

Species in the diatom genus Entomoneis generally exhibit panduriform frustules in a girdle view and have a sigmoid keel. According to AlgaeBase website (Guiry and Guiry 2021), 30 species names have been accepted taxonomically, based on the listed literature under the species name. Species in this genus occupy a wide range of habitats and exhibit a certain amount of morphological diversity. Before Liu et al. (2018) described the freshwater species E. triundulata Bing Liu & D.M. Williams, all species within Entomoneis were considered either marine or brackish (Round et al. 1990). Regarding the morphological diversity of Entomoneis, some species possess three kinds of fibulae: raphe fibulae, keel fibulae and basal fibulae [e.g. E. calixasini Paillès, Blanc-Valleron & Poulin (in Paillès et al. 2014)]. Some possess two kinds of fibulae: raphe fibulae and basal fibulae [e.g. E. paludosa (W. Smith) Reimer (in Osada and Kobayasi 1990a; Dalu et al. 2015)] and others have only raphe fibulae [e.g. E. aequabilis Osada & Kobayasi (Osada and Kobayasi 1991)]. Some species possess unique characters. For example, E. centrospinosa Osada & Kobayasi has 1(0)-3 spines on each side of the central nodule (Osada and Kobayasi 1990b) and E. annagodheae Al-Handal & Mucko has an obliquely transapical fascia (Al-Handal et al. 2020a, b). The striae in *Entomoneis* can be uniseriate (e.g. *E. triundulata* and *E.* annagodheae), biseriate [e.g. E. centrospinosa and E. reimeri Reinke et Wujek (Reinke and Wujek (2013)] or strip-like [e.g. E. aequabilis, E. punctulata (Grunow) Osada & Kobayasi and E. pseudoduplex Osada & Kobayasi (Osada and Kobayasi 1990a)]. Many publications that include scanning electron micrographs often provide only images of low magnification; there are a number of ultrastructural details that require more detailed study (Liu et al. 2018). One example is the outside occlusions of the areolae of Entomoneis.

Round et al. (1990) called the very delicate silica membrane which occludes the pores of many raphid diatoms, a hymen, which is perforated by round or elongate pores ca. 5-10 nm in their shortest diameter. Thus, two types of hymenes can be proposed: Type One is perforated by round pores; Type Two is mainly perforated by elongate (linear) pores - both types of hymenes exist in Entomoneis. Type One has been noted for E. paludosa (Osada and Kobayasi 1990a, p. 170, figs 13-17), Type Two for E. punctulata and E. pseudoduplex (Osada and Kobayasi 1990a, p. 171, figs 26 and 27; p. 172, figs 38 and 39, respectively) and E. aequabilis (Osada and Kobayasi 1991, p. 160, figs. 12 and 13). Round et al. (1990) noted that the whole stria consists of a siliceous membrane in some Entomoneis species (e.g. E. punctulata, E. pseudoduplex and E. aequabilis). Osada and Kobayasi (1990a, p. 165) used "hymen-like strips" to describe the whole stria that consisted of a siliceous membrane in *E. pseudoduplex*. However, a part of a whole stria can consist of a hymen-like strip, such as in *E. paludosa* whose valve middle region is composed of these (Osada and Kobayasi 1990a, p. 170, fig. 10). In the current study, we provide high magnification images of the scanning electron microscope

for three species of *Entomoneis* found in Lake Qinghai, China with the aim of illustrating both types of hymen occlusions.

Lake Qinghai is the largest inland brackish-water lake in China. Its diatom flora has been investigated since 1979 (e.g. Lanzhou Institute of Geology and Chinese Academy of Sciences 1979; Yao et al. 2011; Peng et al. 2013). These studies have provided a list of taxa, but lack useful illustrations (drawings or micrographs) for the taxa observed. However, some interesting new species recently published have been well documented (Peng et al. 2014; 2016; Liu et al. 2020; Deng et al. 2021), confirming that endemic diatom species inhabit this ancient lake. This paper provides further evidence of endemic taxa with the description of two new species of *Entomoneis*.

Materials and methods

Site description

Three sampling sites were chosen from the lakeshore waters of Lake Qinghai (see Liu et al. 2020, p. 116, fig. 1). Geographically, Lake Qinghai is located between longitudes 99°36' and 100°47', latitudes 36°32' and 37°15' in Qinghai Province, China. It is the largest inland brackish-water lake in China. The Lake has ca. 4294 km² surface water area and is ca. 3200 m a.s.l. Its climate belongs to the plateau-continental climate. The average annual temperature is ca. -0.7 °C, the ranges of the average annual precipitation and the average annual evaporation in the Lake region are 319–395 mm and 800–1000 mm, respectively for many years (Luo et al. 2017). More than 50 rivers/ streams run into Lake Qinghai and there is no outlet to discharge the Lake water as Lake Qinghai is hydrologically closed. The surface water evaporating is nearly the sole path for loss of lake water. The lake has an 18.3 m average water depth and maximum of 26.6 m; the average values for alkalinity and pH are 25.6 mmol l⁻¹ and 9.2 respectively (Peng et al. 2014). There is a three-month ice-covered period (middle November to middle February) in Lake Qinghai so that the growth period for diatoms is mainly from May to October.

Sampling

At the three sampling sites in Lake Qinghai (see Liu et al. 2020, p. 116, fig. 1), there are numerous submerged stones with yellow-brown surfaces which indicate many diatoms growing on them. Each stone sampled was placed on a plastic plate, then its surfaces brushed using a toothbrush, with the brushed-off diatom samples being washed on to the plate. The samples were transferred to a 100 ml sampling bottle and fixed with 70% ethanol. Two bottle diatom samples were collected for each sampling site. Together with the sample collection, temperature, pH and conductivity were measured in situ with a portable multimeter (HQ40D, HACH Company).

Methods

The samples were processed (cleaned of organic material) for microscope examination using 10% hydrochloric acid (HCl) and 30% hydrogen peroxide (H_2O_2) . Permanent slides were prepared using the Mounting Medium Naphrax. These slides were examined and the specimens photographed, using a Leica DM3000 light microscope (LM) and a Leica MC190 HD digital camera. The holotype slides are deposited in the Natural History Museum, London, United Kingdom (BM) and isotype slides are kept in the Herbarium of Jishou University, Hunan, People's Republic of China (JIU).

Samples were further examined using a scanning electron microscope (SEM). Several drops of the selected cleaned diatom material were air-dried on to glass coverslips. Coverslips were attached to aluminium stubs using double-sided conductive carbon strip and sputter-coated with platinum (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged using a field emission scanning electron microscope (FE-SEM) Sigma HD (Carl Zeiss Microscopy) available at Huaihua University, China.

Diatom terminology largely follows Ross et al. (1979), Paddock and Sims (1981) and Round et al. (1990), specifically for species in *Entomoneis*, Osada and Kobayasi (1985, 1990a) were followed. We have proposed two types of hymenes, hymen strip and hymen strip region for *Entomoneis* (see below section Discussion, Fig. 14).

Results

Class Bacillariophyceae Haeckel Order Surirellales D.G. Mann Family Entomoneidaceae Reimer Genus *Entomoneis* Ehrenberg

Entomoneis sinensis Bing Liu & D.M. Williams, sp. nov. Figs 1–7, 14

Holotype. Slide BM 81941, the holotype specimen circled on the slide, illustrated here as Fig. 1A; isotype, slide JIU202101, illustrated here as Fig. 1B.

Type locality. China. Qinghai Province, Lake Qinghai, a sampling point near the lakeshore, 36°50'34" N, 99°42'39" E, 3210 m a.s.l., collected by Bing Liu, 19 July 2019.

Description. *LM* (Fig. 1). Frustule panduriform in girdle view (Fig. 1A–F). Frustule dimensions (n = 41): length 22.6–42.6 μ m, width 8.9–14.1 μ m at its centre, 14.6–19.8 μ m at its widest region. Two distinct 8-shaped loops are present in each frustule (indicated in Fig. 1D–F), one 8-shaped loop evident in each valve (Fig. 1G–O). Simple, arcuate junction line discernible in some specimens (indicated in Fig. 1H and I). Costae and striae invisible under LM. Girdle bands numerous.



Figure I. A–O *Entomoneis sinensis* sp. nov., girdle view, LM **A–F** six frustules showing a size diminution series, note the distinctive 8-shaped loops (labelled in Figs **D–F**) **G–O** nine valves showing a size diminution series, note arcuate junction line (labelled in Figs **H** and **I**) **A** micrograph of holotype specimen **B** micrograph of isotype specimen. Scale bar: 20 μm.

SEM, girdle view (Figs 2-4). Frustule panduriform with low keel (Fig. 2A and B; Fig. 3A-F, indicated by fused part). Cells having a 5:2 configuration of girdle bands, i.e. five girdle bands associated with the epivalve and two associated with the hypovalve (Fig. 2C-F, labelled in Fig. 2C and D). Junction line simple, arcuate (Fig. 3A). Two sides of the keel fused so that subraphe canal connects the cell lumen only near the central ending (Fig. 3B and C, arrow, respectively; see also Fig. 7B, arrow) and distal raphe ending (Fig. 3D, arrow). Short bar-like basal fibulae forming junction line (Fig. 3E and F, three arrows, respectively). Each pars media of valvocopula forming 8-shaped loop that is very distinct under LM (Fig. 4A-D). Each costa extending from raphe canal to inconspicuous mantle, warts bearing on each costa (Fig. 4E). Structure of each girdle bands similar, composed of pars exterior, pars media and pars interior (Fig. 4E). Pars media like a sternum, not located at the mid-line but slightly displaced towards pars interior (Fig. 4G). Both pars exterior and interior composed of one row of elongate poroids and each elongate poroid consisting of two costae and a hymen strip between them (thus the elongate poroid is n-shaped), with the n-shaped poroid of pars exterior longer than that of pars interior (Fig. 4F and G).

SEM, *valve view* (Figs 5–7). Valve linear-lanceolate, keel 2-shaped (Fig. 5A, Fig. 6A–C). Costae mostly running from raphe canal to mantle (Fig. 5B), but some



Figure 2. A–F *Entomoneis sinensis* sp. nov., girdle view, SEM **A**, **B** two frustules, note sigmoid girdle bands **C**, **D** central parts from Fig. **A** and **B** respectively, note the 5:2 configuration of girdle bands, i.e. five girdle bands, B1(Valvocopula, VC) to B5, associated with epivalve (EV); two girdle bands, B1(Valvocopula, VC) and B2, associated with hypovalve (HV) **E**, **F** two apices from Fig. **A**. Scale bars: 10 μ m (**A**, **B**); 2 μ m (**C–F**).

bifurcate (Fig. 5B, arrow), some terminating halfway to mantle (Fig. 5C, short costae). Each stria included between two adjacent costae, composed of one hymen strip (Fig. 5A–F). This type of hymen strip belonging to Type Two hymen strip, which is a siliceous membrane strip perforated by two rows of elongate (linear) pores next to the transapical costae and two rows of rounded pores between these two rows of linear



Figure 3. A–**F** *Entomoneis sinensis* sp. nov., girdle view, SEM **A** one broken frustule, note the simple arcuate junction lines **B**–**D** details from Fig. **A** note the fused parts of two sides of the keel and the subraphe canal connecting the cell lumen only near the central (Figs **B** and **C**, arrow, respectively) and the distal raphe ending (Fig. **D**, arrow) **E**, **F** details from Fig. **A** note the short, bar-like basal fibulae (three arrows, respectively). Scale bars: 10 μ m (**A**); 2 μ m (**B**–**F**).

pores (Fig. 14A and B). Mantle inconspicuous (Fig. 5B). Two total rows of separated areolae present along the raphe on both sides of the keel (Fig. 5B, four wavy arrows), but do not extend to the apex (Fig. 5E and F, six arrows, respectively). The hymenes occluding these separated areolae have the same structure as the hymen strip on striae (Fig. 5F, arrows). Stria density 36-43 in 10 μ m (n = 7). Two proximal raphe endings



Figure 4. A–G *Entomoneis sinensis* sp. nov., SEM **A** one valve with associated girdle bands, note the 8-shaped loop formed by the pars media of valvocopula (the pars media indicated by arrowheads and arrows indicating the pars media on the unseen side) **B–D** two valves with girdle bands further showing the 8-shaped loop (labelled in Fig. **D**) **E** middle part of a valve, note the mantle and the three parts of each girdle bands: parts exterior, pars media and pars interior **F** detail from Fig. **E** note the hymen strip occluding the poroid **G** valvocopula in internal view, note the broader pars exterior than pars interior and sternum-like pars media. Scale bars: 10 μ m (**A–D**); 2 μ m (**E–G**).

slightly dilated and a pore-like structure located at the centre of central nodule (Fig. 6D–F, arrow, respectively). Internally, one cell bearing only one lumen, no sub-compartment present (Fig. 7).


Figure 5.A–F *Entomoneis sinensis* sp. nov., valve external view, SEM **A** one whole valve showing the 2-shaped keel outline **B** central part from Fig. **A** note hymen strips, costae, mantle, warts, forked costa (arrow) and one separated row of rounded areolae at each side of the raphe (wavy arrows) **C**, **D** two apices from Fig. **A** note the short costae (two arrows in Fig. **C**) and two costae merging into one (arrow in Fig. **D**) **E**, **F** details showing one separated row of rounded areolae terminating before the apex (six arrows, respectively). Scale bars: 10 μ m (**A**); 2 μ m (**B–F**).

Etymology. Named after China where the species was found (the specific locality is Lake Qinghai).

Ecology and distribution. *Entomoneis sinensis* was found on the stone surfaces in Lake Qinghai. The following environmental parameters were measured in the field.



Figure 6. A–F *Entomoneis sinensis* sp. nov., external view, SEM **A–C** three whole valves, note 2-shaped keel outline **D–F** three middle parts from Figs. **A, B** and **C** respectively, note two proximal raphe endings slightly dilated and a pore-like structure at the centre of central nodule (arrows). Scale bars: 10 µm (**A–C**); 1 µm (**D–F**).

Conductivity was $16296.7 \pm 86.2 \,\mu$ S/cm, pH was 9.14 ± 0.01 and water temperature was $15.5 \pm 0.3^{\circ}$ C. According to above data and because Lake Qinghai is a brack-ish water lake, *Entomoneis sinensis* should be a brackish water diatom species. So far, *E. sinensis* is only found in the type locality and is a dominant species.



Figure 7. A–F *Entomoneis sinensis* sp. nov., valve internal and side views, SEM **A** one valve showing only one lumen (no sub-compartments present) in a cell (double-headed arrow) **B** middle part from Fig. **A** note the canal raphe communicates with the cell interior at the valve centre **C**, **D** two apices from Fig. **A E**, **F** another valve in side view, note the cell lumen. Scale bars: $10 \mu m$ (**A**, **E**); $2 \mu m$ (**B–D**, **F**).

Entomoneis qinghainensis Bing Liu & D.M. Williams, sp. nov. Figs 8–10, 14

Holotype. Slide BM 81942, the holotype specimen circled on the slide, illustrated here as Fig. 8A; isotype, slide JIU202102, illustrated here as Fig. 8B.



Figure 8. A–C *Entomoneis qinghainensis* sp. nov., girdle view, LM **A**, **B** two whole frustules, note the distinctive hymen strip region (labelled in Fig. **C**) **C** epivalve with associated girdle bands, note the hymen strip region and junction line **A** micrograph of holotype specimen **B** micrograph of isotype specimen. Scale bar: 20 µm.



Figure 9. A–C *Entomoneis qinghainensis* sp. nov., frustule view, SEM **A** one complete frustule, note the undulate junction line (indicated by dotted line) and the hymen strip region **B** detail from Fig. **A** note the striae composed of many single areolae near the sternum and two costae merging into one (arrow) **C** detail from Fig. **A** note hymen strips (two arrows). Scale bars: 10 µm (**A**); 2 µm (**B**, **C**).

Type locality. China. Qinghai Province, Lake Qinghai, a sampling point near the lakeshore, 36°50'34" N, 99°42'39" E, 3210 m a.s.l., collected by Bing Liu, 19 July 2019. **Description.** *LM* (Fig. 8). Frustules panduriform in girdle view (Fig. 8A and B). Frustule dimensions (n = 19): length 67.1–116.5 μm, width 21.6–37.5 μm at middle constricted part, 46.6–62.5 μm at widest part. Keel very high. Hymen strip region distinct, like a U-shaped neck pillow, located at the middle of each lobe of valve (in-



Figure 10. A–F *Entomoneis qinghainensis* sp. nov., girdle view, SEM **A** one valve with numerous girdle bands **B**, **C** two apices from Fig. **A** note the hymen strip regions (two double-headed arrows, respectively) **D** central part from Fig. **A** note thickened mantle **E**, **F** two girdle details from Fig. **A** note at least six girdle bands (labelled B1 to B6) associated with the epivalve. Scale bars: $10 \mu m$ (**A**); $5 \mu m$ (**B–F**).

dicated in Fig. 8C). Junction line sinuous with a distinct bulge into the hymen strip region (Fig. 8, see also Fig. 9A). Striae visible under LM, 18-23 in $10 \mu m$. Girdle bands numerous.

SEM, girdle view (Figs 9 and 10). Frustule panduriform, composed of epivalve, girdle bands and hypovalve (Fig. 9A). Junction line confirmed same as LM observation

(Fig. 9A, dotted line). Costae mostly running from raphe canal to mantle, but sometimes two costae merging into one (Fig. 9B, arrow). Hymen strip region composed of costae and hymen strips (Fig. 9A and C, two arrows, respectively; Fig. 10B and C, two double-headed arrows, respectively). This type of hymen strip belongs to Type One hymen strip, which are a siliceous membrane strip perforated by irregularly distributed round pores (Fig. 14C and D). Mantle thickened (Fig. 10B, two arrows). Six girdle bands associated with epivalve (Fig. 10E, labeled B1 to B6). Structure of each girdle bands similar, composed of pars exterior, pars media, and pars interior. The poroids of each girdle band elongate (Fig. 10F).

Etymology. Named after Lake Qinghai, where the species was found.

Ecology and distribution. *Entomoneis qinghainensis* was commonly found on the stone surfaces in Lake Qinghai with *E. sinensis*. According to above data and because Lake Qinghai is a brackish water lake, *E. qinghainensis* should be a brackish water diatom species. So far, *E. qinghainensis* is only found in the type locality and is not a dominant species.

Entomoneis paludosa (W. Smith) Reimer

Figs 11–14

Observation. *LM* (Fig. 11). Frustules panduriform in girdle view (Figs. 11A–D). Frustule dimensions (n = 19): length 34.2–80.7 μ m, width 12.8–21.8 μ m at constricted part, 22.4–30.3 μ m at widest part. Keel high. Hymen strip region distinct, worm-like (i.e. curved from apex to valve centre, widest near distal end), located close to the raphe canal (Fig. 11A–D, indicated in Fig. 11C, see also Fig. 12A, B and D). Junction line slightly sinuous (Fig. 11, see also Fig. 12A). Striae visible under LM, 22–25 in 10 μ m. Girdle bands numerous.

SEM (Figs 12 and 13). Frustule panduriform, composed of epivalve, girdle bands and hypovalve (Fig. 9A and B). Junction line confirmed same as LM observation (Fig. 12A, dotted line). Costae mostly running from raphe canal to mantle. Hymen strip region composed of costae and hymen strips (Fig. 12A and D, two arrows, respectively). This type of hymen strip belongs to Type One hymen strip, which are a siliceous membrane strip perforated by irregularly distributed round pores (Fig. 14E). Mantle thickened (Fig. 12C, arrow). Cells having a 4:2 configuration of girdle bands, i.e. four girdle bands associated with epivalve and two associated with hypovalve (Fig. 12C–E, labelled in Fig. 12E). Fibulae having only two levels: raphe fibulae and basal fibulae (Fig. 13A and B, two arrows, respectively). Internally, one cell bearing only one lumen, no sub-compartment present (Fig. 13C–F).

Ecology and distribution. *Entomoneis paludosa* was commonly found on the stone surfaces in Lake Qinghai with *E. sinensis* and *E. qinghainensis* and it has a global distribution. *Entomoneis paludosa* is a dominant species in the sampling sites.



Figure 11. A–K *Entomoneis paludosa*, girdle view, LM **A–D** four complete frustules, note the hymen strip region (indicated in Fig. **C**) **E–K** seven valves in side view showing size reduced series. Scale bar: 20 μm.



Figure 12. A–E *Entomoneis paludosa*, girdle view, SEM **A** one whole frustule, note the worm-like hymen strip region and the junction line (indicated by the dotted line) **B** another frustule **C–E** details from Fig. **B** note the thickened mantle (Fig. **C**, arrow), worm-like hymen strip region (Fig. **D**, two arrows) and 4:2 configuration of the girdle bands (i.e. four girdle bands associated with epivalve (EV) and two associated with hypovalve (HV) (Fig. **E**). Scale bars: 10 µm (**A**, **B**); 2 µm (**C–E**).



Figure 13. A-F *Entomoneis paludosa*, valve side view, SEM **A-C** three valves in side view, note the basal fibulae and the frustule cavity without sub-compartments (indicated by double-headed arrow) **D-F** details from Fig. **A-C**. Scale bars: 10 µm (**A-C**); 2 µm (**D-F**).

Discussion

Liu et al. (2018) summarised the kinds of keel found in species of the genus *Ento-moneis*. They suggested that *Entomoneis* exhibited two kinds of keel: S-shaped and 2 (reverse)-shaped. Like most species of *Entomoneis*, *E. sinensis* has 2-shaped keel (Fig. 6A–C). *Entomoneis sinensis* has strongly sigmoid girdle bands so that in girdle view, each girdle band crossing appearing decussate (Fig. 2A and B, Fig. 4A and B). Using LM, each valve bears a distinct 8-shaped loop which is formed by the two



Figure 14. A–F Details of hymen and hymen strip in *Entomoneis*, SEM **A**, **B** details of hymen strip of Type Two from *E. sinensis* sp. nov., note the two rows of linear pores (Fig. **A**, two arrows) on either side of the two intermittent rows of rounded pores (Fig. **A**, wavy arrow) **C**, **D** details of hymen strip of Type One from *E. qinghainensis* sp. nov., note the hymen is finely perforated over both the areolae (Fig. **D** arrow) and the hymen strips (Fig. **D**, double-headed arrow) **E** detail of hymen strips (double-headed arrow) **F** detail of hymens from *E. triundulata*, note the hymenes of the areolae in the girdle bands (two arrows). Scale bars: 600 nm (**A–F**).

halves of valvocopula pars media (Fig. 4A–C). No other species in *Entomoneis* bear the 8-shaped loop, so it is considered a unique character. *Entomoneis punctulata, E. pseudoduplex* and *E. aequabilis* all have whole striae (sometimes with a separated single areola near the raphe) occluded by a siliceous strip membrane (Osada and Kob-

ayasi 1990a, 1991). This strip membrane is one type of hymen (Type Two, according to Round et al. 1990). *Entomoneis sinensis* also bear the Type Two hymen strip and here we provide a detailed description: hymen strip of Type Two is a siliceous strip membrane perforated by two rows of linear pores next to transapical costae and two rows of rounded pores between these two rows of linear pores (Fig. 14A and B). The separated areolae close to the raphe (Fig. 5B, wavy arrows) has also this Type Two hymen (see Fig. 5F).

Entomoneis qinghainensis and E. paludosa are similar in many respects. Most important is that they both possess two hymen strip regions in a valve (see Fig. 9A and Fig. 12A). This hymen strip belongs to Type One, which is a siliceous strip membrane perforated by irregularly distributed round pores (Figs. 14C, D and E). These strip hymen regions are positioned between two valve regions consisting of rows of single areolae. So far, there are no other species of Entomoneis that possess this kind of strip hymen region. Entomoneis qinghainensis can be easily distinguished from E. paludosa by the shape of the hymen strip region: the former's hymen strip region is like a U-shaped neck pillow and located nearly at the middle of the valve surface (further away from the raphe canal, see Fig. 9A), whereas the latter's is worm-like (i.e. curved from the apex to the valve centre, widest near the distal end, see Fig. 12A, see also Osada and Kobayasi 1990a, fig. 10; Dalu et al. 2015, figs. 2–8) and located close to the raphe canal (not at the middle of the valve surface). Furthermore, the keel of E. qinghainensis is higher than that of E. paludosa and the stria density of E. qinghainensis is lower than that of E. paludosa (18–23 vs. 22–25 in 10 μ m).

The girdle bands are numerous in the three species described in this paper. *Entomoneis sinensis* has a 5:2 configuration of girdle bands, i.e. five girdle bands associated with the epivalve, two associated with the hypovalve (see Figs. 2C–F). *Entomoneis qinghainensis* has six girdle bands associated with the epivalve (see Fig. 10). *Entomoneis paludosa* has a 4:2 configuration of girdle bands, i.e. four girdle bands associated with the epivalve and two associated with the hypovalve (see Fig. 12). The girdle band poroids have the same hymen structure as those in the valve.

Acknowledgements

We are very grateful to two reviewers and the editors for their careful revisions and constructive comments. This research was supported by the Graduate Scientific Research Innovation Project of Hunan Province [grant number CX20211072].

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RESEARCH ARTICLE



Ligularia monocephala (Asteraceae, Senecioneae), a remarkable new species from Hubei, China

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Academic editor: A. Sennikov | Received 2 January 2022 | Accepted 14 January 2022 | Published 28 January 2022

Citation: Fei W-Q, Deng T, Wang L (2022) *Ligularia monocephala* (Asteraceae, Senecioneae), a remarkable new species from Hubei, China. PhytoKeys 189: 51–60. https://doi.org/10.3897/phytokeys.189.80016

Abstract

Ligularia monocephala, a remarkable new species from Hubei, China, is described and illustrated. It is readily distinguishable in the whole genus by character combination of the reniform to cordate-reniform leaf blades which are palmately-pinnately veined and abaxially purplish red, the solitary and erect capitula, and the pappus which are as long as or slightly longer than tube of the tubular corolla. A detailed description and distribution map of the species are also presented herein.

Keywords

Compositae, Hubei, Shennongjia, taxonomy

Introduction

Ligularia Cass., as the largest genus of tribe Senecioneae in Asteraceae in China, consists of approximately 130 species distributed mainly in eastern Asia (Liu 1989; Liu et al. 1994; Nordenstam 2007; Nordenstam et al. 2009; Liu and Illarionova 2011; Guo and Wang 2021). Since the publication of *Flora of China* (Liu and Illarionova 2011), the updated English version of *Florae Reipublicae Popularis Sinicae* (Liu 1989), extensive works have been done to the taxonomy of this genus at specific level (Fei et al. 2019; Lazkov and Sennikov 2019; Guo and Wang 2021, and references therein).

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During a field expedition in August 2016 to Shennongjia, Hubei, China, the second author was able to discover a unique *Ligularia* population in an alpine region of this area. The plant, at first sight, shows an affinity with *Ligularia hookeri* (C.B. Clarke) Hand.-Mazz. in habit, however, the leaf color and the capitula orientation can easily set them apart. An in-depth survey of herbarium specimens was conducted, resulting in the finding of four gatherings (*Anonymous 662, D.G. Zhang 080826018, Z.E. Zhao 1609* and *X.L. Yu et al. 080078*) all made from Shennongjia, Hubei, China, that are morphologically in conformity with this plant. To precisely decide the identity of these gatherings, we conducted another two field investigations to Shennongjia in September, 2020 and August, 2021, respectively, leading to a better understanding of the variation range of several main morphological characters of this plant. Upon careful observations and comparisons, it was found to be quite different from any other species in the genus in an array of morphological characters. We therefore concluded that this plant represents a hitherto undescribed species, which we describe below.

Materials and methods

For morphological comparisons, we critically examined physical or digitalized herbarium specimens with high-resolution of the genus *Ligularia* at A, BM, CDBI, CSFI, E, GH, HIB, HITBC, HNWP, IBSC, JIU, K, KATH, KUN, LE, NAS, NY, P, PE, S, SZ, W, WU, and WUK (acronyms follow Thiers (2021)). Specimens were collected from Hubei (first in September 2020, second in August 2021) during our several field expeditions. The morphological measurements in the description are based on the *in-situ* observations and dried specimens deposited at IBSC and KUN. Its records of distribution, habitat and phenology are based on both field investigations and specimen records.

Taxonomy

Ligularia monocephala Long Wang, sp. nov.

urn:lsid:ipni.org:names:77254167-1 Figures 1, 2

Diagnosis. Readily distinguishable in the whole genus by the character combination of the reniform to cordate-reniform leaf blades which are purplish red abaxially and palmately-pinnately veined, the solitary and erect capitula, and the pappus which are as long as, or slightly longer, than tube of the tubular corolla.

Type. China. Hubei Province, Shennongjia Forest Department, Shennongding Nature Reserve, Shennonggu valley, 31°26'19.36"N, 110°16'26.46"E, 2681 m a.s.l., on cliffs, 12 July 2021, *W.Q. Fei & H.S. Wu 324* (holotype: IBSC; isotypes: IBSC, KUN).

Description. Perennial herb. Stems solitary, erect, 15-20 cm tall, ca. 3 mm in diam, at base, proximal to median part slightly brown puberulent and white arachnoid to glabrescent, distal part densely brownish pilose. Basal leaves ascending, long petiolate; petiole 5-10 cm long, not winged throughout; leaf blade reniform to cordate-reniform, 3.5-4.5(-6) cm long, 3.5-5(-7) cm wide, herbaceous, adaxially dark green, slightly shortly puberulent to glabrescent, abaxially purplish red, brownish puberulent at first, especially along veins, then becoming glabrescent, base cordate, margin regularly dentate, apex rounded or slightly obtuse; sinus narrow, basal lobes nearly rounded, slightly divergent; vein palmate-pinnate; primary veins 3-5. Stem leaves 4-6(-7). Proximal stem leaves usually 1, petiolate; petioles ca. 3 cm long, basally sheathed; sheath usually more or less broadened; leaf blade reniform to cordate-reniform, slightly smaller than basal leaves. Median stem leaves usually 1, nearly sessile; leaf blade usually less than 3 cm long and 3 cm wide; base slightly or enlarged sheathed. Distal stem leaves usually 2-5, much smaller and reduced, bractlike, lanceolate, 6-10 mm long, 1.5-3 mm wide; margin slightly ciliate or entire. Capitula solitary, erect, 5 cm in diam. including ray florets; bracts 1 or 2, lanceolate to subulate, 3-4 mm long, ca. 1 mm wide. Involucres cylindrical, 11-14 mm high, 9–14 mm in diam., outside shortly brownish puberulent; receptacle densely shortly puberulent outside; phyllaries 9-10, spreading, in 2 rows; outer phyllaries narrowly oblong, 2–2.5 mm wide, apex acute; inner phyllaries oblong, ca. 3 mm wide, margin membranous, apex acute to obtuse. Ray florets 6–9, yellow; lamina oblong to elliptic, 3.0–3.5 cm long, 6–7 mm wide, apex acute, 3-denticulate; tube 5 mm long. Tubular florets numerous, yellow, ca. 2 cm long; tube 5 mm long; limb campanulate, 5-7 mm long; style 1.2 cm long. Achenes (immature) oblong, cylindrical, pale yellow, 3-4 mm long, glabrous. Pappus brown in the upper two-thirds, white (distal part) and purplish red (proximal part) in the lower one-third, 5-7 mm long, as long as, or slightly longer, than tube of tubular corolla.

Etymology. The specific epithet '*monocephala*' alludes to occurrence of solitary capitulum per stem.

Phenology. Flowering from July to August; fruiting in September.

Distribution and habitat. This species is currently known only from Shennongjia of Hubei (Fig. 3). It grows in moist forests or on moist cliffs covered by mosses at elevations of between 2681–3026 m above sea level.

Additional specimens examined (paratypes). CHINA. Hubei: Shennongjia Forest District, Shennongding Scenic Spot, Badongya, on rocky cliffs, 10 July 1987, *Anonymous 662* (HIB); Shennongjia Forest District, Shennongding Scenic Spot, Shennonggu valley, on moist cliffs covered by mosses, 31°26'42.95"N, 110°15'49.88"E, 3026 m a.s.l., 22 September 2020, *L. Wang, X.Q. Guo & Q.E. Yang 4216* (IBSC); ibid., on cliffs, 2852 m, 9 August 2008, *X.L. Yu et al. 080078* (CSFI); Shennongjia Forest District, precise locality unknown, 17 August 2012, *D.G. Zhang 080826018* (JIU); Shennongjia Forest District, precise locality unknown, in rock crevices, 2800 m a.s.l., 2 September 1980, *Z.E. Zhao 1609* (HIB).



Figure 1. Photographs of *Ligularia monocephala* sp. nov. A habitat **B**, C habit. All photographs by Wen-Qun Fei.



Figure 2. Photographs of *Ligularia monocephala* sp. nov. A portion of stem B leaf blade (adaxial surface)
C leaf blade (abaxial surface) D capitulum (top view) E capitulum (side view) F outer phyllaries (abaxial surface) H ray florets I tubular florets. All photographs by Wen-Qun Fei.



Figure 3. Distribution of Ligularia monocephala sp. nov. (black dot).

Discussion

The generic placement of this newly described taxon, which is superficially similar to some species of *Cremanthodium* in the general habit (the gatherings *Anonymous 662*, *D.G. Zhang 080826018*, and *Z.E. Zhao 1609* were, in fact, previously identified on the determination slips as species of *Cremanthodium*), is worthy of detailed remarks. The independent generic status of *Cremanthodium*, the putatively closest ally of *Ligularia*, has been widely accepted in recent checklists or Floras at the national level (e.g. Mathur 1995; Liu 1989; Grierson and Springate 2001; Kress et al. 2003; Liu and Illarionova 2011). Morphologically, the former is generally considered different from the latter by its broadly campanulate or hemispheric (vs. cylindrical or obconic) involucre, although this character is not always applicative in a few species (for example, *C. liangshanicum* L. Wang, C. Ren & Q.E. Yang). Geographically, the former is a high-alpine genus endemic to Sino-Himalayan region with its distribution range locating at 25°–40°N, 75°–104°E (Liu et al. 2002; Wang 2018), while the latter is an Eurasian genus more widely distributed than the former (Liu et al. 1994; Liu et al. 2002). However, the recognition and segregation of *Cremanthodium* has also been

repeatedly questioned. It was once considered as an alpine variant (Wulff 1944) or ecotype (Drury 1967) of *Ligularia*. In addition, recent molecular phylogenetic studies focused on the *LCP complex* (*Ligularia-Cremanthodium-Parasenecio*; Asteraceae, Senecioneae) (Liu et al. 2006; Ren 2012; Ren et al. 2017, 2020) revealed that the two genera are not monophyletic as traditionally defined. They together form three distinct and distantly related clades on chloroplast gene trees, with two clades having the species of the two genera interspersed between each other. The results contradict heavily with Liu's infrageneric classification system (Liu 1982, 1985) of the two genera established based mainly on morphological characters, but seem to be well correlated with the geographical distributions. Based on the above discussion, the newly reported species, characterizing by having cylindrical involucre, and locating at 31°N, 110°E, is here tentatively placed within *Ligularia* on morphological and geographical grounds.

In the genus Ligularia, L. monocephala is tentatively assigned to L. sect. Corymbosae (Franch.) Hand.-Mazz. ser. Retusae S.W. Liu due to its palmate-pinnate leaf venation, lanceolate to subulate bract, and cylindrical involucre. Within this series, it resembles L. phoenicochaeta (Franch.) Hand.-Mazz. to some extent, but differs mainly by the abaxially purplish red (vs. pale green) leaf blades (Fig. 2C), the erect (vs. cernuous) capitula (Fig. 2E), the cylindrical (vs. hemispheric) involucres (Fig. 2E), and the 5-7 mm (1-2 mm) long pappus (Fig. 2I). Ligularia monocephala is also superficially similar to L. jamesii (Hemsl.) Kom. of sect. Ligularia ser. Monocephalae (Nakai) Kitam. and L. hookeri (C.B. Clarke) Hand.-Mazz. (those plants with solitary capitula) of sect. Ligularia ser. Ligularia, particularly in the general habit and the solitary capitula. Morphologically, L. monocephala differs from L. jamesii immediately by the reniform to cordate-reniform (vs. triangular-hastate) leaf blades which are abaxially purplish red (vs. pale green, rarely purplish red) and apically rounded or slightly obtuse (vs. acute or acuminate) (Fig. 2C), the cylindrical (vs. broadly campanulate) involucres (Fig. 2E), the oblong to elliptic (vs. linear-lanceolate) ray laminae which are 6-7 mm (vs. 3-4 mm) wide (Fig. 2H), and the pappus which are as long as or slightly longer than tube of tubular corolla (vs. as long as tubular corolla) (Fig. 2I); and L. monocephala differs from L. hookeri mainly by the abaxially purplish red (vs. pale green, rarely purplish red) leaf blades (Fig. 2C), the erect (vs. cernuous to horizontal) capitula (Fig. 2E), the oblong to elliptic (vs. linear) ray laminae which are 6-7 mm (vs. 1.5-2 mm) wide (Fig. 2H), and the pappus which are as long as, or slightly longer than, the tube of tubular corolla (vs. as long as tubular corolla) (Fig. 2I). A detailed comparison of the four species is given in Table 1.

Acknowledgements

We would like to thank Dr. Chen Ren and Dr. Alexander Sennikov for their constructive suggestions. We are grateful to the curators of A, BM, CDBI, CSFI, E, GH, HIB, HITBC, HNWP, IBSC, JIU, K, KATH, KUN, LE, NAS, NY, P, PE, S,

	L. hookeri	L. jamesii	L. monocephala	L. phoenicochaeta
Stems	distally white arachnoid and	distally white arachnoid-	densely brownish pilose	distally shortly brown
	shortly brown pilose	puberulent		pilose
Basal leaves	leaf blade cordate-sagittate or	leaf blade triangular-	leaf blade reniform to cordate-	leaf blade orbicular-
	reniform, abaxially pale green,	hastate, abaxially pale	reniform, abaxially purplish	reniform, abaxially pale
	rarely purplish red; margin	green; margin sharply	red; margin regularly dentate,	green; margin regularly
	triangularly or coarsely dentate,	dentate, apex acute or	apex rounded or slightly obtuse;	triangular-dentate, apex
	between teeth shortly pilose, apex	acuminate; palmately-	palmately-pinnately veined	rounded; palmately
	rounded; palmately veined	pinnately veined		veined
Capitula	usually solitary, sometimes 2–7(–	solitary; erect	solitary; erect	usually solitary,
	16) arraged in a raceme; cernuous			sometimes 2–4 arranged
	to horizontal			in a lax corymb; cernuous
Involucres	campanulate, 6–8(–10) mm in	broadly campanulate, to	cylindrical, 9–14 mm in diam.,	hemispheric, to 24 mm
	diam., outside shortly brown	1.5 cm, outside white	outside shortly brownish	in diam., outside glabrous
	pilose or glabrous	arachnoid-puberulent	puberulent	
Ray florets	lamina linear, 1.5–2 mm wide	lamina linear-lanceolate,	lamina oblong to elliptic, 6–7	lamina elliptic or oblong-
		3–4 mm wide	mm wide	lanceolate, ca. 2 mm wide
Pappus	brown or pale brown, 6–7 mm	pale yellow, 7–8 mm long,	brown in the upper two-thirds,	purplish brown, 1–2 mm
	long, as long as tubular corolla	as long as tubular corolla	white (distal part) and purplish	long, much shorter than
			red (proximal part) in the lower	tube of tubular corolla
			one-third, 5–7 mm long, as	
			long as or slightly longer than	
			tube of tubular corolla	
Distribution	Shaanxi, Sichuan, Xizang,	Jilin, Liaoning, Nei	Chongqing, Hubei	Yunnan
in China	Yunnan	Mongol	_	

Table I. Differences among Ligularia hookeri, L. jamesii, L. monocephala and L. phoenicochaeta.

SZ, W, WU, and WUK for allowing us to use their scanned images of specimens and for research facilities. Thanks are also given to Mr. Xin-Qiang Guo, and Mr. Hai-Song Wu for field assistance. This work was supported by the National Natural Science Foundation of China (grant no. 31900183), the Biological Resources Programme of the Chinese Academy of Sciences (grant no. KFJ-BRP-017-08), Key Laboratory of Conservation Biology of Golden monkey in Shennongjia of Hubei Province (grant no. SNJGKL202002), the Youth Innovation Promotion Association of Chinese Academy of Sciences (grant no. 2019382), and the Ten Thousand Talents Program of Yunnan Province (grant no. 202005AB160005).

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CHECKLIST

PhytoKeys 189: 61–80 (2022) doi: 10.3897/phytokeys.189.75321 https://phytokeys.pensoft.net



An updated checklist of Mozambique's vascular plants

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Academic editor: T. L.P. Couvreur | Received 15 September 2021 | Accepted 10 January 2022 | Published 28 January 2022

Citation: Odorico D, Nicosia E, Datizua C, Langa C, Raiva R, Souane J, Nhalungo S, Banze A, Caetano B, Nhauando V, Ragú H, Machunguene Jr M, Caminho J, Mutemba L, Matusse E, Osborne J, Wursten B, Burrows J, Cianciullo S, Malatesta L, Attorre F (2022) An updated checklist of Mozambique's vascular plants. PhytoKeys 189: 61–80. https://doi.org/10.3897/phytokeys.189.75321

Abstract

An updated checklist of Mozambique's vascular plants is presented. It was compiled referring to several information sources such as existing literature, relevant online databases and herbaria collections. The checklist includes 7,099 taxa (5,957 species, 605 subspecies, 537 varieties), belonging to 226 families and 1,746 genera. There are 6,804 angiosperms, 257 pteridophytes, and 38 gymnosperms. A total of 6,171 taxa are native to Mozambique, while 602 are introduced and the remaining 326 taxa were considered as uncertain status. The endemism level for Mozambique's flora was assessed at 9.59%, including 278 strict-endemic taxa and 403 near-endemic. 58.2% of taxa are herbaceous, while shrubs and trees account respectively for 26.5% and 9.2% of the taxa. The checklist also includes ferns (3.6%), lianas (1.7%), subshrubs (0.5%) and cycads (0.3%). Fabaceae, Poaceae and Asteraceae are the three most represented families, with 891, 543 and 428 taxa, respectively. The extinction risk of 1,667 taxa is included, with 158 taxa listed as Vulnerable, 119 as Endangered and as 24 Critically Endangered. The geographical distribution, known vernacular names and plants traditional uses are also recorded.

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Keywords

Biodiversity, checklist, flora, Mozambique, taxonomy, vascular plants

Introduction

Mozambique lies on the southeast coast of Africa, between latitudes 10°27'S to 26°52'S and longitudes 40°51'E to 30°12'E, bordered by Tanzania in the north, the Indian Ocean in the east, Zambia to the northwest, Malawi, Zimbabwe, and Eswatini to the west and South Africa to the west and south. The country has a total area of 801,590 km² (Instituto Nacional de Estatística 2020), about 70% of which is covered by forests or other woody vegetation and 26% is included in conservation areas, such as national parks, reserves and game farms (Ministry for the Coordination of Environmental Affairs 2014).

The country is an important area of plant biodiversity (Conde et al. 2014), deriving its plant richness from geomorphological and climatic factors (Darbyshire et al. 2019). Geographically, the Zambezi River, crossing Mozambique from west to east towards the Indian Ocean, roughly bisects the country into two main regions: a southern region, dominated by lowlands, and a northern region which consists of a large plateau. The highland region in Manica Province encompasses the highest point in the country, rising up to 2,436 meters at Mount Binga (Ministry for the Coordination of Environmental Affairs 2014). The country's underlying geology can also be broadly divided into two different regions: the southern region of Mozambique consist of sedimentary rocks (Rutten et al. 2008), whereas the ancient granite rock basement of Africa underlies most of northern and west-central regions (Boyd et al. 2010). The climate is tropical over most of the country, with a sub-tropical climate in the south. Mozambique has two main seasons: a cooler dry season, from April to October and a warmer humid season from October to April (Barbosa et al. 2001). The northern region (Niassa, Cabo Delgado, Nampula and Zambézia provinces) has higher temperatures, with annual temperature averages of 25.5 °C in the coastal area dropping to 18 °C in the uplands. Central Mozambique (Tete, Manica and Sofala provinces) exhibits mean annual temperatures of 25 °C for the coastal lowlands and 20°C for the interior highlands. In the south (Inhambane, Gaza and Maputo provinces) the average annual temperatures vary from 23 °C in the coastal area to 25 °C in the interior (Wils and Lutz 2002). Rainfall distributions fluctuate widely through the country, following a north-south gradient with higher rainfall in the north and in the mountainous areas, where average annual rainfall can be up to 2000 mm. The annual average precipitation is 1030 mm, ranging from 1400 mm/year near the Zambezi basing to 300 mm/year in the south (Uamusse et al. 2017).

According to Burgess et al. (2004), thirteen ecoregions are recorded in Mozambique (Fig. 1). These are included in five main biomes: arid and semi-arid forest; tropical and subtropical rangelands, savannas, shrublands, and woodlands; flooded grasslands and savannas; mountain grasslands and shrublands; mangroves (Ministério da Terra



Figure 1. Mozambique ecoregions (Burgess et al. 2004; Olson 2020).

Ambiente e Desenvolvimento Rural 2015). Such biogeographical complexity results in high plant diversity. Until now, only 6,264 plants species have been recorded (Hyde et al. 2021) and seven broad vegetation communities identified, namely: miombo, woodland, mopane woodland, undifferentiated woodland, afromontane communities, halophytic vegetation, swamp vegetation and coastal mosaic (Bandeira et al. 1996).

According to Darbyshire et al. 2019, Mozambique has a relatively high level of plant endemism, with 572 taxa classified as strict-endemic or near-endemic, which are not evenly distributed throughout the country. Approximately 80% of Mozambique is included in the Zambezian Regional Centre of Plant Endemism (Bandeira et al. 1996), a continental scale phytochorion including the whole of Zambia, Malawi, Zimbabwe, large parts of Angola, Tanzania and Mozambique, and some small parts of Zaire, Namibia, Botswana, and South Africa (White 1983). Focusing on more restricted phytogeographical units, Darbyshire et al. (2019) has proposed four main Centres of Plant Endemism (Fig. 2). The Rovuma Centre of Endemism, previously referred as the Lindi Centre of Endemism (Clarke 1998) and restricted to southeast Tanzania, has recently been extended to the north Mozambique encompassing the coastal area of Cabo Delgado, Nampula and Zambézia provinces (Burrows and Timberlake 2011; Darbyshire et al. 2019). The Maputaland Centre of Endemism, shared with the KwaZulu-Natal province of South Africa and Eswatini, extends from the coastal lowlands of southern Mozambique to the Save River (Van Wyk 1996; Darbyshire et al. 2019). This centre can be subdivided into at least three



Figure 2. Mozambique Centres of Endemism. Modified version from Darbyshire et al. (2019).

sub-centres, such as the Maputaland (sensu stricto), the Lebombo Mountains Centre and the Inhambane Centre (Darbyshire et al. 2019). As part of the Afromontane Archipelagolike Centre of Endemism, Mozambique shares the Chimanimani-Nyanga Centre of Endemism with neighbouring Zimbabwe (Clark et al. 2017; Darbyshire et al. 2019), and includes large part of the Mulanje-Namuli-Ribáuè Centre of Endemism, which extends from southern Malawi to Zambézia and Nampula provinces (Darbyshire et al. 2021).

Despite its high diversity, the Mozambican flora has received only limited research coverage, remaining poorly known (Ministry for the Coordination of Environmental Affairs 2014; Darbyshire et al. 2019). Until the historical Botanic Mission to Mozambique (1942 – 1948) which represents the first study of the country's plant diversity, the Mozambican flora was little known (Conde et al. 2014). The Botanic Mission to Mozambique represented a breakthrough for botanical studies in the country (Ministry for the Coordination of Environmental Affairs 2014), enabling the collection of more

than 7,600 herbarium samples and the description of many families and species (Conde et al. 2014). The country's protracted instability caused by the war of independence (1964 - 1975) and the subsequent civil war (1977 - 1992) resulted in a long period in which biodiversity research was neglected. However, in the last two decades a new impetus in botanical studies has risen (Cheek et al. 2018). In the early 2000s, da Silva et al. (2004) published a preliminary checklist of Mozambique's vascular plants, which included 3,932 indigenous species. The checklist was built on the analysis of specimens from the National Herbarium of Mozambique (LMA) and the Herbarium of Eduardo Mondlane University (LMU) with additional records from literature sources. However, the list was under-representative of Mozambique's plant diversity. Subsequent surveys provided new species and new country records. Timberlake et al. (2011) documented 68 new taxon records for Mozambique during a survey of the coastal dry forests in the Cabo Delgado Province in the northeast Mozambique, while Harris et al. (2011) listed another 31 new taxa for the country. The recent research and programme, such as the ongoing "Flora Zambesiaca" series, has progressively increased the estimated number of species in Mozambique and produced a huge effort to document the country's floristic diversity. According to the Flora of Mozambique website (Hyde et al. 2021), currently the most comprehensive database for plant diversity in Mozambique, 6,264 species are recorded in the country. This figure is expected to grow rapidly, following the increase in botanical expeditions and the resulting new discoveries (Cheek et al. 2018; Darbyshire et al. 2019), marking Mozambique amongst the countries with the highest rate of discovery of new species in continental Africa (Darbyshire et al. 2020). On this basis, considering the crucial role of the national floristic inventory to assess plant conservation, management, and ecological restoration (Brundu and Camarda 2013; Lorite 2016), the need for an updated checklist to summarise the current state of knowledge of Mozambican vascular flora clearly emerges.

This paper presents an updated checklist of Mozambique's vascular plants serving as a basis to guide further botanical research and to support biodiversity conservation planning. For each listed taxon, data such as the nomenclature, taxonomic classification, distribution, local occurrence details, life forms, endemism, and extinction risk are reported. Moreover, considering the key role of indigenous names and traditional uses of plants in botanical studies, frequently conducted through field surveys carried out with local people (de Koning 1993), the known vernacular names and traditional uses of the listed species are reported. Data were collected from large and freely available biodiversity databases, herbarium, museum collections (both national and foreign), and literature sources.

Methods

Data collection and organisation

To compile the present checklist, multiple information sources were examined and combined. An initial list (n = 3,932 species) was obtained, after verification of the taxa's nomenclature, from da Silva et al.'s preliminary checklist (2004). The initial list was

extended using data from the Buffelskloof Herbarium (BNRH, n = 4,266 records), the Royal Botanic Gardens Kew Herbarium (K, n = 7,484 records), the National Herbarium of Mozambique (LMA, n = 22,703 records) and the Eduardo Mondlane University's Herbarium (LMU, n = 2,936 records) (acronyms according to Thiers 2020). Successively, we included taxa described in Mozambique from the following relevant databases of plant diversity and taxonomic research: Global Biodiversity Information Facility (GBIF, https://doi.org/10.15468/dl.gq7jnb, n = 91,832 records), Plants of World Online (POWO, http://www.plantsoftheworldonline.org/ n = 5,639 species), Flora of Mozambique (https://www.mozambiqueflora.com/, n = 6,264 species), Flora Zambesiaca (http://apps.kew.org/efforas/advsearch.do?reset=true, n = 4,482 species), and JSTOR – Global Plants (https://plants.istor.org/, n = 1,846 species). Finally, additional taxa were found through the review of previous studies on Mozambique's flora (de Koning 1993; Timberlake et al. 2007, 2009; Wursten et al. 2017; Bayliss et al. 2010; Müller et al. 2012; Burrows et al. 2018; Darbyshire et al. 2019, n = 4,468 species) and through an extensive review of the most relevant ethnobotanical studies (Krog et al. 2006; Bandeira et al. 2007, 2011; Ribeiro et al. 2010; Silva et al. 2011; Williams et al. 2011; Bruschi et al. 2011, 2014; Conde et al. 2014; Santo-António and Goulão 2015; Moura et al. 2018; Barbosa et al. 2020; Manuel et al. 2020, *n* = 394) to document the traditional knowledge associated with the use of plants in the country.

Overall, a list of 157,576 records was produced, on which a thorough refinement procedure was performed through a Microsoft Excel 2010 spreadsheet. All records were organised by family rank, based on the classification system proposed by APG IV (Angiosperm Phylogeny Group 2016) for the angiosperms, by PPG I (Pteridophyte Phylogeny Group 2016) for the pteridophytes and by Christenhusz et al. (2011) for the gymnosperms. Taxa at rank of form and hybrids were not considered. Different quality filters were applied to remove repeated taxa and to exclude fungi, lichens, algae, bryophytes, and marine species. Finally, a manual refinement was carried out to clean repetition, remove doubtful taxa (labelled as "aff.", "cf." and "sp.") and those whose taxonomic status was uncertain. The resulting intermediate list consisted of 15,605 taxonomic names, 9.9% of the initial collection.

Taxonomic validation

The obtained list was subjected to a taxonomic validation process. Taxonomic rank and plant names were verified and validated with international reference databases: POWO (2021), African Plant Database (2021), The Plant List (2013). Version 1.1 and World Flora Online (2021). Subsequently, data review and validation was performed by floristic experts from the Royal Botanic Gardens Kew, the Botanic Garden Meise and the Buffelskloof Research Centre, which have verified the accepted species name derived from reference databases. Errors and inconsistencies found in the process (such as taxonomic misidentification, geographic errors, and incorrect life form) were assessed and corrected.

Checklist outline

For each entry in the checklist, the taxonomic rank (species, subspecies, variety) is reported. A pragmatic approach was taken when treating the data records for infraspecific taxa (subspecies, varieties), autonyms and inclusive species names. To avoid artificially increasing the overall number of taxa in the checklist, inclusive species names were excluded where possible for species with infraspecific taxa occurring in Mozambique. However, in some cases, we retained inclusive species names where the infraspecific taxon was unclear and the data record added useful distribution information.

Using a modified classification system derived from Darbyshire et al. (2019), we categorised the plants listed in one of the following seven life forms categories: tree, shrub, subshrub, liana, herb, fern, and cycad. For trees and subshrubs, only the succulent subcategory is given, while for shrubs two subcategories, such as succulent and parasitic, were reported. Similarly, for the herbs ten subcategories were reported: aquatic, climber, epiphyte, geophyte, graminoid, parasitic, rhizomatous, seagrass, saprophyte, and succulent. Available life cycle information (annual, biennial and perennial) was also provided.

For each taxon, occurence locality and geographical distribution within the national border were recorded, based on information provided by the literature sources, online databases and herbarium specimen labels. Occurrence localities were organized by province and coded as follows: Maputo City and Province (MP), Gaza (G), Inhambane (I), Manica (MC), Sofala (S), Tete (T), Zambézia (Z), Nampula (NP), Cabo Delgado (CD), and Niassa (NI). If available, data on localities of occurrence, such as protected areas or a specific locality, were also provided. To update the toponyms referring to the colonial period and standardise the Mozambican locality names, a review was conducted through The GeoNames (2021) geographical database (https://www.geonames.org/). Although the geographical information included in the checklist cannot be considered exhaustive, it is provided to support further studies of Mozambican flora.

For endemic and near-endemic species we referred to the criteria in Darbyshire et al. (2019). "Strict-endemic" (E) species were defined as those occurring only within the country borders, while near-endemic (NE) species were designated as those occur in five or fewer localities, besides Mozambique. Endemisms (considering both strict-endemic and near-endemic taxa) were derived from Darbyshire et al. (2019) and through the references databases.

To evaluate the extinction risk of Mozambique vascular plants, The IUCN Red List of Threatened Species (2021) (https://newredlist.iucnredlist.org/) was adopted as the key reference. Taxa were categorised according to the IUCN (2012) categories. An additional remark was included for taxa that need to have Mozambique added to the geographic range in their published IUCN assessments, following the results of this study.

The known vernacular names, obtained from literature sources and herbarium specimen labels, were reported using a simple code composed of the local language and/

or the province where it is spoken. The checklist of vernacular plants names compiled by de Koning (1993), has been adopted as a key reference. Although it cannot be considered exhaustive and updated, it represents the most extensive collection of Mozambique's vernacular plants names. Overall, vernacular names from 18 local languages were reported. All traditional uses of plants recorded in literature sources were included using the following categories: medicine (treatments or remedies for various pathologies), veterinary (animal healthcare), food (preparation of food and beverages, subsistence resources), livestock fodder (plant materials eaten by livestock), cosmetic (personal care), handicraft (production of tools and furniture), fuel (energy supply), ornament (domestic, urban and landscape design), poison (used for hunting purpose) and beliefs (for taxa associated to local beliefs or mystical rituals).

All data were aggregated in a Microsoft Excel 2010 spreadsheet and managed through R software version 3.6.1 (R Core Team 2019).

Results and discussion

The updated checklist of Mozambique vascular plants, presented in Suppl. material 1, accounts for 7,099 taxa (5,957 species, 605 subspecies, 537 varieties), belonging to 226 families and 1,746 genera. These findings, significantly increasing the record of 3,932 vascular plant taxa in Mozambique registered in the previous checklist (da Silva et al. 2004), can be related to the recent increase of botanical exploration in the country (Cheek et al. 2018) and to the availability of freely accessible online botanical databases. The larger group of plants recorded is the angiosperms, 200 families and 1,655 genera, representing 95.8% (6,804) of the listed taxa. Pteridophytes, 20 families and 77 genera, amount to 3.6% (257) of the taxa. Gymnosperms, with 6 families and 14 genera, account for only a very small percentage (0.5%; 38) of the taxa (Fig. 3A).

A total of 6,171 taxa (86.9%) are native to Mozambique, while 602 (8.5%) are introduced, mostly for commercial purposes (Syliver et al. 2020). The remaining 326 taxa (4.6%) are assessed as uncertain status (Fig. 3B).



Figure 3. Floristic patterns for Mozambique's vascular plants. **A** frequency of plant groups **B** geographic origin of taxa.

As seen in other African countries (Braun et al. 2004; Mapaura and Timberlake 2004; Zhou et al. 2017), the three most taxa-rich families in Mozambique are Fabaceae (891 taxa), Poaceae (543) and Asteraceae (428), which also represent the largest families in the world (Zhou et al. 2017). Other well represented families (\geq 100 taxa) are: Rubiaceae (371), Orchidaceae (257), Malvaceae (223), Euphorbiaceae (220), Cyperaceae (218), Acanthaceae (215), Apocynaceae (207), Lamiaceae (205), Asparagaceae (145) and Convolvulaceae (111) (Fig. 4). The most taxa-rich genera (\geq 100 taxa) are *Cyperus* L. (Cyperaceae), *Crotalaria* L. (Fabaceae) and *Indigofera* L. (Fabaceae) accounting for 109, 108 and 102 taxa, respectively.



Figure 4. Most represented families and their endemism rate.

The majority of taxa in the checklist (58.2%) are herbaceous, while shrubs and trees account respectively for 26.5% and 9.2% of the listed taxa. The other life form amount to 6.1% of the listed taxa, divided into: ferns (3.6%), lianas (1.7%), subshrubs (0.5%) and cycads (0.3%). Due to the lack of reliable data, only 1 taxon was not assigned to any life forms category (Fig. 5).

Table 1 shows all life form categories and the growth habit subcategories and life cycle of trees, shrubs, subshrubs, and herbaceous taxa. 2.5% of tree taxa are classified as succulent. Among subshrub taxa just a small number of taxa are classified as succulent (7.9%), while the shrubs include two growth form subcategories: parasitic (3.5%) and succulent (3.0%). Much growth form diversity is found among the herbaceous taxa, with 10 different subcategories (Table 1). As to life cycle, 66.1% of the herbaceous taxa are identified as perennial, 27.5% as annual and only a small percentage (0.1%) as biennial.



Taxa



Life Form	Growth form	Life cycle	Number of taxa
Tree	_	_	651
	Succulent	-	16
Shrub	_	-	1883
	Succulent	-	57
	Parasitic	-	65
Subshrub	_	-	38
	Succulent	-	3
Herb	-	-	4240
	_	Annual	1129
	_	Biennial	4
	_	Perennial	2166
	Aquatic	_	54
	Climber	_	276
	-	Annual	3
	-	Perennial	188
	Epiphyte	-	93
	Geophyte	-	148
	Graminoid	-	84
	_	Annual	25
	_	Perennial	59
	Parasitic	-	14
	_	Annual	4
	_	Perennial	10

Table 1. Life form, growth form and life cycle of the taxa.

Life Form	Growth form	Life cycle	Number of taxa
	Rhizomatous	-	10
	Seagrass	-	9
	Succulent	-	127
	_	Annual	3
	-	Perennial	124
	Saprophyte	-	5
	Unknown	-	10
Liana	-	-	122
Fern	_	-	257
Cycad	-	-	18
Unknown	-	-	1

Regarding endemic and near-endemic plant species, Mozambique has a total of 278 strict-endemic taxa and 403 near-endemics, giving a total endemism level of 9.6%. The increase in the number of strict-endemic and near-endemic taxa compared previous studies (Darbyshire et al. 2019) can be related to the continuous progress in the knowledge of the Mozambique's flora. About 56.1% of the listed strict-endemic taxa are included in ten angiosperm families: Fabaceae (40), Euphorbiaceae (26), Rubiaceae (23), Apocynaceae (12), Malvaceae (12), Lamiaceae (11), Acanthaceae (10), Asphodelaceae (8), Asteraceae (7), Orchidaceae (7). Except for Asphodelaceae, these families are also the most represented families in the vascular flora of Mozambique, showing a congruence between the most species-rich families and those with the highest rate of endemism (Fig. 4). The geographical distribution of endemic taxa in this checklist closely matches the findings of Darbyshire et al. (2019), confirming the importance of Mozambique's Centres of Endemism for flora conservation efforts. Similarly, the mountain areas of the country play a crucial role in the conservation of endemic flora, with the Chimanimani Mountains and Mount Namuli representing the most frequently recorded localities for strict and near endemic taxa, respectively with 137 and 59 taxa. Overall, about 40.2% of endemic taxa (both strict and near endemic) occur in the mountainous areas of the country, consistent with the global pattern of high rates of endemism at high altitudes (Steinbauer et al. 2016).

At the time of compiling this checklist (July 2021), 1,667 of the recorded taxa were registered on the IUCN Red List. Overall, the global extinction risk status for 76.5% of Mozambique's vascular flora is not evaluated (including the taxa categorised as Not Evaluated and those not listed in the IUCN Red List), while a further 0.8% of the taxa are categorised as Data Deficient (Fig. 6A). Such findings highlight the general lack of information on the conservation status of Mozambique's vascular plants. Further studies are urgently needed to identify threatened species and develop proper conservation strategies. About 18.1% of the evaluated taxa are categorized as threatened: 158 Vulnerable, 119 Endangered, and 24 Critically Endangered (Fig. 6B).



Figure 6. Extinction risk of Mozambique's vascular plants. A assessed taxa B IUCN category for the evaluated taxa.

From these data clearly emerge the need to implement effective conservation strategies for Mozambique's flora. According to Darbyshire et al. (2019) the main threat factors for vascular plants in the country are habitat loss and degradation, driven by the recent population growth and the consequent increased pressure on natural ecosystems. The growing commercialisation and the over-exploitation of medicinal plants are also becoming an increasing threat (Bandeira et al. 1996; Senkoro et al. 2020). For example, *Warburgia salutaris* (G. Bertol.) Chiov., one of the most widely used medicinal plants in southern Africa, as a consequence of the increasing commercial demand in the last few years has been subjected to uncontrolled harvesting, resulting in a widespread tree mortality and even in the extinction of local populations in many areas, changing its conservation status to globally Endangered (Senkoro et al. 2020). Moreover, about 75.1% of Mozambique's threatened taxa (Vulnerable, Endangered and Critically Endangered) are endemic to Mozambique (111 strict-endemic; 115 near-endemic) (Fig. 7), highlighting the central responsibility of the country for the conservation of these taxa.

Finally, we have identified 261 of Mozambique's vascular plants assessed in the IUCN Red List of Threatened Species, for which Mozambique is not indicated in the geographical range of distribution. Therefore, there is a need to update these assessments, which provide an invaluable tool for plant conservation.

Based on available occurrences, the geographic distribution of Mozambique's vascular plants is provided in Fig. 8. The distribution patterns identified do not indicate specific latitudinal or regional gradients. Instead, a total of twenty-four occurrence localities, scattered among the province of the country, are recorded. The main occurrence localities (≥ 500 taxa) identified are: the Gorongosa National Park (Sofala province, 740 taxa), the Chimanimani Mountains (Manica, 668), the Serra Gorongosa plateau (Sofala, 549), the Mount Namuli (Zambézia, 536) and the Inhaca Island (Maputo, 534). Other relevant key occurrence localities (ranging from 200 to 400 taxa) are Palma (Cabo Delgado, 316 taxa), Vilanculos (Inhambane, 315 taxa), Serra Chiperone (Zambézia, 298 taxa), Tsetsera & Serra Zuira (Manica, 283), Quiterajo (Cabo Delgado, 255), Mount Mabu (Zambézia, 249), and Serra Choa (Manica, 227 taxa). All occurrence localities are provided in the Suppl. material 1. These localities


Figure 7. Endemism rate among the threatened taxa.

must be considered of high botanical value for floristic study in the country, making their preservation a strategic priority. A large number of taxa are found in the central provinces of the country (Sofala, Manica, Tete, and Zambézia), which host a total of 4,765 taxa. In the South (Maputo, Gaza and Inhambane provinces) 3,292 taxa are recorded, while the North (Nampula, Cabo Delgado and Niassa provinces) counts 3,120 taxa. The most taxa-rich provinces (\geq 2,000 taxa) are Maputo, Manica, Zambézia, and Sofala, accounting for 2,654, 2,474, 2,461 and 2,231 taxa, respectively (Fig. 8). Although the data collected partially fills the previous knowledge gap on the floristic biodiversity of northern Mozambique (da Silva et al. 2004), the southern and central regions of the country still remain the most widely explored. To properly assess the distribution of plant species in Mozambique, further studies conducted equally throughout the country should be undertaken.

The vernacular names of 1,339 vascular plants of Mozambique are reported in this checklist. Considering the various language spoken in the country (According to de Koning (1993), at least eighteen main local languages excluding Portuguese) such ethnobotanical knowledge can play a pivotal role for research on Mozambique's plant biodiversity. Indeed, knowing the local names of plants can facilitate investigations carried out in the field with the support of local indigenous people who lack a formal taxonomic knowledge (de Koning 1993).

The review of available ethnobotanical data for Mozambique's vascular plants resulted in the identification of 773 taxa with traditional uses (Fig. 9), showing the fundamental role played by plants in the livelihood of the Mozambican population. About Sixty



Figure 8. National distribution of occurrence records.



Figure 9. Mozambican vascular plants related to traditional uses.

percent (62.9%) of these plants are used for medicinal purposes. These account for about 6.9% of Mozambique's flora, in line with previous estimates (Bruschi et al. 2011) which highlights the importance of traditional medicine in the Mozambican population's health

care. Other significant traditional uses found are as food supply (34.7%), handicraft production (24.3%) and livestock fodder (12.2%). A smaller number of plants are

production (24.3%), and livestock fodder (12.2%). A smaller number of plants are used as ornamental elements (1.9%), fuel (3.1%), cosmetics (2.1%), in the veterinary field (1.7%), and as poison (0.3%). Finally, 11.4% of traditional plants use is associated to local beliefs. A total of 282 plants (4.0% of the listed taxa) are associated with more than one use, such as *Asparagus africanus* Lam., *Boscia albitrunca* (Burch.) Gilg & Gilg-Ben., *Elaeodendron schlechterianum* (Loes.) Loes., *Euclea divinorum* Hiern., and *Trichilia emetica* Vahl., all having six recorded use categories. Such a wealth of multipurpose taxa highlights the richness and variety of traditional knowledge related to the use of plants in Mozambique, particularly with regard to traditional medicine (Ribeiro et al. 2010).

Conclusion

This is a comprehensive and up to date checklist of Mozambique's vascular plants, summarizing data from relevant literature sources, herbarium collections and authoritative botanical databases. The reported data, including taxonomic classification, biological and morphological attributes, geographical distribution, endemism, extinction risk, and ethnobotanical information, can represent a reliable basis for further botanical studies in Mozambique. In this respect, future efforts should be focused on maintaining the country's floristic knowledge so that it is regularly and frequently updated and easily accessible, in order to support botanical research and plant biodiversity conservation in Mozambique.

Acknowledgements

The authors would like to thank all contributors to this paper. Special thanks to the Mozambique Agricultural Research Institute (IIAM), Eduardo Mondlane University (UEM), Royal Botanic Gardens Kew, and Buffelskloof Research Centre for their contribution to the botanical knowledge of Mozambique. The valuable plant collections hosted in their herbaria, and their efforts to preserve and increase such biodiversity, have allowed us to compile this checklist. The authors would also like to express their warm thanks to the Tropical Important Plant Areas project's team for their efforts in identifying the most important sites for plant diversity in Mozambique, which was a support in carrying out this work. A warm thank you to Alima Ali and Marta Aduge from Eduardo Mondlane University and to Hugo Costa and Eleutério Duarte for their contribution to data collection. Finally, special thanks to Inês Chelene, Camila de Sousa and Tereza Alves from the IIAM for their valuable support in this work. This work has been conducted within the framework of SECOSUD II Project (Conservation and equitable use of biological diversity in the SADC region: from geographic information system (GIS) to Spatial Systemic Decision Support System (SSDSS) – AID: 9695) founded by the Italian Agency for Development Cooperation. The contents of this publication are the exclusive responsibility of the authors and do not necessarily represent the point of view of the Agency.

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Supplementary material I

The updated checklist of Mozambique's vascular plants

Authors: Délcio Odorico, Enrico Nicosia, Castigo Datizua, Clayton Langa, Raquel Raiva, Joelma Souane, Sofia Nhalungo, Aurélio Banze, Belkiss Caetano, Vânia Nhauando, Hélio Ragú, Mário Machunguene Jr., Jónata Caminho, Leonel Mutemba, Efigénio Matusse, Jo Osborne, Bart Wursten, John Burrows, Silvio Cianciullo, Luca Malatesta, Fabio Attorre

Data type: species data

- Explanation note: The updated checklist of Mozambique's vascular plants includes 7,099 taxa (5,957 species, 605 subspecies, 537 varieties), belonging to 226 families and 1,746 genera. For each entry in the checklist, the following available data are reported: nomenclature, taxonomic classification, distribution, local occurrence details, life form and life cycle, endemism, extinction risk, vernacular names and traditional uses.
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Link: https://doi.org/10.3897/phytokeys.189.75321.suppl1

RESEARCH ARTICLE



Valeriana praecipitis (Caprifoliaceae), a species new to science and endemic to Central Chile

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Academic editor: Peter de Lange | Received 5 September 2021 | Accepted 13 October 2021 | Published 4 February 2022

Citation: Villarroel AE, Menegoz K, Le Quesne C, Moreno-Gonzalez R (2022) *Valeriana praecipitis* (Caprifoliaceae), a species new to science and endemic to Central Chile. PhytoKeys 189: 81–98. https://doi.org/10.3897/phytokeys.189.73959

Abstract

The species *Valeriana praecipitis* (Caprifoliaceae), new to science and endemic to the Nuble Region, Central Chile, is formally described. Morphological data support its placement in a new species, clearly different from *V. philippiana*. A detailed description, insights about its habitat and ecology, distribution map and illustration are provided. A table of comparison is also given with the morphological characters discriminating *V. praecipitis* from *V. philippiana*. The species is assessed as Endangered (EN) under the IUCN categories.

Keywords

Andes, biodiversity, cliffs flora, Nuble Region, taxonomy

Introduction

The Valerianaceae family was included in the Caprifoliaceae family by the Angiosperm Phylogeny Group III (APG III 2009). Although some authors continued using Valerianaceae for several years (e.g., Kutschker 2011), the family remained within the Caprifoliaceae in the APG IV (2016). Caprifoliaceae includes about 960 species in approximately 41 genera (Wang et al. 2020), having an almost cosmopolitan distribution, with centres of diversity in eastern North America and eastern Asia (Xu and Chang 2017).

Valeriana is one of the most diverse genera within the Caprifoliaceae, with a worldwide distribution, and centres of diversity in tropical areas of Central-America and to the south along the Andes mountains (Kutschker and Morrone 2012). In South-America, Valeriana species are present in Peru, Bolivia, Brazil, Chile and Argentina (Zuloaga et al. 2008). In Chile, Borsini (1966) described 42 species, while the most recent assessment identified 40 species in the Andes mountains of Chile and Argentina, between latitudes 33° and 56° S (Kutschker 2011). This area was identified as a centre of diversification of Valeriana species (Kutschker and Morrone 2012). In Chile 44 species are accepted, of which 18 are endemic, and interestingly, to date no introduced species has been found so far (Rodríguez et al. 2018). All species are herbaceous (Rodríguez et al. 2018) and many of them are distributed in high-altitude mountainous locations, usually above 1000 m elevation (e.g., V. stricta Clos, V. philippiana Briq.). They can also be restricted to dry soils (e.g., V. corynodes Borsini) or in permanently wet soils (e.g., V. fonckii Phil.). Recently, only one species new to science has been formally described, V. nahuelbutae Penneck. (Penneckamp 2020) and few new geographical locations have been found for other species (e.g., Kutschker 2011; Teillier et al. 2020). Furthermore, for most species little is known about their ecology or ethnobotany, and to this day, only one of the Chilean species have had formal conservation assessments completed according to the IUCN criteria (Valeriana senecioides Phil.; MMA 2021).

The Central Chilean Andes mountains are recognised as a centre for endemism in South-America (e.g., Villagrán and Hinojosa 1997; Arroyo et al. 2006). In addition, the Andean ranges of the Nuble Region were declared as a Biosphere Reserve in 2011 (San Martín 2014; Fig. 1) and this area is of increasing interest since a new genus was recently described (Villarroel et al. 2021). However, several species in the region remain severely threatened (Hechenleitner et al. 2005). Intensive land-use changes due to human activities have affected vast extensions of almost all types of vegetation in Central Chile, including high-Andean habitats (Arroyo et al. 2006). In addition, global climate change is a serious threat to montane habitats that have reduced in size, are geographically isolated, and where the environmental conditions have changed significantly across small distances due to steep slopes (Báez et al. 2016).

In this manuscript we describe a species of *Valeriana* located in the Central Chilean Andes at around 36° S latitude and clearly distinguishable from other species by its remarkable silvery-green basal leaves. In the following sections, a detailed account on how the species was discovered is given as a formal description. In addition, a distribution map, insights about its habitat and ecology, conservation status, and illustrations are provided.

Methods

Herbarium and Fieldwork

During the austral spring-summer between 2015–2021 several botanical explorations were carried out in the Andean ranges within the locality of San Fabián de Alico, Punilla Province, Nuble Region, Chile (Fig. 1), by means of rock-climbing in the Cordillera del Malalcura and Cuernos del Valiente and treks to Laguna del Florido. In addition, seven expeditions were made to Laguna Añil which fell within the scope of our research project entitled "Richness and distribution of the flora along an altitudinal gradient in Bullileo creek, Nuble Region" (Villarroel 2019). During these excursions, a species of *Valeriana* that could not be assigned to any known species for Chile and Argentina was found. Its habitat and characters such as plant size and root system were observed and measured in the field.

Herbarium specimens were collected from Laguna Ańil, and distributed to the herbaria of CONC, EIF, JBN and SGO (acronyms after Thiers 2016). As our preliminary observations of the plant and the key to *Valeriana* genus (Kutschker 2011) suggested similarities with *Valeriana philippiana* Briq., (e.g., basal leaves silvery-green colour and pinnatisect, terminal lobe size equal to bigger, fleshy texture, arranged in an imbricate rosette, fruits pubescent, pappus plumose), we examined the herbarium specimen at SGO to determine the morphological differences between both species. Also, online digital images of specimens available on CONC, E and US herbaria websites were studied, as well as literature (Borsini 1966; Kutschker 2008, 2011). The taxonomic treatment was prepared after examining all available specimens.

Morphological analysis

The morphological study was based on observations and measurement of fresh and dried specimens. Detailed photographs of fresh material were taken in the field to document the overall plant morphology and especially the floral structure. In order to accurately describe the vegetative and reproductive morphology of the collected plants, dry and rehydrated specimens were dissected. Ovary, fruits, flowers and leaf details were photographed with a zoom lens and subsequently observed under a binocular microscope. Terminology for describing *Valeriana* plant parts followed Borsini (1966) and Kutschker (2008, 2011).

Conservation status

The conservation status assessment of the species used the International Union for Conservation of Nature criteria (IUCN Standards and Petitions Committee 2019). The extent of occurrence (EOO) and area of occupancy (AOO) were calculated using GeoCat (Bachman et al. 2011).



Figure 1. Distribution maps of *Valeriana praecipitis* **A** Chile **B** Nuble Region **C** sites of *V. praecipitis* (yellow stars) and extent of occurrence (red line).

Taxonomic treatment

Valeriana praecipitis A.E. Villarroel & Menegoz, sp. nov.

urn:lsid:ipni.org:names:77254613-1 Figures 2, 3, 4

Type. CHILE. Nuble Region, Punilla Province, San Fabián de Alico, Laguna Añil, crevices and small terraces of granite cliffs, 1724 m elevation, 36°32'00.8"S, 71°23'36.1"W, 7 January 2020, *A.E. Villarroel & E. Ponce s.n.*, (holotype SGO!); 1724 m elevation, 36°32'00.8"S, 71°23'36.1"W, 3 February 2021, *A.E. Villarroel & R. Neira* (paratypes EIF!, JBN!); 1650 m elevation, 36°32'2.28"S, 71°23'26.62"W, 7 December 2020, *K. Menegoz & G. Ossa* (paratypes CONC!) (Fig. 2). Laguna Añil is the only site where herbarium specimens were collected.

Diagnosis. The habit and macro-morphology of *Valeriana praecipitis* is similar to *Valeriana philippiana*, but differs by its height (including flower stem) that can reach 65.5 cm (vs. 20 cm), rhizome woody, reaching more than 30 cm long and up to 20 mm diameter (vs. semi-woody, to 14 cm long, to 8 mm diam.), basal leaves pinnatisect to pinnatipartite, up to 26 cm long (vs. pinnatilobed to pinnatisect, to 8 cm long), petiole glabrous (vs. pubescent), lobes 1–35 mm long, 1–24 mm wide (vs. 4–8 × 3–7 mm), upper leaves oblanceolate, 14–40 mm long, 5–19 mm wide, margin entire to irregularly undulate or sinuate, (vs. oblong, 6–10 × 3–5 mm, entire), bracts oblanceolate to oblong, up to 20 mm long (vs. oblong, to 7 mm long), bracteoles spathulate to oblong, 3–7 mm long, entire (vs. oblong, 2.5–4.5 mm, erose), inflorescence a relatively diffuse thyrse or compound dichasial cyme (vs. dense compound dichasial cyme, contracted), corolla up to 4.5 mm long (vs. up to 4 mm), stamens 3 mm long (vs. 2 mm), stigma 0.2 mm long (vs. 0.5 mm), growing on cliffs that remain humid all-year (vs. well-drained rocky soils), and endemic to the Nuble Region (vs. in Chile, *V. philippiana* can be found in Los Lagos, Aysén and Magallanes Regions) (Table 1).

Description. Perennial *herb*, hemicryptophyte, erect or lax when cliff-hanging, simple or branched from the upper part of the taproot, 4–25 cm tall (26–65.5 cm with inflorescence), 4–28.5 cm wide. *Rhizome* is dark brown, thick, circular, simple, sometimes branched, reaching more than 30 cm long, 8–20 mm diameter, vertical to lateral, sometimes stoloniferous, woody, tortuous, rough, fetid. *Secondary-tertiary roots*, numerous, located in the first 3 cm of the upper part of the taproot. *Stem* merging into the taproot, 3.5–6 mm diameter, with short internodes, forming a basal rosette with 9–25 whorled leaves. *Basal leaves* deciduous, silvery-green turning yellow-brown at the end of summer, simple, petiolate, pinnatisect, sometimes becoming gradually pinnatipartite at the apex (mainly young leaves), oblong, generally symmetric; *blade* 3–16 cm long (4–26 cm with petiole), 1.5–6.3 cm wide, glabrous, fleshy, with reticulated veins; *petiole* green turning purple towards the base, canaliculated, up to 14.5 cm long, 3–13 mm wide at the base, 2–7 mm wide at the blade base, entire, glabrous, midrib visible; *lateral lobes* opposite to subopposite, superimposed, orbicular to obovate, base attenuate, apex rounded to retuse, margin entire to slightly undulate and invo-



Figure 2. Holotype of Valeriana praecipitis.



Figure 3. *Valeriana praecipitis* **A** habit **B**, **C** fruit **D** detail of flower **E** stigma **F** bracteole **G** bract **H** upper leave. Drawn by Arón Cádiz-Véliz.

lute, 6–26 per blade; larger lobes located in the centre of the blade, 9–35 × 7–22 mm; smaller lobes located at the base of the blade, $1-15 \times 1-8$ mm; *terminal lobe* orbicular to obovate, 6.5–23.5 × 6–24 mm, base attenuate, apex rounded to obtuse, occasionally retuse, margin entire to irregularly undulate or lobed. *Inflorescence* a relatively diffuse thyrse or terminal compound dichasium, sometimes corymboid. *Floral stem* purple at



Figure 4. *Valeriana praecipitis* **A, B** plants growing in natural habitat **C** rhizome, secondary-tertiary roots **D** basal leaves with lobes detail **E** petioles **F** floral stem, corymboid inflorescence **G** floral stem, thyrse inflorescence **H** upper leaves **I, J** detail of flowers (stamens, style) **K** bracteoles, ovary, calyx **L** dry inflorescence, bracts, bracteoles **M** fruit, pappus. Photographed by Alejandro E. Villarroel and Kora Menegoz.

the base, light green towards the flowers, erect, circular, 28.6-60 cm long, 3.5-6 mm diameter at the base, gradually thinner towards the flowers (1.5–2.9 mm), striated, 5–9 internodes (their length decreasing from base toward the apex), branched in the upper half (2-27 cm long) with 1-6 lateral ascending branch pairs (forming partial inflorescences). Upper leaves green, simple, sessile, oblanceolate, 14-40 × 5-19 mm, opposite, decussate, arranged every 2.7-13.5 cm on the flower stem, leaves' size decreasing from base toward the inflorescence, margin entire to irregularly undulate or sinuate, base decurrent, apex acute to rounded, occasionally retuse, glabrous, less fleshy than basal leaves, reticulated veins. Bracts green, simple, sessile, oblanceolate to oblong, $7-21.2 \times 1-7$ mm, decreasing in size towards the inflorescence, opposite, decussate, margin entire, base decurrent, apex variable (acute, rounded or retuse), glabrous, less fleshy than upper leaves, reticulated veins. Bracteoles green, sometimes turning purple towards the apex, simple, sessile, spathulate to oblong, $3-7.5 \times 0.5-2$ mm, decreasing in size towards the inflorescence, opposite, decussate, margin entire, base decurrent, apex rounded to retuse, glabrous, less fleshy than upper leaves. Flowers hermaphrodite, pentamerous, sessile; *calyx* green and purple at the top, inconspicuous, fused segments forming a wavy ring, 0.3 mm, pubescent, adnate to the infer ovary, accrescent and persistent on fruit modified into feathery structures forming the pappus; corolla 5, fused petals, white, although buds sometimes with purple-pink tinges, infundibuliform, glabrous, 4-4.5 mm wide; corolla tube 3.5-4.5 mm long, base slightly gibbous; *corolla lobes* oblong to obovate, $1-1.5 \times 1-1.7$ mm, perpendicular or slightly inclined in relation to the corolla tube; stamens 3, white, filiform, 3 mm long, exerted, attached in the lower third of the tube; anthers light yellow, ellipsoid, bithecal, dorsifixed, deciduous; *ovary* inferior, green, tricarpellate, trilocular with 1 fertile locule and 2 incipient sterile locules; style 1, white, filiform, 2.2 mm long; stigma trifid, lobed, laminate to lamellate, less than 0.2 mm. Fruit an achene, yellow-green at the base, turning purple towards the apex, ellipsoid, triquetrous, 3×1 mm, pubescent, longitudinally striated on one face, calyx persistent, *pappus* plumose, 0.5 mm diameter at base, bris-

tles 11, purple-reddish colour, 3.5 mm long, with hairs 0.5 mm long. Etymology. The specific epithet refers to cliff faces inhabited by these plants. The name means "Valeriana of cliffs" (latin praeceps = steep place, precipice, dangerous; genitive praecipitis).

Phenology. Flowering from November to December; fruiting from January to February.

Distribution and habitat. Endemic to the Andean ranges of the Nuble Region, Chile (Fig. 1A, B). Specifically, 5 sites are known so far (Fig. 1C): Laguna Añil (1724 m elevation, 36°32'00.8"S, 71°23'36.1"W; 1650 m elevation, 36°32'2.28"S, 71°23'26.62"W); Cuernos del Valiente (1530 m elevation, 36°27'46.31"S, 71°29'14.41"W), Cordillera del Malalcura (1700 m elevation, 36°32'0.23"S, 71°30'7.59"W), Laguna del Florido (1980 m elevation, 36°30'55.07"S, 71°14'1.20"W), and the last at Laguna del Huemul (1970 m elevation, 36°52'40.11"S, 71°29'6.33"W, this locality was found by Eitel Thielemann). The maximum distance between the sites is 38 km. The species usually

grows at high elevations (1530–1980 m), in crevices and small terraces of south, southeast and southwest facing cliffs (Fig. 5). Due to snow melting and low sun exposure, these sites remain humid during the dry season. Two closest weather stations, Punilla (840 m elevation) and Caracol (725 m elevation), indicate that the average annual temperature is 11.8 °C and the average total annual precipitation 2150 mm for the period 1965–2012 (DGA 2018). Although at a lower elevation than *V. praecipitis* altitudinal range, a private pluviometer in the village of San Fabián de Alico (447 m elevation) recorded an annual average precipitation of 1399 mm for the period 2017–2020 (Covarrubias J.C., pers. comm.). In addition, to get a better impression of the local conditions during year 2020 temperature and humidity data loggers HOBO U23 Pro v2 (Onset Computer Corporation, Massachusetts, USA) registered an average annual temperature of 6.7 °C, with a minimum of -2.8 °C, a maximum of 24.1 °C, and an average humidity 60.12% at Laguna Añil; and an average annual temperature of 8.7 °C, with a minimum of -4.5 °C, a maximum of 24.9 °C, and an average humidity of 66.39 % in Cordillera del Malalcura.

Associated vegetation. From a phytogeographical point of view, *Valeriana praecipitis* is part of two vegetational formations and three vegetation belts (Luebert and Pliscoff 2017). The vegetation formation at Laguna Añil, Cuernos del Valiente and Cordillera del Malalcura corresponds to Deciduous forest, and the vegetation belt to the Andean temperate deciduous forest of *Nothofagus pumilio* (Poeppig & Endlicher) Krasser (Nothofagaceae) and *Azara alpina* Poeppig & Endlicher (Salicaceae) (Fig. 5A, B); the vegetation formation in Laguna del Florido corresponds to the Altitude low scrub, and the vegetation belt to the Andean Mediterranean low scrub of *Laretia acaulis* (Cav.) Gillies & Hook. (Apiaceae) and *Berberis empetrifolia* Lam. (Berberidaceae) (Fig. 5C). Finally, Laguna del Huemul is part of the Altitude low scrub formation, and the Temperate Andean low scrub of *Discaria chacaye* (G. Don) Tortosa (Rhamnaceae) and *Berberis empetrifolia* vegetal belt.

Our field observations in Laguna Añil (1724 and 1650 m elevation) indicate a total of 45 species associated with *Valeriana praecipitis*. Some of these species include: *Chiliotrichum diffusum* (G. Forst.) Kuntze, *Senecio spp*. (Asteraceae), *Berberis empetrifolia, Maytenus disticha* (Hook.f.) Urb. (Celestraceae), *Desfontainia fulgens* D. Don (Columelliaceae), *Empetrum rubrum* Vahl ex Willd., *Gaultheria sp., G. poeppigii* DC., *G. pumila* (L.f.) D.J. Middleton, *G. tenuifolia* (Phil.) Sleumer (Ericaceae), *Escallonia alpina* Poepp. ex DC., *E. rubra* (Ruiz & Pav.) Pers., *Rayenia malalcurensis* Menegoz & A.E. Villarroel (Escalloniaceae), *Luzula sp.* (Juncaceae), *Myrceugenia chrysocarpa* (O. Berg) Kausel, *Myrteola nummularia* (Poir.) O. Berg (Myrtaceae), *Nothofagus obliqua* (Mirb.) Oerst., *N. pumilio* (Nothofagaceae), *Codonorchis lessonii* (Brongn.) Lindl. (Orchidaceae), *Saxifraga magellanica* Poir. (Saxifragaceae), *Quinchamalium chilense* Molina (Schoepfiaceae).

Conservation status. *Valeriana praecipitis* is assessed here as Endangered (EN) under the IUCN categories following criteria B2ab(iii). Criterion B2 was selected because its area of occupancy is < 500 km² (estimated at 20 km²). Criterion "a" was selected



Figure 5. Habitat of *Valeriana praecipitis* (red arrow indicates the species) **A**, **B** Laguna Añil **C** Laguna del Florido. Photographed by Alejandro E. Villarroel and Kora Menegoz.

because it is known to exist in two locations (Fig. 1C, north and south of Nuble river). Criterion "b(iii)" was selected because there is a projected decline in the area, extent, and quality of habitat. High Andean plants are very sensitive to global warming, given that migration is limited by the lack of connectivity between summits and its reduced areas on the top, additionally, the 2010–2017 mega-drought in Central Chile, resulted in a significant reduction in precipitation and snow cover – the most severe during the last 1000 years – together with an increase in temperatures for the last decade (Garreaud et al. 2017; Cordero et al. 2019). One of the climate change model scenarios projects an increase of at least 1 °C of the mean temperature for the next 30 years, plus a decrease in winter precipitations of about 30% at the end of the century (Bozkurt et al. 2017). *Valeriana praecipitis* extent of occurrence is ~555 km² (Fig. 1B). The species is not present in any protected area in Chile, and it is not protected by law.

Key to Valeriana species

Key to *Valeriana* species present in the Andes range of the Nuble Region, according to the floristic inventories of Faúndez et al. (1994), Rondanelli et al. (2000), Rodríguez et al. (2008) and Villarroel (2019). Based on Kutschker (2008, 2011).

1	Basal leaves strictly entire
_	Basal leaves strictly divided
_	Basal leaves entire and divided
2	Basal leaves membranaceous
_	Basal leaves sub-fleshy or fleshy
5	Basal leaves fleshy
_	Basal leaves sub-fleshy
6	Basal leaves opaque green colour
_	Basal leaves bright green colour
7	Basal leaves spatulate, ovate or suborbicular, with entire, sinuate or dentate
	margin; inflorescences paniculiform, densely contracted; fruits ellipsoid and glabrous
_	Basal leaves ovate or elliptic, with a markedly sinuate or lobed margin; inflo-
	rescences paniculiform, lax; fruits tightly ellipsoid and densely hirsute
	V. hebecarpa
8	Basal leaves spatulate-obovate, with entire margin; inflorescences spike-like
	and contracted
_	Basal leaves spatulate, with entire to pausidentate margin; inflorescences glo-
	meruliform and contracted
3	Basal leaves fleshy
_	Basal leaves membranaceous
9	Stems hirsute, particularly at the nodes; basal leaf segments ovate to oblong;
	fruits ellipsoid
_	Stems with scarce pubescence; basal leaf segments ovate, oblong, lanceolate- elliptic; fruits ovoid and flat

4	Basal leaves membranaceous	10
_	Basal leaves sub-fleshy	11
10	Stems cylindrical and striated; basal leaf segments ovate-lan	ceolate; fruits
	ovoid or ellipsoid	V. crispa
_	Stems quadrangular with hairy winged edges; basal leaf segr	nents oblong,
	ovate, lanceolate or suborbicular; fruits ellipsoid	V. grandifolia
11	Maximum plant height 80 cm; entire basal leaves oblong, ova	ate or elliptic;
	divided basal leaves pinnatilobate; fruits lageniform	V. laxiflora
_	Maximum plant height 40 cm; entire basal leaves ovate or el	liptic; divided
	basal leaves pinnatisect; fruits ellipsoid	V. obtusifolia

Discussion

Pollen evidence indicates that *Valeriana* is a Holarctic genus that might have arrived from the northern hemisphere, perhaps before the uplifting of the Andes during the Pleistocene (Kutschker and Morrone 2012). The authors found an endemism node area between latitudes 34° and 37° S, suggesting a diversification centre for *Valeriana* after a long period of isolation, caused by the Andes uplift. The same scenario might have occurred with other genera such as *Berberis* L., *Ribes* L., among others, indicating a complex centre of endemism of high conservation value (Moreira-Muñoz and Muñoz-Schick 2007). In the same way the genus *Valeriana* is key to understanding Andean biogeographic history (Kutschker and Morrone 2012), evolutionary processes that occurred in the area could have given rise to locally endemic species such as *V. praecipitis*, *Rayenia malalcurensis* and *Viola chillanensis* Phil. (Violaceae) (RBG Edinburgh 2021).

The discovery of *V. praecipitis* in southern South-America follows the recent discovery of *Rayenia malalcurensis*, a newly described endemic genus and species found in the same area. As mentioned before, since the revision of Kutschker (2011), new locations and a new species were described. These discoveries suggest that both the region and the genus may reveal additional interesting botanic surprises. Although no genetic analysis was performed to determine its phylogenetic affinities, the comparison of morphological characteristics with its closest species shows notable differences in 11 out of 13 sets of characters (Table 1), in addition to a distance between their recorded distributions of approximately 410 km. The species is unmistakable, easy to recognize from other species in the area, especially in spring and summer due to its remarkable silvery-green basal leaves. However, it is difficult to find given the steepness of the sites where it occurs. Its extreme rarity and inaccessibility are undoubtedly the reasons why it has evaded discovery up until the present time.

In addition to consistent morphological differences, *V. praecipitis* stands out from other species of the genus due to its specific ecological habitat. Few other *Valeriana* species are able to grow abundantly in damp rock-cliffs at this high altitude, directly rooted in fissures or small soil pockets (e.g., *V. chilensis* Borsini in Chile or *V. ruizlealii* Borsini in Argentina). Rock-cliffs are challenging habitats with high erosion

Species	V. praecipitis	V. philippiana
Habit	Perennial herb, hemicryptophyte, simple or branched	Perennial herb, hemicryptophyte, simple or branched
Height with	26–65.5 cm	To 20 cm
inflorescence		
Taproot	Circular, reaching more than 30 cm long, 8–20 mm	Circular, reaching 14 cm long, 5-8 mm diameter, some-
rhizome	diameter, sometimes stoloniferous, woody	times stoloniferous, semi-woody
Stem	3.5–6 mm diameter, with very short internodes, forming a basal rosette	3–6 mm diameter, with very short internodes, forming a basal rosette
Basal leaves	Pinnatisect to pinnatipartite, oblong, up to 26 cm long	Pinnatilobed to pinnatisect, oblong, up to 8 cm long
Lobes	Orbicular to obovate, base attenuate, 1–35 × 1–24 mm,	Orbicular to obovate, base attenuate, 4–8 × 3–7 mm,
	overlapped, glabrous, fleshy. In the field, leaf lobes are	overlapped, glabrous, fleshy. In the field, leaf lobes are
	expanded	quite folded
Petioles	Canaliculated, glabrous	Canaliculated, pubescent
Upper leaves	Oblanceolate, 14-40 × 5-19 mm, margin entire to ir-	Oblong, $6-10 \times 3-5$ mm, margin entire
	regularly undulate or sinuate	
Bracts	Oblanceolate to oblong, up to 20 mm long	Oblong, up to 7 mm long
Bracteoles	Spatulate to oblong, 3–7 mm long, margin entire	Oblong, 2.5-4.5 mm long, margin erose
Inflorescence	A relatively diffuse thyrse or compound dichasial cyme	Dense compound dichasial cyme
Flowers	Hermaphrodite; corolla infundibuliform; corolla tube	Hermaphrodite; corolla infundibuliform-campanulate; co-
	3.5-4.5 mm long, base slightly gibbous; corolla lobes ob-	rolla tube 4 mm long, base gibbous; corolla lobes oblong
	long to obovate, 1–1.5 × 1–1.7 mm; stamens 3 mm long,	to obovate, 1.5×1.5 –2 mm; stamens 2 mm long, exerted;
	exerted; ovary incipient sterile locules; style 2.2 mm long;	ovary incipient sterile locules; style 2.5 mm long; stigma
	stigma lobed laminate to lamellate, less than 0.2 mm	lobed lamellate, 0.5 mm
Fruits	Ellipsoid, 3×1 mm, pubescent; pappus plumose, bristles	Ellipsoid, 3–4 × 2 mm, pubescent; pappus plumose,
	11, 3.5 mm long	bristles 11–13, 5–7 mm long

Table 1. Morphological differences between *Valeriana praecipitis* and *V. philippiana*. Based on Borsini (1966) and Kutschker (2008, 2011). In bold: character unique to *V. praecipitis*.

rates, limited soil depth and nutrients availability (Mathaux 2017; March-Salas et al. 2018). Mountain microclimatic conditions at high elevations are also severe due to high insolation and extreme low temperatures (Gale 1972). To adapt to these conditions, the species has a woody rhizome that can be more than 30 cm long and 5–20 mm in diameter, perhaps the largest of any *Valeriana* species. In addition, its deciduous leaves allow this species to avoid freezing temperatures and the presence of snow. These environmental conditions could have triggered the adaptation phenomenon mentioned above, and eventually speciation processes as suggested by Kutschker and Morrone (2012).

Little is known about rare and endangered species growing in the southern Andean cliff ecosystems, and many open questions remain. Future research needs to be done to identify morphological and physiological adaptations to grow under harsh soil and climatic conditions that could shed light on the future of Andean plant communities in the face of climate change. For instance, how new climatic scenarios could affect their area of occupancy? Are they able to migrate? Or more precisely, how extreme and persistent climatic conditions, like the ongoing mega-droughts, might affect the physiological performance of these species, perhaps pressing viable populations' threshold to the edge. The recent finding of *V. praecipitis* and *Rayenia malalcurensis*, in addition to other rare and endemic species, adds to the importance of monitoring and promoting the conservation of these species (Moreno-Gonzalez et al. 2019), especially those of Andean cliff ecosystems.

V. praecipitis is not present in any public protected area in Chile (Fig. 1B). In 2011, the Andean and pre-Andean sectors of the Nuble Region and north of the Biobío Region were declared a UNESCO World Biosphere Reserve (San Martín 2014). All the known subpopulations of *V. praecipitis* are found within this Biosphere Reserve, except the one located in the surroundings of Laguna Añil. However, although they are internationally recognized, Biosphere Reserves protection is not guaranteed by Chilean legislation. Actually, all sites are in privately owned lands with an overall low level of anthropic change. Without any formal protection, the area is highly susceptible to be affected by the construction of hydroelectric dams, mining industry, unregulated animal farming and unsustainable tourism. The high biodiversity levels of this area (e.g., Villarroel 2019), including the presence of narrow range and threatened species such as *V. praecipitis* and *R. malalcurensis* and the high degree of threat of the Andean region, should give priority to the conservation of Andean territory of the Nuble Region. Such conservation priority is ever more urgent in the light of future increasing trends of more frequent and severe fires (e.g., McWethy et al. 2021).

Another threat specific to cliff vegetation is the possible impact that rock-climbing could cause. In Chile (and worldwide) rock-climbing is of increasing interest, attracting more people to the mountains (Bogges et al. 2021), and climbers and hiker's organizations are rightly asking for policy to change to allow for more open access to mountainous areas. However, with a greater number of people accessing mountain cliffs, the risk of negative human impacts would increase (Clark and Hessl 2015). So far, because of their remoteness and inaccessibility, Chilean cliff ecosystems have remained relatively unaltered by human impacts. The lack of access is not only due to a lack of trails, but also because many mountainous areas are privately owned. In the future, the major challenge will be to find the most effective strategy to conserve ecosystems, while maximizing the social benefits of access to mountains.

Additional specimens examined

Valeriana philippiana. **ARGENTINA. Patagonia:** Neuquén Province, Cerro Colouhincul, Mar 1927, Comber E00143957 (E); Río Negro Province, Cerro López, Dic 1928, Cordini 1594532 (US). **CHILE. Los Lagos:** Llanquihue Province, Parque Nacional Vicente Pérez Rosales – Volcán Osorno, 200 m elevation, 41°10'S, 72°30'W, Jan 1986, Gardner 107129 (CONC); Osorno Province, Parque Nacional Puyehue - Volcán Casablanca, 1500 m elevation, 40°47'S, 72°10'W, Jan 1988, Gardner & Knees E00023408 (E); Osorno Province, Mirador Puyehue, Feb 1971, Landrum 107764 (SGO).

Acknowledgements

Fieldwork was financed by FONDECYT N°1181956, and the Instituto de Conservación, Biodiversidad y Territorio, UACh. Alejandro E. Villarroel was supported by CONICYT magister fellowship (22201444). Ricardo Moreno-Gonzalez was supported

by DAAD/BECAS Chile, 2014 (57144001) during early stages of the study design. We express our gratitude to the owners and administrators of the private sites where the plant was found, especially to Elías Acuña and Esau Marin. The authors would like to thank the curators of CONC, EIF, JBN, and SGO, especially Jimena Arriagada. We are indebted to Martin Gardner and Nicolás Lavandero for the support and revision of the manuscript. Thanks to Marco Duretto and an anonymous reviewer for their helpful suggestions during the reviewing process of this manuscript. We are grateful to Arón Cádiz-Véliz for the illustration of the species. Thank you so much to our field partners: Gonzalo Ossa, Eduardo Ponce, Rayen Neira and all members of the Bullileo Team.

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RESEARCH ARTICLE



Capparis (Capparaceae) in Peninsular Malaysia, including a new species and two new varieties

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Academic editor: Karol Marhold | Received 13 December 2019 | Accepted 7 January 2022 | Published 4 February 2022

Citation: Julius A (2022) *Capparis* (Capparaceae) in Peninsular Malaysia, including a new species and two new varieties. PhytoKeys 189: 99–127. https://doi.org/10.3897/phytokeys.189.49367

Abstract

As part of the Flora of Peninsular Malaysia Project, a species checklist of the genus *Capparis* in Peninsular Malaysia is presented here with a total of 11 species, two subspecies and four varieties. A new species and two varieties, endemic to Peninsular Malaysia, are described and illustrated: *Capparis kenaboiensis, C. scortechinii* var. *ruthiae* and *C. trinervia* var. *chungiana. Capparis kenaboiensis* is known from a single site in Negeri Sembilan and is assessed as Vulnerable (VU); *C. scortechinii* var. *ruthiae* from Pahang is Vulnerable (VU); and *Capparis trinervia* var. *chungiana* is known from Negeri Sembilan, Pahang and Selangor and its conservation status is assessed as Near Threatened (NT). A checklist and updated key to the genus in Peninsular Malaysia are provided.

Keywords

Brassicales, conservation, endemics, key, Peninsular Malaysia, sect. Monostichocalyx, taxonomy

Introduction

Capparis L., comprising from 130 to 141 species, is the largest genus in Capparaceae (POWO 2019; Souvannakhoummane et al. 2020) and distributed in the tropical and subtropical regions of the Old World. The genus occupies a wide range of habitats, such as forest margins, coastal vegetation, rocky vegetation, savannahs and thickets. Four sections are usually recognised, viz. *Capparis* sect. *Capparis*, sect. *Busbeckea* (Endl.)

Benth. & Hook.f. (Bentham and Hooker 1862:109), sect. *Monostichocalyx* Radlk. (Radlkofer 1839: 101) and sect. *Sodada* (Forsk.) Endl. (Endlicher 1839: 893). Of these four sections, sect. *Monostichocalyx* is further subdivided into seven groups based on inflorescence characters: Brevispina-, Cataphyllosa-, Grandis-, Moonii-, Seriales-, Subumbellates- and Trinervia-Group (Jacobs 1965).

Generic delimitation and relationships amongst *Capparis* species using morphological and molecular approaches have shown the New World and Old World species of *Capparis* do not form a monophyletic group (Hall 2008; Cardinal-McTeague et al. 2016; Tamboli et al. 2018). The New World taxa, traditionally identified as *Capparis* s.l., differ from Old World *Capparis* by the following traits: absence of prickles, symmetry of flowers, calyx types, calyx and corolla aestivation, different floral nectary structures and different fruit and embryo types (Cornejo and Iltis 2009a). Thus, all species of *Capparis* in the Neotropics belong to other genera, with the species now transferred into several genera (e.g. Cornejo and Iltis 2008, 2009a, 2009b, 2013; Cornejo 2017).

In Peninsular Malaysia, at least ten species and two subspecies were known (Jacobs 1960, 1965; Turner 1995; Kiew et al. 2009) - all members of sect. *Monostichocalyx*, characterised by the well-developed persistent leaves, sepals free in bud and dimorphic in which the outer sepals are larger in size, enclosing the narrower inner pair of sepals (Jacobs 1960: 410). These members were placed into four groups within this section: *Capparis scortechinii* King and *C. trinervia* Hook.f. & Thoms. in the Trinervia-Group; *C. diffusa* Ridl., *C. erycibe* Hallier f., *C. sepiaria* L. and *C. versicolor* Griff. in the Sub-umbellates-Group; *C. pubiflora* DC. in Cataphyllosa-Group; and *C. acutifolia* Sweet, *C. cucurbitina* King and *C. micracantha* DC. in the Seriales-Group. Of these ten species, *C. cucurbitina* was the only endemic to Peninsular Malaysia, being known from Gunung Bubu, Perak.

During a study of *Capparis* specimens for revisionary work on the genus for the Flora of Peninsular Malaysia account, several specimens which were determined as previously-described species (following Jacobs 1965) were critically re-evaluated as two undescribed taxa. These two are clearly distinct in several of their vegetative and reproductive characters from any known species. Another taxon discovered during the 2014 expedition to Kenaboi Forest Reserve (FR), Negeri Sembilan, also represents an undescribed taxon. After morphological comparison with closely related species (see below) and consulting the relevant literature (Jacobs 1960, 1965; Chayamarit 1991; Srisanga and Chayamarit 2004; Zhang and Tucker 2008; Fici 2012, 2016, 2021; Thuong et al. 2013, 2015, 2016, 2017; Fici et al. 2018; Souvannakhoummane et al. 2018; Fici et al. 2020; Fici and Souvannakhoummane 2020; Souvannakhoummane et al. 2020), these taxa are here recognised as three new taxa. Subsequently, in the checklist presented here, there are 11 species of Capparis in Peninsular Malaysia, with two subspecies and four varieties; all taxa are members of sect. Monostichocalyx. In addition to these three new taxa, Peninsular Malaysia now has four endemic taxa of *Capparis*. A key is provided to facilitate identification of the species.

Materials and methods

Morphological observations were made from herbarium material held at BKF, K, KEP, KLU, L, SAN and SING. Specimens deposited in CAL were studied, based on scanned images. Additionally, specimen images from Global Plants JSTOR (http://plants.jstor. org/), Conservatoire & Jardin botaniques de la Ville de Genève. (http://www.ville-ge. ch/musinfo/bd/cjb/chg) and the BioPortal of Naturalis Biodiversity Center (http:// bioportal.naturalis.nl/) were consulted. Floral measurements were made from either rehydrated or fresh material; details are given, based on specimen labels. Flowering and fruiting materials are indicated by 'fl.' and 'fr.', respectively, under the specimens examined section for the new taxa. Specimens were mapped and a conservation assessment of the species undertaken using the IUCN Red List Categories and Criteria (IUCN 2001, 2012) following the guidelines and procedures developed at FRIM for the Malaysia Plant Red List (Chua and Saw 2006).

Taxonomic treatment

Capparis L.

Shrubs, often sprawling or climbing, rarely small trees. Twigs flexuous or straight. **Indumentum** mostly simple hairs, sometimes stellate, often glabrescent. **Stipular thorns** and/or prickles straight or retrorse, divaricate, often well-developed and persistent on main branches, sometimes rudimentary (rarely lacking). *Leaves* alternate or spirally arranged; lamina simple, coriaceous, subcoriaceous or chartaceous, sometimes extra membranous, margin entire, edges flat or recurved, lateral veins ascending regularly, sometimes brochidodromous interlooping near the margin. *Inflorescence* axillary, lateral or terminal; bracts mostly present, but early caducous, rarely to basal bracteoles. *Flowers* solitary or fascicle in the axils or in a series with 2–6-flowered along the twig or arranged in corymbs, umbels or subumbels or paniculate; sepals free in bud, 4, arranged in pairs and opposing sepals equal in size, imbricate or not; petals 4, asymmetrical, the dorsal petals erect and laterally connate at base, lateral ones spreading and free, rather delicate; stamens 6 to numerous, filaments of unequal length, anthers tetrasporangiate, basifixed or dorsifixed, opening lengthwise; ovary 1-locular, on gynophores as long as the stamens or longer, not or very little stretching in fruit, stigma obscure or small, sessile (elsewhere and in Peninsular Malaysia) or in capitate or cushion-shaped (elsewhere, but not in Peninsular Malaysia). Fruit an amphisarca with a subwoody (when young) turning pulpy (when ripe) exocarp, indehiscent (elsewhere and in Peninsular Malaysia) with the exception of Capparis cartilaginea Decne. and C. spinosa L. (a capsular: ripened fruit dehiscent longitudinally), globose, ellipsoid or torulose, exocarp smooth with or without longitudinal ribs (fruit is without longitudinal ribs in Peninsular Malaysia) or tuberculate (elsewhere, but not in Peninsular Malaysia). Seeds few to many rarely 1; embryo tightly coiled with the cotyledons in the centre; cotyledons stipulate, ovate or elliptic, white.

Groups of sect. Monostichocalyx

Capparis sect. *Monostichocalyx*, distributed from Africa (except the northern part) to Asia, extended to Australia and the Pacific, contains the majority of species in the genus with ca. 110 taxa (Jacobs 1965; Srisanga and Chayamarit 2004; Fici 2012, 2021; Thuong et al. 2013, 2015, 2016, 2017; Fici et al. 2018; Souvannakhoummane et al. 2018). Members of this section have been divided into seven groups, but Peninsular Malaysian taxa fall into four groups as mentioned earlier in introduction.

A synoptic key to the four groups of C. sect. *Monostichocalyx* in Peninsular Malaysia (adopted and modified from Jacobs 1965)

1c	Flowers large (sepals 8-15 mm long, torus ca. 5 mm wide), arranged in cor-
	ymbs, umbels to subumbels or racemes. Plants with brown hairs. Twigs angu-
	lar when young, with mostly strong, recurved stipular thorns and/or prickles.
	Leaves with obscure nerves, dull greenish with brown-coloured nerves, longer
	than 6 cm. Stamens 30–75III. Trinervia-Group
1d	Flowers more or less neatly subumbellate, the subumbels axillary and/or ar-
	ranged to panicles. Flowers medium-sized to small (sepals 2-10 mm long).
	Stamens < 70. Ovary 1–3 mm long IV. Subumbellates-Group
1f	Flowers large to small (sepals 3-14 mm long), arranged on a long or short
	leafless bracteates axis or subumbellate. Shoots with cataphylls at the base.
	Hairs 2-armed or hooked or acroscopically curved. Stipular thorns usually
	ascending. Leaves mostly over 8 cm long, mostly dull
1g	Flowers mostly small to medium-sized (sepals 3-15 mm long), serial in supra-
	axillary rows (or sometimes small bundles of cataphylls in their place), exception-
	ally all solitary, but in that case, the twigs red-hairy and the leaves shorter than
	3.5 cm. Indumentum, if present, often (red-)brownishVII. Seriales-Group

Key to the Capparis species in Peninsular Malaysia

(Based on flowering/fruiting specimens)

1	Flowers arranged in a series along the twig just above the leaf axil with up to
	6 flowers
_	Flowers arranged in a short fascicle, umbels to subumbels, racemes or panicu-
	late, either terminal on leafy twigs or axillary in the leaf axil5
2	Stipular thorns absent
_	Stipular thorns present
3	Leaf apex long acuminate, 10-13 mm long. Petals broadly elliptic. Fruit
	torulose
_	Leaf apex mucronate or shortly acuminate, 4–6 mm long. Petals oblanceolate
	or obovate to elliptic. Fruit ellipsoid or oblong4

4	Leaf subcoriaceous to chartaceous, base cordate, apex mucronate. Petals up to
	1.6 cm long. Stamens 15–18 C. micracantha subsp. micracantha
-	Leaf coriaceous, base cuneate, apex shortly acuminate. Petals up to 2.4 cm
	long, stamens > 18 C. micracantha subsp. korthalsiana
5	Inflorescences in axillary fascicles C. pubiflora
-	Inflorescences in terminal and/or axillary racemes, paniculate, umbels or sub-
	umbels6
6	Inflorescences paniculate
_	Inflorescences racemose, umbels or subumbels7
7	Inflorescences strictly racemose, flowers densely or loosely arranged in raceme
_	Inflorescences umbellate, subumbellate and/or flowers arranged racemosely
0	and becoming crowded at the distal part of the inflorescence
8	Flowers loosely arranged in racemes with early caducous leaf-like bracts.
	Lamina surface bullate C. kenaboiensis sp. nov.
-	Flowers densely arranged in racemes and subtended by persistent and con-
0	spicuous leaf-like bracts. Lamina surface smooth
9	Racemes axillary and/or terminal. Leaf-like bracts thick, densely hairy with
	velvety, shiny and rusty hairs abaxially, glabrous adaxially, elliptic, $25-30 \times 10^{-12}$
	10–13 mm. The gynophore densely hairy at base
	C. scortechini var. scortechini
_	Racemes strictly terminal. Lear-like bracts thin, densely hairy with
	tomentose, straw nairs on both surfaces, soon glabrescent abaxially with age,
	harrowly emplic, $(10-)(15-2) \times (1-)(2-6)$ min long. The gynophole glabrous
10	inforgassing and/or suburbala terminal comptimes suillary
10	innorescences in racemes and/or subumbers, terminal sometimes axinary,
	sepais nairy
_	have have have have a second statistical of axillary sometimes terminal, separs gra-
11	Lamina subcoriaceous to coriaceous broadly ovate cometimes ovate elliptic
11	13 16 x 5 5 8 5 cm drying leaves reddish brown rately pale green with pale
	vellow rarely dark red venation on both surfaces intercostal veins obscure
	Inforescence terminal with flowers arranged racemosely and becoming
	crowded at the distal part of the inflorescence stamps $30/40$
	<i>C</i> tringmia var chungiana var pov
_	Lamina chartaceous oblong-elliptic or broadly lanceolate (5-)10-14(-19)
	(2-)35-85 cm drying leaves dull green with brownish main nerves on
	hoth surfaces intercostal veins irregular reticulate and distinct. Inflorescence
	terminal with flowers arranged racemosely and becoming crowded at the
	distal part of the inflorescence, sometimes subumbellate on 3-4 cm long
	peduncles in the axils of the uppermost leaves, stamens $(30-)60-70$
	<i>C. trinervia</i> var. <i>trinervia</i>
12	Twigs obviously flexuous
_	Twigs ± straight

New taxa

Capparis kenaboiensis Julius, sp. nov.

urn:lsid:ipni.org:names:77254614-1 Figures 1, 2, 3

Diagnosis. Vegetatively, this new species resembles *Capparis buwaldae* Jacobs in having bullate leaves with a long acuminate-caudate apex and distinct reticulation, but *C. kenaboiensis* differs from *C. buwaldae* in its terminal (vs. supra-axillary) inflorescences, the absent (vs. present) stem stomata and the smooth (vs. tuberculate) fruit pericarp. The flowers subtended by leaf-like bracts resemble *Capparis scortechinii*, but are early caducous in the new species.

Type. Peninsular Malaysia. Negeri Sembilan: Jelebu, Kenaboi FR, Gunung Besar Hantu, road sides towards Lata Kijang, 3°11.42'N, 100°59.21'E, 459 m alt., 4 Mar 2014 (fl., fr.), *Julius et al. FRI57784* (holotype KEP!; isotypes K!, SAN!, SING!, L!, A!).

Description. Climber 2–8(–12) m long hanging high up on tree. *Twigs* straight, pubescent when young and soon glabrous. *Stipular thorns* retrorse, tips 1–2 mm long. *Leaves* spirally arranged; petioles $8-12 \times ca$. 1 mm, grooved, slender, not thickened, covered with dense, short, silky white hairs; laminas narrowly elliptic, (5.5-)10-11.5 \times (1.5–)3.5–4 cm, chartaceous, bullate, fresh dark green and glossy above, pale green beneath, brownish-green when dry, base acute, margin revolute and entire, apex long acuminate to caudate, with acumen 1-1.5 cm long, glabrous above, hairy beneath, denser on mid-rib and venation; mid-rib sunken above, raised beneath; lateral veins 5-7 pairs, arcuate towards the margin; intercostal veins reticulate, distinct above, prominent beneath. Inflorescences axillary or terminal, racemose, rachis slender and flexuous, conferted towards the top as the buds fall off at bottom part and leave scars, velvety, white hairs throughout; bracts leaf-like, elliptic, $10-25 \times 2-5$ mm, velvety white hairs on both surfaces, early caducous. Flowers loosely arranged, 10-14, buds globose, $4-5 \times 3-5$ mm; pedicels 2–2.5 cm long, whitish; sepals 4, thin, cucullate, whitish-green, except reddish at base inside, keeled, 2 pairs, lanceolate, 6-8 × ca. 5 mm, outer pair larger than inner pair, the outer sepals imbricate, covered with dense, white hairs outside, glabrous inside, the inner pair hairy on keel outside, glabrous inside; petals 4, upper pair pinkish and white along margin and laterally connate at base, lower pair whitish-green and free, elliptic ca. 10×3 mm, inside densely, silky tomentose hairy, outside glabrous, except densely hairy at base, ciliate along the margin; stamens



Figure 1. Distribution of Capparis kenaboiensis.

18–31, unequal in length, filament whitish, 7–20 mm long, glabrous, except hairy at base, anthers dorsifixed, ca. 1.5 mm long, greenish, apex recurved; ovary ellipsoid, ca. 1.5 mm long, greenish, on gynophore 2–2.5 cm long, stigma obscure. *Fruits* young green turning brownish to dark purple when ripe, subglobose to pyriform on slender gynophore, $4.5-5 \times 5.5-6$ cm, pulp pinkish or purplish-red. *Seeds* 1–4, sarcotesta fleshy, yellowish, testa thin and black.



Figure 2. *Capparis kenaboiensis* Julius, sp. nov. **A** infructescence branch **B** inflorescence **C** flower **D** flower with sepals and petals removed exhibiting the stamens and ovary on gynophore **E** stamen **F** the outermost sepals pair **G** the dorsal petals **H** the ventral petals **I** the cross- and longitudinal-section of fruit **J** the stipular thorn close-up. (Drawn by Mohamad Aidil Noordin from *FRI57784*).

Distribution. Endemic in Peninsular Malaysia and known only from the type locality (Fig. 1).

Ecology. Secondary forest margin, in light shade.



Figure 3. *Capparis kenaboiensis* Julius, sp. nov. **A** habitat **B** fruiting branch **C** cross-section of fruit (upper left, the cut ripe fruit) **D** inflorescence **E** flower, front view **F** flower, lateral-abaxial view. (Photographs **A–D** by A. Julius and **E–F** by K. Imin).

Conservation status. *Capparis kenaboiensis* is known from a single locality and is very rare with less than ten individuals found in two subpopulations. The species inhabits a secondary forest margin and by a pathway, which are vulnerable to forest clearing pathways as was observed in 2014 during the botanical survey. Moreover, only two sub-populations were observed during a recent visit in 2019. As the area of occupancy is less than 500 km² and the declining of habitat, this species is assessed as Vulnerable B2ab(iii), following the IUCN Red List Categories and Criteria (IUCN 2001, 2012).

Additional specimens examined. Peninsular Malaysia. Negeri Sembilan: Jelebu, Kenaboi FR, G. Besar Hantu, from Taman Alam Liar towards Trail 1, near pathways, 3°12'N, 100°58'E, 530 m alt., 4 Mar 2014 (fl., fr.), *Julius et al. FRI57797* (KEP!); road sides towards Lata Kijang, 3°11.42'N, 100°59.21'E, 459 m alt., 23 May 2019 (fr.), *Julius et al. FRI 73545* (KEP!).

Notes. *Capparis kenaboiensis* (Figs 2, 3) is characterised by the flowers, loosely arranged in the raceme with each single flower subtended by a leaf-like bract. Vegetatively, *Capparis kenaboiensis* resembles *C. buwaldae* from the Seriales-group, but its short racemose inflorescences subtended by leaves are similar to members of the Subumbellates-Group. Characterised further by the small size of flowers with sepals 6–8 mm long and the plants hairy on young parts, *Capparis kenaboiensis* is best placed within the Subumbellates-Group (Jacobs 1965: 412). Further morphological comparison of this new species with other closely related species is as indicated in the Table 1.

The ripe fruits of this new species are similar to the edible fruits of *Capparis buwaldea* - the smell is like a mangosteen, but the flesh is tasteless.

Characters	Species		
	C. kenaboiensis	C. buwaldae	C. longestipitata
Leaf surface	Bullate	Bullate	Smooth
Leaf shape	Narrowly elliptic	Oblanceolate-elliptic	Oblong to slightly obovate
Leaf size	(5.5–)10–11.5 × (1.5–)3.5–5 cm	6–13(–23.5) × (1.5–)2.5–4.5(–8) cm	5–7 × 2.5–3.5 cm
Leaf base	Acute	Rounded to acute	Rounded
Leaf apex	Long acuminate to caudate,	Long acuminate to caudate, ca.	Acuminate, 0.4-0.7 cm long
	1–1.5 cm long	2 cm long	
Leaf hairs	Glabrous above, hairy beneath,	Glabrous on both surfaces	Glabrous except for mid-rib
	denser on mid-rib and venation		beneath
Leaf colour	Brownish-green	Brownish-green to yellowish-green	Olive green
when dried			
Inflorescences	Simple racemose, axillary or	In a series along the twig, 2–4, or	Compound racemose,
	terminal	supra-axillary	Axillary or terminal
Bracts	Present	Absent	Absent
Sepals shape	Lanceolate, keeled	Ovate	Lanceolate, not keeled
Sepals size	6–8 mm long	3–5 mm long	3–5.5 mm long
Sepals hairs	Densely white hairs outside,	Glabrescent towards margin	Densely greyish puberulous
	glabrous inside		outside
Fruit shape	Subglobose to pyriform	Shortly umbonate at the top and	Unknown
		sometimes at the bottom	
Fruit exocarp	Smooth	Tuberculate	Unknown

Table 1. Morphological comparison of Capparis kenaboiensis, C. buwaldae and C. longestipitata.
Capparis scortechinii var. ruthiae Julius, var. nov.

urn:lsid:ipni.org:names:77254615-1 Figures 4, 5

Diagnosis. Recognised in the genus *Capparis* by the combination of the following characters: the racemes are strictly terminal and compact with flowers subtended by a conspicuous and persistent unguiculate, leaf-like bracts and the smaller $[(13-)16-25 \times 2-8 \text{ mm}]$, greenish, thin leaf-like bracts covered with densely, tomentose, straw hairs on both surfaces soon glabrescent with age and the sepals (inner pair) glabrous along the margin and the filaments and gynophore both entirely glabrous and the petals loosely ciliate along margin.

Type. Peninsular Malaysia. Pahang: Fraser's Hill, 25 Jan 1982 (fl.), *Kiew RK3747* (holotype KLU!).

Description. Climber ca. 1.5 m long or scrambling shrub (height unknown). Twigs straight covered with indumentum of dense, appressed, pale brown hairs soon glabrescent. Stipular thorns retrorse, tips 1-2 mm long. Leaves spirally arranged, but in one plane; petioles $10-15 \times ca$. 10 mm long, slender, young densely hairy with brown hairs soon glabrescent with age; lamina narrowly elliptic or oblong-elliptic, 8.5–11.5(–13.5) × 2.6–3.6 cm, chartaceous to subcoriaceous, drying brownish-green, base cuneate, margin flat and entire, apex acuminate and slightly caudate, with acumen 5–7(–10) mm long, glabrous above, sparsely hairy, denser on mid-rib beneath; mid-rib flat above, raised beneath; lateral veins 4-6 pairs, sunken above, distinct beneath, reddish when dry, looping towards the margin; intercostal veins reddish, finely reticulate, obscure above, distinct beneath. Inflorescences terminal, elongated racemes, rachis stout, straight, 3-8(-10) cm long, densely hairy with pale brown hairs; bracts leaflike, unguiculate, lanceolate, $(13-)16-25 \times 2-8$ mm, densely hairy with pale brown hairs on both surfaces. *Flowers* [in bud] many, compact, buds globose, $4-7 \times 3-7$ mm; pedicels 4–5 mm long, brownish; sepals 4, thin, green, orbicular to ovate, 4–6 × 4-6 mm, outer pair imbricate, densely hairy with appressed pale brown hairs outside, glabrous inside, inner pair with broadly transparent margin, glabrous on both surfaces; petals 4, pinkish, obovate, 6.5-7 × 4.5-5.5 mm, glabrous on both surfaces, except long, silky hairs at base of dorsal pair and dense, short silky hairs at base of ventral pair inside, loosely, ciliate along margin; stamens numerous, unequal length, filament pinkish, ca. 5–6 mm long, anther dorsifixed, ca. 1.5 × 0.5 mm; ovary ovoid, ca. 1.5 × 2.5 mm, on gynophore ca. 4 mm long, glabrous, stigma obscure. Fruits young green, mature fruit n.v., globose on stout gynophore, ca. 9.5 cm in diameter. Seeds many.

Vernacular names. Susoh beruga, kuku lang (Malay).

Distribution. Endemic in Peninsular Malaysia, Pahang, known only from Fraser's Hill and Cameron Highlands (Fig. 4).

Ecology. On degraded hill slopes and open areas along the forest margins, gaps or roadsides, hill forest to lower montane forest at ca. 1280 m elevation.

Etymology. This new variety is named after Dr Ruth Kiew, collector of type specimen and the Flora of Peninsular Malaysia project co-ordinator and consultant.



Figure 4. Distribution of Capparis scortechinii var. ruthiae.

Conservation status. *Capparis scortechinii* var. *ruthiae* was conspicuous and inhabits forest margins, gaps or pathway near roadsides, but has not been relocated after 1993 at the original localities and adjacent areas even though they have been revisited many times. As the estimated extent of occurrence is less than 500 km² and the declining of habitat quality, this variety is assessed as a Vulnerable B2ab(iii), following the IUCN Red List Categories and Criteria (IUCN 2001, 2012).



Figure 5. *Capparis scortechinii* var. *ruthiae* Julius, var. nov. **A** flowering (bud) leafy twig with stipular thorns close-up next to it **B** flower bud close-up **C** flower bud with sepals and petals removed exhibiting the stamens **D** adaxial view of outer sepal (upper) and inner sepal (below) **E** dorsal petals, adaxial view (i) and abaxial view (ii) **F** ventral petals, exhibiting adaxial with hairs at base (left) and abaxial view (right) **G** flower bud dissected exhibiting one elongated stamen and the ovary on gynophore **H** anther, front (right) and back view (left) **I** fruit in cross-section and seeds (below) **J** an unguiculate leaf-like bract **K** venation close-up. (Drawn by Mohamad Aidil Noordin from Kiew *RK*3747 [**A–H, J, L**] and from Henderson *23551* [**I**]: the scale for **C** is similar to **B**).

Notes. Amongst the known members of Trinervia-group, *Capparis scortechinii* var. *ruthiae* (Fig. 5) is closely related to var. *scortechinii* because both are characterised by the compact racemes. However, the new species has racemes strictly in terminal (but axillary rarely terminal in var. *scortechinii*) and the leaf-like bracts are smaller and greenish with tomentose, straw hairs on both surfaces, which is glabrescent with age abaxially (compared to a larger and prominent of velvety, shiny, rusty hairs in var. *scortechinii*). The new variety also differs in its entirely glabrous filament and gynophore (while var. *scortechinii* has the filament and gynophore densely hairy at the base), the petals are loosely ciliate along the margin (but glabrous in var. *scortechinii*), and the inner pair of sepals are glabrous along the margin (but ciliate in var. *scortechinii*).

Flowers with a vestigial and well-developed gynoecium are presented in *Capparis* scortechinii var. scortechinii, but only well-developed gynoecium observed in var. ruthiae.

Additional specimens examined. Peninsular Malaysia. Pahang: one-way road to Fraser's Hill, 25 Jan 1982 (fl.), *Kiew RK1116* (KEP!); Fraser's Hill, 1 Aug 1993 (fl.), *A. Zainuddin AZ4636* (KEP!); Fraser's Hill, Jalan kecil to Gap, 19 Jun 1930 (fl.), *Kalong FMS22381* (KEP!); Fraser's Hill, Richmond, 21 Apr 1955 (fr.), *Purseglove P4248* (L- image!, barcode L1856045) Cameron Highlands, 13 Apr 1930 (fr.), *Henderson SFN23551* (KEP!, SING!).

Capparis trinervia var. chungiana Julius, var. nov.

urn:lsid:ipni.org:names:77254616-1 Figures 6, 7, 8

Diagnosis. Recognised in the genus *Capparis* by the combination of the following characters: the leaves broadly ovate-elliptic, relatively large $(13-16 \times 5.5-8.5 \text{ cm})$ and leaves drying reddish-brown, rarely pale green with pale yellow and rarely dark red venation, the tertiary venation obscure, the inflorescence strictly terminal with flowers arranged racemosely and becoming crowded at the distal part of the inflorescence, the stamens 30–40 and the fruit globose with ca. 11 cm in diameter.

Type. Peninsular Malaysia. Selangor: Kuala Kubu Bharu to Fraser's Hill Road, along the road side, 17 Apr 1971 (fl.), *Mahmud Sidek s.n.* (holotype KLU!).

Description. Woody climber 9–12 m long. *Twigs* straight, covered with velvety, ferruginous hairs and glabrescent with age. *Stipular thorns* recurved downwards, tips 1.5–3 mm long. *Leaves* spirally arranged; petioles $15-20 \times 2-3$ mm, stout, incrassate or thickened, velvety hairy becoming glabrous; lamina broadly ovate sometimes ovate-elliptic, $13-16 \times 5.5-8.5$ cm, coriaceous to subcoriaceous, surface rugose and usually reddish-brown when dry, rarely pale green, base cuneate or occasionally rounded, margin flat and entire, apex acute or shortly cuspidate, with acumen ca. 4 mm long, glabrous on both surfaces; mid-rib flat above, raised beneath; lateral veins 5-7(-8) pairs, flat above, distinct beneath, pale yellow when dry, looping towards the margin; intercostal veins obscure. *Inflorescences* terminal, flowers arranged racemosely and becoming crowded at the distal part of the inflorescence rachis slender and straight;



Figure 6. Distribution of *Capparis trinervia* var. *chungiana*.

bracts caducous. *Flowers* [in bud] 4–7, buds globose, $1-1.8 \times 1.3-1.8$ cm; pedicels 3–3.5 cm long, swollen towards apex, velvety with ferruginous hairs; sepals 4, thick, whitish-green, orbicular, $1.2-1.5 \times 1.2$ cm, outer pair of sepals imbricate, covered with dense, velvety ferruginous hairs outside, glabrous inside, inner pair of sepals glabrous on both surfaces, except silky hairs at base outside; petals 4, cream or white with pinkor dark purple at base; stamens 30–40, unequal in length, anthers yellow; ovary green.



Figure 7. *Capparis trinervia* var. *chungiana* Julius, var. nov. **A** the leafy branch **B** the flowering (bud) leafy twig **C** flower with sepals and petals removed exhibiting the filaments and ovary on gynophore **D** upper pedicel and base of calyx **E** the fruit in cross section **F** stipules close-up. (Drawn by Mohamad Aidil Noordin from *FRI64006* **[A, E, F]** and Mahmud Sidek s.n. **[B–D]**).

Fruits young shiny and green, mature fruit n.v., globose to sub-globose on a stout gynophore, sometimes beaked when young, ca. 9×11 cm. *Seeds* (3–)12–15, sarcotesta whitish, testa thin and whitish-cream.



Figure 8. *Capparis trinervia* var. *chungiana* Julius, var. nov. **A** fruiting twig **B** stipular thorns **C** young fruit on stout gynophore **D** leaf **E** young fruit, cross section. (Photographs by K. Imin).

Vernacular names. Kuku lang (Malay), mentimun (Temuan).

Distribution. Endemic in Peninsular Malaysia, Negeri Sembilan, Selangor and Pahang (Fig. 6).

Ecology. Lowland to lower montane forest, on forest edge or roadsides; 427–1067 m altitude.

Etymology. The new variety is named after Dr Richard Chung Cheng Kong, Curator of the Kepong Herbarium (KEP) and Project Leader of Flora of Malaysia under 10th and 11th Malaysian Plans.

Conservation status. *Capparis trinervia* var. *chungiana* inhabits forest margins, gaps or pathways near to roadsides, but is not a common species. This species has not been relocated in Selangor and Pahang even though the localities and adjacent areas have been revisited. This species, however, has been found and recorded from a new locality in Negeri Sembilan during a botanical survey in 2010, but it could not be relocated from two recent visits in 2014 and 2019. Recent field observations to Negeri Sembilan also show forest clearing pathways near the species habitat. Therefore, this species is assessed as Near Threatened following the IUCN Red List Categories and Criteria (IUCN 2001, 2012).

Additional specimens examined. Peninsular Malaysia. Selangor: Ulu Gombak FR, 259 m (850 ft) alt., 19 Aug 1964 (fr.), *Mohd. Kasim & Mahmud Sidek 624* (KLU!); Gombak Forest, 16th mile, followed path to stream and beyond, 427 m (1400 ft) alt., 12 Jun 1963 (fl.), *M.E.D. Poore 1189* (KLU!); Kepong Plantation Field 9J, hillslope, 10 Jan 1934 (fl.), *Abdul Hamid 33570* (KEP!); Ulu Langat, 30 Mar 1960 (fl., fr.), *Gadoh KL2072* (KEP! 2 sheets); Genting Sempah, 11 Dec 1970 (fl., fr.), *Kochummen FRI16263* (KEP!). Pahang: Fraser's Hill, near gate at gap, roadside, 1067 m (3500 ft) alt., 28 Feb 1979 (fl., fr.), *Kochummen FRI18471* (KEP!); road to Fraser's Hill, climber on Mahang tree beside the road, 914 m (3000 ft) alt., 26 Aug 1981 (fr.), *K.M. Wong FRI32242* (KEP!). Negeri Sembilan: Jelebu, Kenaboi FR, G. Besar Hantu, near Kg. London area, 3°11.00'N, 102°00.76'E, 500 m alt., 6 May 2010 (fr.), *Julius et al. FRI64006* (KEP!).

Notes. The comparatively large flowers, with sepals up to 1.5 cm long, place *Capparis trinervia* var. *chungiana* (Figs 7, 8) in the Trinervia-Group (Jacobs 1965; Srisanga and Chayamarit 2004). Specimens of this new species were determined as *C. erycibe*, no doubt due to the comparatively large leaves, but it differs in having the flowers arranged racemosely and becoming crowded at the distal part of the inflorescence rather than the paniculate arrangement in *C. erycibe*. In addition, *C. erycibe* has smaller flowers with sepals only 4–6 mm long and was placed within the Subumbellates-group (Jacobs 1965: 411).

The flower colour of this species has been described as 'white' or 'cream' with pink or dark purple at base in the label notes. Unfortunately, the open flowers are missing from the specimen sheets. Additionally, there are no new flowering specimens from the recent collections and full details of the flowers, such as measurement of the mature flowers and bracts, remain incomplete. Moreover, the dissected parts of the rehydrated flower buds are in poor condition and mostly do not contain either androecium or gynoecium, the cause of which is unknown. However, this new variety still can be distinguished from other closely related members of the Trinervia-Group (see Table 2 for the comparison details) or species known from Peninsular Malaysia vegetatively. For example, the relatively large fruit (ca. 11 cm in diam.) of *Capparis trinervia* var. *chungiana* is similar to *C. scortechinii* (ca. 11 cm in diam.) and *C. trisonthiae* from Thailand (6–8 cm in diam.), but the latter two differ in their densely racemose and paniculate inflorescences, respectively; and the flowers arranged racemosely and becoming crowded at the distal part of the inflorescence, though has similarity to typical var. *trinervia*, but var. *chungiana* differs by its subcoriaceous to coriaceous leaves with broadly ovate to ovate-elliptic lamina (compared to chartaceous with oblong-elliptic or broadly lanceolate lamina in var. *trinervia*), the drying leaves are reddish-brown, rarely pale green with venation pale yellow, rarely dark red on both surfaces (compared to dull green with brownish main nerves on both surfaces in var. *trinervia*), the intercostal veins are obscure (compared to irregular reticulate and distinct in var. *trinervia*) and the stamens are 30–40 (whereas in var. *trinervia*, (30–)60–70).

Characters		Species	
	C. trinervia var. chungiana	C. trinervia var. trinervia	C. trisonthiae
Stipular thorns	1.5–3 mm long, recurved downwards	1–3(–6) mm long, patent or recurved, mostly downwards, rarely upwards	2–4 mm long, recurved downwards
Petiole	1.5–2 cm long, 2–3 mm in diam., stout	0.7–1.8 cm long, ca. 1.5 mm in diam., slender	ca. 2 cm long, ca. 4 mm in diam., stout
Leaf shape	Broadly ovate to ovate-elliptic	Oblong-elliptic or broadly lanceolate	Broadly ovate
Leaf size	13–16 × 5.5–8.5 cm	(5–)10–14(–19) × (2–)3.5–8.5 cm	(10–)12–18 × (6.5–)8–11 cm
Leaf apex	Acute or shortly cuspidate	Obtuse to shortly acuminate	Retuse to emarginate
Leaf hairs	Glabrous on both surfaces	Glabrescent	Glabrous on both surfaces
Leaf colour	Reddish-brown, rarely pale	Dull green with brownish	Dull green above, brown
when dried	green with pale rarely dark red venation on both surfaces	venation on both surfaces	beneath
Intercostal veins	Obscure on both surfaces	Irregular reticulate, distinct on both surfaces	Irregular reticulate, prominent on both surfaces
Inflorescences	Racemes with flowers becoming crowded at the distal part of the inflorescence	Racemes with flowers becoming crowded at the distal part of the inflorescence or subumbels	A leafy paniculate
Sepals shape	Orbicular	Orbicular	Lanceolate
Sepals size	1.2–1.5 cm long	0.9–1.5 cm long	1.8–2.5 cm long
Sepals hairs	Densely velvety ferruginous	Densely orange-yellow puberulous	Ferruginous puberulous
	hairs abaxially, glabrous	abaxially, glabrous adaxially	abaxially, puberulous
	adaxially		adaxially in the marginal parts
Stamens	30-40	(30–)60–70	140-170
Fruit	Globose, ca. 11 cm in diam.	Globose, 3.5–5 cm in diam.	Ellipsoid, 6-8 cm in diam.

Table 2. Morphological comparison of *Capparis trinervia* var. *chungiana*, *C. trinervia* var. *trinervia* and *C. trisonthiae*.

Checklist of Capparis species in Peninsular Malaysia

(Bt. = Bukit (hill); FR (Forest Reserve); G. = Gunung (mountain); P. = Pulau (islands); WR (Wildlife Reserve))

1. *Capparis acutifolia* Sweet Hort. Brit. 2nd ed. (1830) 585; Jacobs, Blumea 12, 3 (1965) 426, p.p. excluding subsp. *bodinieri* (H.Lév.) Jacobs, subsp. *obovata* Jacobs and subsp. *sabiaefolia* (Hook.f. & Thoms.) Jacobs and subsp. *viminea* (Hook.f. & Thoms.) Jacobs; Chayamarit, Fl. Thailand 5, 3 (1991) 249.

Type. Myanmar [Burma], Kurz 1826 (holotype CAL-photo! barcode CAL0000004987; isotype K!, barcode K000247293).

Distribution. In Peninsular Malaysia, so far collected from Kedah (Gua Labua).

Ecology. Grows on rock crevices in the limestone forest at low altitude ca. 191 m.

Conservation status. This species is rare. It was recently collected in 2008 in a forest area. It is assessed as Endangered B2ab(iii) because the small area of occupancy (< 500 km²), known only from a single locality.

Specimens examined. Peninsular Malaysia. Kedah: Sik, Ulu Muda FR, Gua Labua, limestone hill, 3 Mar 2008, *Rafidah et. al. FRI 55677* (KEP!).

Notes. *Capparis acutifolia* is characterised by the absent of stipular thorns, the inflorescence with 1–2(–5)-flowers in a series along the twig and the extra membranous lamina is ovate with acuminate-caudate apex. There are five subspecies treated under this species (Jacobs 1965), but the other four have all been elevated to species rank: subsp. *sabiaefolia* (Hook.f. & Thoms.) Jacobs and subsp. *obovata* Jacobs as *Capparis sabiifolia* Hook.f. & Thoms. (Chayamarit 1991), subsp. *bodinieri* (H.Lév.) Jacobs as *C. bodinieri* H.Lév. and subsp. *viminea* (Hook.f. & Thoms.) Jacobs as *C. membranifolia* Kurz (Zhang and Tucker 2008; Maurya et al. 2020)

2. *Capparis cucurbitina* **King** J. As. Soc. Beng. 58, 2 (1889) 395; Jacobs, Fl. Malesiana 1, 6 (1960) 85, Blumea 12, 3 (1965) 446.

Type. Peninsular Malaysia, Perak, Ulu Bubu, *King's Coll. 10027* (lectotype designated by Jacobs; CAL-photo!, barcode CAL0000005012, CAL0000005013, CAL0000005014 & CAL0000005015; isolectotypes G, barcode G00237951, K!, barcode K000643986)

Distribution. Endemic in Peninsular Malaysia and restricted to Perak with few collections from Ulu Bubu (*King's Coll. 8824 & King's Coll. 10795*, CAL-photo!), Dindings (*Ridley 10261*, SING!), Parit Forest Reserve (*Symington FMS39490*, SING!) and the type.

Ecology. In dense forest at 152–244 m altitude.

Conservation status. This species is very rare since it has been collected only four times, the last time being in 1899. Despite several visits made more recently to the localities mentioned above, *Capparis cucurbitana* could not be located. As the

estimated extent of occurrence is less than 5000 km² and the quality of habitat is declining, *C. cucurbitina* is assessed as Endangered B1ab(iii), according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012)

Specimen examined. Peninsular Malaysia. Perak: Manjung, Dinding, 1899, *Ridley 10261* (SING!); Kinta, Parit FR, 8 Apr 1935, *Symington FMS39490* (SING!).

Notes. Amongst the species with inflorescence in a series along the stem, *Capparis cucurbitina* can be easily identified by its torulose fruits.

3. *Capparis diffusa* **Ridl.** J. Str. Br. R. As. Soc. 59 (1911) 68; Jacobs, Fl. Malesiana 1, 6 (1960) 81, Blumea 12, 3 (1965) 447, figs 3, 26; Chayamarit, Fl. Thailand 5, 3 (1991) 254.

Type. Peninsular Malaysia, Perlis, Bt. Lagi, *Ridley 15174* (holotype SING! barcode 0056837; isotype K! barcode K000643988).

Distribution. In Peninsular Malaysia, this species found in Perlis, Kedah (Langkawi Islands), Perak (G. Pondok) and Pahang (Bt. Serdam, Gua Kechil).

Ecology. Evergreen forest on limestone rocks and quite common on dry rocky hill slopes.

Conservation status. There are several voucher specimens which have been collected as recently as 2008. None of the habitats is within protected areas. As the estimated extent of occurrence is less than 20,000 km² and the quality of habitat is declining, *Capparis diffusa* is assessed as Vulnerable B1ab(iii), according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012).

Specimens examined. Peninsular Malaysia. Kedah: Langkawi, P. Jerkom, 6°26.67'N, 99°78.33'E, *Stone* 6988 (KLU!). **Perak**: Kuala Kangsar, G. Pondok, Padang Rengas, 4°77.63'N, 100°83.70'E, *Chin 870* (KLU!). **Pahang**: Raub, Bt. Serdam, limestone, near the summit, ground rocky with little soil, vegetation fairly dense scrubby, 3°83.33'N, 101°91.67'E, *Chin 1104* (KLU!); Gua Kechil, 3°83.71'N, 101°93.37'E, *Julius FRI 56286* (KEP!).

Notes. Amongst the species with small leaves, *Capparis diffusa* is distinguished by its terminal or lateral inflorescences on short twigs and the 3–5-flowered in umbellate arrangement, with 1–2 small leaves, sometimes a few umbels united to a small panicle and sessile.

4. Capparis kenaboiensis Julius

5. *Capparis erycibe* Hallier f. Bull. Herb. Bossier 6 (1898) 216; Jacobs, Fl. Malesiana 1, 6 (1960) 75, fig. 8, Blumea 12, 3 (1965) 449; Chayamarit, Fl. Thailand 5, 3 (1991) 251.

Type. Indonesia, Java, Jambi [Tjampea], *Hallier f*. 779A (holotype BO-photo!, acc. no. 134004-134005; isotype L-photo! barcode L0035324). *Synonym: Capparis paniculata* Ridl., J. Fed. Malay St. Mus. 10, 2 (1920) 129. *Type*: Peninsular Malaysia, Kelantan,

near Chaning Estate, Kelantan River, Feb 1917, *Ridley s.n.* (holotype SING!, barcode 0096891]; isotype K!, barcode K000247300).

Distribution. In Peninsular Malaysia, this species is collected from Kelantan (Chaning Woods) and Pahang (Kuala Tembeling).

Conservation status. This species is very rare since it has been collected only twice, the last time being in 1917. Although a fieldwork expedition has been made to the localities, *Capparis erycibe* could not be located. As the estimated of extent occurrence is less than 100 km² and the declining of habitat quality, in which the type locality was converted into an oil palm estate, the species could be assessed as Critically Endangered, according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012). However, the species has also been collected from a National Park, which is a protected area. Therefore, this species is assessed as Near Threatened.

Ecology. In the lowland tropical forests.

Specimens examined. Peninsular Malaysia. Pahang: Jerantut, Tembeling, Kuala Tembeling, Aug 1891, *Ridley s.n.* (SING).

Notes. This species easily recognised by its paniculate inflorescences.

6. *Capparis micracantha* **DC. subsp.** *micracantha* Prod. 1 (1824) 247; Jacobs, Fl. Malesiana 1, 6 (1960) 85, Blumea 12 (1965) 466; Chayamarit, Fl. Thailand 5, 3 (1991) 246.

Type. Indonesia, Java, *Lahaye s.n.* (holotype G-DC! barcode G00203273). **Vernacular names.** *Jambul merak*, *melada* (Malay).

Distribution. In Peninsular Malaysia, it is occurring in Perlis (Kangar, Bt. Papan FR), Penang (P. Badak, P. Tikus), Kedah (Alor Setar, Langkawi), Kelantan (Kota Bharu) and Terengganu (Kuala Terengganu).

Conservation status. This species is widely distributed in the northern part of Peninsular Malaysia and is assessed as Least Concern.

Ecology. On limestone and sandy spots, or riverside at low altitude below 500 m.
Specimens examined. Peninsular Malaysia. Perlis: Kangar, 13 Jul 1936, Corner SFN31557 (KEP!), Ridley s.n. (KEP!). Kedah: Alor Setar, Haniff SFN10449 (SING!), Haniff 10470 (SING!), Ridley 15175 (SING!); Langkawi, P. Batang, Rosdi et al. FRI59928 (KEP!); Sept 1900, Haniff s.n. (SING!); P. Pasir, 2 Mar 1982, Stone 15032 (KEP!); P. Selang, Corner s.n. (SING!); P. Selang, Tanjung Botol, 24 Feb 1970, Stone 9084 (KLU!); P. Singa Besar, 24 Feb 1982, T. & P. 718 (KLU!); P. Timun, 24 Nov 1934, Henderson SFN29124 (KEP!). Penang: Badak Mati, near the beach, Curtis 1762 (SING!); P. Tikus, Mac 1889, Curtis 1762 (SING!). Kelantan: Kota Bharu, Corner s.n. (KEP!). Terengganu: Kuala Terengganu, Holttum 15190 (KEP!); Kg. Padang Negara, Sinclair 39811 (KEP!).

Notes. Capparis micracantha subsp. micracantha has smaller flowers compared to Capparis micracantha subsp. korthalsiana. In addition, it has less than 20 stamens, whereas in subsp. Korthalsiana, the stamens are numerous.

7. *Capparis micracantha* DC. subsp. *korthalsiana* (Miq.) Jacobs Fl. Malesiana 1, 6 (1960) 86. *Synonyms: Capparis korthalsia* Miq., Illustr. (1870) t.17.

Type. Borneo, S Kalimantan, Pulu Lampei, *Korthals s.n.* (holotype L-photo! barcode U0000960); *C. finlaysoniana* Wall. ex Hook. f., Fl. Brit. India 1 (1875) 179. *Type*: Singapore, *Wallich 6992B* (holotype K-W!, barcode K000643985).

Distribution. *Capparis micracantha* subsp. *korthalsiana* is distributed in the southern part of Peninsular Malaysia and it has been collected from Pahang (Rompin) and Johor (Mersing and Masai).

Conservation status. *Capparis micracantha* subsp. *korthalsiana* has been found and collected only four times, the last time being in 1967. With small distribution, the extent of occurrence less than 5000 km² and the declining of habitat, *C. micracantha* subsp. *korthalsiana* could be assessed as Endangered, according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012). However, *C. micracantha* subsp. *korthalsiana* was also found from Pulau Aur, which is a protected habitat under the Marine Park Act. Thus, *C. micracantha* subsp. *korthalsiana* is assessed as Near threatened.

Ecology. Its habitat preference is for wetter forest than that preferred by *C. micra-cantha* subsp. *micracantha*.

Specimens examined. Peninsular Malaysia. Pahang: Rompin, Sg. Aur, SE Pahang Aur FR at Sg. Aur, 11 May 1967, *Whitmore FRI 3689* (KEP!), 20 Apr 1919, *Yeop 3178* (KEP!). Johor: Mersing, P. Aur, Oct 1892, *Fielding s.n.* (SING!); Masai, Hook Linn Estate, 6 Mar 1948, *Mc Caul 38407* (KEP!).

Notes. *Capparis micracantha* subsp. *korthalsiana* differs from *C. micracantha* subsp. *micracantha* in several aspects, but easily distinguished by its inflorescences being supra-axillary, either solitary or in pairs and the stamens are numerous.

8. *Capparis pubiflora* **DC.** Prod. 1 (1824) 246; Jacobs, Fl. Malesiana 1, 6 (1960) 82, figs 16, 17, Blumea 12 (1965) 479; Chayamarit, Fl. Thailand 5, 3 (1991) 258.

Type. Indonesia, Timor, *s.c.*, (holotype P-photo! MNHN-P-P05461890; isotype G-DC! barcode G00207275). *Synonyms: Capparis pubiflora* var. *perakensis* Scort. ex King, J. As. Soc. Beng. 58, 2 (1889) 394, *C. perakensis* (Scort. ex King) Ridl., Fl. Malay Pen. 1 (1922) 124. *Type*: Peninsular Malaysia, Perak, Kuala Dipang FR, *Scortechini 1784* (holotype CAL n.v.; isotype K!, barcode K000643987).

Distribution. In Peninsular Malaysia, it has been found so far from Perak, Kelantan (Gua Musang), Pahang (G. Senyum, Gua Kechil, Bt. Chintamanis and Bt. Cheras) and Selangor (Batu Caves).

Conservation status. *Capparis pubiflora* is found and collected mostly from limestone hills with a recent collection from Gua Musang (*FRI94559*). None of the localities is within protected areas and some limestone hills are active quarries. Therefore, this species is assessed as Near Threatened. **Ecology.** Straggling on floor of a crack in limestone cliff (*Henderson SFN 22319*, *Wan Syafiq FRI94559*), also in damp shady forest (*Scortechini 1784*).

Specimens examined. Peninsular Malaysia. Kelantan: Gua Musang, Felda Perasu, Ktn 84, limestone hill, 26 Sept 2019, *Wan Syafiq FRI94559* (KEP!). PAHANG: Kuantan, Bt. Cheras, 10 Oct 1931, *Henderson SFN25220* (KEP!); Temerloh, G. Senyum, on floor of cleft in limestone clift, 31 Jul 1929, *Henderson SFN22319* (KEP!); Bentung, Bt. Chintamani, 31 Jul 2009, *Julius FRI 56292* (KEP!); Raub, Gua Kechil, 30 Jul 2009, *Julius FRI56289* (KEP!). **Perak**: Kinta, Kuala Dipang FR, *Scortechini 1784* (K!); Kuala Kangsar, Sg. Siput, quarried hills near Jalong, limestone hill, 29 Jan 2018, *Rafidah et al. FRI82018* (KEP!). **Selangor**: Gombak, Batu Caves, 14 Sept 1920, *Burkill SFN6369* (KEP!).

Notes. *Capparis pubiflora* easily recognised by its comparatively large oblanceolate leaves, 13.5–21 × 4–8.5 cm and the axillary fasciculate inflorescences.

9. *Capparis scortechinii* var. *scortechinii* King J. As. Soc. Beng. 58, 2 (1889) 394; Jacobs, Fl. Malesiana 1, 6 (1960) 73, Blumea 12 (1965) 488.

Type. Peninsular Malaysia, Perak, Batang Padang District, *King's Coll. 8083* (holotype CAL; isotype K! barcode K000643959).

Vernacular names. Kuku lang (Malay), akar pengsek, mentimun (Temuan).

Distribution. In Peninsular Malaysia: Penang (Batu Feringgi), Perak (Piah FR), Terengganu (Ulu Brang), Pahang (Taman Negara, G. Benom) and Selangor (Bt. Ranggong, Bt. Putih FR).

Conservation status. *Capparis scortechinii* var. *scortechinii* has been found and collected from at least five states in Peninsular Malaysia, which is considered widely distributed and could be assessed as Least Concern. However, the population has been found and collected mostly from unprotected areas and, thus, this species is assessed as Near Threatened.

Ecology. In rain forest, lowland areas.

Specimens examined. Peninsular Malaysia. Penang: Batu Feringgi, May 1885, *Curtis 239* (SING!). Perak: Hulu Perak, Piah FR, 16 Jul 1967, *Kochummen FRI2464* (KEP!); Batang Padang, *King's collector 8083* (K!). Terengganu: Hulu Terengganu, Sekayu, Bt. Lanjut FR, 20 Sept 1969, *Shing FRI13503* (KEP!); Ulu Berang, Jul 1937, *Moysey SFN33676* (KEP!). Pahang: Temerluh, Krau WR, G. Benom, Ulu Chelon, 24 Mar 1967, *Whitmore FRI3434* (KEP!), logging track from Ulu Cheka to G. Benom, 10 Jan 1978, *Ng FRI27160* (KEP!); Jerantut, Taman Negara, Bt. Terom, near Kuala Keniyum, 5 Mar 1968, *Whitmore FRI8550* (KEP!); Bentung, Ulu Langat FR, Sempadan Looi, 30 Mar 1960, *Gadoh KL2072* (KEP!). Selangor: Petaling, Bt. Cherakah FR, 18 Jul 1986, *FRI33522* (KEP!); Hulu Selangor, Kuala Kubu - Raub Road, along the road side, *Mahmud* s.n. (KEP!); Hulu Langat, Bt. Rangong, Kuala Looi, 13 Mar 1960, *Gadoh KL2044* (KEP!); Hulu Langat, Kuala Pansom, 10 Sept 1958, *Gadoh KL 862* (KEP!); Gombak, Gombak FR, Mile 16, followed path to stream and beyond, 2 Jun 1963, *Poore 1189* (KEP!); ibid., Ulu Gombak FR, edges, 19 Aug 1964, *Mohd. Kasim 625* (KEP!); ibid., Bukit Putih FR, Bkt. Sg. Puteh, 6 Feb 1926, *Mat Yassin 10837* (KEP!); ibid., Kepong Plantation, Field 9J, 10 Feb 1934, *Abdul Hamid FMS33570* (KEP!); ibid., Kepong Plantation, Field 5, 10 Feb 1934, *Abdul Hamid FMS36005* (KEP!).

Notes. Jacobs (1965), in his description of this species, mentions that the gynophore is glabrous, but actually densely hairy at the basal part. The filament is also densely hairy at base. This observation has been confirmed by examining few specimens including the one (*Curtis 239*, SING) cited in his treatment.

Though the inflorescence has been described in the terminal position by Jacobs (1965), but observed only in *King's Coll. 8083* (K), other specimens examined have axillary inflorescences, except in *Curtis 239*, where both axillary and terminal inflorescences occur. Thus, axillary inflorescences are more common in *Capparis scortechinii* var. *scortechinii*.

Two specimens from Fraser's Hill (*Kalong 22381, Pursglove P4248*), cited in Jacobs (1965), have terminal inflorescences, but these belong to *Capparis scortechinii* var. *ruthiae*.

10. Capparis scortechinii var. ruthiae Julius

11. *Capparis sepiaria* L. Syst. Nat., ed. 10. 2: 1071 (1759); Jacobs, Fl. Malesiana 1, 6 (1960) 79, Blumea 12 (1965) 489; Chayamarit, Fl. Thailand 5, 3 (1991) 241.

Type. India, Linnean. Cat. 664.4 (holotype LINN-photo!).

Distribution. In Peninsular Malaysia, so far collected from Kedah (Alor Setar and Kg. Nangka) and Kelantan.

Conservation status. This species is very rare with only four specimens recorded from Kedah and Kelantan and it was last collected in 1933. As the estimated extent of occurrence is less than 5000 km² and the declining of habitat quality, *Capparis sepiaria* is assessed as Endangered B1ab(iii), according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012).

Ecology. In lowland forest.

Specimens examined. Peninsular Malaysia. Kelantan: Gua Musang, Batu Neng, limestone hill, 200–300 m alt., near the summit, on dry rocky terrain, *Chin* 1542 (KLU!); (locality unknown), Mac 1881, *King's collector 1415* (SING!). Kedah: Kampung Nangka, *Holttum 19827* (SING!); Alor Setar, *Mohamed Nur 15176* (SING!).

Notes. Amongst the species with small leaves in this genus, *Capparis sepiaria* is easily recognised by its conspicuously flexuose twigs.

12. *Capparis trinervia* Hook.f. & Thoms. Fl. Brit. India 1 (1875) 175; Jacobs, Fl. Malesiana 1, 6 (1960) 73, Blumea 12 (1965) 500. *Synonym: Capparis kuntslerii* King, J. As. Soc. Beng. 58, 2 (1889) 396; Ridley, Fl. Malay Pen. 1 (1922) 122.

Type. Peninsular Malaysia, Perak, G. Bubu, *King's Coll. 8337* (holotype CAL-photo!, barcode CAL0000004992, CAL0000004993, CAL0000005007; isotype K!, barcode K000643958).

Distribution. In Peninsular Malaysia so far collected from Kedah (Alor Setar) and Kelantan. Recent surveys in 2010 were unable to locate the species.

Conservation status. Data Deficient.

Ecology. Found in hill forest at low altitude, 122–244 m.

Notes. This species is best distinguished from others by combination of several characters: the leaves are trinerved at base and the irregular reticulate venation distinct on both surfaces and the inflorescences are terminal with flowers arranged in subumbels.

13. Capparis trinervia var. chungiana Julius

14. *Capparis versicolor* Griff. Not. Pl. As. (1854) 577; Jacobs, Blumea 12 (1965) 501, fig. 32; Chayamarit, Fl. Thailand 5, 3 (1991) 256.

Type. Myanmar [Burma *Griffith 936*] (holotype K!, barcodes K000247350, K000247351). *Synonym: Capparis larutensis* King, J. As. Soc. Beng. 58 (1889) 393, Jacobs, Fl. Malesiana 1, 6 (1960) 89. *Type*: Peninsular Malaysia, Perak, Larut, *King's Coll. 5103* (holotype CAL-photo!, barcodes CAL0000004975–CAL0000004979; isotype K!, barcode K000643984).

Distribution. In Peninsular Malaysia, recorded so far from Perak (Larut and Kampar).

Conservation status. This species is very rare with only two specimens recorded from Perak and it was last collected in 1898. As the estimated extent of occurrence is less than 5000 km² and the declining of habitat quality, *Capparis versicolor* is assessed as Endangered B1ab(iii), according to the IUCN Red List Categories and Criteria (IUCN 2001, 2012).

Ecology. In thickets or dense forest, clinging on trees, at altitude below 200 m.

Specimen examined. Peninsular Malaysia. Perak: Kinta, Kampar, Sept 1898, *Ridley 9646* (SING!).

Notes. *Capparis versicolor* is amongst the species having relatively small leaves less than 5 cm long. It has a simple corymb inflorescence terminally on a short lateral twig with few leaves below.

Acknowledgements

The study was carried out as part of the Flora of Peninsular Malaysia Project at the Forest Research Institute Malaysia, funded by The Ministry of Science, Technology and Innovation of Malaysia (Project No. 01-04-01-0000 Khas2) and the Ministry of Water, Land and Natural Resources (formerly Ministry of Natural Resources and Environment) of Malaysia under the 10th and 11th Malaysian Plans (SPPII No. P23085100010021 and P23085100018003, respectively). I would like to thank the

Curators of BKF, L, K, KLU, SAN, and SING herbaria for their permission to loan and examine the specimens under their care. The encouragement and useful comments from Dr Richard Chung Cheng Kong and Dr Ruth Kiew are greatly acknowledged. Thanks also to Dr Timothy Utteridge (K), Silvio Fici (SAF) and Xavier Cornejo (GUAY) for reviewing and proof reading my manuscript; to Dr Prasad V.P., Lakshminarasimhan Pakshirajan (CAL) and Abdulrokhman Kartonegoro (BO) for providing some digital type images of *Capparis*; to Dr Lillian Chua (FRIM) for valuable assistance with the conservation assessments; to Wendy Yong Yze Yee for preparing the distribution map for *Capparis kenaboiensis* and *C. scortechinii* var. *ruthiae*; to Mohamad Aidil Noordin (KEP) for preparing the botanical illustration; and to Imin Kamin and Angan Atan (KEP) for assistance in field.

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Supplementary material I

Figure S1–S8

Authors: Avelinah Julius

Data type: map, plate and botanical drawing

- Explanation note: Distribution and description of *Capparis kenaboiensis*, *Capparis scortechinii* var. *ruthiae*, *Capparis trinervia var. chungiana*
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Link: https://doi.org/10.3897/phytokeys.189.49367.suppl1

RESEARCH ARTICLE



Pitcairnia abscondita (Pitcairnioideae, Bromeliaceae), a hidden novelty from north-western Jalisco, Mexico

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Academic editor: Leonardo Versieux | Received 13October 2021 | Accepted 10December 2021 | Published 14 February 2022

Citation: Flores-Argüelles A, López-Ferrari AR, González-Rocha E, Espejo-Serna A (2022) *Pitcairnia abscondita* (Pitcairnioideae, Bromeliaceae), a hidden novelty from north-western Jalisco, Mexico. PhytoKeys 189: 129–139. https://doi.org/10.3897/phytokeys.189.76464

Abstract

Pitcairnia abscondita **sp. nov.**, known until now only from the Municipalities of Cabo Corrientes, Mascota, Puerto Vallarta, San Sebastián del Oeste and Talpa de Allende in the State of Jalisco, Mexico, is here described and illustrated. The new taxon was confused with *P. imbricata* for long time, but differs from this species by its green floral bracts with the apex divergent to spreading (vs. red and appressed) and by the appendiculate at the base chartreuse-green petals (vs. not appendiculate yellow petals). Images and a distribution map of the taxa are presented.

Resumen

Se describe e ilustra *Pitcairnia abscondita* **sp. nov**., conocida hasta ahora únicamente de los municipios de Cabo Corrientes, Mascota, Puerto Vallarta, San Sebastián del Oeste y Talpa de Allende, en el estado de Jalisco, México. El nuevo taxon fue por largo tiempo confundido con *P. imbricata*, pero difiere de la misma por sus brácteas florales verdes con el ápice divergente a extendido (vs. brácteas rojas y adpresas) y por sus pétalos verdes chartreuse con apéndices en la base (vs. pétalos amarillos sin apéndices en la base). Se incluyen imágenes y un mapa de distribución de las especies.

Keywords

Jalisco north coast, Pitcairnia subgenus Pitcairnia

Palabras clave

Costa norte de Jalisco, Pitcairnia subgénero Pitcairnia

Introduction

Pitcairnia L'Hér. (L'Héritier 1789–1790 [1788]) with ca. 409 spp. (Gouda et al. 2021, continuously updated) is the second richest genus in Bromeliaceae and is mainly distributed in South America, but has an important centre of diversification in Mexico. The only species of the family that grows out of America, *P. feliciana* (A. Chev.) Harms & Mildbraed (Harms and Mildbraed 1938), belongs to this genus. Out of the 19 genera of Bromeliaceae present in Mexico (Espejo-Serna et al. 2004; Espejo-Serna 2012; Espejo-Serna and López-Ferrari 2018, here updated), *Pitcairnia* occupies the third place in number of taxa, with 54 species (including the one described here). In addition, the genus is notable for the number of endemic taxa present in the country, which reaches 47 species (87.03% of the total). For Jalisco, we have so far reported 13 species (Espejo-Serna et al. 2004; Espejo-Serna and López-Ferrari 2018, here updated) and, with this new find, that number increases to 14. Of these, *P. singularis* Flores-Arg., Espejo & López-Ferr., (Flores-Argüelles et al. 2017), *P. lokischmidtiae* Rauh & Barthlott (Rauh and Barthlott 1987; see also Rauh 1987) and the new taxon here proposed are restricted to Jalisco.

During the fieldwork for the fulfilment of the Master's Thesis of the first author (Flores-Argüelles 2020), we had the opportunity to collect specimens from one species of *Pitcairnia*, which we could not identify. Recently, reviewing specimens of the family Bromeliaceae collected in Jalisco, we found additional material of this species. After a detailed examination of the specimens, we have not been able to assign it to any of the previously-described species of the genus, so we propose it here as new to science.

Material and methods

Plants were collected in the years 2019 and 2020 in the Municipality of Cabo Corrientes, Jalisco, Mexico. The gathering of the specimens was carried out in accordance with Lot and Chiang (1986). Measurements and description were made from fresh material and herbarium specimens. The morphological terms used in the description are those proposed by Radford et al. (1974) and Scharf and Gouda (2008). The type material was deposited at Herbario Metropolitano Ramón Riba y Nava Esparza, Universidad Autónoma Metropolitana Iztapalapa (UAMIZ). We revised herbarium material of the genus *Pitcairnia* housed at A, C, CHAP, CICY, ENCB, FCME, GH, HEM, IBUG, IEB, MEXU, MICH, MO, P, SERO, UAMIZ, UC, US, XAL and ZEA (ac-

ronyms according to Thiers 2021). To ensure the status of the proposed new species, we revised the protologues, living specimens as well as herbarium specimens and type material of *P. imbricata* (Brongn.) Regel (Regel 1868) and *P. wendlandii* Baker (Baker 1881), the taxa with morphologically most similarities (see Appendix 1). The distribution map of the species was elaborated with the open source geographic information system QGIS (2021), using the data obtained from the herbarium specimen labels.

Taxonomic treatment

Pitcairnia abscondita Flores-Arg., López-Ferr., Gonz.-Rocha & Espejo, sp. nov. urn:lsid:ipni.org:names:77254836-1 Figs 1, 2A–B and 3

Type. MEXICO. Jalisco: municipio Cabo Corrientes, ejido Las Juntas y Los Veranos, santuario las Guacamayas, 20°25.802'N, 105°18.978'W, 600 m a.s.l., bosque de galería, 25 Jan 2020, flowered in cultivation, 12 Jul 2021, A. Flores-Argüelles, G. Contreras-Félix & J. Novoa 1189 (holotype: UAMIZ in two sheets!).

Diagnosis. Similar to *Pitcairnia imbricata*, but differs by the presence of green, widely oblong to widely ovate floral bracts with the apex divergent to spreading (vs. red elliptic with the apex appressed to the rachis), arcuate corollas (vs. erect), appendiculate, chartreuse-green, 9.4–9.8 cm long, petals (vs. yellow, not appendiculate 6.5–6.6 cm long).

Description. Plant terrestrial or rupicolous, growing frequently along streams, aerial portion of the stem inconspicuous, with underground erect rhizomes ca. 5 cm in diam., flowering 100-110 cm tall. Roots fibrous, thin. Rosettes 40-50 cm high, 90-110 cm diam. Leaves 10 to 50, rosulate, monomorphic, pseudopetiolate; sheaths brown to light brown, with a transverse white band at the base, triangular, 5-6 cm long, 5-6 cm wide at the base, strongly nerved, densely white-tomentose abaxially, entire; pseudopetiole 20-40 cm long, ca. 10 mm wide, involute, margins minutely spinose-serrate, densely white-tomentose abaxially at the base; blades green, linear, attenuate towards the apical portion, 90-170 cm long, 2.5-5.5 cm wide at its widest part, with a central longitudinal channel, entire, very sparsely lepidote adaxially, glabrous abaxially. Inflorescence terminal, simple, erect to arched; peduncle green, cylindrical, 70-77 cm long, 0.7-1.4 cm in diam. at the base; peduncle bracts green, foliaceous, erect, the sheaths appressed, the blades becoming progressively reduced distally, narrowly triangular, 4-35 cm long, ca. 3 cm wide at the base, entire, attenuate to long-attenuate, glabrescent to glabrous on both surfaces; spike terete, 10-50 cm long, 3.5-4 cm in diam., rachis wholly covered by the floral bracts; floral bracts foliaceous, appressed and imbricate, green, widely oblong to widely ovate, 49-52 mm long, 30-32 mm wide, the apex acuminate, divergent to spreading in living plants, the margin hyaline, glabrous on both surfaces, much longer than the sepals. Flowers 25-80 per inflorescence, polystichous, zygomorphic, slightly, but conspicuously arcuate-recurved, sessile, acropetalous; sepals free, green, oblong, 28-30 mm long,



Figure 1. *Pitcairnia abscondita* Flores-Arg., López-Ferr., Gonz.-Rocha & Espejo **A** habit **B** detail of the inflorescence **C** flower dissected **D** inflorescence **E** plant in the type locality (*A. Flores-Argüelles et al. 1131*). Photo credits: A. Espejo-Serna.



Figure 2. Flower dissected and detail of the inflorescence **A**, **B** *Pitcairnia abscondita* (*A. Flores Argüelles et al. 1189*) **C**, **D** *P. imbricata* (*A. Espejo et al. 7271*) **E**, **F** *P. wendlandii* (*M.I. Mejía-Marín et al. 146*). Photo credits: A. Espejo-Serna.



Figure 3. Known distribution of Pitcairnia abscondita, P. imbricata and P. wendlandii in Mexico.

9–11 mm wide, ecarinate, nerved, acute and shortly apiculate, glabrous; petals free, chartreuse green, narrowly oblanceolate, 94–98 mm long, 14–19 mm wide, rounded and very shortly apiculate, with an adaxially basal, oblong, ca. 13 mm long × ca. 6 mm wide, erose appendage, almost completely adnate to the petal; stamens all equal in length, shorter than the petals, filaments whitish, filiform, 71–72 mm long; an-thers yellow, linear, 16–17 mm long, basifixed; ovary half superior, greenish-white, ovoid, ca. 15 mm long, ca. 6 mm in diam., glabrous; style linear, arcuate-recurved, ca. 78 mm long; stigma white, conduplicate-spiral (type II sensu Brown and Gilmartin 1984), glabrous. Capsules dark brown when mature, narrowly ovoid in the outline, trigonous in cross section, 19–21 mm long, 6–8 mm in diam., septicidal, glabrous; seeds reddish to light-brown, fusiform, 1–1.5 mm long, long bicaudate, the caudae filiform, ca. 5 mm long each one.

Etymology. The specific epithet refers to the fact that, for a long time, the specimens of this species was "hidden" behind the name *Pitcairnia imbricata* (see McVaugh 1989), to difficulties in identifications of herbarium samples, since the dried specimens of *P. abscondita* can be easily confused with *P. imbricata*.

Distribution and habitat. *Pitcairnia abscondita* is known until now only from the State of Jalisco, in the Municipalities of Cabo Corrientes, Mascota, Puerto Vallarta, San Sebastián del Oeste and Talpa de Allende (Fig. 3), growing rupicolous or terrestrial in wet *Pinus-Quercus* forests, gallery forests and cloud forests often near rivers or streams, at an elevation interval from 400–1,500 m a.s.l. Blooms during July and fructifies from August to September.

Additional specimens examined (paratypes). MEXICO. Jalisco: Municipio Cabo Corrientes: 3-10 km generally east on the road to Mina del Cuale, from the junction 5 km northwest of El Tuito, 850-1,150 m elev., steep mountainsides pine-oak forest on decomposed granitic soils, with Podocarpus, oaks and other deciduous trees in rocky stream valleys, 16-19 Feb 1975, R. McVaugh 26385 (MEXU (two sheets)); MICH (two sheets)); entre El Tuito y Puerto Vallarta, a 20 km de Puerto Vallarta y a 20 km de El Tuito, ca. 450 m elev., bosque de pino-encino, 19 Jul 1976, A. Delgado S. & R. Hernández M. 2617 (MEXU); km 18 camino El Tuito hacia la mina de Zimapán, 960 m elev., bosque mesófilo de montaña, 1 Jun 1985, J.A. Pérez de la Rosa 974 (IBUG); ca. 0.5 km después de Pedro Moreno, rumbo a El Tuito, 637 m elev., 20°24.3833'N, 105°18.2'W, 30 Jul 2003, J. Ceja, A. Espejo, A.R. López-Ferrari, A. Mendoza R. & I. Ramírez M. 1476 (UAMIZ); km 4 del camino El Tuito-Zimapán, 3.5 km al W de la Provincia, 937 m elev., 20°20.9333'N, 105°17.6067'W, bosque de pino-encino con elementos mesófilos, 2 Aug 2011, A. Castro-Castro, J.G. González, R. Guerrero & E. de Castro 2518 (IBUG); km 2 del camino a la mina de Zimapán, 813 m elev., 20°21.0468'N, 105°18.1897'W, bosque de pino-encino, 29 Aug 2019, A. Flores-Argüelles, A.R. López-Ferrari, E. González R., J. Hernández B., R. Hernández C. & A. Espejo 1131 (UAMIZ); camino El Tuitominas de Zimapán, 881 m elev., 20° 21'N, 105°17.7833'W, bosque de pino y encino, 13 Sep 2020, Brunel, E. Ruíz-Sánchez & E. Gándara 901 (IBUG). Municipio Mascota: la Bulera, 9.5 km al WSW de la Estancia, 900 m elev., 20°44.4833'N, 105°0.0333'W, bosque mesófilo de montaña, 2 Apr 2002, P. Carrillo R., E.M. Barba & M. Alcázar 3147 (IBUG, UAMIZ). Municipio Puerto Vallarta: 800 m de Peña Blanca, camino a Talpa de Allende, 1,360 m elev., 20°44.4767'N, 105°01.6467'W, bosque mesófilo de montaña con Magnolia, Clusia, Calophyllum, Chamaedorea, Chryosophila, Cecropia, Quercus, 22 Feb 1998, R. Ramírez D., F. Cupul, H. Hernández, J. Fonseca & F. Rodríguez Z. 5252 (IBUG); Ojo de Agua, 6 km al SE de Vallejo, 1,190 m elev., 20°31.337'N, 105°11.6212'W, bosque de encino con Quercus magnoliifolia, Brahea sarukhanii, Bejaria mexicana, 21 Jan 2013, A. Flores-Argüelles & R. Romero 652 (IBUG, ZEA). Municipio San Sebastián del Oeste: los Ojos de Agua, ca. 3.7 km en línea recta al SW de la Estancia de los Landeros, 1,280 m elev., 20°44.665'N, 104°55.9017'W, bosque de galería con Podocarpus, Hedyosmum, Saurauia, 24 Jul 2014, P. Carrillo R., D. Cabrera-Toledo, L.A. Ortega-Valencia & L.M. Valadez-Sandoval 7439 (IBUG). Municipio Talpa de Allende: steep mountains 11-12 miles south of Talpa de Allende, in the headwaters of a west branch of Río de Talpa, 1,200 m elev., 20°14.5833'N, 104°46.7757'W, barranca above a rapid clear stream, in dense Forest of Quercus, Carpinus, Distylium, Magnolia, Podocarpus, with pine forest on the ridges above, 18-19 Oct 1960, R. McVaugh 20441 (MICH); brecha Talpa-La Cuesta, 1,400 m elev., bosque mesófilo de montaña, 29 Jul 1990, R. Ramírez D. & R.G. Tamayo 2139 (IBUG); 15 km al SW de Talpa, 5 km de Aranjuez, 2 km al NNE de la estación de microondas, 1,320 m elev., 20°18.05'N, 104°53.3'W, bosque de pino-encino con asociación de Pinus douglasiana, P. jaliscana, P. oocarpa, Quercus magnoliifolia, Q. elliptica, 16 Jul 2009, L.M. González-Villarreal, J.A. Pérez de la Rosa & G. Vargas 5180 (IBUG).

Comments. In herbarium specimens, *Pitcairnia abscondita* superficially resembles *P. imbricata* and/or *P. wendlandii*. However, the new taxon differs from these two

	P. abscondita	P. imbricata	P. wendlandii
Leaf blades (cm)	90–170 × 4.5–5.5	70–120 × 5.5–6	50-110 × 4-4.5
Floral Bracts (mm)	widely oblong to widely ovate, green, divergent to spreading at the apex; 49–52 × 30–32	elliptic, red, appressed at the apex; 35–57 × 13–24	elliptic, red to greenish-red, divergent to spreading at the apex; $65-66 \times 22-25$
Flowers	arcuate-recurved	straight	straight
Sepals (mm)	oblong, acute apiculate; 28–30 × 9–11	oblong apiculate; 21–22 × ca. 8	narrowly triangular, acute; 20–21 × ca. 3
Petals (mm)	narrowly oblanceolate, chartreuse green; 94–98 × 14–19	narrowly oblong, yellow; 65–66 × 12–13	narrowly oblong, to linear, yellow- greenish; 69–70 × 9–10
Anthers (mm)	16–17	ca. 11	13-14
Distribution (Mexico)	Jalisco	Chiapas, Guerrero, Jalisco, Oaxaca, Puebla, Veracruz	Chiapas, Guerrero, Oaxaca

Table 1. Comparative features of *Pitcairnia abscondita* with *P. imbricata* and *P. wendlandii* (see also Figs 1 and 2).

species by the characters shown in Table 1 and Fig. 2. In fact, the Jalisco material that McVaugh (1989) included in his Flora Novo-Galiciana as *P. imbricata*, actually belongs to the new species.

The flowers of *Pitcairnia abscondita* last only one night, opening between 7.30 and 9.30 pm and remain that way during the night, starting to close at 7.30 in the morning, being completely closed at 9.00 am, so they are likely associated with a pollination syndrome by moths or bats, different from *P. imbricata* which, due to its red floral bracts and yellow flowers of diurnal anthesis, is associated with an ornithophilic pollination syndrome (Proctor et al. 1996).

As far as we know, the plants of *P. abscondita* are not used by the inhabitants of the region, so we think that the species has no immediate human pressure; however and due to the lack of detailed information about the precise distribution of the species, we suggest the inclusion of the new taxon in the Not Evaluated (NE) category of the IUCN (2020).

Acknowledgements

We thank Walter Till, Eric Gouda and two anonymous reviewers for their critical review and improvement of the manuscript. Rodrigo Hernández-Cárdenas, Lizetth Jimena Hernández-Barón, Gerardo Contreras-Félix and Jorge Novoa-Ramos helped us in the fieldwork. The curators of the following Herbaria gave us all the facilities to consult their collections: A, C, CHAP, CICY, ENCB, FCME, GH, HEM, IBUG, IEB, MEXU, MICH, MO, P, SERO, UAMIZ, UC, US, XAL and ZEA.

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Appendix I

Specimens examined.

1. Pitcairnia imbricata (Brongn.) Regel

MEXICO. CHIAPAS: Municipio Berriozábal, C.R. Beutelspacher 29652 (HEM), D.E. Breedlove & R.F. Thorne 30896 (MEXU); municipio de Chilón, D.E. Breedlove 34566 (ENCB; municipio El Bosque, D.E. Breedlove & R.L. Dressler 29831 (MEXU, MO); municipio La Trinitaria, D.E. Breedlove 56560 (ENCB); municipio Ocosingo, E. Martínez S. 17047 (CICY, MEXU), E. Martínez S. 17610 (MEXU, MO), E. Martínez S. et al. M-21912 (MEXU), G. Aguilar M. et al. 6924 (UAMIZ), P.E. Valdivia Q. 2418 (XAL). GUERRERO: MUNICIPIO Atoyac de Álvarez, C.A. Granados et al. 370 (MEXU), J.C. Soto N. & F. Solórzano G. 12815 (MEXU, UAMIZ), P. Tenorio L. et al. 487 (MEXU, MO), V.C. Aguilar J. 642 (FCME), Y. Ramírez-Amezcua et al. 905 (IEB); municipio Chilpancingo de los Bravo, E. Matuda 38683 (ENCB, FCME, MEXU), E. Matuda & colaboradores 38711 (MEXU), H.E. Moore Jr. 5112 (GH, UC, US), H.E. Moore Jr. 8130 (MEXU, US), H.E. Moore Jr. & C.E. Wood Jr. 4705 (A, MICH, US), H. Kruse 798 (FCME, MEXU), J. Ceja et al. 1678 (IEB, UAMIZ), L.A. Kenoyer C246 (GH), R.M. Fonseca 1725 (FCME); municipio Mochitlán, G. Espinosa F. 316 (FCME). JALISCO: Municipio Casimiro Castillo, R. Cuevas G. et al. 6547 (ZEA); municipio Cuautitlán de García Barragán, A. Vázquez & R. Zúñiga 4457 (ZEA); municipio Villa de Purificación, G. Morales et al. 116 (IBUG, ZEA), J.L. Rodríguez et al. 297 (ZEA), L. Guzmán H. et al. 5239 (ZEA). OAXACA: Municipio San Felipe Usila, G. Ibarra M. et al. 3748 (MEXU, MO); municipio San Juan Bautista Valle Nacional, A.R. López-Ferrari et al. 3196 (UAMIZ), R. Torres C. & L. Cortés A. 7250 (MEXU); municipio San Juan Juquila Vijanos, X. Munn et al. 8 (MEXU); municipio San Miguel Quetzaltepec, B. Rendón A. et al. 1391 (UAMIZ); municipio Santiago Comaltepec, A. Mendoza R. et al. 280 (UAMIZ), A. R. López-Ferrari et al. 2117 (UAMIZ), J. Santana & L. Pacheco 913 (UAMIZ), J. Utley & K. Burt-Utley 6739 (MEXU), W.L. Graham 1414 (MICH); municipio Santiago Jocotepec, B.P. Reko 4128 (US); municipio Totontepec Villa de Morelos, R. Torres C. & L. Cortés A. 10441 (MEXU). PUEBLA: Municipio Chignautla, F. Liebmann 7953 (C, MO); municipio Cuetzalan del Progreso, J.L. Contreras J. 6134 (CHAP); municipio Hueytamalco, W. López-Forment s. n. (MEXU); municipio Huitzilan de Serdán, G. Toriz A. et al. 621 (CHAP, MEXU); municipio Tepango de Rodríguez, J. García-Cruz et al. 1413 (UAMIZ); municipio Xochitlán de Vicente Suárez, I.N. Gomez-Escamilla & B.E. Téllez-Baños 190 (UAMIZ); municipio Yaonáhuac, P. Tenorio L. et al. 14066 (MEXU). VERACRUZ: Municipio Atzalan, F. Ventura 17297 (ENCB, IEB, MEXU, XAL); municipio Catemaco, A. Gómez-Pompa 5148 (XAL), S. Sinaca 44 (MEXU), S. Sinaca 176 (MEXU, MO), W. Boege 3183 (MEXU): municipio Fortín, E. Bourgeau 1778 (MO, P); municipio Pajapan, J.I. Calzada 10899 (XAL), M. Nee & J.I. Calzada 22725 (GH, XAL), M. Nee et al. 24975 (GH); municipio San Andrés Tuxtla, S. Sinaca C. et al. 989 (MEXU); municipio Soteapan, J.I. Calzada et al. 11439 (XAL), M. Cházaro & P. Sánchez 3545 (XAL), T.P. Ramamoorthy et al. 3891 (CICY, MEXU); municipio Tlapacoyan, F. Miranda 3305 (MEXU); F. Ventura 13131 (ENCB, IEB); municipio Yecuatla, C. Gutiérrez B. & M. Cházaro B. 1572 (XAL).

2. Pitcairnia wendlandii Baker

MEXICO. CHIAPAS: Municipio Acacoyagua, E. Matuda 17729 (MEXU, MO), N. Martínez-Meléndez 916 (HEM); municipio Ángel Albino Corzo, M.A. Pérez Farrera 1166 (HEM); municipio Jiquipilas, D.E. Breedlove 23970 (ENCB), M.A. Pérez Farrera 463 (HEM); municipio La Concordia, D.E. Breedlove 40123 (MEXU), G. del C. López H.159 (HEM), J. Martínez-Meléndez 641 (UAMIZ), J. Martínez-Meléndez 1344 (HEM), N. Martínez-Meléndez 224 (HEM), R.J. Hampshire & A. Reyes-García 1260 (MEXU), R. Martínez-Camilo 789 (HEM); municipio Mapastepec, J.I. Calzada et al. 9006 (MEXU, XAL), M. Heath & A. Long 1166 (MEXU); municipio Tonalá, M.A. Pérez-Farrera 425 (HEM); municipio Unión Juárez, E. Martínez S. & A. Reyes 20310 (IEB, MEXU, MO); municipio Villa Comaltitlán, C.R. Beutelspacher 22799 (HEM); municipio Villa Corzo, F. Hernández-Najarro 792 (HEM), H. Gómez D. 623 (HEM), J. Martínez-Meléndez 462 (HEM), J. Martínez-Meléndez 490 (HEM), J. Martínez-Meléndez 1618 (HEM), M.A. Pérez Farrera 488 (HEM), M.A. Pérez Farrera 709 (HEM). Guerrero: Municipio San Luis Acatlán, F. Lorea 4884 (FCME). OAXACA: Municipio Asunción Ixtaltepec, R. Torres C. & C. Martínez R. 6087 (MEXU, MO); municipio Guevea de Humboldt, R. Torres C. & C. Martínez R. 5948 (MO); municipio Putla, E. Solano C. & J.C. Gutiérrez H. 4244 (UAMIZ); municipio San Juan Colorado, M.I. Mejía Marín et al. 146 (UAMIZ), M.I. Mejía-Marín et al. 325 (UAMIZ), M.I. Mejía-Marín et al. 704 (UAMIZ), M.I. Mejía-Marín et al. 720 (UAMIZ), M.I. Mejía-Marín et al. 802 (UAMIZ), M.I. Mejía-Marín et al. 900 (UAMIZ), M.I. Mejía-Marín et al. 928 (UAMIZ); municipio San Mateo Río Hondo, A. García-Mendoza & F. Martínez 2687 (MEXU, MO); municipio Santa Catarina Juquila, A. Espejo et al. 7271 (UAMIZ); municipio Santa Cruz Itundujia, A. Nava Z. et al. 2027 (SERO), K. Velasco G. et al. 2144 (SERO); municipio Santiago Lachiguiri, sin colector s. n. (MEXU).

RESEARCH ARTICLE



Goodyera medogensis (Orchidaceae), a new species from Tibet, China

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Academic editor: J. Farminhão | Received 31 October 2021 | Accepted 28 January 2022 | Published 14 February 2022

Citation: Tong Y-H, Sun M, Wang B-M, Tian H-Z (2022) *Goodyera medogensis* (Orchidaceae), a new species from Tibet, China. PhytoKeys 189: 141–154. https://doi.org/10.3897/phytokeys.189.77374

Abstract

A new species of *Goodyera* (Orchidaceae) from Tibet, China, *G. medogensis*, is described and illustrated here. Molecular phylogenetic results based on one nuclear (ITS) and two plastid markers (*matK* and *trnL-F*) support the recognition of *G. medogensis* as a new species in *Goodyera* subsection *Reticulum*. Morphologically, the novelty is most similar to *G. biflora*, *G. vittata* and especially to *G. hemsleyana*, but differs by the thick grid lines of the reticulations with a diffused margin on the adaxial surface of the leaf blades, the inflorescence with more flowers, the obliquely obovate-rhombic petals, the yellow or yellowish labellum without a lamella on the blade, and the shorter trichomes on the floral bracts, sepals and ovary. Finally, a key to the species of *Goodyera* subsect. *Reticulum* in China is also provided.

Keywords

Cranichideae, Jewel orchid, morphology, new species, Orchidoideae, phylogeny

Introduction

The genus *Goodyera* R. Br. (Orchidaceae) belongs to the subtribe Goodyerinae (Pridgeon et al. 2003; Chase et al. 2015), which includes three major subdivisions: the *Pachyplectron* clade, the *Goodyera* clade and the *Cheirosylis* clade (Chen et al. 2019).

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^{*} These authors contributed equally.

Different phylogenetic studies have revealed that *Goodyera* is polyphyletic (Juswara 2010; Hu et al. 2016; Chen et al. 2019), which has led Pace (2020) to subsequently propose a new generic arrangement for the *Goodyera* clade including 11 genera: *Goodyera s.s., Cionisaccus* Breda, *Aspidogyne* Garay, *Microchilus* C. Presl, *Kreodanthus* Garay, *Lepidogyne* Blume, *Hylophila* Lindl., *Platylepis* A. Rich., *Eucosia* Blume, *Erythrodes* Blume, *Salacistis* Rchb. f. and *Paorchis* M. C. Pace. Since the available molecular phylogenetic results are based on few markers, and the quality and representativeness of samples are limited, in this study, we still accept a broad concept of *Goodyera*.

Goodyera s.l. consists of about 99 species distributed in Africa (Mozambique), Western Indian Ocean Islands, Asia, Southwest Pacific islands, Northeast Australia, Europe, Macaronesia (Madeira), North and Central America and the Caribbean (Chen et al. 2009; Zhou et al. 2020; Govaerts et al. 2021; Thiv et al. 2021). It is characterized by an elongate creeping rhizome, a cymbiform lip with a concave-saccate hypochile, sectile pollinia and one stigma.

There are 36 accepted species of *Goodyera* in China, of which 15 species are endemic (Chen et al. 2009; Guan et al. 2014; Jin and Yang 2015; Zhou et al. 2016; Liu et al. 2019; Wang et al. 2020; Zhou et al. 2020), and only *G. repens* (L.) R. Br. and *G. brachystegia* Hand.-Mazz. are distributed to the north of the Yangtze River. During a botanical survey in Medog County, Tibet Autonomous Region in January 2021, a few living plants with greenish reticulated leaves, resembling those of *Macodes* (Blume) Lindl., were collected and cultivated in Kunming Botanical Garden. Plants bloomed vigorously with many flowers in August. We also found flowering individuals in the field at the same time. The novelty is similar to *G. hemsleyana* King & Pantl. at first glance. However, it has greenish flowers with a yellow lip, while *G. hemsleyana* has pink flowers with a white lip. Besides, the reticulations on the leaf blades of the two species are also different.

Based on molecular phylogenetic evidence and a detailed examination of the morphological characters of our materials, the relevant types and a comprehensive array of additional herbarium specimens of similar species, we concluded that the plants discovered in Medog County in January 2021 represented a new species to science. It is described and illustrated here as *G. medogensis* H. Z. Tian, Y. H. Tong & B. M. Wang and included in a key to the species of *Goodyera* subsect. *Reticulum* in China.

Materials and methods

Based on our field observations of *Goodyera* in China in the past ten years, we identified three species that were closely related to the novelty, viz. *G. hemleyana*, *G. biflora* (Lindl.) Hook. f. and *G. vittata* Benth. ex Hook. f. (Fig. 1). Accordingly, we checked the types and additional material of these three taxa together with other pertinent specimens at HSNU, IBSC, K, KUN, LBG and PE. Photographs of specimens housed at AMES, AU, BR, CAL, CSFI, M and P were also examined. Among the target taxa, *G. biflora* is by far the most well-represented species in herbaria, and we did not find any

additional specimens of the new species. Furthermore, we examined the distribution of multiple characters (e.g. leaf reticulation, flower number and indumentum, color and margin of the labellum) in all species of *Goodyera* subsect. *Reticulum* in China, and these observations were summarized in the form of an identification key.



Figure 1. Goodyera medogensis and three morphologically similar species in situ **A** G. medogensis (from Medog, Tibet) **B** G. hemsleyana (from Malipo, Yunnan) **C** G. biflora (from Medog, Tibet) **D** G. vittata (from Yingjiang, Yunnan). Photographs **A**, **C**, **D** by Huai-Zhen Tian **B** by Chao Hu.

Voucher specimens of *G. medogensis* were collected in Medog County and preserved at the herbarium of South China Botanical Garden, Chinese Academy of Sciences (IBSC) and East China Normal University (HSNU). Fresh leaves used for molecular analyses were preserved in silica gel. The morphological description of the novelty is based on living material. Measurements were performed with a ruler (0.5 mm accuracy), and small plant parts were observed and measured under a stereo microscope (Mshot-MZ101).

To study the phylogenetic position of the new species within the genus *Goodyera*, three DNA fragments, viz. internal transcribed spacer (ITS) and two plastid DNA regions (*matK* and *trnL-F*), were selected for building the phylogenetic tree based on previous studies (Hu et al. 2016; Zhou et al. 2020). In total, 33 species represented by 80 samples of *Goodyera* were analyzed with one sample of *Zeuxine flava* (Wall. ex Lindl.) Benth. ex Hook. f. as the outgroup. All sequences were downloaded from Gen-Bank except those of the new species. Species names and GenBank accession numbers are provided in the supplementary materials (Suppl. material 1: Table S1).



Figure 2. Phylogenetic tree of *Goodyera* species inferred by Bayesian and maximum likelihood analyses based on the nrDNA (ITS) dataset. Posterior probability (PP) ≥ 0.50 in BI analysis and bootstrap (BS) % values ≥ 50 in ML analysis are indicated above the branches. Dashes mean the nodes are not supported, i.e. the BS value < 50% in the ML analysis or PP < 0.50 in the BI analysis. The samples of the new species are highlighted in orange, and other species of sect. *Reticulum* are highlighted in bold. The clade of *Goodyera* sect. *Reticulum* is highlighted with the green rectangle.
Total genomic DNA of the new species was extracted from silica gel-dried leaves using a modified CTAB method (Doyle and Doyle 1987). Polymerase Chain Reaction (PCR) amplification was carried out on TAKARA TP600 thermocycler (TAKARA BIO INC, Japan) using 25 µl reactions containing 12.5 µl $2\times$ Taq PCR Master Mix (HuaGene, China), 8.5 µl ddH₂O, 1.5 µl of each primer (10 µM) and 1 µl target DNA template. Detailed information of primers of relevant DNA fragments used in PCR amplification and sequencing, as well as the procedures of PCR, can be found in Suppl. material 1: Table S2. The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1%), colored with GoldView I (Solarbio, China). Clearly distinguishable bands were recorded, and then the corresponding PCR products were sequenced by Shanghai HuaGene Biotech Co., Ltd (Shanghai, China).

Sequences were firstly assembled and edited with Seqman (DNA STAR package, Madison, WI, F USA) and then adjusted manually. Phylogenetic analysis was conducted using PhyloSuite ver. 1.2.2 (Zhang et al. 2020). Sequences were aligned with MAFFT (Katoh and Standley 2013). Ambiguously aligned fragments were removed using



Figure 3. Phylogenetic tree of *Goodyera* species inferred by Bayesian and maximum likelihood analyses based on the cpDNA (*matK* + *trnL-F*) datasets. Posterior probability (PP) \ge 0.50 in BI analysis and boot-strap (BS) % values \ge 50 in ML analysis are indicated above the branches respectively. Dashes mean the nodes are not supported, i.e. the BS value < 50% in the ML analysis or PP < 0.50 in the BI analysis. The samples of the new species are highlighted in orange, and other species of sect. *Reticulum* are highlighted in bold. The clade of *Goodyera* sect. *Reticulum* is highlighted with the green rectangle.

Gblocks (Talavera and Castresana 2007) with all parameters at their default settings. Next, *matK* and *trnL-F* were concatenated as well as ITS, *matK* and *trnL-F* respectively to two datasets. Thus, three datasets were constructed in total: the cpDNA dataset (*matK* and *trnL-F*), the nrDNA dataset (ITS) and the nr+cpDNA dataset (ITS, *matK* and *trnL-F*). The three datasets were analyzed by using Bayesian inference (BI) and maximum likelihood (ML) methods respectively. Best-fit evolutionary models for Mrbayes and IQ-TREE were selected under the Bayesian Information Criterion (BIC) using ModelFinder (Kalyaanamoorthy et al. 2017). The best-fit models for the Maximum likelihood (ML) analysis are K80+R2 (ITS) and K3Pu+F+R2 (cpDNA, nr+cpDNA), and for Bayesian inference (BI) they are K2P+G4 (ITS) and GTR+F+G4 (cpDNA, nr+cpDNA).

Based on these models, the Maximum Likelihood (ML) analysis was performed with IQ-TREE (Nguyen et al. 2015) for 10000 ultrafast (Minh et al. 2013) bootstraps, and Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist



Figure 4. Phylogenetic tree of *Goodyera* species inferred by Bayesian and maximum likelihood analyses based on the nr + cpDNA (including ITS, *matK* and *trnL-F*) datasets. Posterior probability (PP) \ge 0.50 in BI analysis and bootstrap (BS) % values \ge 50 in ML analysis are indicated above the branches respectively. Dashes mean the nodes are not supported, i.e. the BS value < 50% in the ML analysis or PP < 0.50 in the BI analysis. The samples of the new species are highlighted in red, and other species of sect. *Reticulum* are highlighted in bold. The clade of *Goodyera* sect. *Reticulum* is highlighted with the green rectangle.

et al. 2012). BI analysis consisted of two simultaneous runs and four simultaneous Markov Chain Monte Carlo (MCMC) chains, and ran for 3000000 generations with chain sampling every 1000 generations. The average deviation of split frequencies fell below 0.01, and initial 25% of sampled data were discarded as burn-in. The phylogenetic trees were visualized and modified in FigTree version 1.4.3 (Rambaut 2016).

Results

Our ML and BI phylogenetic trees constructed from the three datasets showed that our four samples of *G. medogensis* cluster into one separate subclade (Figs 2–4) which is nested in the clade of *Goodyera* subsection *Reticulum* S. W. Chung & C. H. Ou (Hu et al. 2016) consisting of eight other species, viz. *G. biflora, G. hachijoensis* Yatabe, *G. hemsleyana, G. hispida* Lindl., *G. malipoensis* Q. X. Guan & S. P. Chen, *G. pusilla* Bl., *G. vittata* and *G. yamiana* Fukuy.. Thus, the results of phylogenetic analyses support the recognition of *G. medogensis* as a new species belonging to the subsection *Reticulum*, and has close relationship with *G. biflora, G. hemsleyana* and *G. vittata* (Fig. 1).

Taxonomic treatment

Goodyera medogensis H. Z. Tian, Y. H. Tong & B. M. Wang, sp. nov. urn:lsid:ipni.org:names:77254838-1 Figs 5, 6

Type. China. Tibet Autonomous Region: Medog County, Renqingbeng Temple, under evergreen broad-leaved forest, cultivated at Kunming Botanical Garden, 3 August 2021 (fl.), *B. M. Wang TYH-2523* (holotype: IBSC, isotype: HSNU).

Diagnosis. Similar to *G. hemsleyana*, but distinguished by the greenish thick reticulations on the adaxial surface of leaf blades with diffused margin (vs. white thin reticulations with clear margin), inflorescence with more flowers ((6-)12–15 vs. 4–10), petals obliquely obovate-rhombic (vs. obliquely ovate-oblong), labellum yellow or yellowish (vs. white, with light greenish to pinkish tinge at apex) without lamella on blade (vs. with a low bi-lamellate callus), and floral bracts, sepals as well as ovary with shorter trichomes.

Description. Terrestrial herb, 12–25 cm tall. Rhizome 4–6 cm long, 1.5–3 mm in diam., greenish, rooting at nodes. Roots fleshy, 0.7–7 cm long, yellowish brown, with minute root hairs. Stem erect, terete, 4–9 cm long, 2.7–4 mm in diam., pale green, glabrous, with few sheaths at base formed by withered bases of petioles. Leaves 3–7; petiole 1.1–1.6 cm long, sheathing at base; lamina ovate, $1.9-4.2 \times 1.5-2.6$ cm, obtuse at base, acute at apex, adaxially green to bluish green with greenish-white reticulations, grid lines thick, margin diffused, transverse ones 5–7, abaxially pale green, 5–7-veined. Inflorescence a terminal raceme, laxly (6–)12–15-flowered, spirally arranged, pubescent; peduncle 4–6.5 cm long, pubescent, with 2–3 sheathing bracts; sheathing bracts $1.5-1.9 \times 0.6-0.8$ cm, oblong-lanceolate, acute at apex, pale green, clasping, more or



Figure 5. *Goodyera medogensis* **A** habit **B** inflorescence **C** flower, lateral (left) and front (right) view, with the arrow showing close-up of trichomes on abaxial surface of sepals **D** sepals **E** petals **F** column with labellum, anther and ovary **G** labellum **H** pollinarium **I** column with part of the ovary. Photographs by Yi-Hua Tong.

less pubescent, especially on the margin, 3–5-veined; rachis 3.5–7 cm long, pubescent. Floral bracts ovate-lanceolate, $1.4-1.7 \times 0.5-0.6$ cm, acuminate to acute at apex, pale green, longer than ovary, sometimes shortly ciliate at margins, pubescent abaxially, trichomes multicellular, up to 0.8 mm long, glabrous adaxially, 3-veined. Flowers resupinate, opening weakly, 8-10 mm long. Sepals 1-veined, acute at apex, olive greenish, with reddish or brownish tinge when old, with dense clavate trichomes outside, trichomes up to 0.5 mm long; dorsal sepal ovate-lanceolate, $8-9 \times 2-3$ mm, forming a hood with the petals; lateral sepals ovate-lanceolate, $8-9 \times 3.5-4$ mm. Petals obliquely obovate-rhombic, $8-8.5 \times 3-3.5$ mm, acuminate to acute at apex, white, with reddish or brownish tinge at central part, glabrous, 1-veined. Labellum oblong-ovate, 6-7 mm long, yellow or yellowish; hypochile with a sac ca. $1.5-2.5 \times 2.5-3.5$ mm, with glandular hairs inside; epichile oblong to oblong-lanceolate, entire, $5-6.5 \times 2-3$ mm, margins slightly undulate, obtuse to subacute at apex. Column cylindrical, ca. 1 mm long; rostellum 3.5-4 mm long, bifid, acuminate at apex. Stigma suborbicular, ca. 1 × 1 mm, entire. Anther yellowish brown, ovate, ca. 2.5 × 1.5 mm. Pollinarium 3–3.5 mm long; pollinia 2, oblong-obclavate, 0.8-1 mm long, dull yellowish white, sectile, bifid; caudicles 1.5–1.7 mm long; viscidium narrowly ovate-oblong, 2–3× ca. 0.5 mm, acute at apex, membranous. Ovary plus pedicel terete, 6-7.5× ca. 2 mm, pale green, twisted, pubescent, trichomes blunt, multicellular, consisting of 5-7 cells. Fruit not seen.

Etymology. The species epithet refers to the type locality, Medog County.

Vernacular name. 墨脱斑叶兰 (Chinese pinyin: mò tuō bān yè lán).

Distribution and habitat. This species is currently known only from Medog County, Tibet, China. It grows under evergreen broad-leaved forests at elevations of 1600–2300 m.

Conservation status. During our three surveys in January, June and August 2021, *Goodyera medogensis* was found in the forests of Medog Town and Beibeng Township of Medog County, where we counted a minimum of 200 individuals. However, since the population assessment of this species in the whole Medog County has not been made, conservation status of this new species is best classified as 'Data Deficient' (DD) (IUCN Standards and Petitions Committee 2019). It occurs within a conservation area, and no threats from logging, tourism or poaching have been recorded until now.

Phenology. Flowering in July-August.

Additional specimens examined. *Goodyera medogensis* (paratypes): China: Tibet Autonomous Region, Medog County, Bari village, elev. 1750 m, 23 June 2021, cultivated in East China Normal University, 10 August 2021 (fl.), *J. Huang & M. Sun 21062310* (HSNU); ibid., Medog village, 19 August 2021, *H. Z. Tian et al. 21081914* (HSNU); ibid., Gelin village, 21 August 2021, *H. Z. Tian et al. 21082102* (HSNU).

Goodyera hemsleyana: India: Senchal, 2100 m, July 1892, *Pantling 215* (lectoype: CAL000000625 (photo); isolectotypes: CAL000000624 (photo), K00387611, BR0000006573508 (photo), M0226196 (photo), AMES00090573 (photo), P00333538 (photo)).

Goodyera biflora: Nepal: sin. loc., Wallich, Cat. no. 7379 (holotype: K000364600; isotype: K001127259). INDIA: sin. loc., 1900, J. F. Duthie 24164 (K); Mussorie, July 1901, P. W. Mackinnon 25408 (AMES02091486, photo); Himachal Pradesh, Shimla,



Figure 6. *Goodyera medogensis* **A** habit **B** leaf **C** bract **D** flower, front view **E** flower, lateral view **F** sepals **G** petals **H** column with labellum, anther and ovary **I** labellum **J** pollinarium **K** column with part of the ovary. Scale bars: 3 cm (**A**); 2 cm (**B**); 5 mm (**C**–**I**); 4 mm (**K**); 2 mm (**J**). Drawn by Jun Cai.

1524 m, 30 June 1886, *H. Collett. 325* (К); Himalaya, 1981 m, 1844, *M. P. Edgeworth* 58 (К). Снима: Tibet, Gyirong Valley, Tsangpo Valley, 2743 m, 6 August 1935, *F. Kingdom-Ward* 12159 (К); Tibet, Bome, 2 July 1952, *P. C. Tsoong* 6699 (PE00339319,

PE00339320); Anhui, Jinzhai, 1600 m, 6 August 1986, Plant Resource Team D0062 (PE); Guangdong, Fengkai, 16 June 1974, Yue Seven Four 5295 (IBSC0627265); Guangdong, Xinyi, 15 April 1931, S. P. Ko 51307 (IBSC0627264, IBSC0627266, IBSC0627267, PE00339323, PE00339324); Guangdong, Ruyuan, 27 November 1957, L. Teng 5866 (IBSC0627260); Guizhou, Fanjing Mt., 10 September 1987, China-USA Scientific Research Team s.n. (PE00339322); Guizhou, Rongjiang, 10 July 1974, Anonymous 74-913 (IBSC0627261); Henan, Xin County, 10 August 2013, C. S. Zhu, S. J. Li, X. L. Hou, S. X. Zhu, J. M. Li 130808106 (AU066898, photo); Henan, Shangcheng, 19 June 1984, Plant Resource Research Team D0546 (PE00850731); Hubei, Huanggang, 2 October 2018, X. X. Zhu, L. L. Shi, S. S. Duan, M. J. Hu, Q. Lü ZXX18494 (KUN1444572, photo); Hubei, Shiyan, 8 July 2013, S. L. Li GanQL486 (KUN1458343, photo); Hunan, Chengbu, 22 May 2020, L. Wu, W. J. Liu, C. F. Deng 10403 (CSFI071975, photo); Hunan, Sangzhi, 600 m, 22 June 1991, Q. Lin 714 (IBSC0627263); Hunan, Sangzhi, 11 June 2019, X. Li, C. F. Deng, J. L. Li 190611132 (CSFI072175, photo); Hunan, Cili, 2 October 1984, G. X. Xing & Q. Xia 05571 (PE00339318); Sichuan, Hechuan to Mabian, 1934, S. L. Sun 5590 (PE00339322); Sichuan, s. d., P. C. Tsoong 3981 (PE01849749); Yunnan, Wenshan, 25 September 1958, H. T. Tsai 58-8126 (KUN0022202); Zhejiang, West Tianmu, 2 July 1925, D. *X. Zhang* 266 (LBG00108146).

Goodyera vittata: India: Sikkim Himalaya, J. D. Hooker 336 (holotype: K000364605); Singalelah Range, 2438 m, July 1896, R. Pantling 410 (AMES02091540, photo). CHINA: Tibet, Zayu, 25 July 1980, 2100 m, Z. C. Ni, Y. Z. Wang, D. Ci et al. 0757B (PE00339514); Yunnan, Yingjiang, 14 August 2012, H. Z. Tian & C. Hu 706 (HSNU).

Key to species of Goodyera subsect. Reticulum in China

1	Inflorescence with more than 20 flowers; dorsal sepal less than 6 mm long2
_	Inflorescence with less than 20 flowers; dorsal sepal more than 6 mm long6
2	Leaves green, without white or pale green venation on adaxial surface
	G. yamiana
_	Leaves with white or pale green venation on adaxial surface
3	Margins of epichile and petals irregularly denticulate G. pusilla
_	Margins of labellum and petals entire
4	Flowers glabrous G. hachijoensis
_	Flowers pubescent
5	Flower diameter 6–7 mm G. malipoensis
_	Flower diameter 3–4 mm G. hispida
6	Inflorescence mostly with 2 flowers, sometimes up to 6; dorsal sepal 20-25
	mm longG. biflora
_	Inflorescence mostly with 4–15 flowers; dorsal sepal 3–14 mm long7
7	Leaves adaxially with a white band along midvein
_	Leaves adaxially with white or greenish reticulate venation

8	Leaves adaxially with thin and white reticulations with clear margins; labellum
	white, with light greenish to pinkish tinge at apex G. hemsleyana
_	Leaves adaxially with thick and greenish reticulations with diffused margins;
	labellum yellow or yellowish G. medogensis

Discussion

The new species has the typical features of *Goodyera* sect. *Reticulum*, i.e., the leaves have reticulations on adaxial surface, and lateral sepals are not reflexed backwards. According to Hu et al. (2016), sect. *Reticulum* is one of the four sections of *Goodyera*, and can be further divided into two subsections, viz., *G.* subsect. *Reticulum* S. W. Chung & C. H. Ou and *G.* subsect. *Foliosum* S. W. Chung & C. H. Ou. Based on morphology, *G. medogensis* is probably most closely related to *G. hemsleyana*. However, considering the conflicting results shown by nrDNA and cpDNA, the position of the new species within subsect. *Reticulum* remains elusive.

Acknowledgements

We are grateful to Mr. Zhen Liu (Medog Forestry Bureau) and Mr. Xi-Xian Wang (China Railway 12th Bureau Group) for their help during field trips. Mr. Jun Cai is thanked for preparing the beautiful line-drawing. We also thank the curators and staff of the herbaria mentioned above for facilitating the checking of specimens and Dr. Pankaj Kumar (Kadoorie Farm & Botanic Garden) for his valuable comments. This study was funded by the National Natural Science Foundation of China (grant no. 31870180), Biological Resources Program, Chinese Academy of Sciences (KFJ-BRP-017-33) and Biodiversity Survey, Observation and Assessment Program of Ministry of Ecology and Environment of China.

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Supplementary material I

Table S1, S2. Supplementary file of *Goodyera medogensis* (Orchidaceae), a new species from Tibet, China

Authors: Yi-Hua Tong, Mei Sun, Bing-Mou Wang, Huai-Zhen Tian

Data type: phylogenetic

- Explanation note: Details of materials for phylogenetic study and primers for sequence amplication and sequencing.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/phytokeys.189.77374.suppl1

RESEARCH ARTICLE



Silene vanchingshanensis (Caryophyllaceae) a new species from Southwest China

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Academic editor: G. P. G. del Galdo | Received 21 December 2021 | Accepted 1 February 2022 | Published 15 February 2022

Citation: Yang F, Liu J-L, Li P-P, Wang H-C (2022) *Silene vanchingshanensis* (Caryophyllaceae) a new species from Southwest China. PhytoKeys 189: 155–162. https://doi.org/10.3897/phytokeys.189.79631

Abstract

Silene vanchingshanensis (Caryophyllaceae), a new species from Fanjingshan Mountain in Guizhou (southwest China) is described and illustrated. It is morphologically similar to *S. morrisonmontana* and *S. hupehensis*, from which it can be easily distinguished by having pubescent stems usually 10–15 cm long, linear-oblanceolate leaves 3–6 cm × 3–6 mm, often 2–5-flowered cymes, pink or violet petals and narrowly ovoid capsules.

Keywords

Endemism, Guizhou, Silene, Silene morrisonmontana, Silene hupehensis

Introduction

Silene L. (*Sileneae* DC., Caryophylloideae Arnott, Caryophyllaceae Juss.) is the largest genus of the carnation family, comprising 700 to 870 species (Mabberley 2017; Jafari et al. 2020), mostly occurring in temperate regions and subtropical mountains of the Northern Hemisphere (Zhou et al. 2001; Oxelman et al. 2011). The centre of its species diversity is observed in Western Asia and the Mediterranean area, but areas of Central Asia are also highly diverse (see, for example, Jafari et al. 2020). Taxonomically, *Silene* represents a notoriously difficult genus, having a high species-richness, widespread distribution, broad morphological variations and the complex genetic background. Its

generic delimitation has been controversial (Oxelman and Lidén 1995; Jafari et al. 2020) with some authors lumping many members into the genus (e.g. Greuter 1995; Desfeux and Lejeune 1996; Jafari et al. 2020), whereas others support separation of *Agrostemma, Atocion, Eudianthe, Heliosperma, Petrocoptis* and *Viscaria* (e.g. Oxelman and Lidén 1995; Oxelman et al. 1997, 2001; Popp and Oxelman 2004; Frajman et al. 2009a, b; Greenberg and Donoghue 2011). In addition to taxonomic research, the genus *Silene* is also difficult from the nomenclatural point as highlighted, for example, by Iamonico (2018, 2021).

Concerning China, the first comprehensive revision of the genus *Silene* was carried out by Tang (1996) who recognised 131 species (including two subspecies and 17 varieties). In the most recent treatment by Zhou et al. (2001), 110 species were accepted, of which 67 are endemic. *Silene* taxa can be found throughout the country, mostly being found in the north-western and south-western provinces, with more than 60 species in the Hengduan Mountains (Zhou 1983; Wu 1993; Zhuang 1995; Tang 1996; Wu et al. 2003).

As part of the taxonomic revision of *Silene* in the Sino-Himalayan Region for the *Flora of Pan-Himalayas*, an undescribed species was found and is proposed here.

Materials and methods

The new species was studied both in the field and at herbaria. The collections housed at CDBI, KUN, PE, PYU, XTBG and YUKU (acronyms according to Thiers 2022), as well as digital images available at JSTOR Global Plants (http://plants.jstor.org/) and at the Chinese Virtual Herbarium (http://www.cvh.ac.cn/), were examined. Pertinent taxonomic literature (e.g. Xiao and Xie 1982; Zhuang 1995; Tang 1996; Zhou et al. 2001) were extensively consulted. Morphological studies were carried out on dried material under a stereomicroscope (Olympus SZX2, Tokyo, Japan) and measurements were made using a ruler and a metric vernier caliper.

Taxonomy

Silene vanchingshanensis C.Y.Wu ex Huan C. Wang & Feng Yang, sp. nov. urn:lsid:ipni.org:names:77254897-1 Figs 1–3

Type. China. Guizhou Province: Jiangkou County, summit of the Fanjingshan Mountain, 27°54'51"N, 108°41'35"E, steep cliffs or rock crevices, alt. 2,450–2,500 m, 10 July 2021, *Feng Yang & Jing-Li Liu JK12775* (holotype YUKU02074621!; isotypes YUKU02074622!, YUKU02074623!, YUKU02074624!, YUKU02074625!, YUKU02074643!).

Diagnosis. Silene vanchingshanensis is similar to S. morrisonmontana, from which it differs by its shape and size of leaves (linear-oblanceolate, $3-6 \text{ cm} \times 3-6 \text{ mm}$ vs. linear, $2-7 \text{ cm} \times 2-3 \text{ mm}$), cymes (often 2–5-flowered vs. usually solitary) and colour of petals (pink or violet vs. white).



Figure 1. Silene vanchingshanensis (Drawn from the holotype by Jing-Li Liu) A habit B petal C pistil D calyx.

Description. Herbs perennial. Rhizomes slender, creeping, branched. Stems caespitose, ascending, 10–15 cm long, slender, pubescent, usually with clustered sterile shoots at the base. Basal leaves linear-oblanceolate, 3–6 cm long, 3–6 mm wide, base cuneate layout@pensoft.net, attenuate into petiole, connate, cylindrical, apex acuminate, margin ciliate, mid-vein prominent; cauline leaves usually 4–6 pairs, sessile, lanceolate to linear-oblanceolate, 2–4 cm long, 3–4 mm wide, apex acuminate, margin ciliate. Cymes often 2–5-flowered, flowers rarely solitary. Flowers slightly nodding; pedicel densely hairy, 8–25 mm long; bracts lanceolate, 10–15 mm long, ca. 2 mm wide. Calyx campanulate, ca. 12 mm long, 5–8 mm in diameter, base rounded, longitudinal veins violet, converging at apex, veins hairy; calyx teeth narrowly triangular, 3–4 mm long, margin ciliate, apex acute to acuminate. Androgynophore 2–3 mm long, pubescent. Petals pink or violet, 1.5–2.0 cm long; claws saccate-oblanceolate, ciliate at base; auricles orbicular, sometimes obscurely laciniate; limbs exserted beyond calyx, obovate, 6–9 mm long, bifid, rarely deeply lobed to middle; lobes narrowly elliptic or ovate, sometimes with one obtuse tooth on each lateral side; coronal scales



Figure 2. *Silene vanchingshanensis* **A** habit **B** flower (front view) **C** basal leaves **D** cauline leaves and bracts **E** basal leaves **F** flower (side view, showing the calyx and pedicel) **G** dissected flower (showing the androgynophore and claws) **H** petal (showing the claw, auricles and coronal scales) **I** pistil and androgynophore **J** stamens, pistil and androgynophore **K** calyx after anthesis **L** immature capsule.

flabellate, ca. 1 mm long, white with a little tint of violet, laciniate at apex. Stamens 10, slightly exserted, filaments hairy at base. Styles usually 3, sometimes 5, included. Capsule narrowly ovoid, 12–15 mm long, slightly equal to persistent sepals. Seeds reniform.

Phenology. Flowering and fruiting times from July to September.

Etymology. The specific epithet *vanchingshanensis* is derived from the type locality "Vanchingshan", a variant name for Fanjingshan Mountain.

Distribution and habitat. *Silene vanchingshanensis* is currently known only from *locus classicus* (Fanjingshan Mountain), a famous scenic resort in Guizhou, southwest China. The species grows on cliffs or rock crevices of the mountain summit at elevations of 2,100–2,500 m.

Taxonomic notes. The name *Silene vanchingshanensis* was first proposed by the Chinese botanist Zhengyi Wu in 1988 on the label of a KUN specimen (*C. P. Jian et al. 32080*). However, no name was formally published.

According to the taxonomic treatment of Chinese Silene by Tang (1994), S. vanchingshanensis should be assigned to Silene sect. Morrisonmontanae C. L. Tang (synonymised in Silene sect. Physolychnis (Benth.) Bocquet in Jafari et al. (2020)) due to its cymes 2–5-flowered and campanulate calyx. S. vanchingshanensis is mostly similar to S. morrisonmontana (Hayata) Ohwi & H.Ohashi, but differs from the latter by its leaves linear-oblanceolate (vs. linear), 3–6 cm \times 3–6 mm (vs . 2–7 cm \times 2–3 mm), cymes (1–) 2–5-flowered (vs. usually solitary) and petals pink or violet (vs. white). The distribution areas of these two species are separated: S. vanchingshanensis is endemic to Guizhou, while S. morrisonmontana is only found in Taiwan. S. vanchingshanensis is also similar to S. hupehensis C. L. Tang, but clearly differs from the latter by its stems with hairs, 10–15 cm (vs. glabrous, 10–30 cm) long, leaves linear-oblanceolate (vs. narrowly linear), 3–6 cm \times 3–6 mm (vs. 5–8 cm \times 2–3.5 mm) and capsule narrowly ovoid (vs. ovoid), 12–15 mm (vs. 6–8 mm) long. A detailed morphological comparison between these three species is summarised in Table 1.

Characters		Species	
	S. vanchingshanensis	S. morrisonmontana	S. hupehensis
Stems (cm)	10–15	10–15	10-30
Leaves (cm × mm)	linear-oblanceolate, 3–6 × 3–6	narrowly linear, $2-7 \times 2-3$	narrowly linear, $5-8 \times 2-3.5$
Inflorescence	often 2–5-flowered cymes, rarely solitary	flowers solitary	often 2–5-flowered cymes, rarely solitary
Calyx (cm × mm)	campanulate, $1.2 \times 5-8$, veins hairy	cylindrical-campanulate, swollen, 1.4–1.8 × 10–12, veins hirsute	campanulate, $1.2-1.5 \times 3.5-7$, veins glabrous
Petals	pink or violet, limbs obovate, 6–9 mm long, bifid, lobes narrowly elliptic or ovate	white, limbs obovate, 4–6 mm long, shallowly bifid; lobes narrowly elliptic or ovate	pink, limbs obovate or broadly ovate, 7–9 mm long, shallowly bifid, lobes nearly orbicular
Fruit	narrowly ovoid, 12–15 mm	narrowly ovoid, 8–10 mm	ovoid, 6–8 mm
Distribution	Guizhou, southwest China	Taiwan, east China	Gansu, Henan, Hubei, Shaanxi, Sichuan, central to southwest China

Table 1. Morphological comparison of S. vanchingshanensis, S. morrisonmontana and S. hupehensis.



Figure 3. An isotype of *Silene vanchingshanensis* (YUKU 02074643).

Additional specimens examined. *Silene vanchingshanensis* (paratypes). CHINA. Guizhou: Jiangkou County, Fanjingshan Mountain, 15 August 2003, *S. Z. He et al.* 0308038 (GZTM), ibid., Jingding, alt. 2,150 m, 25 September 1963, *C. P. Jian et al.* 32080 (KUN), ibid., collection time unknown, *s. n.* 51495 (IBSC).

Silene morrisonmontana. CHINA. Taiwan: Hsinchu City, Wufeng village, Sheipa National Park, Tapachienshan, 24°27'47"N, 121°15'29"E, on shady rocky slope, alt. ca. 3,400 m, 7 September1993, C. L. Huang et al. 103 (HAST), Taichung City, Wuling, on route from 369 Lodge to Hsuehshan Peak, alt. ca. 3,884 m, 2 August 1991, D. S. HSU & Moore, S. J. 723 (HAST), Nantou County, Jenai village, Chilailishan, alt. 3,330 m, 4 September 1998, T. Y. A. Yang No. 11253 (PE).

Silene hupehensis. CHINA. Henan: Luanchuan County, Laojunshan, 24 July 2006, Chang-Shan Zhu 2006100 (HITBC). Hubei: Shengnongjia Forest District, along the road between Guanmenshan and Xiaoshennongjia, 31°30'N, 110°30'E, 10 September 1980, 1980 Sino-Amer. Exped. No 973 (PE). Shaanxi: Mei County, Taibai Mountain, Fangyang temple, hillsides and meadows, alt. 3,000 m, 9 August 1977, You-Hao Guo & Zhi-Xing Hu 489 (IBSC). Sichuan: Shimian County, on the way from Jiziping to Xishan, 29°04'29"N, 102°11'22"E, alt. 2,961 m, 31 July 2007, Ji-Pei Yue Yue-07160 (KUN).

Acknowledgements

We would like to thank the curators and staff of the herbaria from which specimens have been used in this study and to thank Dr Duilio Iamonico and Richard Rabeler for their critical comments on the manuscript. This research was supported by the National Natural Science Foundation of China (grant no: 31960040) and the Second Tibetan Plateau Scientific Expedition and Research (STEP) programme (2019QZKK0502).

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