

Species discrimination of novel chloroplast DNA barcodes and their application for identification of *Panax* (Aralioideae, Araliaceae)

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Abstract

Certain species within the genus *Panax* L. (Araliaceae) contain pharmacological precious ginsenosides, also known as ginseng saponins. Species containing these compounds are of high commercial value and are thus of particular urgency for conservation. However, within this genus, identifying the particular species that contain these compounds by morphological means is challenging. DNA barcoding is one method that is considered promising for species level identification. However, in an evolutionarily complex genus such as *Panax*, commonly used DNA barcodes such as *nrITS*, *matK*, *psbA-trnH*, *rbcL* do not provide species-level resolution. A recent *in silico* study proposed a set of novel chloroplast markers, *trnQ-rps16*, *trnS-trnG*, *petB*, and *trnE-trnT* for species level identification within *Panax*. In the current study, the discriminatory efficiency of these molecular markers is assessed and validated using 91 reference barcoding sequences and 38 complete chloroplast genomes for seven species, one unidentified species and one sub-species of *Panax*, and two outgroup species of *Aralia* L. along with empirical data of *Panax* taxa present in Vietnam via both distance-based and tree-based methods. The obtained results show that *trnQ-rps16* can classify with species level resolution every clade tested here, including the highly valuable *Panax vietnamensis* Ha et Grushv. We thus propose that this molecular marker to be used for identification of the species within *Panax* to support both its conservation and commercial trade.

Keywords

DNA barcode, *Panax* genus, *Panax vietnamensis*, *petB*, *trnE-trnT*, *trnQ-rps16*, *trnS-trnG*

Introduction

The genus *Panax* L. is well-known in culinary and medicinal traditions in many countries including China, Korea, Japan, and Vietnam. Its species produce ginsenosides, also known as ginseng saponins with strong antioxidant, antidiabetic, antitumor, and neuroprotective activities (Jung et al. 2017; Patel and Rauf 2017). Due to their high commercial demand, many species within *Panax* have been over-harvested and are at risk of extinction (Case et al. 2007; McGraw et al. 2013; Manzanilla et al. 2018). In Vietnam, there are three species of *Panax* including *Panax vietnamensis* Ha et Grushv., *Panax stipuleanatus* H.T.Tsai et K.M.Feng, and *Panax bipinnatifidus* Seem., all recorded and classified as endangered. Of these species *P. vietnamensis* is endemic to Vietnam and is considered to have the highest medical potential and is therefore the most commercially valuable (Nguyen 2005; Nguyen et al. 2007). Two varieties of *P. vietnamensis*, *Panax vietnamensis* var. *fuscidiscus* K.Komatsu, S.Zhu et S.Q.Cai and *Panax vietnamensis* var. *langbianensis* N.V.Duy. V.T.Tran et L.N.Trieu, are also present in Vietnam (Phan et al. 2013; Nong et al. 2016). Recently, an unidentified sample of *Panax* discovered on Puxailaileng Mountain of Nghe An Province was also reported by Phan et al. (2014) and referred hereafter as “*Panax* sp. Puxailaileng”.

Historically, morphological methods have been used to identify ginseng species, though this is challenging due to how similar different ginseng species can appear. Incorrect identification can lead to unintentional or intentional mislabeling and adulteration with low-quality ginsengs, and ultimately affect the consumers’ health and damage the providers’ integrity. Recently, molecular methods have been shown to be efficient for solving problems related to species identification. However, the most commonly used barcoding sequences are challenging to use in the genus *Panax*, because these often lack sufficient variability to unambiguously identify the species (Komatsu et al. 2001; Janzen et al. 2009; Hollingsworth et al. 2011; Zuo et al. 2011; Li et al. 2015). According to Zuo et al. (2011) *rpoC1*, *rbcL*, and *rpoB* were the low discriminatory with only four to eight variable sites. The region *psbK-psbI* had the higher discriminatory ability but low chance of successful sequencing (Janzen et al. 2009). Furthermore, *psbA-trnH* sequence analysis was reported inaccurate because of the complicated microevolution (Li et al. 2015). The attempts using above loci along with *matK*, *trnD* and *ycf1* for identifying species in the genus *Panax* also were unable to completely solve the challenge (Komatsu et al. 2001; Shi et al. 2015). Other newly proposed InDel (Nguyen et al. 2017) and dCAPS markers (Nguyen et al. 2020) were also developed but the requirement for multi-locus analyses is time-consuming and labor-intensive. This raises the need to develop better molecular markers for identification of the species within *Panax* (Shneyer 2009; Li et al. 2015; Manzanilla et al. 2018).

Previously performed an *in silico* analysis indicated that the chloroplast DNA markers *trnQ-rps16*, *trnE-trnT*, *petB*, and *trnS-trnG* had high species identification potential within the genus *Panax* (Manzanilla et al. 2018) and could be used in routine classification processes. In the present study, we assess and experimentally test the discriminatory efficiency of these commonly used and novel chloroplast markers in classifying species of *Panax* with an emphasis on those distributed in Vietnam.

Materials and methods

Plant materials

Leaf samples of five taxa belonging to the genus *Panax* were collected in the North and Central Vietnam (Table 1, Fig. 1). These included twenty-two samples of *P. vietnamensis* from eighteen distinct populations distributed on Ngoc Linh Mountain in Quang Nam and Kon Tum Provinces, samples of *P. vietnamensis* var. *fuscidiscus* and *Panax* sp. *Puxailaileng* collected in their natural habitats from Lai Chau and Nghe An Provinces, respectively, as well as, *P. stipuleanatus* and *P. bipinnatifidus* gathered from Lao Cai Province. All specimens were morphologically identified by plant taxonomists Nguyen Tap and Nguyen Quoc Binh using identification keys (Ha and Grushvitzky 1985; Nguyen 2005; Phan et al. 2013; Tran et al. 2016) and deposited at the Vietnam National Museum of Nature (VNMN). *P. vietnamensis* var. *langbianensis* is narrowly distributed in Lang Bian Mountain of Southern Vietnam and we were not able to collect samples from it. All lab and bioinformatics work was conducted at the Institute of Genome Research, Vietnam Academy of Science and Technology.

Table 1. Sample collection information.

Sample ID	Collector	Collection date	Collected location		
			Coordinates	District	Province
<i>P. vietnamensis</i>					
TL25	Luong Duc Toan	10/16/2017	15°01.17'N, 108°00.76'E	Nam Tra My	Quang Nam
CP13	Luong Duc Toan	10/16/2017	15°01.40'N, 108°03.10'E	Nam Tra My	Quang Nam
TN22	Luong Duc Toan	10/16/2017	15°00.94'N, 108°03.08'E	Nam Tra My	Quang Nam
D42	Le Thi Thu Hien	09/28/2018	15°00.94'N, 108°02.58'E	Nam Tra My	Quang Nam
D43	Le Thi Thu Hien	09/28/2018	15°00.94'N, 108°02.58'E	Nam Tra My	Quang Nam
D11	Le Thi Thu Hien	09/28/2018	15°00.94'N, 108°02.58'E	Nam Tra My	Quang Nam
D6	Le Thi Thu Hien	09/28/2018	15°00.94'N, 108°02.58'E	Nam Tra My	Quang Nam
Q1	Le Thi Thu Hien	09/28/2018	15°02.53'N, 108°02.72'E	Nam Tra My	Quang Nam
B42	Le Thi Thu Hien	09/28/2018	15°03.11'N, 107°97.97'E	Nam Tra My	Quang Nam
ML043	Luong Duc Toan	10/11/2017	15°03.20'N, 107°97.90'E	Nam Tra My	Quang Nam
TL27	Luong Duc Toan	10/11/2017	15°03.18'N, 107°97.91'E	Nam Tra My	Quang Nam
TT15	Luong Duc Toan	10/11/2017	14°96.41'N, 108°10.05'E	Nam Tra My	Quang Nam
TR2	Luong Duc Toan	10/11/2017	15°07.73'N, 108°00.76'E	Nam Tra My	Quang Nam
PL073	Luong Duc Toan	10/11/2017	15°27.50'N, 107°87.90'E	Phuoc Son	Quang Nam
TG07	Luong Duc Toan	10/11/2017	15°79.20'N, 107°25.90'E	Tay Giang	Quang Nam
NLay1	Le Thi My Hao	10/11/2017	14°59.60'N, 108°14.80'E	Tu Mo Rong	Kon Tum
MR3	Le Thi My Hao	10/11/2017	14°97.08'N, 107°99.90'E	Tu Mo Rong	Kon Tum
TX1	Le Thi My Hao	10/11/2017	14°96.10'N, 107°95.40'E	Tu Mo Rong	Kon Tum
MR7	Le Thi My Hao	10/11/2017	14°97.10'N, 107°89.50'E	Tu Mo Rong	Kon Tum
NL1	Le Thi My Hao	10/11/2017	15°06.20'N, 107°94.40'E	Dak Glei	Kon Tum
X1	Le Thi My Hao	10/11/2017	15°07.60'N, 107°83.20'E	Dak Glei	Kon Tum
MH1	Le Thi My Hao	10/11/2017	15°73.00'N, 107°54.43'E	Dak Glei	Kon Tum
<i>P. vietnamensis</i> var. <i>fuscidiscus</i>					
SLC	Nguyen Tien Dung	07/31/2015	22°20.00'N, 103°42.40'E	Sin Ho	Lai Chau
<i>Panax</i> sp. <i>Puxailaileng</i>					
SNA	Nguyen Tien Dung	12/07/2015	19°53.06'N, 104°33.89'E	Ky Son	Nghe An
<i>P. stipuleanatus</i>					
TTH	Nguyen Tien Dung	08/26/2015	22°40.86'N, 103°80.67'E	Sa Pa	Lao Cai
<i>P. bipinnatifidus</i>					
SVD	Nguyen Tien Dung	08/26/2015	22°40.86'N, 103°80.67'E	Sa Pa	Lao Cai

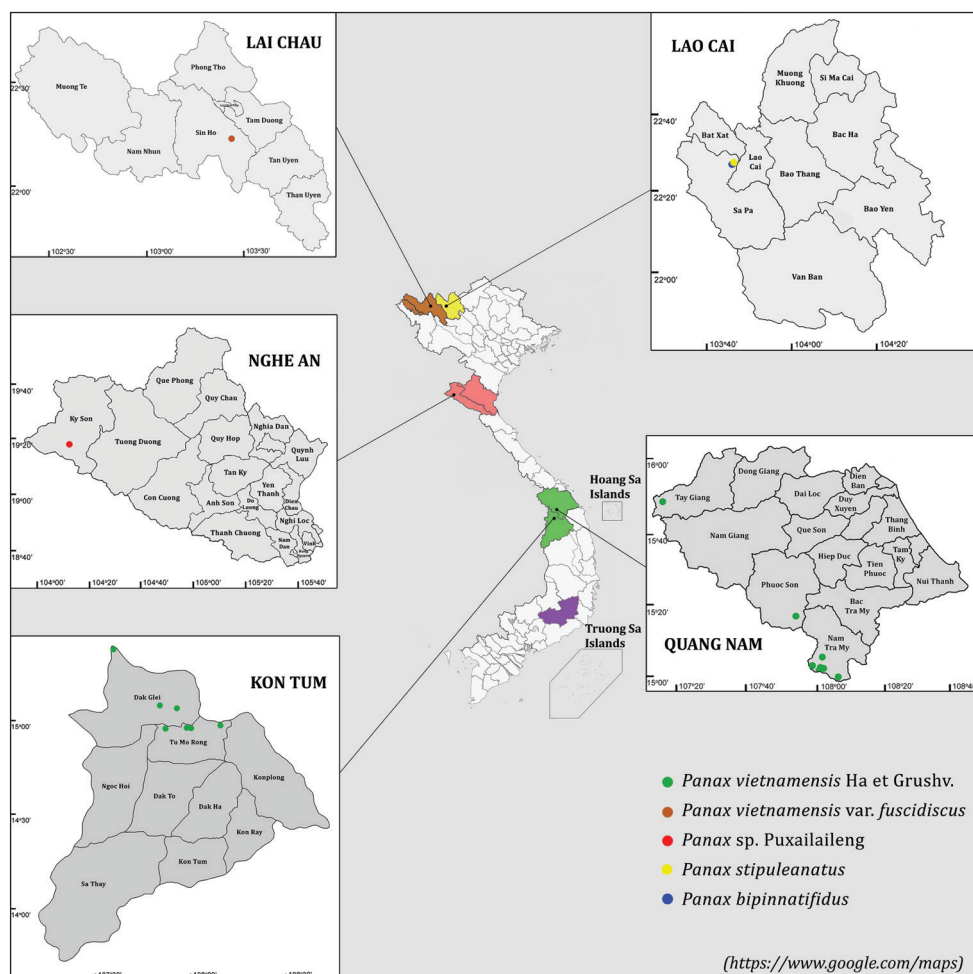


Figure 1. Distribution of *Panax* in Vietnam and sample locations. *P. vietnamensis* (green) collected in Quang Nam and Kon Tum Provinces. *P. vietnamensis* var. *fuscidiscus* (brown) collected in Lai Chau Province. *Panax* sp. *Puxailaileng* (pink) collected in Nghe An Province. *P. bipinnatifidus* (blue) and *P. stipuleanatus* (yellow) collected in Lao Cai Province. The natural distribution of *P. vietnamensis*, *P. vietnamensis* var. *fuscidiscus*, and *Panax* sp. are marked as green, brown, and pink, respectively. The wild habitat for *P. bipinnatifidus* and *P. stipuleanatus* is shown in yellow, and the purple area represents the distribution region of *P. vietnamensis* var. *langbiangensis* (not included in this study).

DNA extraction, amplification, and sequencing of previously used and novel markers

Total genomic DNA was extracted from leaf specimens using GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) with the provided protocol. The concentration of genomic DNA was determined using a NanoDrop Spectrophotometer 2000 (Thermo Fisher Scientific, USA). Primer pairs for amplification of *psbA*-

Table 2. Primers used in this study.

Region	Primer name	Sequence (5'-3')	Approximate amplicon length (bp)
ITS	ITS_AB_101	ACGAATTCATGGTCCGGTGAAGTGTTCG	650
ITS	ITS_AB_102	TAGAATTCCCCGGTTTCGCTCGCCGTAC	650
<i>matK</i>	MatK_F1A	ACYGTATTTATGTTTACGACG	750
<i>matK</i>	MatK_R1A	TCCATHTDGAAATCTTGGTTCA	750
<i>psbA-trnH</i>	PsbA_trnH_PF	ACCCGGTCTTAGTGTATACGAG	390
<i>psbA-trnH</i>	PsbA_trnH_PR	TTCCTGCTTGATCCACTTGG	390
<i>rbcL</i>	RbcL_PF	AGTGTGGATTCAAGCTGGTG	550
<i>rbcL</i>	RbcL_PR	TGGTTGTGAGTTTACGTTCT	550
<i>trnQ-rps16</i> (1)	Pv_trnQ_rps16_F	GAAGATTAGGTCCTTAGTCGTTTCG	590
<i>trnQ-rps16</i> (1)	Pv_trnQ_rps16_R	GATTCAGCATTCCCAGAGAATTGG	590
<i>trnS-trnG</i> (2)	Pv_trnS_trnG_F	GCCGCTTTAGTCCACTCAGC	660
<i>trnS-trnG</i> (2)	Pv_trnS_trnG_F	GTGTTGACATTTTTCGTGGGGG	660
<i>petB</i> (3)	Pv_petB_F	AATATTCAGACCTCGCGGCC	580
<i>petB</i> (3)	Pv_petB_R	GGCTCAAGCAAAACACCCAA	580
<i>trnE-trnT</i> (4)	Pv_trnE_trnT_F	GAGTGGTTGGTCCGTCAGAA	520
<i>trnE-trnT</i> (4)	Pv_trnE_trnT_R	CATGCGCTTACTCTACCGCT	520

trnH, *matK* and *rbcL* regions were designed based on available sequences deposited in GenBank, and for ITS region primers were designed as previously reported (Sun et al. 1994). Primers for the four barcodes identified *in silico* were designed based on the chloroplast genome sequence of *P. vietnamensis* (Manzanilla et al. 2018) and are shown in Table 2. Each target DNA region was amplified in a PCR volume of 20 μ L containing 1X DreamTaq buffer, 200 mM of each dNTP, 2.5 μ M of each primer, 1.5 mM $MgCl_2$, 0.75 units of Dream Taq DNA polymerase (Thermo Fisher Scientific, USA) and 50 ng of template DNA. PCR amplification was performed on a Mastercycler (Eppendorf, Germany) using the following conditions: 2 min at 94 °C initial denaturation, 35 amplification cycles (30 s at 94 °C denaturation, 20 s at 55–62 °C annealing, and 1 min at 72 °C extension), 5 min at 72 °C extension, and a final hold at 4 °C. The amplified products were purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) as described by the manufacturer, then screened on a 1% agarose gel. Purified products were sequenced using ABI 3500 Genetic Analyzer applying BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA).

Nucleotide matrix construction

Raw sequencing data were checked for quality and cleaned using BioEdit version 7.0.9 (Hall 1999). Ambiguous nucleotides and poor signal regions were removed to avoid incorrect alignment in further analyses. A nucleotide matrix was assembled for both individual and concatenated markers. A matrix from the newly obtained and 91 reference barcoding sequences, and 38 complete chloroplast genomes representing seven species of *Panax* (*P. vietnamensis*, *P. stipuleanatus*, *P. bipinnatifidus*, *Panax ginseng* C.A.Mey, *Panax japonicus* (T.Nees) C.A.Mey, *Panax notoginseng* (Burkill) F.H.Chen ex C.Y.Wu et K.M.Feng, *Panax quinquefolius* L.), one unidentified species of *Panax*

(*Panax* sp. Puxailaileng), and one sub-species of *Panax* (*P. vietnamensis* var. *fuscidiscus*), and two species of *Aralia* L. (*Aralia elata* (Miq.) Seem. and *Aralia undulata* Hand.-Mazz. in Broterus) used as outgroup (Suppl. material 1) were globally aligned using MAFFT version 7.407 (Katoh et al. 2002) followed by local re-alignment with MUSCLE version 3.8.1551 (Edgar 2004). Manual adjustments were made when necessary to improve the matrix. Variable sites, Parsimony informative (PI) sites, mean pairwise distances, and intra/interspecific mean distances were calculated based on nucleotide matrix by MEGAX software (Kumar et al. 2018).

Genetic distance-based methods for species discrimination

Pairwise summary and pairwise explorer modules in TaxonDNA version 1.8 (Meier et al. 2006) were used to calculate the distribution of intra/interspecific pairwise distances for barcoding gap analysis in order to analyze the space between intra- and interspecific distances using the Kimura-2-parameter (K2P) nucleotide substitution model. The Best Match/ Best Close Match (BM/ BCM) modules in TaxonDNA were also used to assess species discrimination power of the analyzed markers using the K2P distance as a model. Thresholds for the best close match were computed from the pairwise summary.

Tree-based method for species discrimination

The best substitution model for each matrix was searched for using the jModelTest2 (Darriba et al. 2012). Phylogenetic trees based on Maximum Likelihood (ML) method were constructed by both RAxML version 8.2.10 (Stamatakis 2014) and IQTREE version 1.6.12 (Bui et al. 2020). Two species *A. undulata* and *A. elata*, from the sister genus *Aralia* were used as outgroups. ML tree searches were performed with bootstrap calculation at 1000 bootstrap replicates. ML trees were then used to perform species delimitation using mPTP version 0.2.4 (Kapli et al. 2017) with two Markov chain Monte Carlo (MCMC) runs, one million steps for each run, and Likelihood ratio test set to 0.01.

Results

Amplification and sequencing efficiency

To evaluate the species discrimination efficiency for both the commonly used as well as newly proposed DNA markers for *Panax* we assessed the amplification success as well as the amplicon lengths. Bidirectional Sanger DNA sequencing of each fragment showed the amplicon lengths to be as follows: ITS 618–619 bp, *matK* 751 bp, *psbA-trnH* 352–361 bp, *rbcL* 521 bp, *trnQ-rps16* 575–590 bp, *trnS-trnG* 648–658 bp, *petB* 576–577 bp, and *trnE-trnT* 490–514 bp. ITS and *matK* did not amplify efficiently despite optimization of PCR amplification conditions, while other chloroplast regions were easily amplified. Despite some challenges, both PCR amplification and sequencing were successful for all regions (Table 3).

Table 3. Amplification and sequence information for all analyzed markers and their combinations.

Marker	Amplification/ Sequencing success rate (%)	Matrix size (bp)	Variable sites (%)	No. of PI sites	Mean pairwise distance	Intraspecific distances (mean)	Interspecific distances (mean)
ITS	100/ 100	623	17.17	75	0.0259	0.0000 –0.0292 (0.0107)	0.0082 –0.0400 (0.0261)
<i>matK</i>	100/ 100	751	4.26	29	0.0054	0.0000 –0.0016 (0.0003)	0.0000 –0.0216 (0.0091)
<i>psbA-trnH</i>	100/ 100	362	10.22	27	0.0175	0.0000 –0.0029 (0.0010)	0.0000 –0.0297 (0.0212)
<i>rbcl</i>	100/ 100	521	2.50	11	0.0061	0.0000 –0.0007 (0.0002)*	0.0019 –0.0101 (0.00615)
<i>trnQ-rps16</i> (1)	100/ 100	657	6.54	35	0.0116	0.0000 –0.0025 (0.0007)	0.0067 –0.0222 (0.0131)
<i>trnS-trnG</i> (2)	100/ 100	674	5.34	22	0.0068	0.0000 –0.0027 (0.0005)	0.0017 –0.0133 (0.0082)
<i>petB</i> (3)	100/ 100	591	5.58	30	0.0164	0.0000 –0.0025 (0.0004)	0.0013 –0.0340 (0.0196)
<i>trnE-trnT</i> (4)	100/ 100	614	13.84	16	0.0075	0.0000 –0.0004 (0.0001)	0.0039 –0.0274 (0.0108)
1+2	100/ 100	1331	5.94	57	0.0090	0.0000 –0.0021 (0.0006)	0.0047 –0.0167 (0.0105)
1+3	100/ 100	1248	6.09	65	0.0139	0.0000 –0.0025 (0.0006)	0.0040 –0.0251 (0.0164)
1+4	100/ 100	1271	10.07	51	0.0096	0.0000 –0.0014 (0.0004)	0.0054 –0.0238 (0.0120)
2+3	100/ 100	1265	5.45	52	0.0112	0.0000 –0.0014 (0.0005)	0.0017 –0.0210 (0.0135)
2+4	100/ 100	1288	9.39	38	0.0071	0.0000 –0.0017 (0.0003)	0.0034 –0.0195 (0.0093)
3+4	100/ 100	1205	9.79	46	0.0121	0.0000 –0.0013 (0.0003)	0.0025 –0.0240 (0.0154)
1+2+3	100/ 100	1922	5.83	87	0.0113	0.0000 –0.0016 (0.0005)	0.0036 –0.0196 (0.0134)
1+2+4	100/ 100	1945	8.43	73	0.0086	0.0000 –0.0016 (0.0005)	0.0045 –0.0199 (0.0106)
1+3+4	100/ 100	1862	8.65	81	0.0119	0.0000 –0.0017 (0.0004)	0.0040 –0.0213 (0.0146)
2+3+4	100/ 100	1879	8.20	68	0.0101	0.0000 –0.0011 (0.0004)	0.0027 –0.0186 (0.0127)
1+2+3+4	100/ 100	2536	7.77	103	0.0104	0.0000 –0.001 (0.0005)	0.0037 –0.0181 (0.0128)

*Markers with the barcoding gap were displayed in bold format. Grey = common barcoding markers, Green = novel barcoding markers; Yellow = combinations of two novel markers; Orange = combinations of three novel markers; Dark grey = combination of all four novel markers.

Nucleotide matrix

The nucleotide matrices for the amplified markers and complemented with the 89 reference barcoding sequences and 36 complete chloroplast genomes from the seven species of *Panax*, one unidentified species and one sub-species of *Panax* present in GenBank, showed that the matrix sizes ranged from 362 to 751 bp for individual markers and 1205 to 2536 bp for concatenated markers (Table 3). Among all individual and concatenated markers, ITS possessed the highest proportion of variable

sites (17.17%), followed by *trnE-trnT* (13.84%), *psbA-trnH* (10.22%), and *trnQ-rps16* and *trnE-trnT* combined (10.07%). ITS also had the most divergence (0.0259) when calculating the mean pairwise distances for each barcode, followed by *psbA-trnH* (0.0175) and *petB* (0.0164) (Table 3). Higher numbers of variable sites and pairwise distances indicate higher species divergence, though a previous study has suggested that the proportion of variable sites may not affect a marker's classification ability (Manzanilla et al. 2018).

Species discrimination power assessment for different markers

Genetic distance-based and sequence similarity-based analyses

Distance-based classification methods rely on intraspecific and interspecific distances to set a threshold to distinguish distinct species. In this study, genetic distances were calculated between individuals both within and between species using MEGAX and Pairwise Explorer (TaxonDNA). Due to the complexity in the species group consisting of *P. bipinnatifidus* and *P. stipuleanatus*, these two species were treated as a single group when calculating pairwise distances and assessing the species classification ability of different markers. For interspecific distances, MEGAX computed the average distance of all pairwise distances between each two species while TaxonDNA returned all the distances for every pair of sequences. According to the distances obtained from MEGAX a barcoding gap exists in *rbcL*, *trnQ-rps16*, *trnE-trnT*, and all combined markers (Table 3). However, distribution analysis of pairwise distances in TaxonDNA shows that there was no barcoding gap in eight individual and eleven concatenated marker pairs (Fig. 2). The overlap of intraspecific and interspecific distance distribution was mainly due to the complex genetic distances inside *P. ginseng* species and similarity between them and *P. quinquefolius*. High divergence within species and low diversity between species in a complex genus like *Panax* results in difficulties in setting threshold values for species discrimination using distance-based methods.

BM/BCM analysis from TaxonDNA discriminates species based on similarity between sequences. For separated barcodes, analysis results showed that *trnS-trnG* and *rbcL* regions had the strongest discriminatory power with 100% correct identification for both BM calculations, followed by *trnE-trnT* (98.76%), *trnQ-rps16* (97.53%), and ITS (93.82%). BCM analysis returned more stringent calculations of successful identified sequences than BM with 100% for *trnS-trnG*, 98.76% for *trnE-trnT*, 96.87% for *rbcL*, and 95.06% for *trnQ-rps16*. Markers that had the lowest identification success rate were *petB* (BM: 72.83%, BCM: 71.60%), *matK* (BM: 62.50%, BCM: 60.93%), and *psbA-trnH* (BM: 60.93%, BCM: 60.93%). Combinations made from the four newly proposed markers were also estimated for species identification tests. Discriminatory abilities of concatenated markers were observed to be slightly better than most separated barcodes. Combinations 2+3, 2+4, 3+4, and 2+3+4 showed correct classification rates of 100% for both BM and BCM calculations (Fig. 3).

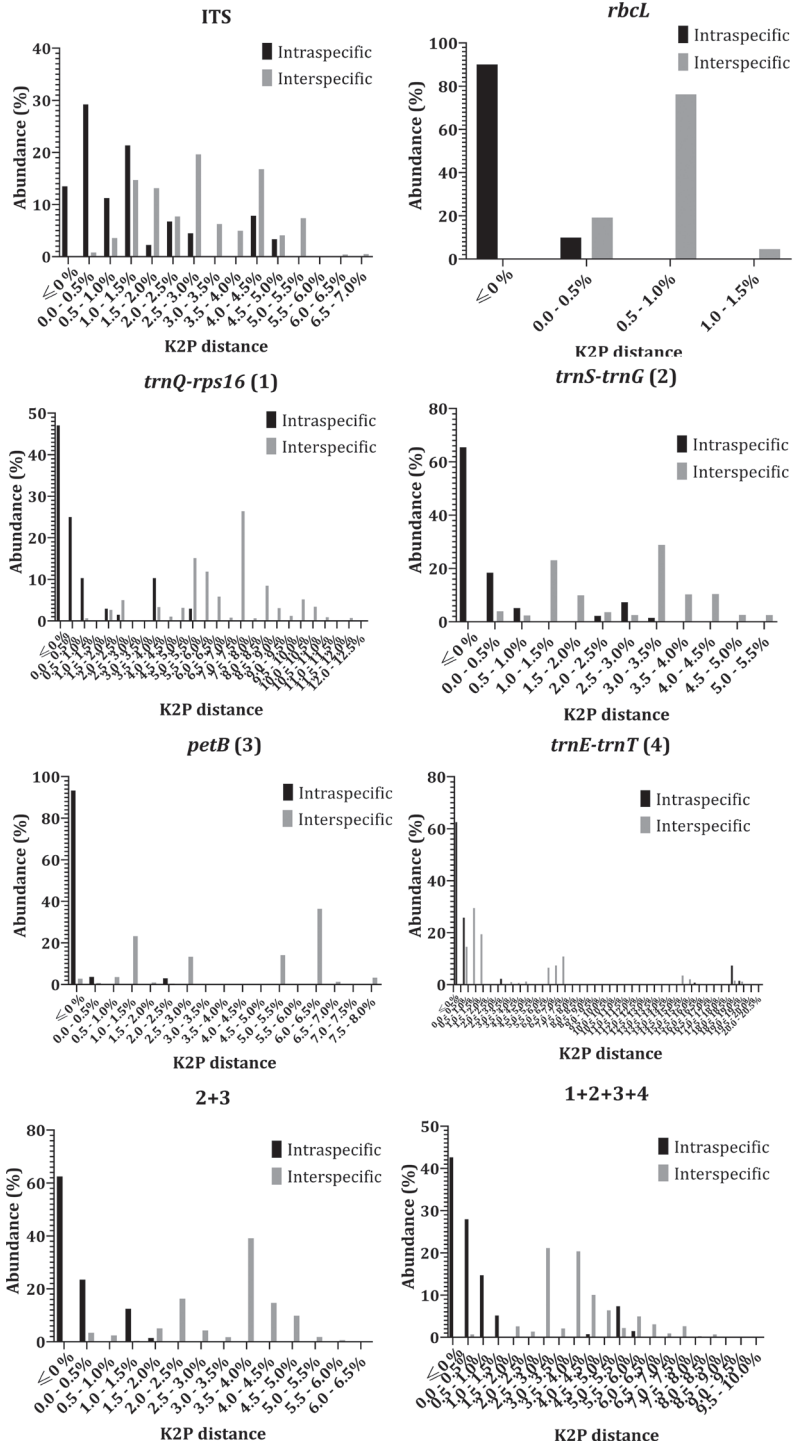


Figure 2. Percent relative abundance in distribution of intra/interspecific K2P pairwise distances estimated for markers.

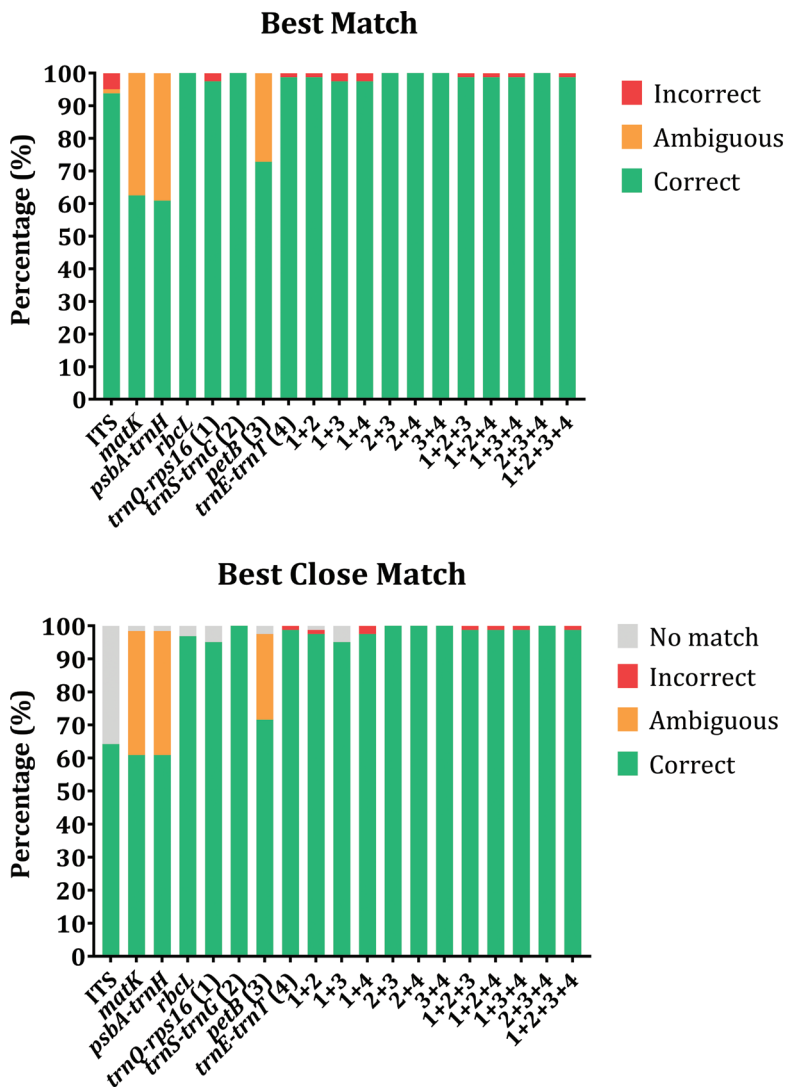


Figure 3. Successful identification rates among analyzed barcodes by Best Match and Best Close Match function.

Tree-based analysis

Both separate and concatenated matrices were used to reconstruct ML trees. We found that most of the markers could separate most of the clades with strong bootstrap support, with the exception of *P. bipinnatifidus* and *P. stipuleanatus*. These sister species had poor branch structure and weak support values. The taxonomic circumscription of *P. bipinnatifidus* has been controversial. Recent studies from Nong et al. (2016),

Pham et al. (2020) suggested identifying the leaflet ginseng, which was previously recorded as *P. bipinnatifidus* in Vietnam, as *P. stipuleanatus*. Based on morphological characteristics and ITS region, Wen and Zimmer (1996) suggested that the division of the leaflet does not warrant recognition of a novel species or variety. We therefore grouped these species into one group since the initial analysis steps and clade complexity excluded further classification tests. High intraspecific divergence in *P. ginseng* resulted in this clade being divided into two subgroups in the phylogenetic analysis. In contrast, although there are nucleotide differences between sequences of *P. vietnamensis* TX1, *P. vietnamensis* var. *fuscidiscus* SLC, and other samples of *P. vietnamensis*, our analysis clearly showed that all samples of *P. vietnamensis*, and a taxon *Panax* sp. Puxailaileng belonged to the same clade with strong support (Fig. 4). Phylogenetic trees were also used to estimate the species delimitation using mPTP. The results indicated that among eight individual markers only *trnQ-rps16* region could classify all six clades of *Panax* with strong support. Other regions performed more poorly in delimitation of tested taxa and only weakly supported speciation between *P. ginseng* and *P. quinquefolius* and/or *P. vietnamensis* and *P. japonicus*. The ITS region is an exception as its nucleotide sequence has the highest variability among species leading to over-splitting of sequences into many small subgroups. The excessive segregation limited the discriminatory ability of this ITS marker at species level. For concatenated barcodes, 1+4, 1+2+4, 1+3+4, and 1+2+3+4 showed the ability to classify all six clades in the genus *Panax* (Figs 4, 5).

Selecting markers for identification of *Panax* spp.

Incongruence between genetic distance-based, sequence similarity-based and tree-based methods has led to difficulties in choosing robust markers for species discrimination in complex genera like *Panax*. Here we examined the identification abilities of two methods for four newly proposed markers and combinations thereof in comparison with four commonly used barcodes (Fig. 5). Distance-based methods failed to detect the barcoding gap between intraspecific and interspecific distances for analyzed markers due to the complex divergence in sister species *P. ginseng* and *P. quinquefolius*. *RbcL* had the least overlapping intra/interspecific distances, but also had the lowest variation in pairwise distance. This leads to low resolution in species classification. Barcoding analysis based on sequence similarity showed high correct identification percentages for two of the four common barcodes and three of the four novel barcodes. All concatenated markers showed high identification power, but this was not the case for classification results calculated by tree-based methods. The highest identification rates were observed in *trnQ-rps16*, 1+4, 1+2+4, 1+3+4, and 1+2+3+4 regions with mPTP analysis. Other robust markers identified by TaxonDNA had lower species resolution in mPTP. We thus propose that *trnQ-rps16* is the best single marker for species identification in the *Panax* genus since it provides the best classification resolution in both sequence similarity-based and tree-based analyses.

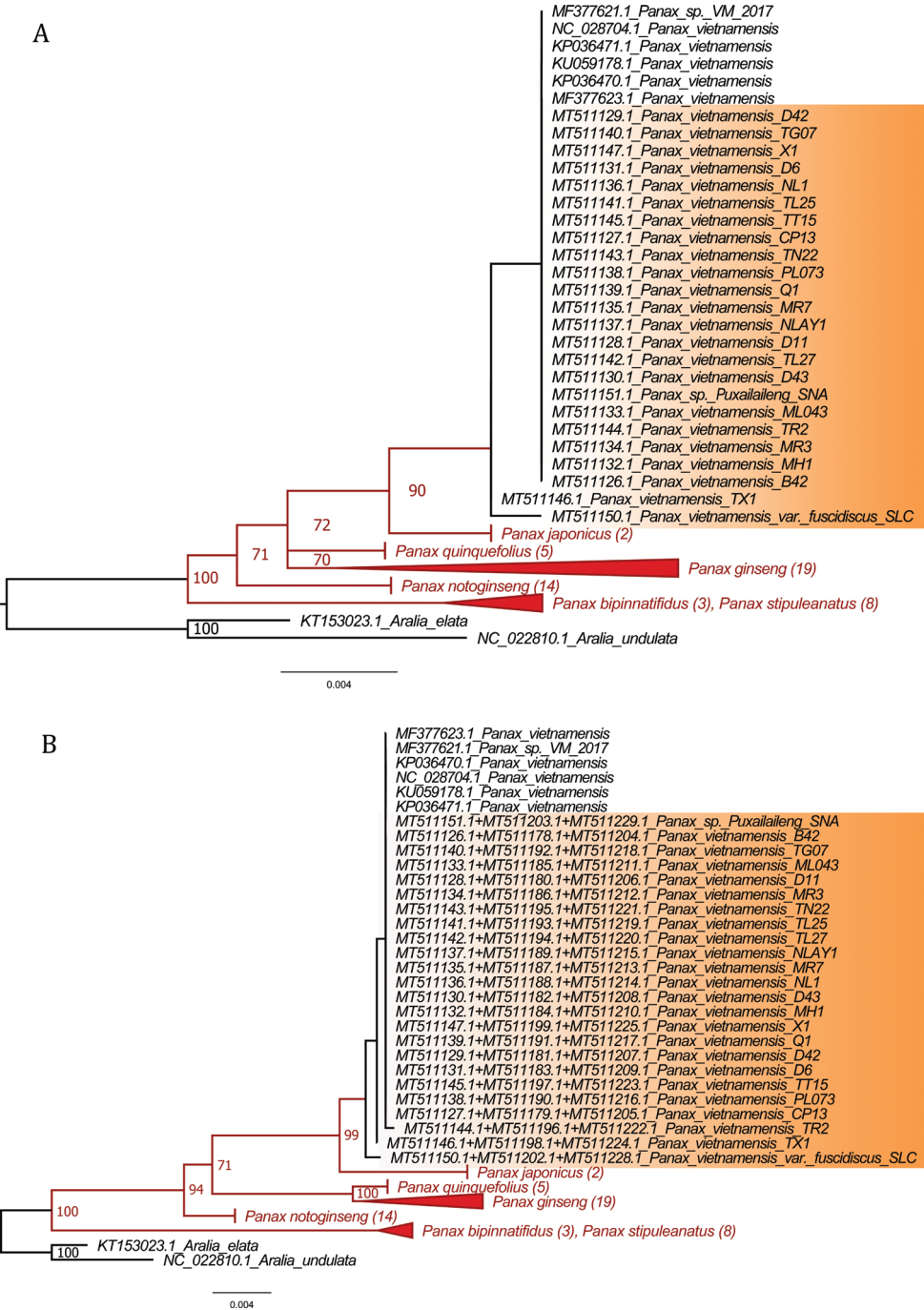


Figure 4. Results of mPTP species delimitation analysis for several markers based on ML trees **A** Species delimitation for marker *trnQ-rps16* **B** Species delimitation for the combination of markers 1+3+4. Bootstrap values are displayed on the branches. The red branches represent supported species delimitations. Sequences highlighted in orange originate from this study.

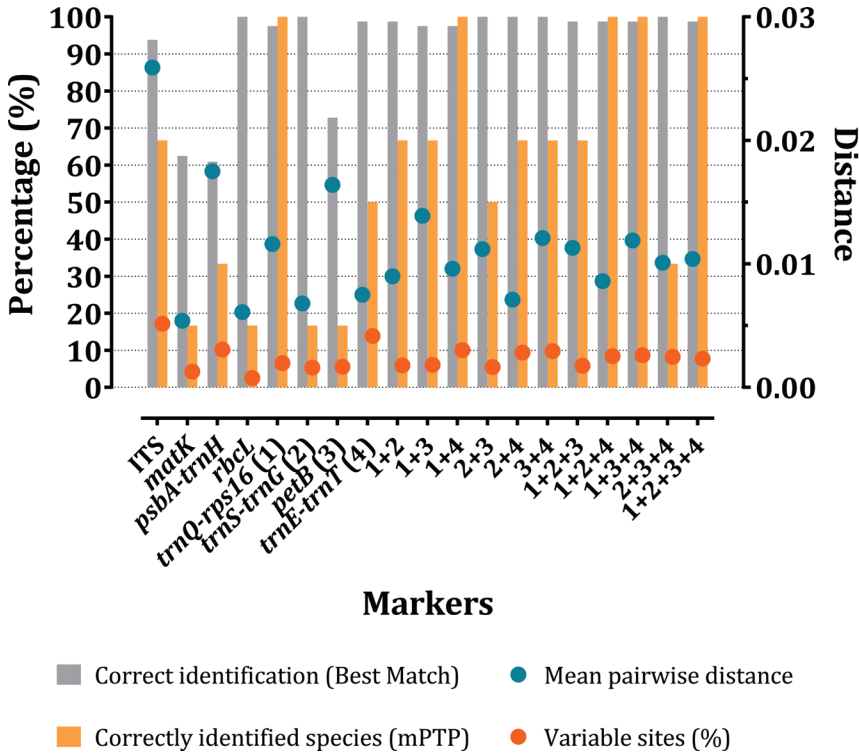


Figure 5. Percentage of variable sites, mean pairwise distances, and correct classification percentages of all markers and combinations

Discussion

Phylogenetic studies on *Panax* using different DNA barcodes, different reference sequences or samples have resulted in conflicting tree topologies and clade placements for several species (Wen and Zimmer 1996; Komatsu et al. 2001; Zuo et al. 2011; Ali et al. 2012; Le et al. 2017; Manzanilla et al. 2018). For example, two sister species *P. bipinnatifidus* and *P. stipuleanatus* were separated into two distant clades based on the combined dataset of six markers by Zuo et al. (2011). Meanwhile, these two above taxa could not be separated into two clades using the ITS region alone (Wen and Zimmer 1996). This inability to resolve a clear sister relationship between these two species was also supported by the four novel markers assessed in our study. Similarly, *P. vietnamensis* was reported to belong to the same clade as *Panax japonicus* var. *major* (Burkill) C.Y.Wu et Feng, *Panax pseudoginseng* subsp. *himalaicus* H.Hara based on 18S rRNA and *matK* (Komatsu et al. 2001), and to be closely related to *Panax zingiberensis* C.Y.Wu et Feng and *Panax wangianus* S.C.Sun based on ITS2 (Ali et al. 2012), *P. notoginseng* based on ITS, *matK*, *rbcL*, *psbA-trnH*, and 18S rRNA (Le et al. 2017), as well as *P. japonicus* based on *in silico* data of four potential markers (Manzanilla et al. 2018). With regard to an unidentified sample *Panax* sp. Puxailaileng, our phylogenetic

trees based on comprehensive datasets of the marker *trnQ-rps16* or combined markers 1+3+4 (Fig. 4) obviously revealed this taxon belonged to the same clade with all samples of *P. vietnamensis*. Relevant bootstrap values at 90–99 by Maximum Likelihood method indicated that confidence intervals were eligible for genetic correlation of these samples. The obtained results on these novel markers are congruent with or different from previous studies. *Panax* sp. Puxailaileng was suggested to be *P. vietnamensis* based on its morphological characteristics and ITS-rDNA sequence, though further studies are still needed to unambiguously resolve its identity (Tran et al. 2016). Similarly, morphology and molecular-based phylogenetic analyses suggested *Panax* sp. Puxailaileng found in the wild in Ky Son District, Nghe An Province were *P. vietnamensis* var. *fuscidiscus* (Pham et al. 2020). In another study, samples of *Panax* sp. collected from Puxailaileng Mountain were reported to be closely related to *P. stipuleanatus* based on the commonly use markers ITS-rDNA and *matK* (Vu et al. 2020). Present results based on empirical data support the results of the *in silico* study by Manzanilla et al. (2018). *P. vietnamensis* is closely related to *P. japonicus*. This apparent contradiction with other studies might result from differences in dataset structures, the number of species and taxa included, and classification methods. Indeed, different approaches can return different results in DNA barcoding analyses (Wen and Zimmer 1996; Komatsu et al. 2001; Zuo et al. 2011; Ali et al. 2012; Le et al. 2017; Manzanilla et al. 2018). The genetic distances calculated in MEGAX suggest that barcoding gaps exist in the markers *rbcL*, *trnQ-rps16*, *trnE-trnT*, and all concatenated markers, whereas TaxonDNA showed overlap between intra and interspecific distances in all analyzed markers (Table 3, Fig. 2). This incongruence could make a tremendous difference in the output of the analyses. Nevertheless, results obtained from the BM/BCM module in TaxonDNA are similar to distances calculated in MEGAX. In general, the discriminatory power assessed in TaxonDNA is higher than in the tree-based method mPTP (Fig. 5). Especially for *rbcL*, distance-based methods provide the highest percentage of correctly identified species while the tree-based method gave the lowest percentage of correctly identified species. The low number of variable sites within some markers can explain the differences in results between the two methods (Fig. 5). Highly conserved regions with low percentages of variable sites might lead to sound results when calculating genetic distances, but might make the construction of a phylogenetic tree challenging. This limitation for distance-based methods can sometimes result in biologically meaningless results (Meier et al. 2008; Ross et al. 2008). However, the comparison between the two methods generated several common similarities for the marker *trnQ-rps16* and a number of different marker combinations with high discriminatory power, e.g., 1+4, 1+2+4, 1+3+4, 1+2+3+4 (Fig. 5).

In the present study, the discriminatory power of four chloroplast markers proposed by Manzanilla et al. (2018), for *Panax* spp. was evaluated. The highly valuable *P. vietnamensis* species and collected samples from eighteen distinct populations across the country were analyzed. The obtained results did not show any significant differences between the populations, which suggests that *trnQ-rps16* marker is appropriate for identification at the species level and does not lead to misidentifications of *P. vietnamensis* regardless of

their origins or geographical distributions. Only small differences between experimental and *in silico* analyses were observed. The *in silico* study included only a limited number of *P. vietnamensis* accessions, and this may explain why the empirical data contains more variable sites in *P. vietnamensis*. Our results support the use of *trnQ-rps16* as a single molecular marker for species identification in the genus *Panax*. An advantage of using a single region for species identification is the reduced time and effort necessary for routine analyses, though it can be combined with other markers (*petB*, *trnE-trnT*) when necessary if a single marker is not enough to unambiguously provide a species-level identification. Beside its discriminatory power, *trnQ-rps16* can also be easily amplified.

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References

- Ali MA, Al-Hemaid F, Lee J, Choudhary R, Al-Harbi N (2012) Assessing nrDNA ITS2 sequence based molecular signature of ginseng for potential use in quality control of drug. *African Journal of Pharmacy and Pharmacology* 6: 2767–2774. <https://academicjournals.org/journal/AJPP/article-abstract/DE65DA736356>
- Bui QM, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Case MA, Flinn KM, Jancaitis J, Alley A, Paxton A (2007) Declining abundance of American ginseng (*Panax quinquefolius* L.) documented by herbarium specimens. *Biological Conservation* 134(1): 22–30. <https://doi.org/10.1016/j.biocon.2006.07.018>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Ha TD, Grushvitzky IV (1985) A new species of the genus *Panax* (Araliaceae) from Vietnam. *Botanicheskii Zhurnal* 70: 519–522.

- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium Series. [London]: Information Retrieval Ltd., c1979–c2000. https://www.academia.edu/2034992/BioEdit_a_user_friendly_biological_sequence_alignment_editor_and_analysis_program_for_Windows_95_98_NT
- Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. *PLoS ONE* 6(5): e19254. <https://doi.org/10.1371/journal.pone.0019254>
- Janzen DH, Hallwachs W, Blandin P, Burns JM, Cadiou JM, Chacon I, Dapkey T, Deans AR, Epstein ME, Espinoza B, Franclemont JG, Haber WA, Hajibabaei M, Hall JP, Hebert PD, Gauld ID, Harvey DJ, Hausmann A, Kitching IJ, Lafontaine D, Landry JF, Lemaire C, Miller JY, Miller JS, Miller L, Miller SE, Montero J, Munroe E, Green SR, Ratnasingham S, Rawlins JE, Robbins RK, Rodriguez JJ, Rougerie R, Sharkey MJ, Smith MA, Solis MA, Sullivan JB, Thiaucourt P, Wahl DB, Weller SJ, Whitfield JB, Willmott KR, Wood DM, Woodley NE, Wilson JJ (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources* 9(S1): 1–26. <https://doi.org/10.1111/j.1755-0998.2009.02628.x>
- Jung J, Lee NK, Paik HD (2017) Bioconversion, health benefits, and application of ginseng and red ginseng in dairy products. *Food Science and Biotechnology* 26(5): 1155–1168. <https://doi.org/10.1007/s10068-017-0159-2>
- Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T (2017) Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* (Oxford, England) 33: 1630–1638. <https://doi.org/10.1093/bioinformatics/btx025>
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Komatsu K, Zhu S, Fushimi H, Qui TK, Cai S, Kadota S (2001) Phylogenetic analysis based on 18S rRNA gene and *matK* gene sequences of *Panax vietnamensis* and five related species. *Planta Medica* 67: 461–465. <https://doi.org/10.1055/s-2001-15821>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Le TH, Nguyen NL, Bui MM, Ha HH, Huynh TTH, Nong VH, Ha VH, Le TTH (2017) Application of DNA barcodes in identification of ginseng samples in the genus *Panax* L. *Vietnam Journal of Biotechnology* 15: 63–72. <https://doi.org/10.15625/1811-4989/15/1/12321>
- Li MR, Shi FX, Zhou YX, Li YL, Wang XF, Zhang C, Wang XT, Liu B, Xiao HX, Li LF (2015) Genetic and epigenetic diversities shed light on domestication of cultivated ginseng (*Panax ginseng*). *Molecular Plant* 8(11): 1612–1622. <https://doi.org/10.1016/j.molp.2015.07.011>
- Manzanilla V, Kool A, Nguyen NL, Nong VH, Le TTH, De Boer H (2018) Phylogenomics and barcoding of *Panax*: Toward the identification of ginseng species. *BMC Evolutionary Biology* 18(1): 1–14. <https://doi.org/10.1186/s12862-018-1160-y>
- McGraw JB, Lubbers AE, Van der Voort M, Mooney EH, Furedi MA, Souther S, Turner JB, Chandler J (2013) Ecology and conservation of ginseng (*Panax quinquefolius*) in a changing world. *Annals of the New York Academy of Sciences* 1286(1): 62–91. <https://doi.org/10.1111/nyas.12032>

- Meier R, Shiyang K, Vaidya G, Ng PK (2006) DNA barcoding and taxonomy in *Diptera*: A tale of high intraspecific variability and low identification success. *Systematic Biology* 55(5): 715–728. <https://doi.org/10.1080/10635150600969864>
- Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology* 57(5): 809–813. <https://doi.org/10.1080/10635150802406343>
- Nguyen T (2005) The species of *Panax* L. in Vietnam. *Journal of Medicinal Materials* 10(3): 71–76.
- Nguyen TB, Tran DL, Nguyen T, Vu VD, Nguyen NT, Nguyen VT, Nguyen KK [Eds] (2007) Vietnam Red Data Book. Part II. Plants. Natural Science and Technology Publisher.
- Nguyen VB, Park HS, Lee SC, Lee J, Park JY, Yang TJ (2017) Authentication markers for five major *Panax* species developed via comparative analysis of complete chloroplast genome sequences. *Journal of Agricultural and Food Chemistry* 65(30): 6298–6306. <https://doi.org/10.1021/acs.jafc.7b00925>
- Nguyen VB, Vo NLG, Waminal NE, Park HS, Kim NH, Jang W, Lee J, Yang TJ (2020) Comprehensive comparative analysis of chloroplast genomes from seven *Panax* species and development of an authentication system based on species-unique single nucleotide polymorphism markers. *Journal of Ginseng Research* 44(1): 135–144. <https://doi.org/10.1016/j.jgr.2018.06.003>
- Nong VD, Le NT, Nguyen DC, Tran VT (2016) A new variety of *Panax* (Araliaceae) from Lam Vien Plateau, Vietnam and its molecular evidence. *Phytotaxa* 277(1): 47–58. <https://doi.org/10.11646/phytotaxa.277.1.4>
- Patel S, Rauf A (2017) Adaptogenic herb ginseng (*Panax*) as medical food: Status quo and future prospects. *Biomedicine and Pharmacotherapy* 85: 120–127. <https://doi.org/10.1016/j.biopha.2016.11.112>
- Pham TN, Pham TH, Nguyen QN, Phan VT, Nguyen MK, Dinh DL (2020) A molecular phylogeny of *Panax* L. genus (Araliaceae) based on ITS-rRNA and *matK* support for identification of *Panax* species in Vietnam. *VNU Journal of Science: Medical and Pharmaceutical Sciences* 36(2): 91–99. <https://doi.org/10.25073/2588-1132/vnumps.4198>
- Phan KL, Le TS, Phan KL, Vu DD, Pham VT (2013) Lai Chau ginseng *Panax vietnamensis* var *fuscidiscus* K.Komatsu, S.Zhu et S.Q.Cai. Morphology, distribution and conservation status. Proceedings of the 2nd VAST-KAST Workshop on Biodiversity and Bio-active compound. Natural Science and Technology Published House, 65–73.
- Phan KL, Tran TVT, Nguyen TT, Phan KL, Nguyen TL, Nguyen TL, Dang XM (2014) Morphological and molecular characteristics of *Panax* sp. (Araliaceae) from Phu Xai Lai Leng Mountain, Nghe An Province, Vietnam. *Journal of Biology* 36(4): 494–499. <https://doi.org/10.15625/0866-7160/v36n4.5212>
- Ross HA, Murugan S, Sibon Li WL (2008) Testing the reliability of genetic methods of species identification via simulation. *Systematic Biology* 57(2): 216–230. <https://doi.org/10.1080/10635150802032990>
- Shi FX, Li MR, Li YL, Jiang P, Zhang C, Pan YZ, Liu B, Xiao HX, Li LF (2015) The impacts of polyploidy, geographic and ecological isolations on the diversification of *Panax* (Araliaceae). *BMC Plant Biology* 15(1): e297. <https://doi.org/10.1186/s12870-015-0669-0>
- Shneyer V (2009) DNA barcoding is a new approach in comparative genomics of plants. *Russian Journal of Genetics* 45(11): 1267–1278. <https://doi.org/10.1134/S1022795409110027>

- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* (Oxford, England) 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89(1): 26–32. <https://doi.org/10.1007/BF00226978>
- Tran NL, Nguyen TD, Nguyen TT, Le TTH, Ngo HL, Nguyen DN, Tran QT, Hoang NN, Phung VH (2016) Results of study on sam Puxailaileng in high mountain, Nghe An Province, Nghe An. *Journal of Science and Technology* 12: 7–11.
- Vu DD, Tran TVT, Phan KL, Nguyen MT, Nguyen TTH, Nguyen TH, Phan KL (2020) Using ITS-rDNA and *matK* gene nucleotide sequences for identification ginseng species in *Panax* in Phu xai lai leng, Ky Son, Nghe An. *Journal of Biotechnology* 18(1): 75–85. <https://doi.org/10.15625/1811-4989/18/1/15267>
- Wen J, Zimmer EA (1996) Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6(2): 167–177. <https://doi.org/10.1006/mpev.1996.0069>
- Zuo Y, Chen Z, Kondo K, Funamoto T, Wen J, Zhou S (2011) DNA barcoding of *Panax* species. *Planta Medica* 77(02): 182–187. <https://doi.org/10.1055/s-0030-1250166>

Supplementary material I

NCBI accession numbers of DNA barcoding sequences, and complete chloroplast genomes used in this study.

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Data type: NCBI accession numbers of DNA sequences and complete chloroplast genomes

Explanation note: The NCBI accession numbers of newly obtained and 91 reference barcoding sequences, and 38 complete chloroplast genomes representing seven *Panax* species, one unidentified species and one sub-species of *Panax*, and two out-group *Aralia* species.

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Carex malipoensis (Cyperaceae), a new species from southeast Yunnan, China

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Abstract

Carex malipoensis, a new species from southeast Yunnan, China, is here described and illustrated. It is morphologically similar to *C. trichophylla* in sect. *Euprepes*, but differs from it by its longer inflorescences and peduncles, pendulous spikes, hispidulous female glumes, densely hispidulous utricles, and longer nutlets.

Keywords

Carex sect. *Euprepes*, *Carex trichophylla*, morphology, taxonomy

Introduction

As one of the largest angiosperm genera, *Carex* Linnaeus (1753) (Cyperaceae) comprises an extremely high diversity of about 2000 species (Roalson et al. 2021; WCSP 2021). The genus has a Cosmopolitan distribution. It was placed within the tribe Cariceae and divided into four subgenera by Kükenthal (1909): subg. *Psyllophora* (Degland 1828) Petermann (1849), subg. *Vigneastra* (Tuckerman 1843) Kükenthal (1899), subg. *Vigneae* (P. Beauv. ex Lestiboudois 1819) Petermann (1849) and subg. *Carex*. This classification of Kükenthal had been widely adopted by subsequent researchers for a long time. Recent molecular phylogenetic studies revealed that Cariceae is a natural group, and the previously recognized genera of this tribe, i.e., *Cymophyllus* Mack. ex Britton & A.Br.,

Kobresia Willdenow, *Schoenoxiphium* Nees von Esenbeck, and *Uncinia* Persoon, should be merged into *Carex* (Yen and Olmstead 2000; Global *Carex* Group 2015; Starr et al. 2015; Lévillé-Bourret et al. 2018; Martín-Bravo et al. 2019; Villaverde et al. 2020; Larridon et al. 2021; Roalson et al. 2021). A framework of the combined giant genus was urgently needed to increase our understanding of *Carex*. In order to solve this problem, Villaverde et al. (2020) conducted a robust phylogeny of *Carex* based on molecular data (308 nuclear exon matrices, 543 nuclear intron matrices and 66 plastid exon matrices) and six clades were recognized. Accordingly, Villaverde et al. (2020) proposed an updated infrageneric classification of *Carex* and classified it into six subgenera: subg. *Siderosticta* M.J. Waterway, subg. *Carex*, subg. *Euthyceras*., subg. *Psyllophorae*, subg. *Uncinia* (Pers.) Peterm. and subg. *Vigneae*. However, the classification within the subgenera still remained unresolved, so a more systematic and friendly infrageneric classification system was required. A framework infrageneric classification of *Carex* was proposed recently which divided *Carex* into 62 formally named Linnean sections and 49 informal groups based on the current phylogenetic knowledge of *Carex* (Roalson et al. 2021).

A total of 527 *Carex* were recorded in Flora of China (Dai et al. 2010), the Catalogue of Life China (Chen and Zhang 2018) recorded 593 species, which represents the most complete and update list of the genus in China. The number of species of *Carex* continues growing in China as more new species have been reported in recent years (Lu and Jin 2018; Zhang et al. 2018; Jin et al. 2020; Lu et al. 2020; Yang and Liu 2020).

During our field investigations between 2016 and 2018, we collected specimens of an unknown species of *Carex* in Malipo County, southeast Yunnan Province, China. After careful morphological studies, examination of herbarium specimens and relevant literature, we concluded that it can be assigned to *Carex* sect. *Euprepes* based on a combination of some morphological characters: cauline leaves well-developed; leaf blades and involucre bracts elliptic to linear-elliptic, with prominent transverse veins; complex branched inflorescence; spikes androgynous; and presence of utriculiform cladophylls (Jiménez-Mejías et al. 2016) at the base of spikes. Molecular phylogenetic studies indicated that *C.* sect. *Euprepes* belongs to the core *Carex* clade (Starr et al. 2015; Villaverde et al. 2020), and the most recent infrageneric classification framework placed it with the Indica Clade together with species traditionally placed in sections *Euprepes* and *Mapaniifoliae* (Roalson et al. 2021). Consisting of seven species, sect. *Euprepes* are restricted to South and Southeast Asia (Nelmes 1955). Only one species of the section, *C. zizaniifolia* Raymond (1959), was previously reported from China, distributed in southeast Yunnan Province (Dai et al. 2010; Chen and Zhang 2018). However, after our research on this section, we conclude that our new collections are different from all known species and represent a species new to science. We describe and illustrate it here below.

Materials and methods

The new species was compared morphologically with specimens of other taxa of *Carex* sect. *Euprepes* from the following public herbaria A, BM, E, GH, HNU, IBSC, K, KUN, HUH, MO, MT, NY, P, PE, and US [acronyms follow Theirs (continuously

updated)] as well as our new collections across China (especially with material collected from southeast Yunnan and neighboring area; herbarium specimens kept in KUN). Meanwhile, protologues and other related taxonomic literature were collated and reviewed. The characters' data come from specimens measurements and the prologue (*C. atrivaginata* Nelmès and *C. tricophylla* Nelmès (1955), *C. euprepes* Nelmès and *C. laosensis* Nelmès (1939), *C. tavoyensis* Nelmès (1948), *C. zizaniifolia* Raymond and *C. poilanei* Raymond (1959)). The terminology used by Kükenthal (1909) for the morphological description of *Carex* species was adopted here. The distribution of the new species was compiled from the herbarium specimen records and our own collections, and shown on the distribution map.

Results

A detailed morphological comparison of the potential new species and the seven species of *C. sect. Euprepes* is summarized in Table 1. Morphologically, the new species is most similar to *C. tricophylla* but can be distinguished by the characteristics of culms (70–105 cm long, 2–4 mm thick, sides ribbed in the new species vs. culms 45–65 cm long, 1–1.5 mm thick, sides concave in *C. tricophylla*); leaf sheaths (1.5–7 cm long vs. 1.2–2 cm long); inflorescences (20–45 cm long, peduncles up to 9 cm long vs. 3–15 cm long, more or less exserted), spikes (15–33 mm long, pendulous vs. 7–10 mm long, erect), female glumes (hispidulous vs. glabrous), utricles (densely hispidulous vs. glabrous below, adpressed-hispid above), and nutlets (4–4.5 mm long vs. 2.25–2.5 mm long). The new taxon can be distinguished from all the other seven species in this section by their culms (length, cross-sectional shape, sides and indumentum); leaves (shape, transverse veins), sheath (length, indumentum and the appendage of mouth); inflorescence bigger (20–45 × 3–5 cm in the new taxa vs. 3–25 × 1–5 cm in the other species) or with different shape (oblong in the new taxon vs. oblong, narrowly oblong or triangular-ovate in other species); peduncles longer (up to 9 cm long in the new taxon vs. scarcely or slightly exserted in other species; only *C. atrivaginata* has relative long peduncles not exceeding 5 cm); and the female glumes, utricles and nutlets (differences in shape, size and indumentum).

Taxonomy

***Carex malipoensis* Yuan Y. Li & H. Peng, sp. nov.**

urn:lsid:ipni.org:names:77235050-1

Figures 1, 2

Type. CHINA. Yunnan Province, Malipo County, Mengdong Village, Xiangchunping, 22°54'36.77"N, 104°38'54.09"E, alt. 1850 m, 5 December 2016, E.D. Liu *et al.* LiuED6425 (holotype: KUN! Barcode 1433368; isotype: KUN! Barcode 1347669).

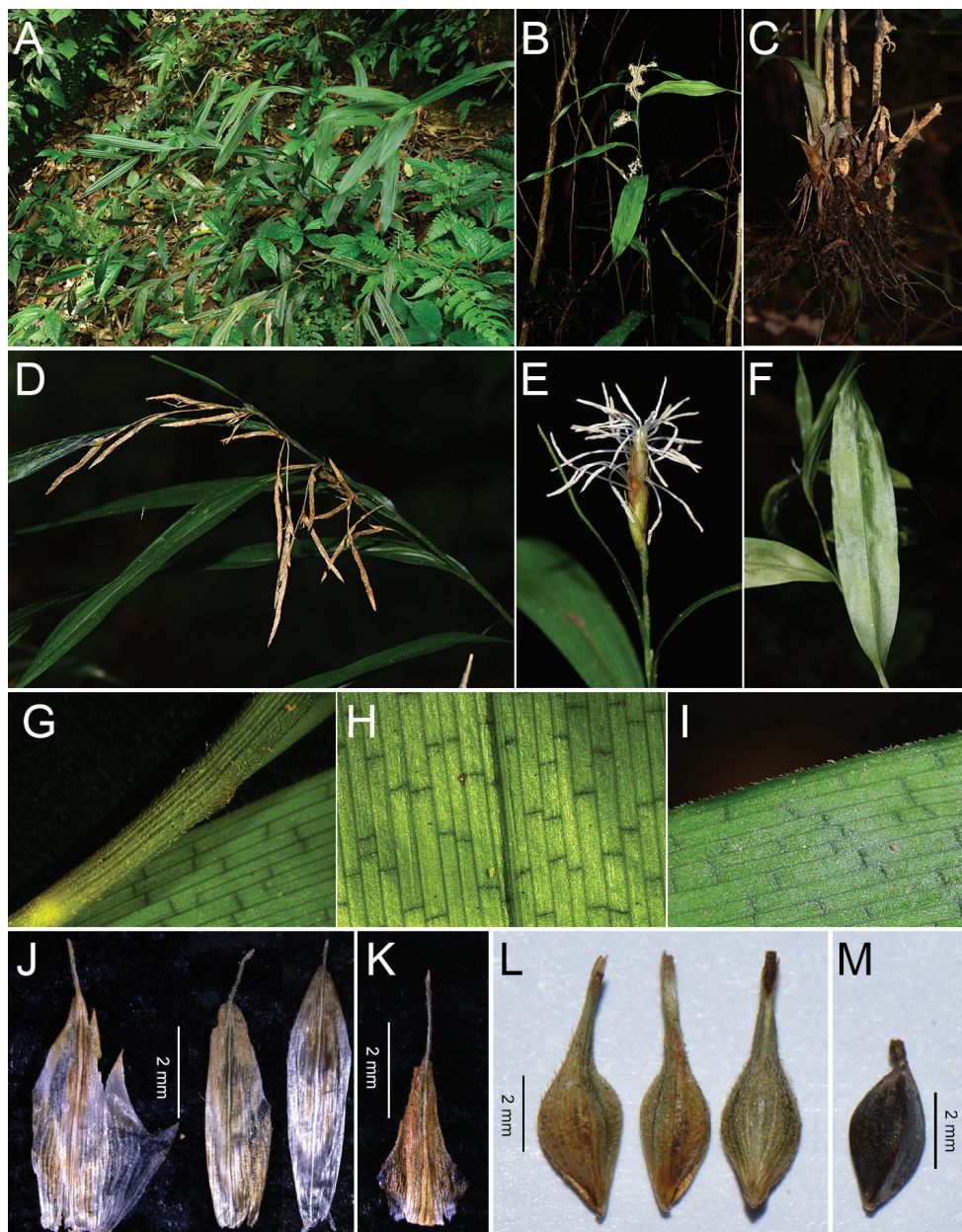


Figure 1. Morphology of *Carex malipoensis* **A** habitat **B** plant **C** rhizome **D** inflorescence **E** spike **F** leaf blade **G** sheath **H** the transverse veins **I** leaf margin **J** male glumes **K** female glume **L** utricles **M** achene. Scale bars: 2 mm. Photographed by X.X. Zhu (**A**, **D**, **E** *LiuED6425*, *LiuED5912* KUN), Y.P. Chen (**B**, **C**, **F**, **I** *Y.P. Chen & L.Q. Jiang MLP01* KUN), and Y. Y. Li (**G**, **H**, **J–K** *Y.P. Chen & L.Q. Jiang MLP01* KUN; **L**, **M** *LiuED6425* KUN).

Table 1. Morphological comparisons between *Carex malipoensis* and species in *C.* sect. *Euprepes*.

Characters	<i>C. malipoensis</i>	<i>C. atrivaginata</i>	<i>C. euprepes</i>	<i>C. lasensis</i>	<i>C. poilanei</i>	<i>C. tavoyensis</i>	<i>C. tricophylla</i>	<i>C. zizaniifolia</i>
Culm								
Length	70–105 cm	65 cm	70 cm	unknown	70–105 cm	unknown	45–65 cm	60–70 cm
Thick	2–4 mm	3 mm	3 mm	2 mm	2–3 mm	2–2.5 mm	1–1.5 mm	2 mm
Transverse angle	obtuse	acute	obtuse	obtuse	acute	acute, obtuse to subacute	obtuse	obtuse
Sides	ribbed	concave	concave	concave	concave	ribbed, slightly twisted	concave	concave
Indumentum	hispidulous	glabrous	glabrous	glabrous	glabrous	glabrous	hispidulous upward	glabrous
Leaf								
Number	3–5	7	4–6	1–4	6–8	unknown	6 or more	8
Shape	elliptic	narrowly linear-lanceolate	elliptic	narrowly linear-lanceolate	narrowly linear-lanceolate	Linear-elliptic	elliptic, apex attenuated	narrowly elliptic or lanceolate
Length	12–28 cm	13–16 cm	17–24 cm	20–30 cm	18–30 cm	25–28 cm	12–18 cm	15–25 cm
Width	2–3 cm	1–1.3 cm	3–4.5 cm	8–15 mm	1–2 cm	2.3–3 cm	1.2–2 cm	2–2.5 cm
Indumentum	glabrous, sparsely hispidulous on undersurface midrib	glabrous, apex hispidulous	scabrid along veins undersurface	glabrous	glabrous	scabrid along veins undersurface	scabrid along veins undersurface	scabrid along veins
Leaf sheath								
Length	1.5–7 cm	1.5–5 cm	1–2 cm	1.5–2 cm	2–5 cm	unknown	1.5–2 cm	1.4–2 cm
Indumentum	hispidulous	glabrous	glabrous below, hispidulous above	hispidulous	glabrous	glabrous	hispidulous	glabrous
Appendage of sheath mouth	not developed	not developed	prominent	small	small	small	prominent	small
Inflorescence								
Shape	oblong	oblong	narrowly oblong	narrowly oblong	triangular-ovate	narrowly oblong	narrowly oblong	narrowly oblong
Length	20–45 cm	8–10 cm	10–22 cm	3.5–13.5 cm	12–20 cm	15–25 cm	3–15 cm long	5–6 cm
Width	3–5 cm	2–5 cm	3–4 cm	1–2.5 cm	3–6 cm	1–2 cm	1–2 cm	1–1.5 cm
Peduncles	exserted	exserted	scarcely or slightly exserted	scarcely exserted	exserted	scarcely exserted	exserted	scarcely exserted
Spikes								
Length	15–30 (45) mm	10–25 mm	8–10 mm	5 mm	8–18 mm	5–9 mm	7–10 mm	5–6 mm
Male part vs female part	much longer	much longer	equal	longer	much longer	slightly longer	longer	slightly longer
Utricles								
Shape	ovate-elliptic	ovate-elliptic	elliptic	broadly ellipsoid or ellipsoid-obovoid	ovate or rhomboid-ovate	ellipsoid or obovoid-ellipsoid	obovoid or ellipsoid-obovoid	ovate-elliptic

Characters	<i>C. malipoensis</i>	<i>C. atrivaginata</i>	<i>C. euprepes</i>	<i>C. laosensis</i>	<i>C. poilanei</i>	<i>C. tavoyensis</i>	<i>C. trichophylla</i>	<i>C. zizaniifolia</i>
Length	6–7.5 mm	6–7.5 mm	4–4.5 mm	4–4.5 mm	4–5 mm	4–5 mm	6–6.5 mm	ca. 3 mm
Indumentum	densely hispidulous	densely hispidulous	glabrous, margins hispidulous	glabrous	unknown	glabrous below, adpressed-hispid above	glabrous below, adpressed-hispid above	densely hispidulous
nutlets	ca. 4 mm	ca. 4 mm	2.25–2.5 mm	2.25–2.8 mm	unknown	ca. 2.3 mm	2.25–2.5 mm	immature

The characters marked “unknown” for the missing description of the protologue or the original materials incomplete.

Diagnosis. The new species is most similar to *C. trichophylla* Nelmes (1955), but differs in inflorescences 20–45 cm long (vs. shorter than 15 cm in *C. trichophylla*), peduncles up to 9 cm long (vs. more or less exserted in *C. trichophylla*), spikes pendulous (vs. erect in *C. trichophylla*), female glumes hispidulous (vs. glabrous in *C. trichophylla*), utricles densely hispidulous (vs. glabrous below, adpressed-hispid above in *C. trichophylla*), and nutlets 4–4.5 mm long (vs. 2.25–2.5 mm long in *C. trichophylla*).

Description. Rhizomes elongate. Culms tufted, 70–105 cm long, 2–4 mm in diam, obtusely trigonous, hispidulous, basal sheaths dark brown. Leaves 3–5, basal and cauline, loosely arranged; leaf blade elliptic, 12–28 × 2–3 cm, transverse veins prominent, margin hispidulous-villous, base round to cuneate, apex acute, greyish green when dried; sheaths 1.5–7 cm long, hispidulous, mouth hairy, not developed into prominent appendage. Involucral bracts leaf-like, longer than inflorescence, sheathing; panicles 20–45 × 3–5 cm, with 12–16 branches, single or binate; peduncles up to 9 cm long, reduced toward apex, tenuous, glabrous or slightly pubescent; inflorescence axes sharply trigonous, hairy on edges; bractlets glumiform, ca. 5 mm long, apex awned, awns 4–5 mm long; cladophylls utriculiform, 3–4 mm long; spikes bisexual, androgynous, 15–30 (–45) mm long; male part of spike much longer than female part; male part densely many flowered, ca. 2.5 mm wide; female part fewer flowered, 3–4.5 mm wide; male glumes oblong-lanceolate, 6–9 × 1.5–2 (–3.2) mm, awned; female glumes oblong-lanceolate, 3.8–4.2 mm long, pale brown, green at middle, apex acute, midrib extending into a scabrid awn. Utricles ovate-elliptic, 6–7.5 mm long, green to brown, veined, densely hispidulous, apex attenuating into a long beak, ca. 3 mm long, orifice oblique. Nutlets ca. 4 × 1.8–2 mm, dark brown, obovate-elliptic, trigonous. Stigmas 3.

Phenology. Flowering from November to December, and fruiting in May.

Distribution. The new species is currently known from Malipo County in south-east Yunnan at the Sino-Vietnamese border (Fig. 3).

Habitat. The new species usually grows in evergreen broad-leaved forests at altitudes of 1100–1850 m.

Etymology. The specific epithet refers to Malipo County of Yunnan Province, China, from where the type specimens were collected.

Common name (assigned here). Ma Li Po Tai Cao (麻栗坡臺草; Chinese name).



Figure 2. Line drawing of *Carex malipoensis* **A** habit **B** male glume **C** female glume **D** utricles **E** achene **F** part of blade, show the transverse veins. Scale bars: 6 cm (**A**); 2 mm (**B–E**). Drawn by Yuan Luo from the type specimen.

Additional specimens examined (paratypes). CHINA. Yunnan: Maguan County, Ching-kou Loa-chün-shan, 7 December 1947, *K.M. Feng* 13677 (KUN0368409; KUN1263725); Malipo County, Mengdong Village, 4 December 2016, *E.D. Liu et al.* *LiuED* 6336 (KUN1433717), *LiuED*6403 (KUN1433131); Malipo County, Laojun-shan, Bailingyan, 13 May 2017, *E.D. Liu et al.* *LiuED*5912 (KUN1340680); Malipo County, Tianbao Town, Bajiaoping Village, 1 December 2018, *Y.P. Chen & L.Q. Jiang* *MLP01*(KUN).



Figure 3. Distribution map of *Carex malipoensis* (▲) and *C. trichophylla* (●).

Specimens examined of other species. *Carex atrivaginata*: VIETNAM. Chapa: Pételot, 3179 (P00277787, P00277788); E. Poilane, 27084 (MT00072452, MT00072458); *C. euprepes*: LAOS. Tawiang, Chiengkwan: 2 April, 1932, Kerr, 20927 (BM001172101, K000291207, K000291208, K000291210, NY04059693, P00282617); *C. laosensis*: LAOS. Pak Munung, Wieng chan: 22 April, 1932, Kerr, 21202 (K000291209, K000291210, P00284722); *C. poilanei*: LAOS. Phong Saly: 6 September, 1941, E. Poilane, 25984 (MT00117677); E. Poilane, 32994 (MT00072475); *C. tavoyensis*: MYANMAR. Padachaung, Tavoy. 3 April, 1921, P.T. Russell, 1935 (K000999214); *C. trichophylla*: VIETNAM. Chapa (Fig. 3): 1 July, 1930, Pételot, 5325 (GH00027549, P00302178, P00302179, P00302180, US00087306); *C. zizaniifolia*: CHINA. Yunnan, Pingbian: 1934, H. T. Tsai 62809 (A00027543, IBSC0653006, KUN0368701, PE00030290).

Conservation status. The new species is currently known from Maguan and Malipo Counties in Yunnan, China. Only six collections have been recorded since 1947. It may be classified as Endangered (EN) or Vulnerable (VU) according to the IUCN Red List criteria (IUCN 2012). However, collections of *Carex* are often deficient, and a solid suggestion is needed based on a comprehensive investigation about the new species. Therefore, we suggest to characterize the conservation status of *C. malipoensis* as Data Deficient (DD) at present.

Discussion

In comparison with other species of *C.* sect. *Euprepes*, *C. malipoensis* is morphologically most similar to *C. trichophylla*. Both species have obtusely trigonous culms and elliptic and subpetioleate leaf blades, inflorescences big and loose, and utricles longer than 6 mm, all characters that differ from all the remaining species of *C.* sect. *Euprepes*. Despite their shared similarities, *C. malipoensis* can be distinguished from *C. trichophylla* in the length of inflorescences and peduncles, and indumentum of sheath and utricles. Specifically, *C. malipoensis* has much longer culms and spikes, and larger leaves, panicles, and achenes compared with that of *C. trichophylla*. Moreover, the female glumes and utricles are hispidulous in *C. malipoensis*, but glabrous in *C. trichophylla*.

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References

- Chen WL, Zhang SR (2018) Seed Plants. Catalogue of Life China. Science Press, Beijing, 49–91.
- Dai LK, Liang SY, Zhang SR, Tang YC, Koyama T, Tucker GC (2010) *Carex* Linnaeus. In: Wu ZY, Raven PH, DY H (Eds) Flora of China. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis, 293–294.
- Degland JV (1828) *Carex*. In: Loiseleur-Deslongchamps JLA (Ed.) Flora Gallica 2. 2nd edition. J. B. Baillière, Paris, 281–313.
- Global *Carex* Group (2015) Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): A new broader circumscription. Botanical Journal of the Linnean Society 179(1): 1–42. <https://doi.org/10.1111/boj.12298>
- IUCN (2012) IUCN Red List Categories and Criteria, Version 3.1. 2 ed. Gland, Switzerland and Cambridge, UK, 32 pp.
- Jiménez-Mejías P, Luceno M, Wilson KL, Waterway MJ, Roalson EH (2016) Clarification of the Use of the Terms Perigynium and Utricle in *Carex* L. (Cyperaceae). Systematic Botany 41(3): 519–528. <https://doi.org/10.1600/036364416X692488>
- Jin XF, Liu YD, Lu YF, Sun WY, Wang H (2020) Notes on *Carex* (Cyperaceae) from China (VI): The identity of *Carex retrofracta* Kuk. (sect. *Confertiflorae*). Phytotaxa 429(2): 135–147. <https://doi.org/10.11646/phytotaxa.429.2.4>

- Kükenthal G (1899) Die *Carex* vegetation des außertropischen Südamerika (ausgenommen Paraguay und Südbrasilien). Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 27: 485–563.
- Kükenthal G (1909) Cyperaceae-Caricoideae. In: Engler HGA (Ed.) Das Pflanzenreich. Wilhelm Engelmann, Leipzig, 824 pp.
- Larridon I, Zuntini AR, Lévillé-Bourret É, Barrett RL, Starr JR, Muasya AM, Villaverde T, Bauters K, Brewer GE, Bruhl JJ, Costa SM, Elliott TL, Epiawalage N, Escudero M, Fairlie I, Goetghebeur P, Hipp AL, Jiménez-Mejías P, Sabino Kikuchi IAB, Luceño M, Márquez-Corro JI, Martín-Bravo S, Maurin O, Pokorny L, Roalson EH, Semmouri I, Simpson DA, Spalink D, Thomas WW, Wilson KL, Xanthos M, Forest F, Baker WJ (2021) A new classification of Cyperaceae (Poales) supported by phylogenomic data. Journal of Systematics and Evolution 59(4): 852–895. <https://doi.org/10.1111/jse.12757>
- Lestiboudois TG (1819) Essai sur la famille des Cypéracées. Didot Jeune, Paris, 46 pp.
- Lévillé-Bourret É, Starr JR, Ford BA (2018) Why are there so many sedges? Sumatroskirpeae, a missing piece in the evolutionary puzzle of the giant genus *Carex* (Cyperaceae). Molecular Phylogenetics and Evolution 119: 93–104. <https://doi.org/10.1016/j.ympev.2017.10.025>
- Linnaeus C (1753) Species Plantarum 2. Impensis Laurentii Salvii, Stockholm, 561–1200.
- Lu YF, Jin XF (2018) Notes on *Carex* (Cyperaceae) from China (V): New species, combination and subspecies from Zhejiang. Phytotaxa 372(3): 203–211. <https://doi.org/10.11646/phytotaxa.372.3.3>
- Lu ZC, Su YL, Lu YF, Jin XF (2020) *Carex pingleensis* (*Carex* sect. *Mitratae*), a new species of Cyperaceae from Guangxi, China. Taiwania 65: 391–395. <https://doi.org/10.6165/tai.2020.65.391>
- Martín-Bravo S, Jiménez-Mejías P, Villaverde T, Escudero M, Hahn M, Spalink D, Roalson EH, Hipp AL, Global Carex Group (2019) A tale of worldwide success: Behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. Journal of Systematics and Evolution 57: 695–718. <https://doi.org/10.1111/jse.12549>
- Nelmes E (1939) Notes on *Carex*: VIII. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 6: 305–306 <https://doi.org/10.2307/4111742>
- Nelmes E (1948) A New Species of *Carex* from Burma. Kew Bulletin 3(1): 67–68. <https://doi.org/10.2307/4118923>
- Nelmes E (1955) The Genus *Carex* in Indo-China, including Thailand and Lower Burma. Mémoires du Muséum National d'Histoire Naturelle Nouvelle Série Série B, Botanique. Editions Du Muséum, Paris, 103–108.
- Petermann WL (1849) Deutschlands Flora. Wigand, Leipzig, 668 pp.
- Raymond M (1959) Carices Indochinenses necnon Siamenses. Memoires de Jardin Botanique de Montreal 53: 36–37.
- Roalson EH, Jiménez-Mejías P, Hipp AL, Benítez-Benítez C, Bruederle LP, Chung K-S, Escudero M, Ford BA, Ford K, Gebauer S, Gehrke B, Hahn M, Hayat MQ, Hoffmann MH, Jin X, Kim S, Larridon I, Lévillé-Bourret É, Lu Y, Luceño M, Maguilla E, Márquez-Corro JI, Martín-Bravo S, Masaki T, Míguez M, Naczi RFC, Reznicek AA, Spalink D, Starr JR (2021) A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. Journal of Systematics and Evolution 59(4): 726–762. <https://doi.org/10.1111/jse.12722>

- Starr JR, Janzen FH, Ford BA (2015) Three new, early diverging *Carex* (Cariceae, Cyperaceae) lineages from East and Southeast Asia with important evolutionary and biogeographic implications. *Molecular Phylogenetics and Evolution* 88: 105–120. <https://doi.org/10.1016/j.ympev.2015.04.001>
- Thiers B (continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. <http://sweetgum.nybg.org/ih> [accessed 12 August 2021]
- Tuckerman E (1843) *Enumeratio methodica Caricum quarundam I. Riggs, Schenectadiae*, 27 pp.
- Villaverde T, Jiménez-Mejías P, Luceño M, Waterway MJ, Kim S, Lee B, Rincón-Barrado M, Hahn M, Maguilla E, Roalson EH, Hipp AL (2020) A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogenetic tree. *Botanical Journal of the Linnean Society* 194(2): 141–163. <https://doi.org/10.1093/botlinnean/boaa042>
- WCSP (2021) World checklist of selected plant families. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. <http://wcsp.science.kew.org/> [accessed 12 August 2021]
- Yang HB, Liu GD (2020) *Carex ledongensis* (Cyperaceae), a new sedge from Hainan Isl. Of South China. *Phytotaxa* 461(2): 72–78. <https://doi.org/10.11646/phytotaxa.461.2.1>
- Yen AC, Olmstead RG (2000) Molecular systematics of Cyperaceae tribe Cariceae based on two chloroplast DNA regions: *ndhF* and *trnL* intron-intergenic spacer. *Systematic Botany* 25(3): 479–494. <https://doi.org/10.2307/2666691>
- Zhang SR, Zhang J, Liu ZY, Qu S, Han RG (2018) *Carex nodosa* (Cyperaceae), a new species of *C.* sect. *Occlusae* from southwest China. *Nordic Journal of Botany* 36(8): e01900. <https://doi.org/10.1111/njb.01900>

Notes on *Carex* (Cyperaceae) from China (VIII): five new species and a new variety from southern and south-western China

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Abstract

Our field surveys and specimen examination of *Carex* from southern to south-western regions in China resulted in the discovery of five new species and one new variety, which are here named as *Carex brevihispida* X.F.Jin & Y.F.Lu (in sect. *Surculosae*), *C. puberulitriculata* Y.F.Lu & X.F.Jin (sect. *Clandestinae*), *C. paratatsiensis* Y.F.Lu & X.F.Jin (sect. *Aulocystis*), *C. huanjiangensis* S.Yun Liang ex Y.F.Lu & X.F.Jin (sect. *Decorae*), *C. liangiana* X.F.Jin & Y.F.Lu and *C. thibetica* Franch. var. *angustifolia* X.F.Jin & Y.F.Lu (sect. *Rhomboidales*).

Keywords

Carex, China, Cyperaceae, new species, new variety, sedge, taxonomy

Introduction

Carex L. (Cyperaceae: tribe Cariceae), a morphologically diverse genus with about 2000 accepted species (Govaerts et al. 2021; Roalson et al. 2021), differs from the other genera within the family Cyperaceae by having unisexual flowers and a partially or completely enclosed prophyll, which is termed a utricle here. Most recent molecular phylogenetic studies of tribe Cariceae have demonstrated the genus *Carex* could be separated into four to six clades (Yen and Olmstead 2000; Roalson et al. 2001, 2021; Starr et al. 2008; Waterway et al. 2009, 2015; Jung and Choi 2013; Yano et al.

2014; Jiménez-Mejías et al. 2016; Martín-Bravo et al. 2019; Villaverde et al. 2020), in contrast to the earlier traditional classifications, based on morphology (Kükenthal 1909; Egorova 1999; Dai et al. 2000; Dai et al. 2010). As a result, *Kobresia* Willd., *Schoenoxiphium* Nees, *Cymophyllus* Mack. and *Uncinia* Pers. were merged into *Carex*, making the circumscription of *Carex* broader and equal to tribe Cariceae (Waterway et al. 2015; Roalson et al. 2021).

China is incredibly rich in species diversity of *Carex* and 527 recorded species are distributed from southern to northern regions and grow in various habitats, such as growing in forest, on grassland, in wetland or in sand (Dai et al. 2010). We carried out a taxonomic study of *Carex* from China since 2008. Recently, our field surveys and specimen examination of *Carex* have resulted in the discovery of several new taxa (Zhou and Jin 2014; Jin et al. 2015a, 2015b; Chen and Jin 2015; Lu and Jin 2017, 2018). In the present study, we describe another five distinctive new species and one new variety from the southern and south-western regions of China.

Materials and methods

Over 20000 collections of *Carex* from East Asia, which were preserved in 27 Herbaria (alphabetically BM, CDBI, E, FJFC, FNU, GXMI, HGAS, HHBG, HNNU, HTC, HZU, IBK, IBSC, K, KUN, KYO, LBG, LE, N, NAS, P, PE, SZ, TI, WUK, ZJFC, and ZM), were examined. Our study is mainly based on these Herbarium collections and the descriptions for the new species and variety were also derived from the collected specimens. Width of leaves, length of glumes, utricles and nutlets were all measured from mature collections and descriptions of indumentum, colour of glumes, utricles and nutlets were observed from these specimens as well. These new taxa were critically compared with the type specimens of the relatives.

Taxonomic treatment

1. *Carex brevibispida* X.F.Jin & Y.F.Lu, sp. nov.

urn:lsid:ipni.org:names:77235053-1

Figure 1A–G

Latin diagnosis. *Haec species est affinis C. kwangsiensi* F.T. Wang & Tang ex P.C. Li, a qua foliis caulinis, bracteis et pedunculis secundariis omnibus dense brevi-hispidis, squamis pistillatis 3–3.5 mm longis, dorso sparse pubescentibus vel subglabris differt.

Type. CHINA. Guangxi: Baise, Youjiang Dist., Daleng, Mount Dawangling, 23°44'29.95"N, 106°23'28.85"E, by stream under forest, alt. 770 m, 26 Apr 2019, X.F. Jin, W.J. Chen, X. Cai & Y.L. Xu 4408 (holotype: ZM; isotypes: ZJFC, ZM).

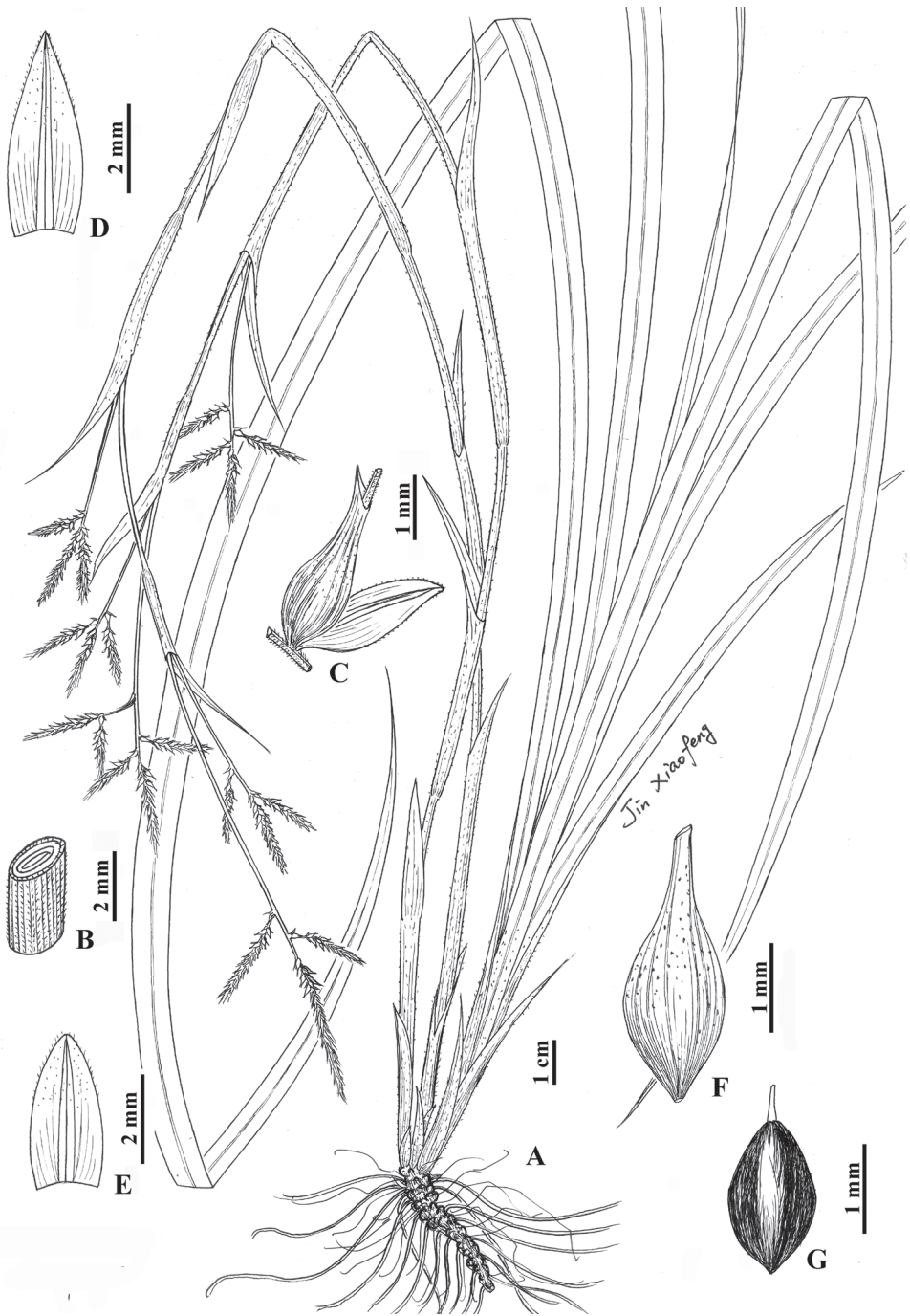


Figure 1. *Carex brevihispida* sp. nov. **A** habit **B** part of culm **C** cladophyll **D** staminate glume **E** pistillate glume **F** utricle **G** nutlet (Drawn by Xiao-Feng Jin; based on holotype: *Xiao-Feng Jin et al.* 4408 in ZM).

Rhizomes woody, thick, moniliform, creeping or obliquely ascending. Culms pseudolateral, 40–65 cm tall, ca. 2 mm thick, trigonous, base with grey-brown sheath. Leaves basal and cauline; basal leaves longer than or almost equal to culms, several ones forming a high shoot, blades flat or slightly revolute at margin, 5–7.5 mm wide, adaxially glabrous, abaxially glabrous or densely short-hispidulous along mid-ribs, scabrous on both surfaces and margins; cauline leaves spathe-like, lower ones rarely shortly leaf-like, purple-red when fresh, pale red-brown when dried, densely short-hispidulous. Bracts spathe-like, densely short-hispidulous. Panicle compound; inflorescence branches corymbose, single or binate, 3–5 cm long, 1.5–4.5 cm wide, with 2–5 spikes, rarely solitary; peduncles of inflorescence branches slender, densely short-hispidulous, exerted from bract sheath; inflorescence axes acutely trigonous, densely short-hispidulous; bractlets scale-like, lanceolate, 3–5 mm long, apex obtuse, glabrous, purple-red spotted; cladophylls utriculiform, ca. 2.5 mm long, distinctly thinly veined, orifice obliquely truncate. Spikes exerted from cladophylls, obliquely or horizontally patent, androgynous, 1–4.5 cm long, staminate part clavate or oblong, densely flowered, longer than or almost equal to pistillate part, pistillate part sparsely 1–8-flowered. Staminate glumes narrowly ovate-elliptic or elliptic-lanceolate, yellow-brown or brown, 3–4.5 mm long, apex acuminate or obtuse, upper margin ciliate, with yellow 3-veined costa. Pistillate glumes ovate or elliptic-ovate, yellow-brown, 3–3.5 mm long, apex obtuse, upper margin ciliate, abaxially sparsely pubescent or glabrous, with yellow 3-veined costa. Utricles brown-green, ovoid, obtusely trigonous, 2.5–3 mm long, obliquely patent, densely purple-red papillose, distinctly thinly veined, sparsely pubescent along veins, base with 0.2–0.3 mm long stipe, apex gradually contracted into a ca. 0.7 mm long beak, orifice obliquely truncate. Nutlets tightly enveloped, grey-brown, ovoid, trigonous, 1.7–2 mm long, base with ca. 0.3 mm long stipe; style base slightly thickened; stigmas 3.

Etymology. The specific epithet ‘*brevihispida*’ refers to the culms, inflorescence bracts and peduncles of inflorescence branches that are all densely short-hispidulous.

Phenology. Flowering and fruiting is from late March to late April.

Additional specimens examined. CHINA. Guangxi: Baise, Youjiang Dist., Daleng, Mount Dawangling, 23°44'29.95"N, 106°23'28.85"E, by stream under forest, alt. 770 m, 26 Apr 2019, *X.F. Jin, W.J. Chen, X. Cai & Y.L. Xu* 4406 (ZM); the same locality, on cliff under forest, alt. 774 m, 26 Apr 2019, *X.F. Jin, W.J. Chen, X. Cai & Y.L. Xu* 4399 (ZJFC, ZM).

Conservation status. Near threatened (NT). The new species is currently known from the type locality, Mount Dawangling and grows by stream under forest. Tourists in the scenic region may interfere with the new species (IUCN 2019).

Notes. The new species, *Carex brevihispida*, has spathe-like cauline leaves and bracts and lateral culms, which morphologically belongs to sect. *Surculosae* in subg. *Vigneastr*a (Dai et al. 2010). Recent phylogenetic hypotheses revealed the *Siderostictae* clade includes all species traditionally placed in sections *Siderostictae*, *Hemiscaposae* and *Surculosae* (Yano et al. 2014; Martín-Bravo et al. 2019; Villaverde et al. 2020; Roalson et al. 2021). It is morphologically similar to *Carex kwangsiensis*, but differs in having cauline leaves, bracts and secondary peduncles all densely short-hispidulous, pistil-

late glumes 3–3.5 mm long, dorsally sparsely pubescent or nearly glabrous. In *Carex kwangsiensis*, the cauline leaves, the bracts and the secondary peduncles are pubescent and the pistillate glumes are 2–2.5 mm long and dorsally pubescent.

2. *Carex puberuliutriculata* Y.F.Lu & X.F.Jin, sp. nov.

urn:lsid:ipni.org:names:77235054-1

Figure 2A–E

Latin diagnosis. *Affinis* *C. pseudohumili* F.T. Wang & Y.L. Chang ex P.C. Li, a qua utriculis superne puberulis, apice pubescentibus, squamis pistillatis atro-purpureis vel brunneis manifeste longioribus, nucibus obovoideis recedit.

Type. CHINA. Sichuan: Baoxing, Fengtongzhai Natural Reserve, from Sandaoniupeng to Yuanyanyao, roadside grasses, alt. 3400–3700 m, 9 Jul 2017, X.H. Xiong 1129 (holotype: ZM; isotypes: ZJFC, ZM).

Rhizomes short, woody. Culms central, caespitose, 6.5–20 cm tall, obtusely trigonous, smooth, base with yellow-brown or dark brown fibrous sheaths. Leaves almost equal to or shorter than culms; blades flat, 0.7–1.5 mm wide, margin slightly revolute, scabrous, apex curved or slightly circinate. Bracts spathe-like, shorter than inflorescence, base with 0.3–1.2 cm long sheaths. Spikes 2–4, upper ones aggregated, sometimes with lowest one exerted from basal culms; terminal spike staminate, oblong or clavate-cylindrical, 1.1–2 cm long, 1.5–3 mm wide, base with 0.8–1.2 cm long peduncles; lateral spikes pistillate, oblong or ovoid, 0.6–1 cm long, 3–3.5 mm wide, densely 7–15-flowered, peduncles erect, exerted from sheaths, 0.5–1.9 cm long. Staminate glumes obovate, purple-black or brown, 4–4.5 mm long, apex acute or obtuse, with pale yellow 1-veined pubescent costa excurrent into a mucro. Pistillate glumes ovate, purple-black or brown, 2.3–2.5 mm long, apex acute or obtuse, with yellow-brown 1- or 3-veined pubescent costa excurrent into a 0.5–1 mm long scabrous awn. Utricles pale yellow-brown, obovoid, obtusely trigonous, 3–3.3 mm long, membranous, obliquely patent, puberulent on upper part, laterally 2-veined, inconspicuously thinly veined, base cuneate and shortly curved stipitate, apex abruptly contracted into a ca. 0.3 mm long beak, orifice emarginate or obliquely truncate. Nutlets tightly enveloped, pale yellow, obovoid, trigonous, 2.2–2.3 mm long, base shortly stipitate, apex beakless; style base not thickened; stigmas 3.

Etymology. The specific epithet ‘puberuliutriculata’ refers to the puberulent utricles of the new species.

Phenology. Flowering and fruiting is in early July.

Additional specimen examined. CHINA. Sichuan: Baoxing, Fengtongzhai Natural Reserve, from Sandaoniupeng to Yuanyanyao, in grasses, alt. 3400–3700 m, 9 Jul 2017, X.H. Xiong 1128 (ZJFC, ZM).

Conservation status. Least Concern (LC). The new species is a common grass in the meadow of Fengtongzhai at an elevation from 3300 to 4000 m. Local animal grazing may have an impact on this species (IUCN 2019).

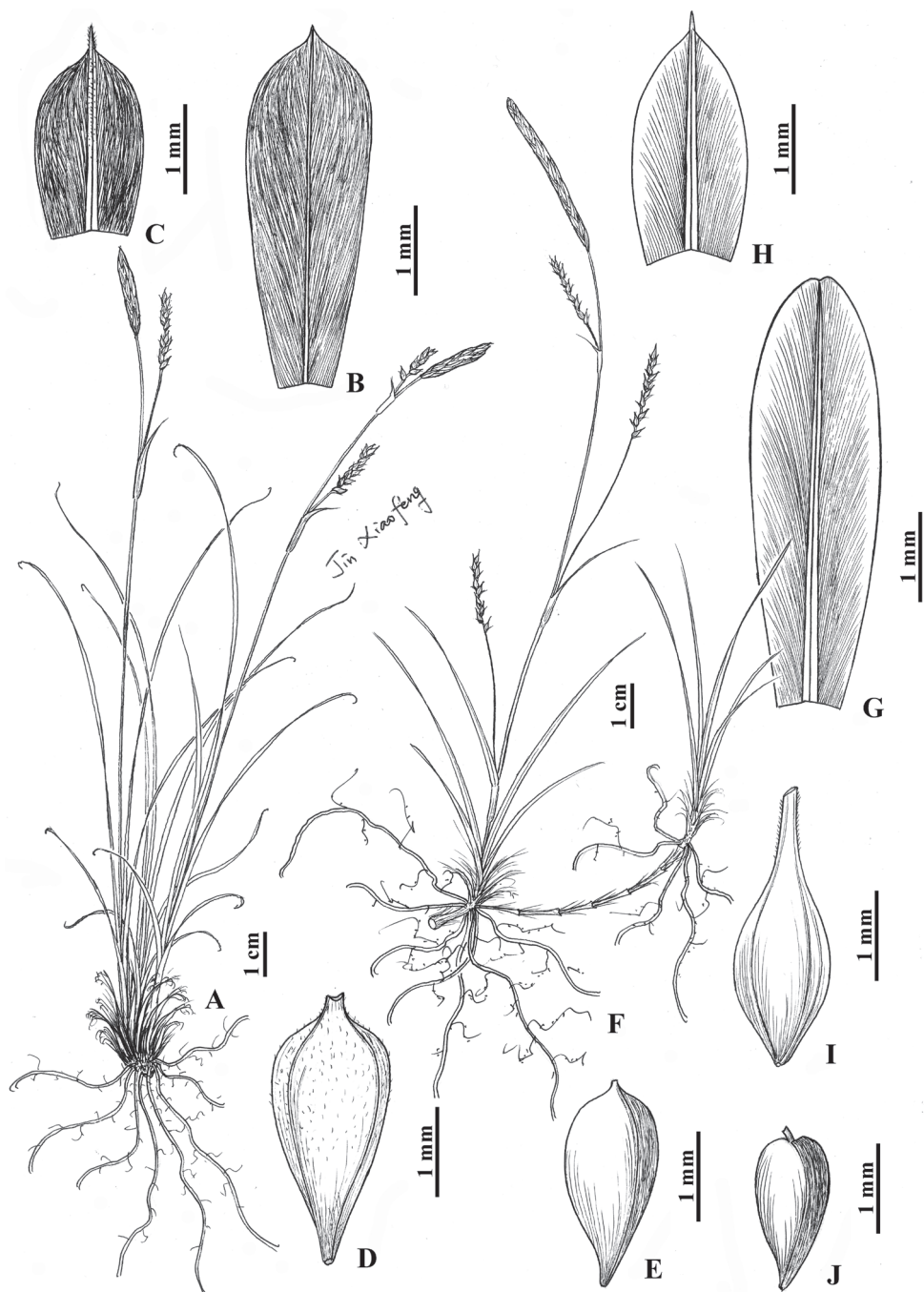


Figure 2. A–E *Carex puberuliutriculata* sp. nov. A habit B staminate glume C pistillate glume D utricle E nutlet F–J *Carex paratatsiensis* sp. nov. F habit G staminate glume H pistillate glume I utricle J nutlet (Drawn by Xiao-Feng Jin; based on holotype: X.H. Xiong 1129 for *C. puberuliutriculata* in ZM and holotype: X.H. Xiong 999A for *C. paratatsiensis* in ZM).

Notes. The new species is somewhat morphologically similar to *Carex pseudohumilis* in having hairy utricles, inconspicuous beaks and leaves curved or slightly circinate at the apex (Dai et al. 2000, 2010), but it differs from the latter in having pistillate glumes purple-black or brown, utricles puberulent on upper part and longer than pistillate glumes and nutlets obovoid. Herein, it is placed in sect. *Clandestinae*, which is part of the poorly resolved Hallerianae-Digitatae clade (Roalson et al. 2021).

3. *Carex paratatsiensis* Y.F.Lu & X.F.Jin, sp. nov.

urn:lsid:ipni.org:names:77235056-1

Figure 2F–J

Latin diagnosis. *Haec species nova C. tatiensi* (Franch.) Kük. *affinis est, sed utriculis brevioribus, 3–3.2 mm longis, membranaceis, squamis pistillatis ovatis vel late ovatis, nucibus obovoideis, stigmatibus 2 vel 3 differt.*

Type. CHINA. Tibet: Mêdog, Dayandong, 29°25'45.54"N, 95°02'58.37"E, in thickets on slope, alt. 2950 m, 7 Jun 2017, X.H. Xiong 999A (holotype: ZM; isotypes: ZJFC, ZM).

Rhizomes slender, long, woody, long-stoloniferous. Culms central, 14–30 cm tall, slender, obtusely trigonous, lower part smooth and upper part scabrous, base with red-brown fibrous sheaths. Leaves shorter than culms; blades flat, 1–2 mm wide, margin scabrous. Bracts shortly leaf-like or uppermost setaceous, shorter to longer than inflorescence, base with 0.5–2 cm long sheaths. Spikes 2–4, remote; terminal 1 or 2 spikes staminate, narrowly cylindrical, 1–3.5 cm long, 1–2.5 mm wide, base with 0.3–6 cm long peduncles; lateral spikes pistillate, single or rarely binate, cylindrical, 0.8–2.7 cm long, 2.5–4 mm wide, 8–18-flowered, peduncles erect, slender, 0.3–7.5 cm long, exerted from sheaths. Staminate glumes obovate-lanceolate, red-brown, 5–5.5 mm long, apex acute or emarginate, with yellow 3-veined costa excurrent into a mucro. Pistillate glumes ovate or broadly ovate, red-brown, 2.5–2.8 mm long, margin whitish hyaline, apex acute or emarginate, with yellow-brown 3-veined costa excurrent into a mucro. Utricles red-brown and yellow-green below, ellipsoid, obtusely trigonous, 3–3.2 mm long, membranous, obliquely patent, inconspicuously several thinly veined, base cuneate and shortly stipitate, apex gradually contracted into a ca. 1 mm long beak, orifice truncate or 2-lobed with minute teeth, margin barbate. Nutlets tightly enveloped, yellow, obovoid, trigonous, 1.8–2 mm long, apex with ca. 0.3 mm long curved beak; style base not thickened; stigmas 2 or 3.

Etymology. The specific epithet 'paratatsiensis' refers to the similarity with *Carex tatsiensis*.

Phenology. Flowering and fruiting is in early June.

Additional specimen examined. CHINA. Tibet: Mêdog, Dayandong, 29°25'45.54"N, 95°02'58.37"E, in thickets on slope, alt. 2950 m, 7 Jun 2017, X.H. Xiong 999B, 999C (ZJFC, ZM).

Conservation status. Data Deficient (DD). There is inadequate information for distribution and population status and we could not make a direct assessment of its risk of extinction now (IUCN 2019).

Notes. This new species is similar to *Carex tatsiensis*, which was placed in sect. *Aulocystis*, but differs in having utricles shorter (3–3.2 mm long), membranous, pistillate glumes ovate or broadly ovate and nutlets obovoid with 2 or 3 stigmas. Based on the phylogenetic hypotheses, the traditional taxonomic section *Aulocystis* was polyphyletic and clustered with some species of sect. *Clandestinae*, which made it a heterogeneous group (Roalson et al. 2021). In some descriptions of *Carex tatsiensis* (Dai et al. 2000, 2010), the utricles were described as membranous, but our examination showed the utricles of *C. tatsiensis* are thin-coriaceous or coriaceous (Jin and Lu 2020).

4. *Carex huanjiangensis* S.Yun Liang ex Y.F.Lu & X.F.Jin, sp. nov.

urn:lsid:ipni.org:names:77235058-1

Figure 3A–H

Latin diagnosis. *Haec species C. perakensi C.B. Clarke affinis est, sed squamis staminatis et pistillatis fulvis, utriculis 9–10 mm longis, glabris, rostris margine serrulatis, stylis basi glabris differt.*

Type. CHINA. Guangxi: Huanjiang, Dongxing Town, Jiupengtun, by stream, alt. 1200 m, 22 May 1989, *Beijing Exped 894059* (holotype: PE).

Rhizomes dark brown, woody, stiff, sometimes stoloniferous, with black-brown fibrous roots. Culms central, loosely caespitose, 40–80 cm tall, trigonous. Leaves basal and cauline; basal leaves longer than or almost equal to culms, blades 5–12 mm wide, coriaceous, apex acuminate, margin scabrous; cauline leaves 1 or 2, sometimes absent, slightly longer than inflorescence, blades 5–9 mm wide, coriaceous, apex acuminate, margin scabrous. Bracts leaf-like, longer than or slightly longer than inflorescence, 2.5–8 mm wide, with the lowest sheath to 2 cm long, upper sheaths shorter or sheath absent. Panicle compound, 15–28 cm long, 2 inflorescence branches in each bract sheath, rarely single; inflorescence branch 5–8.5 cm long, base pedunculate; bractlets scale-like, broadly ovate-round, yellow-brown, 4–5 mm long, ca. 4.5 mm wide, apex obtuse or mucronate, with yellow 1-veined costa. Spikes 13–46, sessile, 4–7 in a racemose; terminal spikes narrowly ovate or ovate-elliptic, 13–17 mm long, 6–7 mm wide, base with 3–6 pistillate flowers, staminate part 6–8 mm long; lateral spikes elliptic-lanceolate, 7–12 mm long, 2–3.5 mm wide, base with single pistillate flower, staminate part 5–8 mm long. Staminate glumes narrowly obovate or obovate-elliptic, yellow-brown, 6.5–7 mm long, apex acuminate or obtuse, with yellow 1-veined costa. Pistillate glumes broadly ovate, yellow-brown, 6.5–7 mm long, apex obtuse, with yellow 1-veined costa excurrent into a 0.5–1 mm long scabrous awn. Utricles pale brown, obovoid, compressed trigonous, 9–10 mm long, obliquely patent, glabrous, abaxially, and adaxially with 9 or 10 veins, respectively, apex gradually contracted into

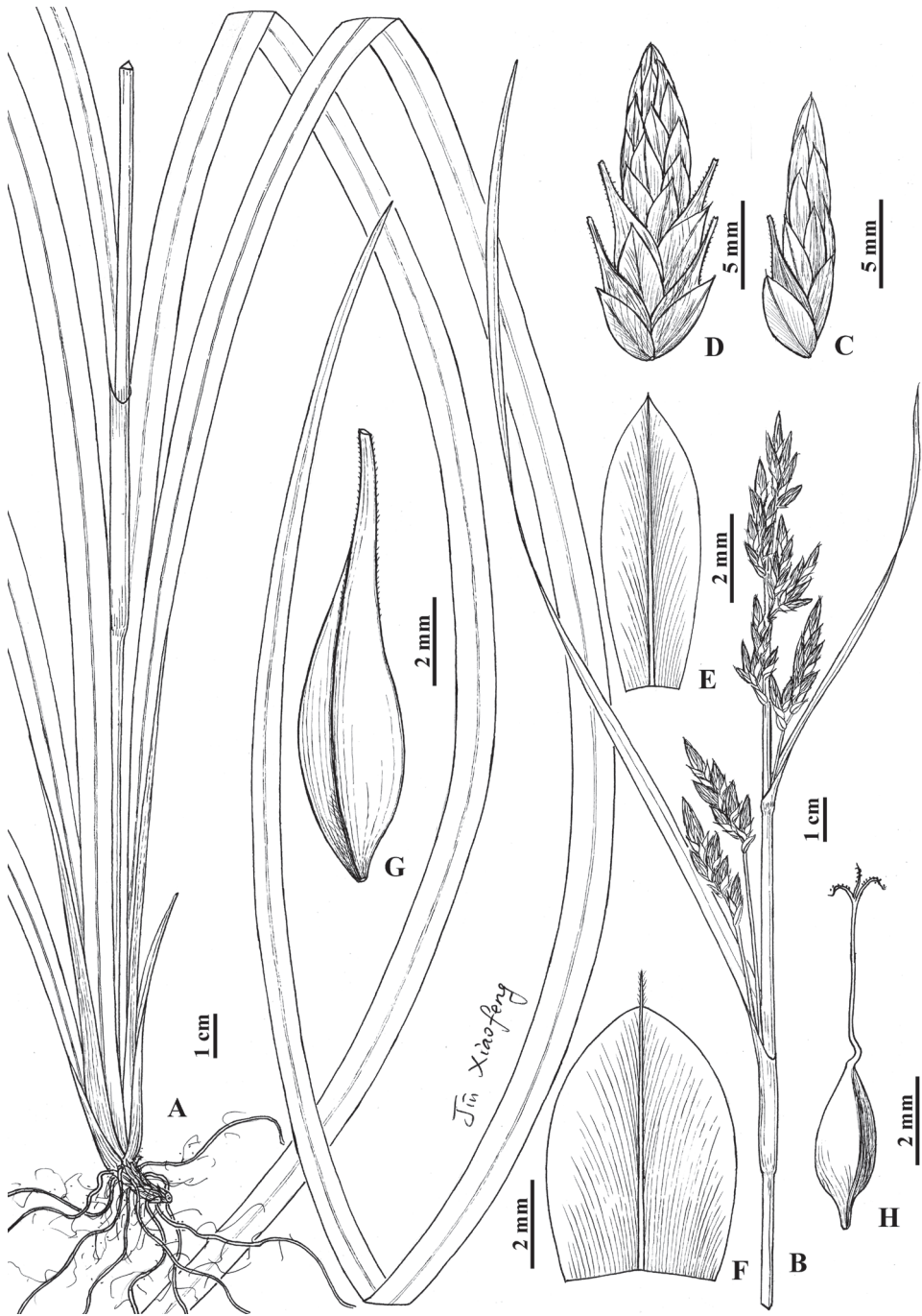


Figure 3. *Carex huanjiangensis* sp. nov. **A** lower part of habit **B** upper part of habit **C** lateral spike **D** terminal spike **E** staminate glume **F** pistillate glume **G** utricule **H** nutlet (Drawn by Xiao-Feng Jin; based on holotype: Beijing Exped 894059 in PE).

a 3–3.5 mm long erect beak, orifice obliquely truncate, margin barbate. Nutlets loosely enveloped, grey-brown, narrowly ovoid, trigonous, ca. 3.5 mm long, base with ca. 0.7 mm long stipe; style base curved, not thickened; stigmas 3.

Etymology. The specific epithet ‘huanjiangensis’ refers to the type locality of this new species, Huanjiang County of Guangxi Zhuang Autonomous Region.

Phenology. Flowering and fruiting is in early to late May.

Additional specimens examined. CHINA. Guangxi: Huanjiang, Jiuren Forestry Farm, on slope under forest, alt. 1450 m, 21 May 1989, *Beijing Exped.* 892928 (PE); the same locality, alt. 980 m, 21 May 1989, *Beijing Exped.* 893056 (PE); on slope under forest, alt. 1000 m, 25 May 1989, *Beijing Exped.* 895076 (PE); by stream, alt. 700 m, 25 May 1989, *Beijing Exped.* 895011 (PE); Rongshui, Xiangcaopeng, by stream, alt. 1000 m, 2 May 1989, *Beijing Exped.* 892115 (PE).

Conservation status. Least Concern (LC). The new species grows on slopes under the forests in Huanjiang and Rongshui Counties, where are seriously disturbed by local people (IUCN 2019).

Notes. Based on phylogenetic analyses, the large *Decora* clade includes most species in sections *Decorae* and *Indicae* (Roalson et al. 2021). Herein, we identified the former section as lacking utriculiform cladophylls, whereas they are present in section *Indicae*. This new species is similar to *Carex perakensis*, but differs in having both staminate and pistillate glumes yellow-brown, utricles longer (9–10 mm long), glabrous, with beak margins barbate and styles glabrous. In *Carex perakensis*, the glumes are pale yellow-brown, the utricles are 4.5–6 mm long, densely hispidulous and styles are sparsely barbate.

5. *Carex liangiana* X.F.Jin & Y.F.Lu, sp. nov.

urn:lsid:ipni.org:names:77235060-1

Figure 4A–E

Latin diagnosis. *Species nova est affinis C. diplodo Nelmes, a qua spicis terminalibus androgynis, squamis pistillatis ovatis, apice breviter et scabrose aristatis, utriculis glabris, nucibus apice erostris facile differt.*

Type. CHINA. Sichuan: Kangding, Zhonggucun, Wachang, 30°15'16.72"N, 101°52'47.75"E, in grass along stream, alt. 3061 m, 1 Aug 2019, X.F. Jin, Y.F. Lu & X.H. Xiong 4518 (holotype: ZM; isotypes: ZJFC, ZM)

Rhizomes creeping, woody, stiff, with many brown fibrous roots. Culms central, loosely caespitose, 25–60 cm tall, acutely trigonous, smooth, glabrous, with single bract-like leaf near base. Leaves basal, far shorter than culms; blades less than 12 cm long, flat, 2.5–5.5 mm wide, coriaceous, apex acuminate, margin slightly scabrous. Lower bracts shortly leaf-like, longer than spikes, upper ones setaceous, shorter than spikes, all bracts sheathed; sheaths 0.5–2.5 cm long. Spikes 4 or 5 in a racemose, terminal spike androgynous, clavate-cylindrical, 1.5–3 cm long, 2–2.5 mm wide, base

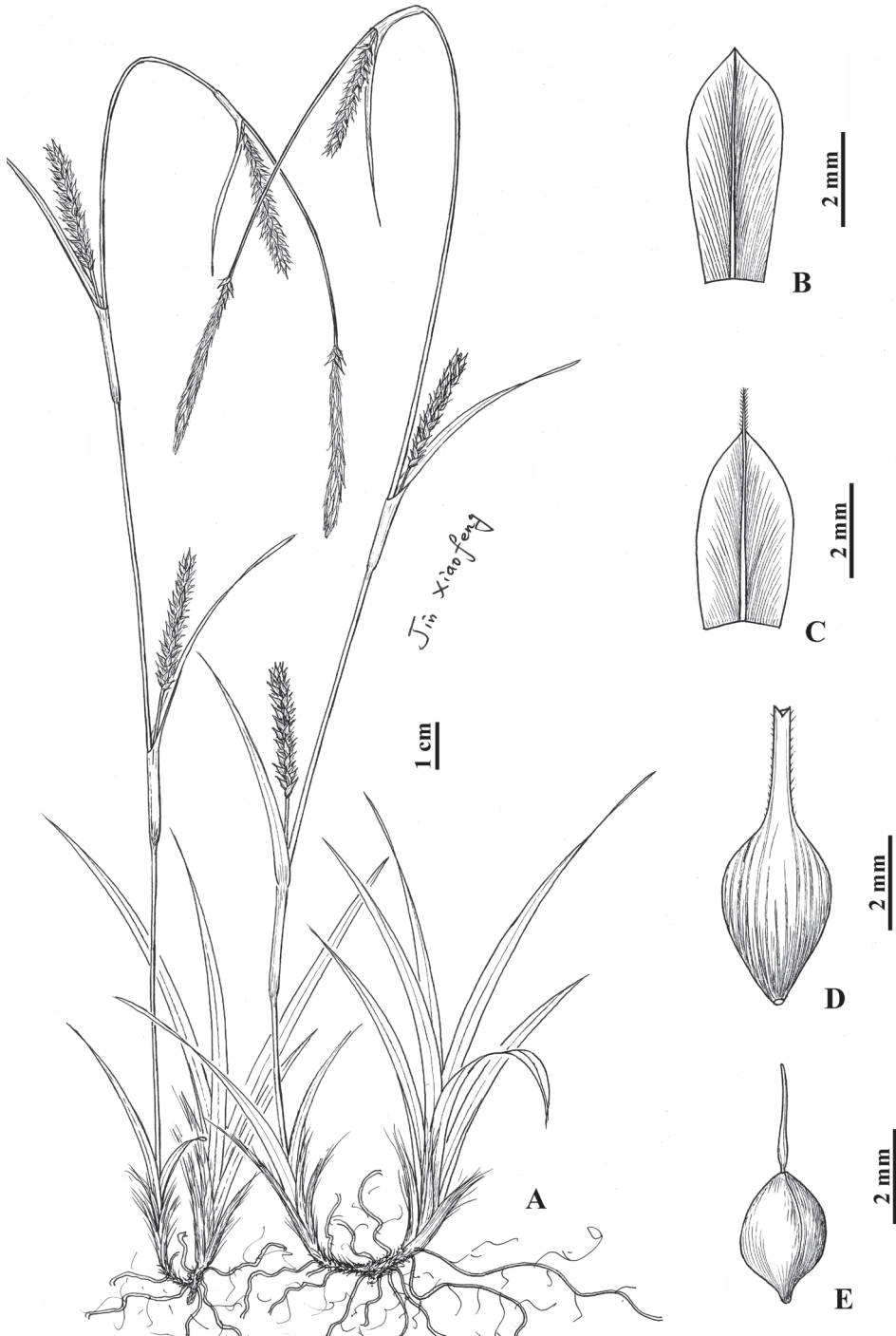


Figure 4. *Carex liangiana* sp. nov. **A** habit **B** staminate glume **C** pistillate glume **D** utricle **E** nutlet
(Drawn by Xiao-Feng Jin; based on holotype: X.F. Jin, Y.F. Lu & X.H. Xiong 4518 in ZM).

with 1–6 pistillate flowers and a 1–2.5 cm long peduncle, lateral spikes pistillate, sometimes with 3–6 staminate flowers at apex, cylindrical, 1–2.5 cm long, 5–5.5 mm wide, 8–24-flowered, lower 1 or 2 peduncles exerted from sheaths, upper ones enclosed. Staminate glumes obovate, yellow-brown, 4.5–5 mm long, apex acute, with yellow 1-veined costa excurrent into a mucro. Pistillate glumes ovate, yellow-brown, margin whitish hyaline, 4–4.3 mm long, apex acute or obtuse, with yellow 1-veined costa excurrent into a 0.5–1 mm scabrous awn. Utricles yellow-brown, ovoid, obtusely trigonous, 5–6 mm long, obliquely patent, abaxially thinly 11–14-veined, adaxially thinly 8- or 9-veined, glabrous, apex gradually contracted into a 2–2.5 mm long beak, orifice 2-lobed with short teeth, margin barbate or smooth. Nutlets tightly enveloped, yellow-brown, broadly ovoid, trigonous, 2.3–2.5 mm long, base with a stipe ca. 0.3 mm long; style base slightly thickened; stigmas 3.

Etymology. The specific epithet 'liangiana' is in honour of Prof. Song-Yun Liang, who is a Chinese researcher on the taxonomy of Cyperaceae and Liliaceae.

Phyeology. Flowering and fruiting is from late June to early August.

Additional specimens examined. CHINA. Sichuan: Kangding, Zhonggucun, Wachang, 30°15'16.72"N, 101°52'47.75"E, in grass along stream, alt. 3061 m, 1 Aug 2019, *X.F. Jin, Y.F. Lu & X.H. Xiong* 4512 (ZJFC, ZM); the same locality, in grass, alt. 3370 m, 29 Jul 1963, *W. Sichuan Exped. (K.C. Kuan & W.T. Wang)* 505 (PE). Gansu: Zhouqu, Wuping Dist., Shatan Forestry Farm, in wetland, alt. 2300 m, 26 Jun 1964, *P.C. Kuo* 5115 (WUK).

Conservation status. Vulnerable, VU B2aC1 (IUCN 2019). This new species is known from two localities, Kangding of Sichuan Province and Zhouqu of Gansu Province, the area of occupancy is less than 10000 km² and the estimated individuals are less than 5000 individuals in the two populations.

Notes. It is a remarkable species in sect. *Rhomboidales* with the terminal spikes androgynous and the nutlets not beaked at the apex. It is somewhat similar to *Carex diplodon* in the shape of the utricles and nutlets, but the new species has culms that are loosely tufted with elongate rhizomes and the nutlets neither concave nor excavated on the faces, which differentiates it from the majority of species in sect. *Rhomboidales* (Jin and Zheng 2013; Jin et al. 2014). Further phylogenetic study is needed to establish its relationships.

6. *Carex thibetica* Franch. var. *angustifolia* X.F.Jin & Y.F.Lu, var. nov.

urn:lsid:ipni.org:names:77235062-1

Figure 5A–E

Latin diagnosis. *A var. thibetica differt foliis 4–6.5 mm latis, spicis staminatis 1.5–2 mm latis, utriculis longioribus 9–12 mm longis.*

Type. CHINA. Hunan: Dongan, Shunhuangshan Forestry Park, Butterfly Valley, by stream under forest, alt. 680 m, 21 May 2017, *H. Wang* 1434 (holotype: ZM; isotypes: ZJFC, ZM).

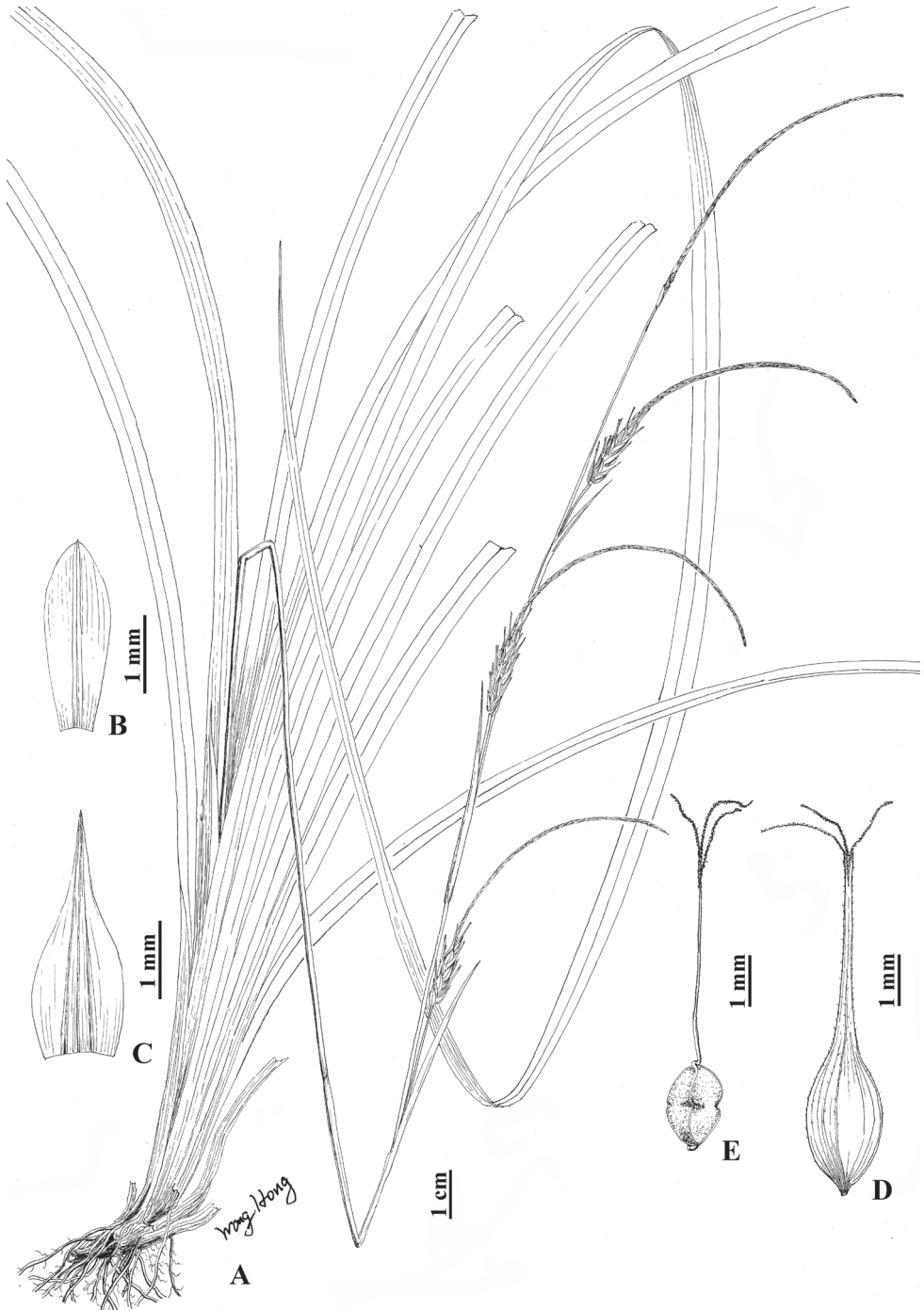


Figure 5. *Carex thibatica* var. *angustifolia* var. nov. **A** habit **B** staminate glume **C** pistillate glume **D** utricule **E** nutlet (Drawn by Hong Wang; based on holotype: *H. Wang* 1434 in ZM).

Rhizomes short or elongate, woody, stiff, with many brown fibrous roots. Culms lateral, loosely caespitose, 20–55 cm tall, obtusely trigonous, smooth, glabrous. Leaves basal, slightly shorter to longer than culms; blades flat, 4–6.5 mm wide, coriaceous, apex acuminate, lower surfaces and margin slightly scabrous. Bracts shortly leaf-like, shorter than spikes, sheathed; sheaths 1.5–2.5 cm long. Spikes 3 or 4 in a racemose; terminal spike staminate, narrowly linear-cylindrical, 3.5–8 cm long, 1.5–2 mm wide, base with 4–8 cm long peduncles; lateral spikes androgynous, staminate part longer than pistillate part, cylindrical, 4–9 cm long, 7–10 mm wide, 8–20-flowered (pistillate), with peduncles exerted from sheaths. Staminate glumes lanceolate or lanceolate-oblong, pale yellow-green, 7–7.5 mm long, apex acuminate, with pale brown 1-veined costa. Pistillate glumes narrowly ovate, pale green-brown or brown, 8.5–11 mm long, apex acuminate, with green or brown-green 3-veined costa. Utricles yellow-brown or brown, rhombic-ovoid, obtusely trigonous, 9–12 mm long, slightly longer than pistillate glumes, nearly erect or obliquely patent, distinctly thinly veined, sparsely puberulent on upper part, apex abruptly contracted into a 4.5–6 mm long beak, orifice 2-lobed with long teeth, margin barbate. Nutlets tightly enveloped, brown, rhombic-ovoid, trigonous, 3–4 mm long, base with a ca. 0.5 mm long curved stipe, apex abruptly contracted into a coiled short beak, with 3 angles constricted at middle and sides concave above and below; style base slightly thickened; stigmas 3.

Etymology. The variety epithet ‘angustifolia’ refers to the narrower leaves (4–6.5 mm wide) than the typical variety (6–17 mm wide).

Phenology. Flowering and fruiting is from mid-April to late May.

Additional specimens examined. CHINA. Hunan: Dongan, Shunhuangshan Forestry Park, Butterfly Valley, by stream under forest, alt. 680 m, 21 May 2017, *H. Wang 1433* (ZJFC, ZM); the same locality, alt. 730 m, 19 Apr 2018, *W.J. Chen 2394* (HTC, ZM), *2395* (ZJFC, ZM), *2396* (ZJFC, ZM). Suining, Huangsang Natural Reserve, Quyougu, under forest, alt. 755 m, 26 Apr 2014, *J.J. Zhou & Z.P. Song 1404223* (CSFI); the same locality, Banchong, under forest, alt. 906 m, 2 May 2014, *J.J. Zhou & Z.P. Song 1405022* (CSFI).

Conservation status. Least Concern (LC). The new variety is known from four localities in southern Hunan Province, but two of them are seriously disturbed by local people (IUCN 2019).

Notes. The new variety differs from the typical variety in having the leaves narrower, 4–6.5 mm in width, terminal staminate spikes 1.5–2 mm in width and utricles longer, 9–12 mm long. It differs from *Carex thibatica* var. *pauciflora* in having lateral spikes with densely 8–20 pistillate flowers and utricles longer, 9–12 mm long.

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References

- Chen WJ, Jin XF (2015) *Carex huangshanica* (sect. *Rhomboidales*, Cyperaceae), a new species from Anhui, eastern China. *Phytotaxa* 233(3): 281–287. <https://doi.org/10.11646/phytotaxa.233.3.5>
- Dai LK, Liang SY, Tang YC, Li PC (2000) *Carex* L. In: Dai LK, Liang SY (Eds) *Flora Reipublicae Popularis Sinicae* (Vol. 12). Science Press, Beijing, 56–518.
- Dai LK, Liang SY, Zhang SR, Tang Y, Koyama T, Tucker GC (2010) *Carex* L. In: Wu ZY, Raven PH, Hong DY (Eds) *Flora of China* (Vol. 23). Acoraceae through Cyperaceae: Cyperaceae. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 285–461.
- Egorova TV (1999) The sedges (*Carex* L.) of Russia and adjacent states (within the limits of the former USSR). St. Petersburg State Chemical-Pharmaceutical Academy, St. Petersburg and Missouri Botanical Garden, St. Louis, 772 pp.
- Govaerts R, Jiménez-Mejías P, Koopman J, Simpson D, Goetghebeur P, Wilson K, Egorova T, Bruhl J (2021) World Checklist of Cyperaceae. Facilitated by the Royal Botanic Gardens, Kew. <http://wccsp.science.kew.org/>
- IUCN (2019) Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommittee. <https://www.iucnredlist.org/resources/redlistguidelines> [accessed on 15 March 2021]
- Jiménez-Mejías P, Hahn M, Lueders K, Starr JR, Brown B, Chung KS, Escudero M, Ford BA, Ford KA, Gebauer S, Gehrke B, Hoffmann M, Jung J, Kim S, Luceño M, Maguilla E, Martín-Bravo S, Míguez M, Molina A, Naczi RFC, Reznicek AA, Villaverde T, Waterway MJ, Wilson K, Jin XF, Yang JC, Zhang SR, Hipp AL, Roalson EH (2016) Megaphylogenetic specimen-level approaches to the *Carex* (Cyperaceae) phylogeny using barcode regions ITS, ETS, and matK: Systematics implications. *Systematic Botany* 41: 500–518. <https://doi.org/10.1600/036364416X692497>
- Jin XF, Lu YF (2020) sect. *Aulocystis* In: Jin XF, Zhang SR (Eds) *Flora of Pan-Himalaya Vol 12(2) Cyperaceae II*. Science Press, Beijing, 212–250.
- Jin XF, Zheng CZ (2013) Taxonomy of *Carex* sect. *Rhomboidales* (Cyperaceae). Science Press, Beijing, 237 pp.
- Jin XF, Zhou YY, Hipp A, Jin SH, Oda J, Ikeda H, Yano O, Nagamasu H (2014) Nutlet micromorphology of *Carex* section *Rhomboidales* sensu Kükenenthal (Cyperaceae) and its systematic implications. *Botanical Journal of the Linnean Society* 175(1): 123–143. <https://doi.org/10.1111/boj.12149>
- Jin XF, Cen JM, Hahn M, Lu YF, He JJ (2015a) Notes on *Carex* (Cyperaceae) from China (II): A taxonomic revision of sect. *Radicales* (Kük.) Nelmes. *Phytotaxa* 217(2): 117–132. <https://doi.org/10.11646/phytotaxa.217.2.2>

- Jin XF, Chen WJ, Cen JM (2015b) Notes on *Carex* (Cyperaceae) from China (III): The description of four new species. *Phytotaxa* 231(2): 165–174. <https://doi.org/10.11646/phytotaxa.231.2.5>
- Jung J, Choi HK (2013) Recognition of two major clades and early diverged groups within the subfamily Cyperoideae (Cyperaceae) including Korean sedges. *Journal of Plant Research* 126(3): 335–349. <https://doi.org/10.1007/s10265-012-0534-2>
- Kükenthal G (1909) Cyperaceae-Caricoideae. In: Engler A (Ed.) *Das Pflanzenreich* (Vol. 20). Wilhelm Engelmann, Leipzig, 1–824.
- Lu YF, Jin XF (2017) *Carex sinosupina* sp. nov. (sect. *Lamprochlaenae*, Cyperaceae) from Gansu, north central China. *Nordic Journal of Botany* 35(5): 573–577. <https://doi.org/10.1111/njb.01565>
- Lu YF, Jin XF (2018) Notes on *Carex* (Cyperaceae) from China (V): New species, combination and subspecies from Zhejiang. *Phytotaxa* 372(3): 203–211. <https://doi.org/10.11646/phytotaxa.372.3.3>
- Martín-Bravo S, Jiménez-Mejías P, Villaverde T, Escudero M, Hahn M, Spalink D, Roalson EH, Hipp AL, Global Carex Group (2019) A tale of worldwide success: Behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. *Journal of Systematics and Evolution* 57: 695–718. <https://doi.org/10.1111/jse.12549>
- Roalson EH, Columbus JT, Friar EA (2001) Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and trnT-L-F (cpDNA) region sequences: Assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. *Systematic Botany* 26: 318–341.
- Roalson EH, Jiménez-Mejías P, Hipp AL, Benítez-Benítez C, Bruederle LP, Chung K-S, Escudero M, Ford BA, Ford K, Gebauer S, Gehrke B, Hahn M, Hayat MQ, Hoffmann MH, Jin X-F, Kim S, Larridon I, Léveillé-Bourret É, Lu Y-F, Luceño M, Maguilla E, Márquez-Corro JJ, Martín-Bravo S, Masaki T, Míguez M, Naczi RFC, Reznicek AA, Spalink D, Starr JR, Uzma, Villaverde T, Waterway MJ, Wilson KL, Zhang S-R, Global Carex Group (2021) A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. *Journal of Systematics and Evolution* 59(4): 726–762. <https://doi.org/10.1111/jse.12722>
- Starr JR, Harris SA, Simpson DA (2008) Phylogeny of the unispicate taxa in Cyperaceae tribe Cariceae II: The limits of *Uncinia*. In: Naczi RFC, Ford BA (Eds) *Sedges: Uses, diversity and systematics of the Cyperaceae*. Monographs in Systematic Botany from the Missouri Botanical Garden 108: 243–267.
- Villaverde T, Jiménez-Mejías P, Luceño M, Waterway MJ, Kim S, Lee B, Rincón-Barrado M, Hahn M, Maguilla E, Roalson EH, Hipp AL, Wilson KL, Larridon I, Gebauer S, Hoffmann MH, Simpson DA, Naczi R F C, Reznicek AA, Ford BA, Starr JR, Park J, Escudero M, Martín-Bravo S, Global Carex Group (2020) A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogeny. *Botanical Journal of the Linnean Society* 194(2): 141–163. <https://doi.org/10.1093/botlinnean/boaa042>
- Waterway MJ, Hoshino T, Masaki T (2009) Phylogeny, species richness, and ecological specialization in Cyperaceae tribe Cariceae. *Botanical Review* 75(1): 138–159. <https://doi.org/10.1007/s12229-008-9024-6>

- Waterway MJ, Bruhl JJ, Wilson KL, Ford BA, Starr JR, Jin XF, Zhang SR, Gebauer S, Hoffmann MH, Gehrke B, Yano O, Hoshino T, Masaki T, Ford KA, Chung KS, Jung J, Kim S, Escudero M, Luceño M, Maguilla E, Martín-Bravo S, Míguez M, Villaverde T, Molina A, Simpson DA, Bruederle LP, Hahn M, Hipp AL, Rothrock PE, Reznicek AA, Naczi RFC, Thomas WW, Jiménez-Mejías P, Roalson EH, Alverson WS, Cochrane TS, Spalink D (2015) Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): A new broader circumscription. *Botanical Journal of the Linnean Society* 179(1): 1–42. <https://doi.org/10.1111/boj.12298>
- Yano O, Ikeda H, Jin XF, Hoshino T (2014) Phylogeny and chromosomal variations in East Asian *Carex*, Siderostictae group (Cyperaceae), based on DNA sequences and cytological data. *Journal of Plant Research* 127(1): 99–107. <https://doi.org/10.1007/s10265-013-0578-y>
- Yen AC, Olmstead RG (2000) Molecular systematics of Cyperaceae tribe Cariceae based on two chloroplast DNA regions: *ndhF* and *trnL* intron-intergenic spacer. *Systematic Botany* 25(3): 479–494. <https://doi.org/10.2307/2666691>
- Zhou YY, Jin XF (2014) Notes on *Carex* (Cyperaceae) from China: Three new species. *Phytotaxa* 164(2): 133–140. <https://doi.org/10.11646/phytotaxa.164.2.7>

Molecular phylogeny and taxonomy of the *Hydrangea serrata* complex (Hydrangeaceae) in western Japan, including a new subspecies of *H. acuminata* from Yakushima

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Abstract

According to the contemporary classification of *Hydrangea* native to Japan, *H. serrata* is a polymorphic species including six varieties. We discovered a plant identified as *H. serrata*, but morphologically distinct from previously known varieties, in Yakushima island where approximately 50 endemic species are known. To determine the relationship of this plant with previously known varieties, we examined morphology and constructed a highly resolved phylogeny of *H. serrata* and its relatives using three chloroplast genomic regions, *rbcL*, *trnL* intron, *psbA-trnH*, and two nuclear genomic regions, ITS1 and ITS2, and Multiplex ISSR genotyping by sequencing (MIG-seq). Based on these morphological and phylogenetic observations, we describe *Hydrangea acuminata* subsp. *yakushimensis* **subsp. nov.** as a newly discovered lineage in Yakushima, Japan and propose *Hydrangea minamitanii* **stat. nov.** and *Hydrangea acuminata* subsp. *australis* **stat. nov.** which were previously treated as varieties of *H. serrata*.

Keywords

cpDNA, DNA barcoding, F_{ST} , island, ITS, MIG-seq, threatened plants

Introduction

Hydrangea L. s. lat. is a genus of Hydrangeaceae, comprising approximately 200 species distributed in East and Southeast Asia and the New World (De Smet et al. 2015). Based on molecular phylogenetic studies, De Smet et al. (2015) proposed a broad circumscription of *Hydrangea* by absorbing the other eight genera of tribe Hydrangeae. Under this proposal, *Cardiandra* Siebold & Zucc., *Deinanthe* Maxim., *Pileostegia* Hook. f. & Thomson, *Platycrater* Siebold & Zucc., and *Schizophragma* Siebold & Zucc., which have been recognized in the representative flora of Japan (Kitamura and Murata 1979; Satake et al. 1999; Ohba 2017), are reduced to *Hydrangea* s. lat. In contrast, Ohba and Akiyama (2016) preferred to retain these genera and proposed generic segregation of most of the sections and subsections of *Hydrangea* s. lat. proposed by De Smet et al. (2015). In this study, we follow the broad circumscription of *Hydrangea* by De Smet et al. (2015) that retains species widely known as “hydrangea,” including *H. macrophylla* (Thunb.) Ser. and *H. serrata* (Thunb.) Ser., under the genus name of *Hydrangea*.

In 2005, we discovered a plant of the genus *Hydrangea* from a mountain-top area of the Yakushima Island, a small island with an area of 504.88 km² and a maximum peak of 1,936 m in elevation, part of which is designated as a UNESCO Man and the Biosphere Reserve (Okano and Matsuda 2013). The Yakushima Island is a center of plant endemism in Japan, harboring approximately 45 endemic species, including *Hydrangea grosseserrata* Engl. (Masamune 1934; Hotta 1974; Yahara et al. 1987). Whereas *H. grosseserrata* grows in evergreen forests at lower elevations, the newly discovered plant of *Hydrangea* is restricted to the mountain-top. In addition, they are morphologically distinct from *H. grosseserrata*. Although the flora of Yakushima has been well studied by the classic work of Masamune (1934) and a subsequent work of Yahara et al. (1987), recent field surveys discovered six additional new species endemic to this island: *Oxygyne yamashitae* Yahara & Tsukaya (Burmanniaceae, Yahara and Tsukaya 2008), *Carex mochomuensis* Katsuy. (Katsuyama 2009), *Haplopteris yakushimensis* C.W. Chen & Ebihara (Pteridaceae, Chen et al. 2014), *Dryopteris protobissetiana* K. Hori & N. Murak. (Dryopteridaceae, Hori et al. 2015), *Lecanorchis tabugawaensis* Suetsugu & Fukunaga (Orchidaceae, Suetsugu and Fukuhara 2016), and lastly *Sciaphila yakushimensis* Suetsugu, Tsukaya & H. Ohashi (Triuridaceae, Suetsugu et al. 2016). Considering the high endemism of the flora of Yakushima, we suspected that the plant of *Hydrangea* could be a new taxon. In this study, we compared the newly discovered plant with a morphologically similar species by molecular phylogenetic analysis and morphological observations.

The newly discovered plant is morphologically identified as *Hydrangea serrata* in having ovate-oblong petals, distinct peduncles, and oblong leaves, based on the key and description of Ohba (2017). According to Yamazaki (2001) and Ohba and Akiyama (2013), *H. serrata* is a polymorphic species, including six varieties, but the plant discovered from a mountain-top area of the Yakushima Island appeared to be

different from those varieties. Among these six varieties, the following three varieties are distributed on the main island of Kyushu located 60 km north of Yakushima: *H. serrata* var. *acuminata* (Siebold & Zucc.) Nakai, var. *australis* T. Yamaz., and var. *minamitanii* H. Ohba. To examine the genetic divergence of the newly discovered plant from the three varieties of *H. serrata* distributed on the Kyushu Island, we reconstructed phylogenetic trees of *H. serrata* and its relatives using three chloroplast genomic regions, *rbcL*, *trnL* intron, *psbA-trnH*, and two nuclear genomic regions, ITS1 and ITS2, and Multiplex ISSR genotyping by sequencing (MIG-seq; Suyama and Matsuki 2015).

A previous molecular phylogenetic study was performed on *H. serrata* and its relatives using *rbcL*, *matK*, and Random Amplified Polymorphic DNA (RAPD) markers (Uemachi et al. 2014), but this study did not examine var. *australis* and var. *minamitanii*. Uemachi et al. (2014) revealed that *H. serrata* var. *serrata* diverged to the western and eastern groups in Japan, corresponding to *H. serrata* var. *acuminata* and *H. serrata* var. *serrata* s. str., respectively.

Our new molecular phylogenetic analysis covered all the lineages distributed in Kyushu, including the newly discovered lineage from Yakushima, *H. serrata* var. *acuminata*, var. *australis*, and var. *minamitanii* from western Japan, as well as var. *angustata* (Franch. & Sav.) H. Ohba and var. *serrata* s. str. from eastern Japan. The results supported the treatment of the former three varieties as *H. acuminata* subsp. *acuminata*, *H. acuminata* subsp. *australis*, and *H. minamitanii*, respectively, and treating the newly discovered lineage as a new subspecies of *H. acuminata*.

Materials and methods

Field surveys

We carried out field studies in Yakushima Island of Kagoshima Prefecture and five additional prefectures, including Fukuoka, Miyazaki, Kochi, Mie, and Shizuoka. In total, we collected 24 samples consisting of 10 species with five infraspecific taxa of *Hydrangea* for DNA isolation (Table 1): *H. acuminata* subsp. *acuminata* from four localities (Fig. 1), *H. acuminata* subsp. *australis* from two localities (Fig. 1), *H. acuminata* subsp. *yakushimensis* described below (Fig. 1), *H. macrophylla*, *H. minamitanii*, *H. serrata* var. *angustata*, and *H. serrata* var. *serrata* of sect. *Macrophyllae* (E. M. McClint.) Y. De Smet & Samain (De Smet et al. 2015); *H. grosseserrata*, *H. kawagoeana*, *H. luteovenosa*, and *H. scandens* of sect. *Chinenses* Y. De Smet & Samain; and *H. hirta* of sect. *Hirtae* Y. De Smet & Samain. These three sections belong to the monophyletic group *Hydrangea* II (De Smet et al. 2015). As outgroups, we included *H. davidii* Franch., *H. indochinensis* Merr., and *H. febrifuga* (Lour.) Y. De Smet & Granados (*Dichroa febrifuga* Lour.) collected in Vietnam (Table 1), where we carried out a series of field studies (Middleton et al. 2019; Nagahama et al. 2021). In each sample, a small leaf piece was cut out, placed in a tea bag, and dried with silica gel in a zip-lock bag.

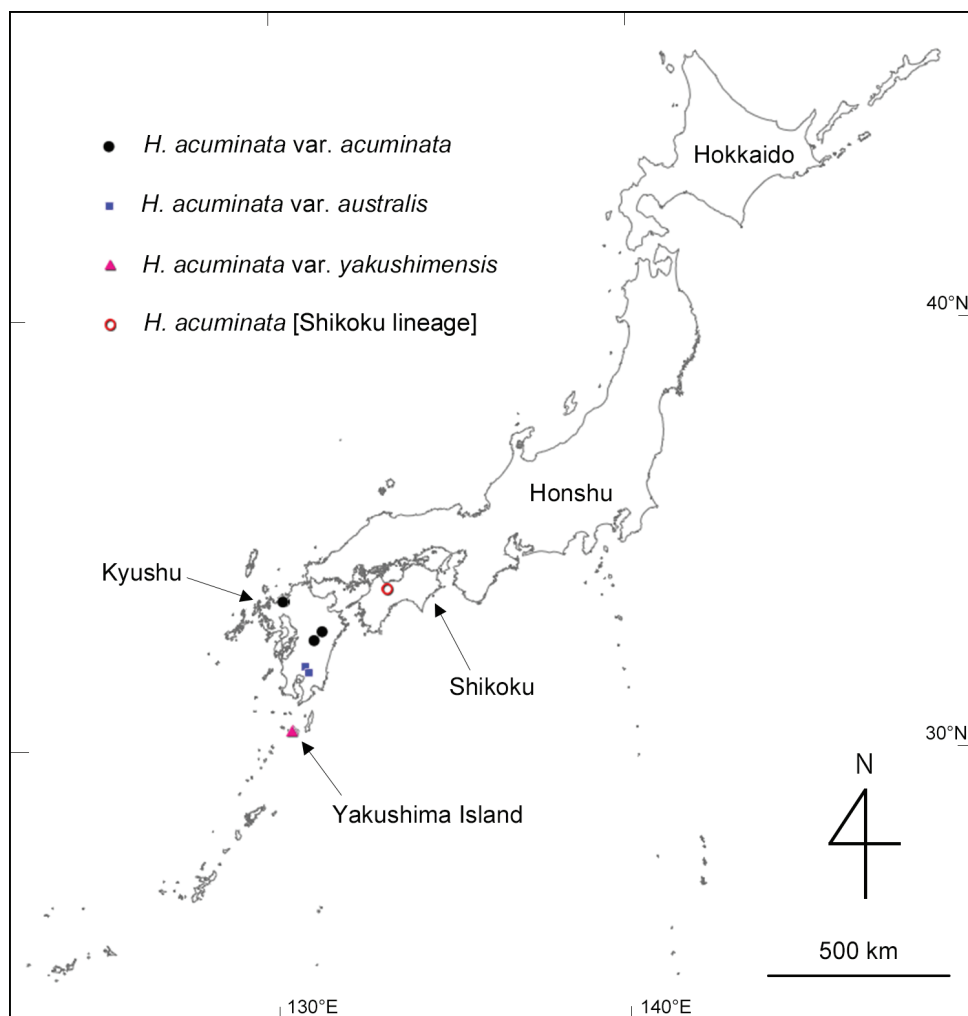


Figure 1. Localities of *Hydrangea acuminata* subsp. *acuminata* (including Shikoku lineage), subsp. *australis*, and subsp. *yakushimensis* where DNA samples and voucher specimens were collected in this study. The map was produced from Chiriin Chizu Vector (<https://maps.gsi.go.jp/vector/>).

DNA isolation, genome-wide Single Nucleotide Polymorphism (SNP) genotyping, and construction of phylogenetic trees

Total DNA was extracted from the dried leaves using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Multiplex ISSR genotyping by sequencing (MIG-seq, Suyama and Matsuki 2015) was used for *de novo* SNP detection. Briefly, a MIG-seq library was prepared by a two-step PCR amplification process based on the protocol detailed by Suyama et al. (2022). The amplicons in the size range of 300–800 bp were purified and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using an MiSeq Reagent Kit v3 (150 cycles, Illumina). We

Table 1. Samples used in molecular phylogenetic analyses.

Scientific name	Voucher ID	Locality	Coordinates
<i>Hydrangea (Dichroa)</i> sp.	V8372	Bidoup Nui Ba, Vietnam	12.16016944, 108.5364333
<i>Hydrangea acuminata</i> [Shikoku lineage]	TGK0472	Ino, Kochi	33.781458, 133.188252
<i>Hydrangea acuminata</i> [Shikoku lineage]	JPN3301	cultivated, Fukuoka	33.55545001, 130.1939861
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	JPN0330	Mt. Ihara, Fukuoka	33.48363400, 130.2638410
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	JPN0433	Mt. Raizan, Fukuoka	33.48293333, 130.2204444
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	JPN2336	Mt. Oyaji, Miyazaki	32.77326944, 131.3367306
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	JPN2063	Mt. Shiraiwa, Miyazaki	32.56233100, 131.1113540
<i>Hydrangea acuminata</i> ssp. <i>australis</i>	JPN0908	Mt. Karakuni, Miyazaki	31.93438888, 130.8600000
<i>Hydrangea acuminata</i> ssp. <i>australis</i>	JPN3192	Miyakonojyo, Miyazaki	31.78877222, 130.9603278
<i>Hydrangea acuminata</i> ssp. <i>yakushimensis</i>	JPN1708	Yakushima, Kagoshima	30.372031, 130.504266
<i>Hydrangea acuminata</i> ssp. <i>yakushimensis</i>	JPN1799	Yakushima, Kagoshima	30.34255555, 130.4810000
<i>Hydrangea davidii</i>	V4997	Fansipan, Vietnam	22.34225, 103.7764167
<i>Hydrangea grosseserrata</i>	JPN0528	Yakushima, Kagoshima	30.34619444, 130.3918750
<i>Hydrangea grosseserrata</i>	JPN0652	Yakushima, Kagoshima	30.26264444, 130.5800944
<i>Hydrangea hirta</i>	JPN2415	Mt. Amagi, Shizuoka	34.86201944, 139.0215139
<i>Hydrangea indochinensis</i>	V4959	Fansipan, Vietnam	22.34755555, 103.7721944
<i>Hydrangea kawagoeana</i>	TG00879	Suwanose-jima, Kagoshima	29.62290600, 129.69778900
<i>Hydrangea luteovenosa</i>	JPN0378	Mt. Ihara, Fukuoka	33.48294444, 130.2541972
<i>Hydrangea luteovenosa</i>	JPN0901	Mt. Karakuni, Miyazaki	31.93438888, 130.8600000
<i>Hydrangea luteovenosa</i>	JPN1982	Mt. Osuzu, Miyazaki	32.29758800, 131.4459520
<i>Hydrangea macrophylla</i>	JPN3302	cultivated, Fukuoka	33.55545001, 130.1939861
<i>Hydrangea macrophylla</i>	JPN3303	cultivated, Fukuoka	33.55545001, 130.1939861
<i>Hydrangea minamitanii</i>	JPN1983	Mt. Osuzu, Miyazaki	32.29758800, 131.4459520
<i>Hydrangea minamitanii</i>	TG01200	Aya, Miyazaki	32.03053900, 131.21502800
<i>Hydrangea scandens</i>	JPN1980	Mt. Osuzu, Miyazaki	32.29758800, 131.4459520
<i>Hydrangea scandens</i>	JPN2931	Kihoku, Mie	34.18644999, 136.1858528
<i>Hydrangea serrata</i> var. <i>angustata</i>	JPN2404	Izu City, Shizuoka	34.96862800, 138.8459450
<i>Hydrangea serrata</i> var. <i>serrata</i>	JPN2980	Osugi-dani, Mie	34.21346388, 136.1650250

skipped the sequencing of the first 17 bases of reads 1 and 2 (SSR primer regions and anchors) using “DarkCycle”. Low-quality reads and extremely short reads containing adapter sequences were removed using Trimmomatic 0.39 (Bolger et al. 2014). Stacks 2.41 pipeline software (Catchen et al. 2013; Rochette et al. 2019) was used to obtain individual genotypes with the following parameters: minimum depth of coverage required to create a stack (m) = 3, maximum distance between stacks (M) = 2, maximum mismatches between loci when building the catalog (n) = 2. Three different filtering criteria were applied for quality control of the SNP data. First, any SNP site where one of two alleles had less than three counts was filtered out because it is difficult to distinguish polymorphisms from sequencing errors when the minor allele count of SNPs is too low (Roesti et al. 2012). Second, loci containing SNPs with high heterozygosity ($Ho \geq 0.6$) were removed because excess heterozygosity may have resulted from artificial loci built from several paralogous genomic regions. Third, SNPs with a genotyping rate of < 50% were eliminated. Using the third criterion, the SNPs that were retained by 14 or more samples remained in the SNP dataset.

Maximum likelihood phylogeny based on SNPs was inferred using software RAxML 8.2.10 (Stamatakis 2014). We used a GTRCAT model and performed 1,000 replicates of parallelized tree search bootstrapping. Based on the clades of the MIG-seq tree, we estimated pairwise F_{ST} values for each clade using the POPULATIONS program in Stacks.

Sequencing and phylogenetic analysis of chloroplast and nuclear genomic regions

The chloroplast and nuclear genomic regions were sequenced using the next generation sequencing (NGS) technique (Suyama et al. 2022). First, three chloroplast genomic regions, *rbcl*, *trnL* intron, and *psbA-trnH*, and two nuclear genomic regions, ITS1 and ITS2, were simultaneously amplified using the Multiplex PCR Assay Kit Ver. 2 (Takara Bio, Kusatsu, Japan) (first PCR reaction). The first primers consisted of tail sequences and locus-specific primers (Suyama et al. 2022). Second, the products from the first PCR reaction were purified and used for the second PCR. The second PCR was conducted using primer pairs including tail sequences, adapter sequences for Illumina sequencing, and the index sequence to identify each individual sample. Third, the second PCR products from each sample were mixed, and sequencing was performed using an Illumina MiSeq platform with an MiSeq Reagent Nano Kit v2 (500 cycles, Illumina). We skipped the sequencing of the first three bases of reads 1 and 2 (anchor region for the 2nd PCR primer) using the “DarkCycle” option of the MiSeq system. Both ends of the fragments and index sequences were read by paired-end sequences (reads 1 and 2) and index sequencing. The number of bases read was 251 bases for both read 1 and read 2.

The sequences of the five regions were determined using Claident pipeline (Tanabe and Toju 2013, <http://www.claident.org/>, Tanabe, A.S., Claident, Date of access: 05/01/2021). First, raw MiSeq BCL data were converted into FASTQ data using the BCL2FASTQ program provided by Illumina, and raw FASTQ data were demultiplexed based on index and primer sequences using the clsplitseq program in Claident. Subsequent analysis was performed per region per individual. In ITS1 and ITS2, we merged paired-end reads because reads 1 and 2 overlapped. In *rbcl*, *trnL* intron, and *psbA-trnH*, we independently analyzed reads 1 and 2 because the length of the sequenced reads was too short to merge reads 1 and 2. Second, the low-quality 3' tails were trimmed and the low-quality sequences were filtered out using the clfilterseq program. Third, the noisy and chimeric sequences were removed using the ccleanseq program. Fourth, the remaining reads were clustered with a cut-off sequence similarity of 99%. An operational taxonomic unit (OUT) that had the most observed reads within the individual was treated as a representative OTU sequence.

Multiple alignments of the chloroplast and nuclear genomic regions were performed using the program MAFFT 7.313 (Katoh and Standley 2013), and alignment columns containing gaps were trimmed using a heuristic selection method based on similarity statistics of trimAl 1.4.rev15 (Capella-Gutiérrez et al. 2009). We used Kakusan 4.0 (Tanabe 2011) to find suitable nucleotide substitution models and partitioning strategies for the nucleotide datasets. The chloroplast and nuclear genomic regions were independently run through Kakusan. The corrected Akaike Information Criterion (AICc; Sugiura 1978) was used to compare nonpartitioned, partitioned _ equal _ mean _ rate, and separate models. The nonpartitioned model (GTR + Γ) proved optimal for both the chloroplast and nuclear genomic regions. Maximum likelihood phylogenies were inferred using RAxML 8.2.10 (Stamatakis 2014), whereby 1,000 replicates of parallelized tree search bootstrapping were conducted.

Table 2. The specimens used for measurements of nine morphological traits.

Taxa	Specimen ID	Herbaria
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	KAG161334, KAG161335, KAG161336, KAG161337, KAG161338, KAG161344, KAG161345, KAG161348, KAG161349, KAG161350	KAG
<i>Hydrangea acuminata</i> ssp. <i>acuminata</i>	Fujii 117037	KYO
<i>Hydrangea acuminata</i> ssp. <i>australis</i>	KAG023305, KAG083840, KAG083882, KAG086731, KAG161312, KAG161315, KAG161327, KAG161377	KAG
<i>Hydrangea acuminata</i> ssp. <i>australis</i>	Fujii 18200, Fujii 178001	KYO
<i>Hydrangea acuminata</i> ssp. <i>yakushimensis</i>	Yahara et al. 791, 792, 793–1, 793–2, 793–3, 793–4, 1103, 1104, 1105, JPN1799	FU

Morphological observations

Using the specimens listed in Table 2, we measured the following leaf traits using the largest leaf: leaf blade length, leaf blade width, petiole length, leaf apex length, leaf teeth length, and the number of teeth on one side of the leaf margin. Leaf teeth length was measured as the height from the line between two bases of a tooth to the tip of the tooth, for the highest tooth of the largest leaf. We also measured the corymb length, corymb width, and capsule length for fruiting specimens.

Data resources

All raw MIG-seq data were deposited at the DDBJ Sequence Read Archive (DRA) with accession number DRA011509. The demultiplexed raw reads of ITS and cpDNA regions were deposited at the DDBJ Sequence Read Archive (DRA) with accession number DRA011510. All sequences of ITS and cpDNA regions were registered to DNA Data Bank of Japan (DDBJ) under accession nos. LC657594–LC657817.

Results

Phylogenetic and population genetic analyses using MIG-seq

A total of 22,106,838 raw reads ($789,530 \pm 47,627$ reads per sample) were obtained, and after quality control, 20,944,147 reads ($748,005 \pm 45,296$ reads per sample) remained. After *de novo* SNP detection and filtering, the dataset had 1,746 SNPs from 685 loci.

In the MIG-seq tree (Fig. 2), nine *Hydrangea* species were clustered into three clades corresponding to sect. *Macrophyllae* (*H. acuminata*, *H. macrophylla*, *H. minamitanii*, and *H. serrata*), sect. *Chinenses* (*H. grosseserrata*, *H. kawagoeana*, *H. luteovenosa*, and *H. scandens*), and sect. *Hirtae* (*H. hirta*). In the *Macrophyllae* clade, *H. minamitanii* was sister to the clade including the other three species and monophylies of both *H. minamitanii* and the latter clade were supported by 100% bootstrap values. Among the latter three species, the clade including *H. macrophylla* and *H. serrata* was

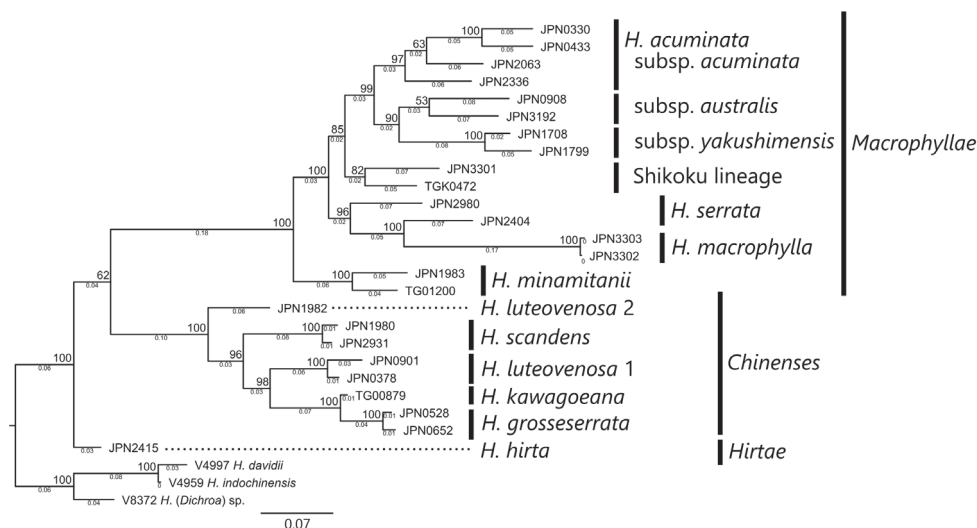


Figure 2. Molecular phylogenetic tree reconstructed using MIG-seq. Bootstrap values are shown on the nodes, and branch lengths are shown on the internodes. Branch length represents the average number of substitutions per SNP site.

supported by a 96% bootstrap value and sister to the clade of *H. acuminata* supported by an 85% bootstrap value. Within *H. acuminata*, the Shikoku lineage was sister to a clade supported by a 99% bootstrap value including subsp. *acuminata*, subsp. *australis*, and subsp. *yakushimensis*, and the sister relationship of subsp. *australis* and subsp. *yakushimensis* was supported by a 90% bootstrap value. Even after the separation of *H. acuminata*, *H. serrata* was not monophyletic. The samples of *H. serrata* from Mie (JPN2980; var. *serrata*) and Shizuoka (JPN2404; var. *angulata*) were clustered with *H. macrophylla* but not sister to each other, and the sister relationship of *H. serrata* var. *angulata* and *H. macrophylla* was supported by a 100% bootstrap value. Similarly, *H. luteovenosa* was not monophyletic. Whereas *H. luteovenosa* 1 was sister to a clade including *H. kawagoeana* and *H. grosseserrata*, *H. luteovenosa* 2 was sister to a clade including all the other samples of sect. *Chinenses*.

The degree of genetic differentiation measured by F_{ST} (Table 3) was 0.251 between *H. acuminata* subsp. *acuminata* and subsp. *australis*, 0.316 between subsp. *acuminata* and subsp. *yakushimensis*, and 0.437 between subsp. *australis* and subsp. *yakushimensis*. Among the closely related species of sect. *Macrophyllae*, F_{ST} was 0.553 between *H. macrophylla* and *H. serrata*, 0.317–0.514 between *H. acuminata* and *H. serrata*, and 0.452–0.652 between *H. acuminata* and *H. macrophylla*. *Hydrangea minamitanii* is differentiated from *H. acuminata* subsp. *acuminata*, subsp. *australis*, subsp. *yakushimensis*, Shikoku lineage, *H. serrata*, and *H. macrophylla* in F_{ST} values of 0.340, 0.470, 0.546, 0.439, 0.480, and 0.657, respectively. Between species of sect. *Chinenses*, F_{ST} varied from 0.395 (*H. kawagoeana* vs. *H. grosseserrata*) to 0.632 (*H. grosseserrata* vs. *H. scandens*). Between sections, F_{ST} varied from 0.454 (*H. luteovenosa* 2 of sect. *Chinenses* vs. *H. acuminata* subsp. *acuminata*) to 0.814 (*H. hirta* vs. *H. macrophylla*).

Table 3. The degrees of genetic differentiation between taxa measured by F_{ST} .

		<i>Hirtae</i>		<i>Chinenses</i>				<i>Macrophyllae</i>					
		<i>H. grosseserrata</i>	<i>H. kawagoeana</i>	<i>H. luteovenosa</i> 1	<i>H. scandens</i>	<i>H. luteovenosa</i> 2	<i>H. minamitanii</i>	<i>H. acuminata</i> ssp. <i>acuminata</i>	<i>H. acuminata</i> ssp. <i>australis</i>	<i>H. acuminata</i> ssp. <i>yakushimensis</i>	<i>Shikoku lineage</i>	<i>H. serrata</i>	<i>H. macrophylla</i>
<i>Hirtae</i>	<i>H. hirta</i>	0.739	0.696	0.700	0.693	0.624	0.724	0.511	0.654	0.720	0.637	0.715	0.814
<i>Chinenses</i>	<i>H. grosseserrata</i>	–	0.395	0.580	0.632	0.616	0.749	0.590	0.705	0.769	0.711	0.735	0.808
<i>Chinenses</i>	<i>H. kawagoeana</i>	–	–	0.473	0.561	0.524	0.768	0.578	0.697	0.736	0.695	0.734	0.792
<i>Chinenses</i>	<i>H. luteovenosa</i> 1	–	–	–	0.544	0.510	0.729	0.574	0.681	0.728	0.687	0.702	0.775
<i>Chinenses</i>	<i>H. scandens</i>	–	–	–	–	0.551	0.722	0.574	0.684	0.759	0.695	0.711	0.787
<i>Chinenses</i>	<i>H. luteovenosa</i> 2	–	–	–	–	–	0.501	0.454	0.594	0.679	0.565	0.598	0.720
<i>Macrophyllae</i>	<i>H. minamitanii</i>	–	–	–	–	–	–	0.340	0.470	0.546	0.439	0.480	0.657
<i>Macrophyllae</i>	<i>H. acuminata</i>	–	–	–	–	–	–	–	0.251	0.316	0.257	0.317	0.452
	ssp. <i>acuminata</i>												
<i>Macrophyllae</i>	<i>H. acuminata</i> ssp. <i>australis</i>	–	–	–	–	–	–	–	–	0.437	0.405	0.441	0.606
<i>Macrophyllae</i>	<i>H. acuminata</i> ssp. <i>yakushimensis</i>	–	–	–	–	–	–	–	–	–	0.453	0.514	0.652
<i>Macrophyllae</i>	<i>Shikoku lineage</i>	–	–	–	–	–	–	–	–	–	–	0.364	0.585
<i>Macrophyllae</i>	<i>H. serrata</i>	–	–	–	–	–	–	–	–	–	–	–	0.553

Phylogenetic tree reconstructed using ITS sequences

A total of 111,216 reads (3,972 ± 299 reads per sample, ITS1) and 81,988 reads (2,928 ± 155 reads per sample, ITS2) were obtained. After gaps were trimmed, the total length of the sequences was 635 bp (ITS1: 267 bp, ITS2: 368 bp). In the ITS tree (Fig. 3), sect. *Macrophyllae* was supported by a 92% bootstrap value, and sect. *Chinenses* was supported by an 85% bootstrap value. In sect. *Macrophyllae*, only three branches were supported by bootstrap values larger than 80%: a clade including *H. acuminata* and *H. minamitanii* was supported by an 88% bootstrap support, *H. acuminata* subsp. *yakushimensis* was supported by 97%, and *H. macrophylla* was supported by 94%. In sect. *Chinenses*, a clade including *H. kawagoeana* and *H. grosseserrata* was supported by a 91% bootstrap value, and another clade including *H. luteovenosa* 1 and *H. scandens* was supported by a 90% bootstrap value. *Hydrangea luteovenosa* 1 and *H. luteovenosa* 2 were not sister to each other.

Phylogenetic tree reconstructed using cpDNA sequences

A total of 20,290 reads (725 ± 68 reads per sample, *rbcL*), 18,724 reads (669 ± 68 reads per sample, *trnL* intron), and 20,194 reads (721 ± 72 reads per sample, *psbA-trnH*) were obtained. After gaps were trimmed, the total length of the sequences was 1,354 bp. The sequenced lengths of each region were 222 bp and 227 bp (read 1 and 2 of *rbcL*), 228 bp and 228 bp (read 1 and 2 of *trnL* intron), and 226 bp and 223 bp (read 1 and 2 of *psbA-trnH*). In the cpDNA tree reconstructed using these sequences (Fig. 4), the monophyly of sect. *Macrophyllae* was supported by a 96% bootstrap value, and the two lineages of sect. *Chinenses* and *H. hirta* were polychotomous.

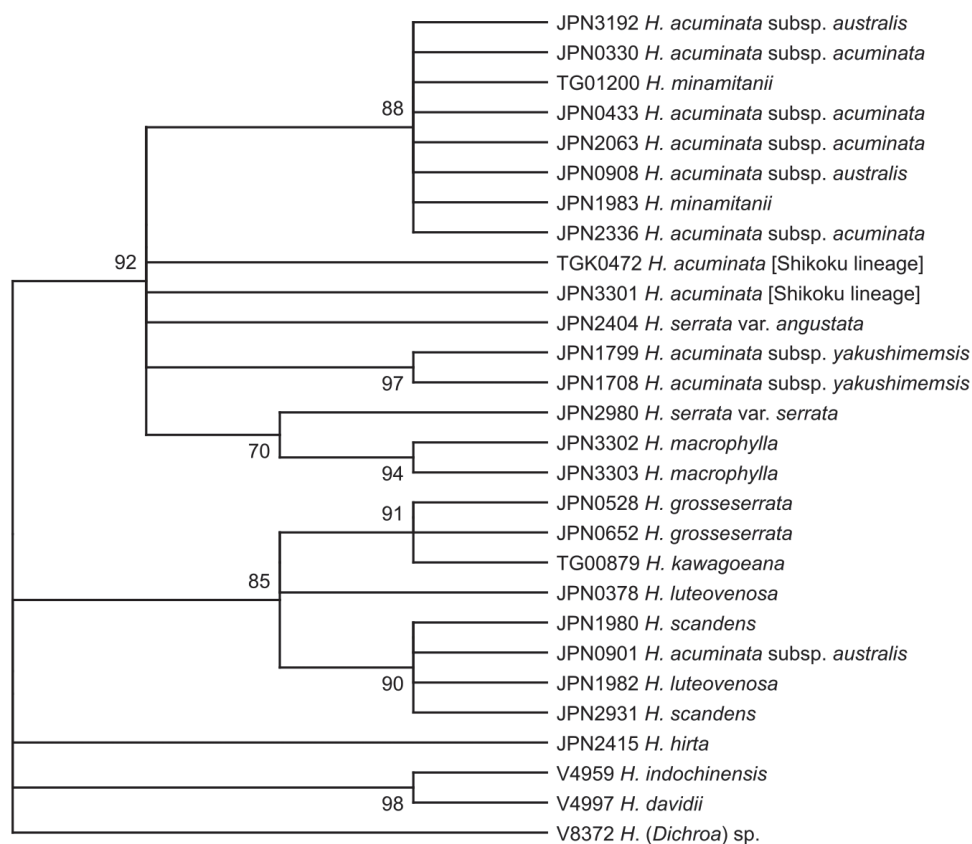


Figure 3. Molecular phylogenetic tree reconstructed using ITS sequences. Bootstrap values are shown on the nodes. Nodes supported by less than 70% bootstrap values are not shown.

Morphological observations

Morphologically, *H. acuminata* subsp. *yakushimensis* is similar to subsp. *acuminata* in having blue-colored flowers: fertile flowers with blue-colored petals, stamens, and sterile flowers with blue-colored calyces (Fig. 5). However, *H. acuminata* subsp. *yakushimensis* is distinct from subsp. *acuminata* in that the upper leaf surface is glabrous except on veins (vs. sparsely hairy), the lower leaf surface is glabrous or only slightly hairy except for tufted hairs at axils of lateral veins (vs. sparsely hairy), and capsules are shorter than 2.7 mm (vs. 3.2 mm or longer; Fig. 6; Table 4; Table 5). In addition, *H. acuminata* subsp. *yakushimensis* is different from subsp. *acuminata* in that the number of teeth along each margin of the largest leaf exceeds 27 (vs. 27 or fewer in subsp. *acuminata*), the length of leaf serrations of the largest leaf exceeds 3 mm (vs. 1.0–2.9 mm), and the width of infructescence attains to 7–12 cm (vs. 3.2–8.7 cm).

Phylogenetically, *H. acuminata* subsp. *yakushimensis* is sister to subsp. *australis*. Morphologically, *H. acuminata* subsp. *yakushimensis* is similar to subsp. *australis* in

having leaves larger than subsp. *acuminata* but distinguished with leaves glabrous adaxially except veins (vs. sparsely hairy in subsp. *australis*; Table 5) and capsule less than 3 mm long (Table 4).

Discussion

The discovery of *H. acuminata* subsp. *yakushimensis* is surprising because Yakushima is a well-botanized island, and *H. acuminata* subsp. *yakushimensis* has conspicuous, blue-colored flowers. This discovery illustrates that botanical surveys in the mountain-top area of Yakushima still remain insufficient, most likely because of its steep topography. In fact, our recent surveys resulted in the discovery of not only *H. acuminata* subsp. *yakushimensis* but also an additional new taxon of *Stellaria* (Caryophyllaceae) (Yahara et al. 2021b). Further field surveys including researchers who have more experience climbing mountains and steep cliffs could result in the discovery of even more undescribed taxa from the mountain-top area of Yakushima.

Using RAPD and the sequences of *rbcL* and *matK*, Uemachi et al. (2014) showed that *H. serrata* var. *serrata* s. lat. diverged to western and eastern groups, corresponding to *H. acuminata* and *H. serrata* var. *serrata* s. str., respectively. However, Uemachi et al. (2014) did not examine *H. acuminata* subsp. *australis*, *H. acuminata* subsp. *yakushimensis*, and *H. minamitanii*. The MIG-seq tree (Fig. 2) revealed that *H. minamitanii* is sister to the clade including *H. acuminata*, *H. serrata*, and *H. macrophylla*. *Hydrangea minamitanii* is differentiated from the other species of sect. *Macrophyllae* with F_{ST} values from 0.340 to 0.657, and this difference was equivalent to the F_{ST} variation between the species of sect. *Chinenses* from 0.395 (*H. kawagoeana* vs. *H. grosseserrata*) to 0.632 (*H. grosseserrata* vs. *H. scandens*). These findings support the treatment of *H. minamitanii* as a distinct species.

In contrast, the F_{ST} between *H. acuminata* subsp. *acuminata* and subsp. *australis* (0.251) is lower than the above values (0.340 to 0.657) observed between the species, supporting the treatment as two subspecies. Similarly, the F_{ST} between *H. acuminata* subsp. *acuminata* and subsp. *yakushimensis* was 0.316, which is considered to be at the

Table 4. Measurements for nine morphological traits of *H. acuminata* ssp. *acuminata*, ssp. *australis*, ssp. *yakushimensis*, and *H. minamitanii*.

	<i>H. a. ssp. yakushimensis</i>	<i>H. a. ssp. acuminata</i>	<i>H. a. ssp. australis</i>	<i>H. minamitanii</i>
Leaf length	12.5±1.5 (10.2–14.4) cm	10.4±2.8 (10.4–15.6) cm	13.4±1.6 (10.1–15.4) cm	12.6±1.1 (12–14) cm
Leaf width	6.2±0.9 (4.6–7.5) cm	4.3±1.1 (2.8–6.2) cm	7.7±1.4 (5.4–9.7) cm	6.2±0.9 (5.0–6.9) cm
Petiole length	2.8±1.0 (1.2–5.3) cm	2.1±0.8 (0.9–3.5) cm	2.8±1.3 (0.9–5.0) cm	3.0±1.2 (2.2–4.5) cm
Leaf apex length	1.7±0.5 (1.0–2.4) cm	1.8±0.7 (0.9–2.9) cm	1.7±0.5 (0.7–2.5) cm	1.5±0.5 (0.8–1.9) cm
Leaf teeth length	2.8±1.1 (1.0–5.0) mm	1.8±0.6 (1.0–2.9) mm	3.0±1.0 (1.0–5.0) mm	3.1±0.8 (2.2–4.2) cm
No of teeth	28.5±7.1 (15–42)	21.2±6.1 (9–27)	28.5±7.1 (15–42)	28.8±4.9 (23–35)
Corymb length	4.3±1.5 (2.0–7.0) cm	4.1±1.5 (1.9–6.3) cm	5.8±2.2 (3.0–9.2) cm	5.1±0.9 (4.5–6.3) cm
Corymb width	6.7±2.7 (3.9–11.8) cm	5.5±1.9 (3.2–8.7) cm	8.9±2.5 (6.2–12.8) cm	7.9±0.9 (7.2–9.2) cm
Capsule length	2.4±0.2 (2.2–2.7) mm	3.9±0.6 (3.2–5.1) mm	4.3±0.4 (3.8–4.9) mm	4.6±1.0 (3.9–5.3) mm

Table 5. Morphological comparison between *H. acuminata* ssp. *acuminata*, ssp. *australis*, ssp. *yakushimensis*, and *H. minamitanii*.

	<i>H. a. ssp. yakushimensis</i>	<i>H. a. ssp. acuminata</i>	<i>H. a. ssp. australis</i>	<i>H. minamitanii</i>
Upper surface of lamina	glabrous	sparsely hairy	sparsely hairy	glabrous
Upper surface of veins	hairy	hairy	hairy	hairy
Lower surface of lamina	glabrous	sparsely hairy	densely curled hairy	glabrous
Lower surface of veins	glabrous or glabrescent	sparsely hairy	densely curled hairy	glabrous or glabrescent
Axils of lateral veins	hairs densely tufted	hairs not densely tufted	hairs not densely tufted	hairs densely tufted
Petiole	glabrous	hairy	densely hairy	glabrous
Young shoot	glabrous	hairy	densely hairy	glabrous
Calyx of showy flower	blue	blue	blue	pink or white

subspecies level, and the F_{ST} between subsp. *australis* and subsp. *yakushimensis* (0.437) was slightly higher. Differences between *H. acuminata* subsp. *acuminata* and subsp. *australis* are smaller, not only genetically, but also morphologically: JPN0908 collected at 1700-m elevation on Mt. Karakuni was identified as subsp. *australis* in the MIG-seq tree, but is morphologically very similar to subsp. *acuminata*, suggesting hybridization or intergradation between subsp. *acuminata* and subsp. *australis*.

In the MIG-seq tree (Fig. 2), *H. acuminata* subsp. *yakushimensis* was sister to *H. acuminata* subsp. *australis* distributed in the southern part of Kyushu mainland (Kagoshima Pref. and the southern part of Miyazaki Pref.). There are other cases where the endemic plants of Yakushima have related taxa in southern Kyushu. For example, *Asarum* (Araceae, Okuyama et al. 2020), *Mitella* (Saxifragaceae, Okuyama et al. 2005), and *Rhododendron* (Ericaceae, Minamitani et al. 2018) have all been reported as showing this pattern. The sister relationship between *H. acuminata* subsp. *yakushimensis* and subsp. *australis* provided another case which supported the phytogeographical similarity between the endemic flora of Yakushima and the flora of the southern Kyushu mainland.

The MIG-seq tree (Fig. 2) showed that the Shikoku lineage was distinct from a clade including three subspecies of *H. acuminata* distributed in Kyushu. This finding agrees with the results of Uemachi et al. (2014), showing that the samples from Shikoku were distinct for both *rbcL* and *matK* sequences from other “western subgroups” corresponding to *H. acuminata*. We did not find differences in *rbcL* sequences between the Shikoku lineage and other samples of *H. acuminata*, which is most likely because we determined shorter sequences of *rbcL* than did Uemachi et al. (2014): 449 bp. vs 1257 bp. The MIG-seq tree and the results described by Uemachi et al. (2014) suggest that the Shikoku lineage may be treated as a fourth subspecies of *H. acuminata*. However, further morphological and molecular phylogenetic studies, using more samples from Shikoku, are needed to conclude the taxonomic treatment of the Shikoku lineage.

The MIG-seq tree (Fig. 2) also showed that *H. serrata* was not monophyletic if *H. macrophylla* was separated as a species; the sample of *H. serrata* var. *angulata* was sister to *H. macrophylla*, and the sample of *H. serrata* var. *serrata* was basal to this sister

pair. This result suggests that *H. serrata* includes multiple species even after *H. acuminata* and *H. minamitanii* are separated. *Hydrangea serrata* s. lat. is widely distributed from Kyushu to Hokkaido, the northern-most island of Japan. Our samples were limited to the area of western Japan on the Pacific side and did not include *H. serrata* var. *jezoensis*. Further studies of populations in central and northern Japan, including more samples of *H. serrata* var. *angulata*, var. *serrata*, and var. *jezoensis*, are needed to revise the taxonomy of the complex that has been treated as *H. serrata* s. lat.

It is notable that *H. luteovenosa* 1 and *H. luteovenosa* 2 were not sister to each other in both MIG-seq and ITS trees. In the MIG-seq tree which has a higher resolution than the ITS tree, *H. luteovenosa* 2 (JPN1982 collected from Mt. Osuzu, Miyazaki Pref.) was basal to a clade including *H. scandens*, *H. luteovenosa* 1 (JPN0378 collected from Mt. Ihara, Fukuoka Pref.), *H. kawagoeana*, and *H. grosseserrata*. It is likely that *H. luteovenosa* contains two cryptic species. To test this possibility, further studies with more samples of *H. luteovenosa* are needed.

This study demonstrated the usefulness of MIG-seq to obtain finely resolved phylogenetic trees for closely related species and infraspecific taxa in taxonomically complicated groups such as *Hydrangea*. Compared with the ITS and cpDNA trees, where only a few branches were supported by bootstrap values larger than 90%, most branches in the MIG-seq tree were supported by bootstrap values larger than 90%. In the ITS tree, the monophyly of *H. acuminata* subsp. *yakushimensis* was supported by the 97% bootstrap value, but the monophyly of *H. acuminata* subsp. *acuminata* and subsp. *yakushimensis* was ambiguous; the cluster of *H. acuminata* subsp. *acuminata* and *H. minamitanii* with the bootstrap value 88% was weakly consistent with the MIG-seq tree topology. The resolution of the MIG-seq tree is even higher than that of the RAPD tree for the *H. serrata* complex obtained by Uemachi et al. (2014). Other recent studies using MIG-seq on *Hosta* (Yahara et al. 2021a) and *Stellaria* (Yahara et al. 2021b) have also demonstrated its usefulness in resolving taxonomic complexity and describing new taxa. As this method is more applicable to a small number of poor-quality samples than RAD-seq (Binh et al. 2018; Strijk et al. 2020; Zhang et al. 2020), it is expected to be used for taxonomic studies of many groups for which reliable phylogenetic relationships could not be reconstructed by conventional molecular phylogenetic methods.

Key to related species

- 1 Calyces of marginal showy flowers, petals of fertile flowers, and stamens always pink or white.....**2**
- Calyces of marginal showy flowers, petals of fertile flowers, and stamens light blue when flowering.....**3**
- 2 Leaves glabrous adaxially except veins and glabrous abaxially except for tufted hairs at axils of lateral veins. Distributed in Kyushu ***H. minamitanii***
- Leaves more or less hairy adaxially and abaxially. Distributed in Honshu ***H. serrata***

- 3 Leaves glabrous adaxially except veins. Capsules 2.7 mm or shorter *H. acuminata* subsp. *yakushimensis*
 – Leaves hairy adaxially. Capsules 3.2 mm or longer 4
 4 Leaves usually sparsely hairy abaxially, hair not curled. Leaf width less than 6.2 cm *H. acuminata* subsp. *acuminata*
 – Leaves usually densely hairy abaxially, hair curled. Leaf width often 6.2 cm or larger *H. acuminata* subsp. *australis*

Taxonomy

Hydrangea acuminata

Hydrangea acuminata Siebold & Zucc., Fl. Jap. 1: 110, t. 56, 57-I (1839); Ohba & Akiyama in Bull. Natl. Mus. Nat. Sci., Ser. B, 39: 178 (2013).

Type. JAPAN, Higo Province, Kyushu (*L0043373*, the lectotype designated by Ohba and Akiyama (2013)).

Hydrangea acuminata subsp. *acuminata*

Hortensia serrata var. *acuminata* (Siebold & Zucc.) H. Ohba & S. Akiyama, J. Jap. Bot. 91: 347 (2016).

Hydrangea macrophylla (Thunb.) Ser. var. *acuminata* (Siebold & Zucc.) Makino, Ill. Fl. Nippon: 484, f. 1451 (1940), nom. tant.

Japanese name. Sawa-ajisai, Nishino-yama-ajisai.

Distribution and habitats. *Hydrangea acuminata* subsp. *acuminata* is widely distributed on the main island of Kyushu, and usually grows on the soil near streams and often on cliffs, and sometimes in disturbed habitats.

Note. Ohba and Akiyama (2016) treated this species as a variety of *Hortensia serrata*. However, our phylogenetic analysis described below supports the treatment of it as a distinct species.

Hydrangea acuminata subsp. *yakushimensis* Yahara & Tagane, subsp. nov.

urn:lsid:ipni.org:names:77248597-1

Figures 4, 5

Diagnosis. *Hydrangea acuminata* subsp. *yakushimensis* is different from subsp. *acuminata* in that it has smaller capsules, 2.2–2.7 mm long with calyx tube 1.2–1.4 mm long and projected apical part including persistent style 1.0–1.3 mm (vs. capsules 3.2–5.1 mm

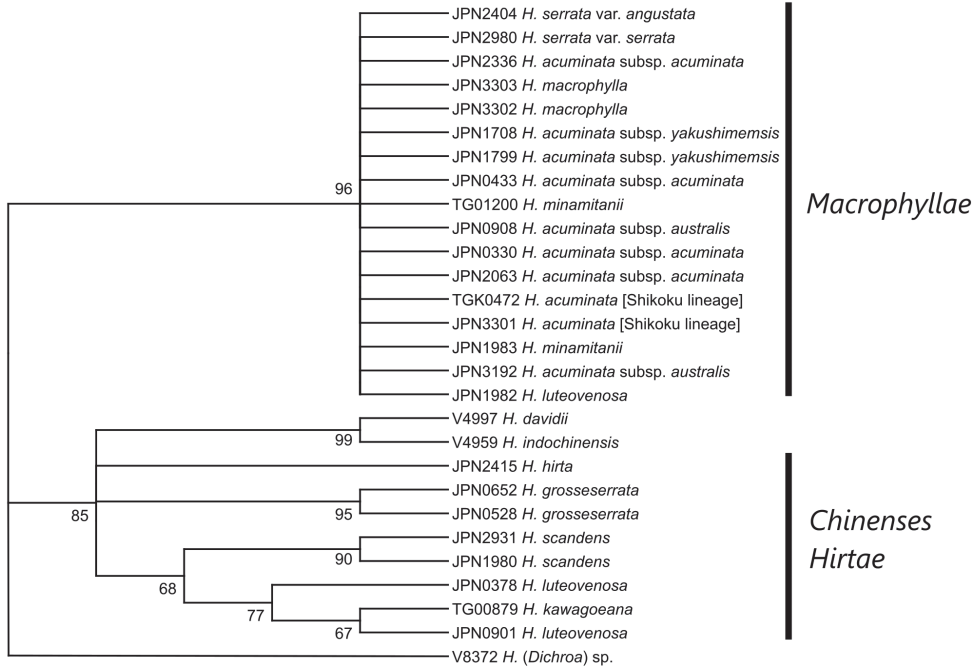


Figure 4. Molecular phylogenetic tree reconstructed using cpDNA sequences. Bootstrap values are shown on the nodes. Nodes supported by less than 60% bootstrap values are not shown.

long with calyx tube 1.6–3.4 mm and projected apical part including persistent style 1.5–2.0 mm), a larger infructescence attaining to 7×12 cm (vs. attaining to 6.3×8.7 cm), leaves glabrous adaxially except veins (vs. hairy) and glabrous or only slightly hairy abaxially except for tufted hairs at axils of lateral veins (vs. hairy overall on abaxial surface).

Type. JAPAN. Kagoshima Pref.:Yakushima Migitani, on cliff along stream, $30.34255555^{\circ}\text{N}$, $130.48100000^{\circ}\text{E}$, 1520 m elevation, 9 September 2020, with fruits, *K. Fuse JPN1799* (holotype: KYO!).

Description. Shrubs 1–1.5 m tall. First year's twigs green when fresh, with dark purple lenticels, glabrous, terete. Old twigs pale brown; bark not peeled off. Leaves opposite; petioles purplish green, 1.7–3 cm long, glabrous; leaf blade adaxially green, abaxially light green when fresh, pale green when dried, elliptic, $9\text{--}12 \times 4.6\text{--}6.4$ cm, papery, adaxially glabrous except veins which are covered with minute hairs, abaxially glabrous or only sparsely hairy except for tufted hairs at axils of lateral veins, secondary veins 6–9 on each side of mid-vein, adaxially slightly sunken, abaxially slightly elevated, base broadly cuneate, apex long acuminate, margin serrate, teeth 2–3 mm high, 13–31 along each side of the margin. Inflorescences corymbose cymes, 2–7 cm long, 4–12 cm in diam., densely pubescent, apex flat to slightly arcuate, 3–5-branched; the longest internode of each branch 1.5–2.5 cm long, densely pubescent; infructescence attaining to 7×12 cm. Marginal showy flowers light blue, on pedicel 1–2 cm long; sepals 3 to 5, rhomboid-elliptic, $0.8\text{--}1.4 \times 0.5\text{--}1.1$ cm, glabrous, apex obtuse, base rounded to cuneate, margin entire. Fertile flowers protandrous, light blue.

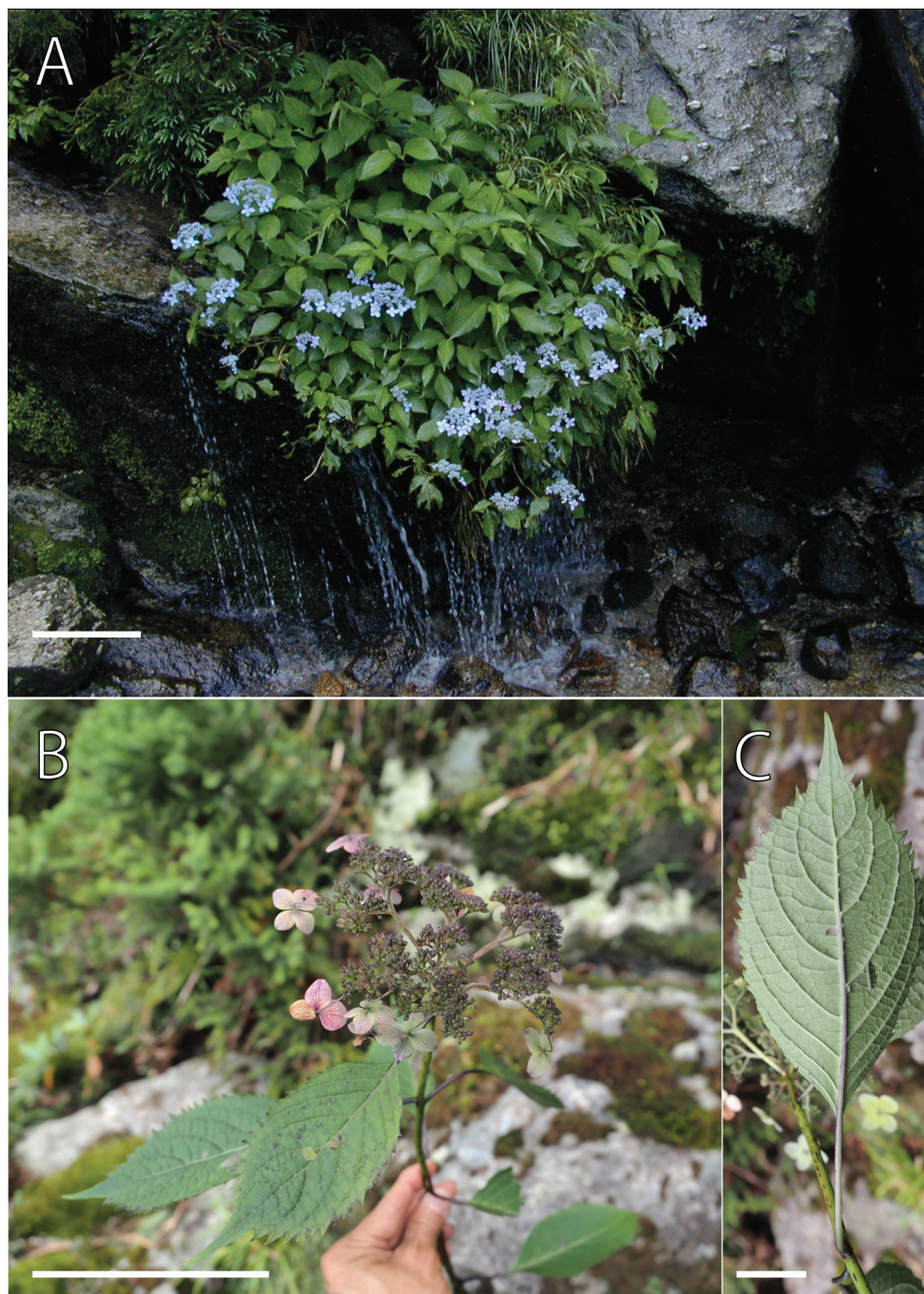


Figure 5. *Hydrangea acuminata* subsp. *yakushimensis* Yahara & Tagane **A** a tree growing on cliff along stream **B** a fruiting twig of the specimen JPN1799 (holotype) **C** lower leaf surface of the specimen JPN1799. Scale bars: 20 cm (**A**); 10 cm (**B**); 2 cm (**C**).

Male-stage flowers on pedicel 1–1.8 mm long; calyx tube funnel-shaped, ca. 1 mm long, 0.8 mm in diam., lobes 5, triangular, 0.5 × 0.4 mm, apex acute; petals 5, light blue, elliptic, 2–2.2 × 1 mm, glabrous, apex acute; stamens 10, light blue, subequal, filaments 1.5–3 mm long, glabrous, anthers white, globular, 0.6 mm in diam.; ovary nearly 1/2 superior, style 3, connate at base, slightly spreading, dark blue, ca. 0.7 mm long, stigma flat. In female-stage flowers, petals and stamens fallen off; ovary nearly 1/2 superior; calyx tube light blue, ca. 1 mm long; style darker blue, spreading, ca. 1 mm long; capsules 2.2–2.7 mm long; calyx tube subglobose, 1.2–1.4 mm long, 1.5–2 mm in diam., projected apical part including persistent styles 1.5 mm long. Seeds light brown, elliptic, 0.8 × 0.5 mm, not winged.

Japanese name. Yakushima-ruri-ajisai.

Phenology. Flowers were collected in July and August, and fruits were collected in September.

Distribution and habitat. Yakushima (Yaku Island), Japan (endemic). The distribution of *H. acuminata* subsp. *yakushimensis* is restricted to cliffs along streams at Yakushima. It mainly grows in the mountain-top area from 1520 to 1750 m, but one population occurs at an elevation of 575 m, along the Miyanoura River.

Etymology. The specific epithet is derived from the type locality, Yakushima.

IUCN Conservation status. Endangered (EN) based on criterion D; the population size is above 50, but less than 250.

Additional specimens examined. JAPAN. Kagoshima Pref., Yakushima: Mt. Nagata, on cliff, 30.343799°N, 130.492056°E, 1750 m elevation, 2 August 2005, with flowers, *T. Yahara*, *S. Tagane*, *K. Fuse* & *T. Saito* 0791 (FU!); Kamisamano-kubo, on cliff, 30.343799°N, 130.492056°E, 1750 m elevation, 2 August 2005, with flowers, *T. Yahara*, *S. Tagane*, *K. Fuse* & *T. Saito* 0792 (FU!); ditto, with flowers, *T. Yahara*, *S. Tagane*, *K. Fuse*, *T. Saito* 0793 (FU!); Nemachino-kubo, on cliff, 30.345465°N, 130.49468230°E, 1740 m elevation, 12 July 2006, sterile, *S. Tagane* & *K. Fuse* 1065 (FU!); Migitani, on cliff along stream, 30.3425555°N, 130.4810000°E, 1520 m elevation, 13 July 2006, with flowers, *S. Tagane* & *K. Fuse* 1103, 1104, 1105 (FU!); Sensuikyo, 30.372031°N, 130.504266°E, 575 m elevation, 31 August 2020, sterile, *K. Fuse* JPN1708 (FU!).

***Hydrangea acuminata* subsp. *australis* (T. Yamaz.) Yahara, stat. nov.**

urn:lsid:ipni.org:names:77248598-1

Hydrangea serrata var. *australis* T. Yamaz., J. Jap. Bot. 76: 175 (2001). **Type.** JAPAN.

Kagoshima Pref., Mt. Takakuma, 11 August 1942, *T. Yamazaki* s.n. (TI).

Hortensia serrata var. *australis* (T. Yamaz.) H. Ohba & S. Akiyama in Ohashi et al., Wild Fl. Jap. rev. ed. 4: 166 (2017), comb. nud.

Japanese name. Nangoku-yama-ajisai.

Distribution and habitats. *Hydrangea acuminata* subsp. *australis* is widely distributed at lower elevations in the Kagoshima Prefecture and the southern part of the Miyazaki Prefecture of the Kyushu Island and usually grows in disturbed places along

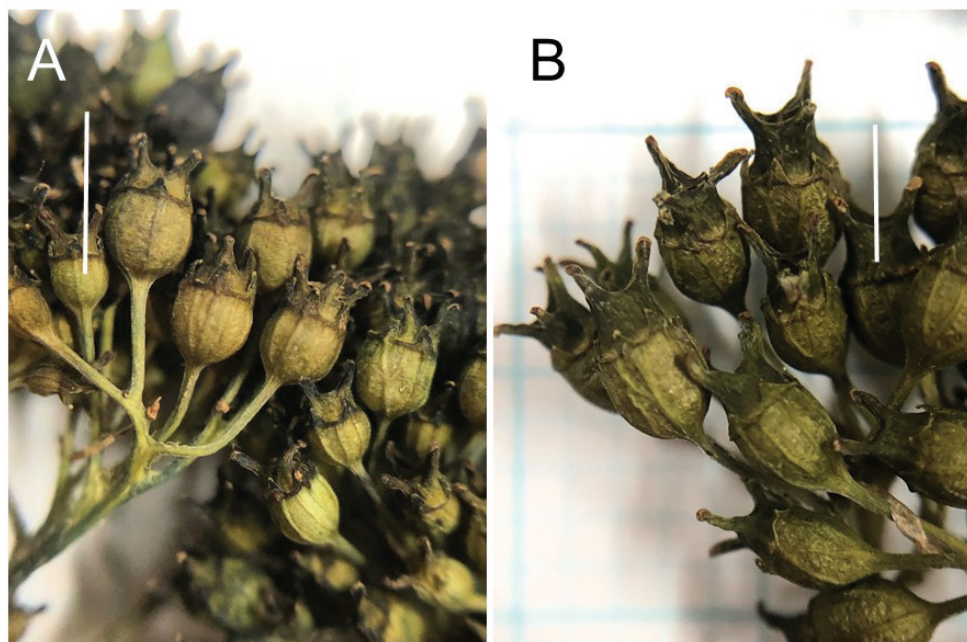


Figure 6. Fruits of *Hydrangea acuminata* subsp. *yakushimensis* Yahara & Tagane **A** and subsp. *acuminata* **B** Specimen: JPN1799 (holotype) **A** JPN2063 **B**. Scale bars: 3 mm.

the margins of evergreen forests or *Cryptomeria* plantations. JPN0908 was collected on a volcanic cliff at 1700 m elevation on Mt. Karakuni and was identified as subsp. *australis* in the MIG-seq tree (Fig. 2).

Note. *Hydrangea acuminata* subsp. *australis* is distinguished from subsp. *acuminata* mainly by its larger and wider leaves often exceeding 6.2 cm wide (vs. not exceeding 6.2 cm), having more serrations along margin (22–43 vs. 9–27) and dense curled hair on the lower surface of lamina. However, JPN0908, was identified as subsp. *australis* in the MIG-seq tree, which is morphologically similar to subsp. *acuminata* in having smaller leaves, fewer serrations, and sparser pubescence on the lower surface of the leaf. This specimen might be of hybrid origin between subsp. *australis* and subsp. *acuminata*.

Representative specimens examined. JAPAN. Kagoshima Pref.: Kagoshima City, 22 July 2002, with flowers, *K. Maruno* s.n. (KAG 083840!); Shibushi City, 4 June 2002, with fruits, *K. Maruno* s.n. (KAG 083882!); Aira City, 11 July 2004, *K. Maruno* s.n. (KAG 086731!); Kimotsuki Town, 300 m elevation, 20 July 1986, with fruits, *S. Hatusima* 41199 (KAG 161312!); Kirishima City, 450 m elevation, 22 November 1986, with fruits, *S. Hatusima* 41920 (KAG 161315!); Mt. Nokubi, 700 m elevation, 12 July 1987, with flowers, *S. Hatusima* 42447 (KAG 161327!).

***Hydrangea minamitanii* (H. Ohba) Yahara, stat. nov.**

urn:lsid:ipni.org:names:77248599-1

Hydrangea serrata (Thunb.) Ser. var. *minamitanii* H. Ohba in J. Jap. Bot. 64: 199 (1989); Ohba & Akiyama, Bull. Natl. Mus. Nat. Sci., Ser. B, 39: 179 (2013).

Type. JAPAN. Miyazaki Pref., Saito City, *T. Minamitani* 26304 (TI).

Hortensia serrata var. *minamitanii* (H. Ohba) H. Ohba & S. Akiyama, J. Jap. Bot. 91: 347 (2016).

Japanese name. Hyuga-ajisai.

Note. *Hydrangea minamitanii* and *H. acuminata* ssp. *acuminata* often grow close, within 100 m of each other, but the former grows on wet cliffs along streams, and the latter grows on soil along forest margin. *Hydrangea minamitanii* is distinct from *H. acuminata* in having leaves glabrous abaxially except tufted hairs at axils of lateral veins, glabrous petioles, and glabrous young shoots (Table 5). No intermediates have been discovered in localities where two species grow. *Hydrangea minamitanii* is similar to *H. acuminata* subsp. *yakushimensis* in growing on cliffs along streams and having leaves glabrous on both surfaces except veins and tufted hairs at axils of lateral veins, but they are distinguished by their capsule size (3.9–5.3 mm or longer in *H. minamitanii* vs. 2.2–2.7 mm in *H. acuminata* subsp. *yakushimensis*) and flower colors (pink or white flowers vs. blue flowers). Whereas *H. acuminata* subsp. *yakushimensis* is endemic to the Yakushima island, *H. minamitanii* is restricted to the mountains of central and eastern Kyushu, mainly in the Miyazaki Prefecture.

Additional specimens examined. JAPAN. Miyazaki Pref.: Mt. Osuzu, 500 m elevation, 20 October 1960, with fruits, *S. Sako* 3285 (KAG 161375!); ditto, 500 m elevation, 28 July 1971, with flowers, *S. Hatusima* & *S. Sako* 32643 (KAG 161376!); ditto, 11 July 1976, with flowers, *T. Minamitani* 22630 (KAG 161378!); Aya Town, 73 m elevation, 24 October 2019, with fruits, *S. Tagane* 1200 (KAG 128616!).

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with permission from the local offices of Forestry Agency: Mt. Shiraiwa of Kyushu-chuo-sanchi National (Kokutei) Park, Mt. Oyaji of Sobo-katamuki National (Kokutei) Park, Mt. Karakuni of Kirishima National (Kokuritsu) Park, Osugi-dani of Yoshino-kumano National (Kokuritsu) Park, and Mt. Amagi of Fuji-hakone National (Kokuritu) Park. We thank the Ministry of Environment's Rare Species Conservation Promotion Office for their help in obtaining collection permits. We would like to thank Editage (www.editage.com) for English language editing. This study was supported by the Environment Research and Technology Development Fund (JP-MEERF20204001) of the Ministry of the Environment, Japan, and partly by JSPS KAKENHI grant number 21K06307.

References

- Binh HT, Ngoc NV, Tagane S, Toyama H, Mase K, Mitsuyuki C, Suyama Y, Yahara T (2018) A taxonomic study of *Quercus langbianensis* complex based on morphology, and DNA barcodes of classic and next generation sequences. *PhytoKeys* 95: 37–70. <https://doi.org/10.3897/phytokeys.95.21126>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* (Oxford, England) 30(15): btu170. <https://doi.org/10.1093/bioinformatics/btu170>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25: 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An analysis tool set for population genomics. *Molecular Ecology* 22(11): 3124–3140. <https://doi.org/10.1111/mec.12354>
- Chen CW, Ebihara A, Chiou WL, Li CW (2014) *Haplopteris yakushimensis* (Pteridaceae, Vittarioideae), a new species from Yakushima Island, Japan. *Phytotaxa* 156(4): 229–234. <https://doi.org/10.11646/phytotaxa.156.4.5>
- De Smet Y, Mendoza CG, Stefan Wanke S, Goetghebeur P, Samain MS (2015) Molecular phylogenetics and new (infra)generic classification to alleviate polyphyly in tribe Hydrangeae (Cornales: Hydrangeaceae). *Taxon* 64(4): 741–753. <https://doi.org/10.12705/644.6>
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* (San Francisco, Calif.) 12: 12–15.
- Hori K, Ebihara A, Nakato N, Murakami N (2015) *Dryopteris protobissetiana* (Dryopteridaceae), a new diploid sexual species of the *Dryopteris varia* complex (Subg. Erythrovariae, Sect. Variae) from Yakushima, Kagoshima, Japan. *Acta Phytotaxonomica et Geobotanica* 66: 47–57. <https://doi.org/10.18942/apg.KJ00009868505>
- Hotta M (1974) Distribution and Differentiation of Plants. Sanseido, Tokyo, 12 pp. [In Japanese]
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>

- Katsuyama T (2009) A new species of the genus *Carex* (Cyperaceae) from Yakushima Island, Japan. *Journal of Japanese Botany* 84: 8–12. http://www.jjbotany.com/pdf/JJB_084_008_012.pdf
- Kitamura S, Murata G (1979) Colored Illustration of Woody Plants of Japan 2. Hoikusha, Osaka, 545 pp.
- Masamune G (1934) Floristic and geobotanical studies on the island of Yakushima. *Memoirs of the Faculty of Science and Agriculture. Taihoku Imperial University* 11: 1–637.
- Middleton DJ, Armstrong K, Baba Y, Balslev H, Chayamarit K, Chung RCK, Conn BJ, Fernando ES, Fujikawa K, Kiew R, Luu HT, Aung MM, Newman MF, Nobuyuki T, Tagane S, Thomas DC, Tran TB, Utteridge TMA, van Welzen PC, Widyatmoko D, Yahara T, Wong KM (2019) Progress on Southeast Asia's Flora projects. *Gardens' Bulletin (Singapore)* 71(2): 267–319. [https://doi.org/10.26492/gbs71\(2\).2019-02](https://doi.org/10.26492/gbs71(2).2019-02)
- Minamitani T, Kadota Y, Yonekura K (2018) A classification of the genus *Rhododendron* sect. *Brachycalyx* (Ericaceae) in Japan (1). *Journal of Japanese Botany* 93(2): 75–103.
- Nagahama A, Tagane S, Zhang M, Ngoc NV, Binh HT, Cuong TQ, Nagamasu H, Toyama H, Tsuchiya K, Yahara T (2021) *Claoxylon langbianense* (Euphorbiaceae), a new species from Bidoup-Nui Ba National Park, southern Vietnam. *Acta Phytotaxonomica et Geobotanica* 72: 275–280. <https://doi.org/10.18942/apg.202016>
- Ohba H (2017) Hydrangeaceae. In: Ohashi H, Kadota Y, Murata J, Yonekura K (Eds) *Wild Flowers of Japan*, revised edition 4. Heibonsha, Tokyo, 157–172. [In Japanese]
- Ohba H, Akiyama S (2013) A revision of the species of *Hydrangea* (Hydrangeaceae) described by Siebold and Zuccarini, Part 1. *Bulletin of the National Museum of Nature and Science. Series B, Botany* 39(4): 173–194. https://www.kahaku.go.jp/research/publication/botany/download/39_4/BNMNS_B39-4_173-194.pdf
- Ohba H, Akiyama S (2016) Generic segregation of some sections and subsections of the genus *Hydrangea* (Hydrangeaceae). *Journal of Japanese Botany* 91(6): 345–350. http://www.jjbotany.com/pdf/JJB_091_345_350.pdf
- Okano T, Matsuda H (2013) Biocultural diversity of Yakushima Island: Mountains, beaches, and sea. *Journal of Marine and Island Cultures* 2(2): 69–77. <https://doi.org/10.1016/j.imic.2013.11.008>
- Okuyama Y, Fujii N, Wakabayashi M, Kawakita A, Ito M, Watanabe M, Murakami N, Kato M (2005) Nonuniform concerted evolution and chloroplast capture: Heterogeneity of observed introgression patterns in three molecular data partition phylogenies of Asian *Mitella* (Saxifragaceae). *Molecular Biology and Evolution* 22(2): 285–296. <https://doi.org/10.1093/molbev/msi016>
- Okuyama Y, Goto N, Nagano AJ, Yasugi M, Kokubugata G, Kudoh H, Qi Z, Ito T, Kakishima S, Sugawara T (2020) Radiation history of Asian *Asarum* (sect. *Heterotropa*, Aristolochiaceae) resolved. *Annals of Botany* 126(2): 245–260. <https://doi.org/10.1093/aob/mcaa072>
- Rochette NC, Rivera-Colón AG, Catchen JM (2019) Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology* 28: 4737–4754. <https://doi.org/10.1111/mec.15253>
- Roesti M, Salzburger W, Berner D (2012) Uninformative polymorphisms bias genome scans for signatures of selection. *BMC Evolutionary Biology* 12(1): e94. <https://doi.org/10.1186/1471-2148-12-94>

- Satake Y, Watari S, Hara H, Tominari T [Eds] (1999) Wild Flowers of Japanese Trees 1. Heibonsha, Tokyo.
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* (Oxford, England) 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Strijk JS, Binh HT, Ngoc NV, Pereira JT, Slik F, Sukri RS, Suyama Y, Tagane S, Wiering JJ, Yahara T, Hinsinger DD (2020) Museomics for reconstructing historical floristic exchanges: Divergence of stone oaks across Wallacea. *PLoS ONE* 15(5): e0232936. <https://doi.org/10.1371/journal.pone.0232936>
- Suetsugu K, Fukunaga H (2016) *Lecanorchis tabugawaensis* (Orchidaceae, Vanilloideae), a new mycoheterotrophic plant from Yakushima Island, Japan. *PhytoKeys* 73: 125–135. <https://doi.org/10.3897/phytokeys.73.10019>
- Suetsugu K, Tsukaya H, Ohashi H (2016) *Sciaphila yakushimensis* (Triuridaceae), a new mycoheterotrophic plant from Yakushima Island, Japan. *Journal of Japanese Botany* 91: 1–6. http://www.jjbotany.com/pdf/JJB_091_1_6.pdf
- Sugiura N (1978) Further analysts of the data by akaike's information criterion and the finite corrections. *Communications in Statistics – Theory and Methods* 7: 13–26. <https://doi.org/10.1080/03610927808827599>
- Suyama Y, Hirota SK, Matsuo A, Tsunamoto Y, Mitsuyuki C, Shimura A, Okano K (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. *Ecological Research*. 37: 171–181. <https://doi.org/10.1111/1440-1703.12270>
- Suyama Y, Matsuki Y (2015) MIG-seq: An effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Scientific Reports* 5(1): e16963. <https://doi.org/10.1038/srep16963>
- Tanabe AS (2011) Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multi-locus sequence data. *Molecular Ecology Resources* 11: 914–921. <https://doi.org/10.1111/j.1755-0998.2011.03021.x>
- Tanabe AS, Toju H (2013) Two new computational methods for universal DNA barcoding: a benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants. *PLoS ONE* 8: e76910. <https://doi.org/10.1371/journal.pone.0076910>
- Uemachi T, Mizuhara Y, Deguchi K, Shinjo Y, Kajino E, Ohba H (2014) Phylogenetic relationship of *Hydrangea macrophylla* (Thunb.) Ser. and *H. serrata* (Thunb.) Ser. evaluated using RAPD markers and plastid DNA sequences. *Journal of the Japanese Society for Horticultural Science* 83(2): 163–171. <https://doi.org/10.2503/jjshs1.CH-092>
- Yahara T, Tsukaya H (2008) *Oxygyne yamashitae*, a new species of Thismiaceae from Yaku Island, Japan. *Acta Phytotaxonomica et Geobotanica* 59: 57–68. https://www.jstage.jst.go.jp/article/apg/59/2/59_KJ00005012322/_pdf
- Yahara T, Ohba H, Murata J, Iwatsuki K (1987) Taxonomic review of vascular plants endemic to Yakushima Is., Japan. *Journal of the Faculty of Science, University of Tokyo. Sect. 3. Botany* 14: 69–119. <http://www.biology.kyushu-u.ac.jp/~ecology/yahara/publication/Yahara&al1987Yakushima.pdf>

- Yahara T, Hirota SK, Fuse K, Sato H, Tagane S, Suyama Y (2021a) Validation of *Hosta alata* (Asparagaceae) as a new species and its phylogenetic affinity. *PhytoKeys* 181: 79–93. <https://doi.org/10.3897/phytokeys.181.64245>
- Yahara T, Hirota SK, Fuse K, Sato H, Tagane S, Suyama Y (2021b) A new subspecies of *Stellaria alsine* (Caryophyllaceae) from Yakushima, Japan. *PhytoKeys* 187: 177–188. <https://doi.org/10.3897/phytokeys.187.64023>
- Yamazaki T (2001) A new variety of *Hydrangea serrata* (Thunb.) Ser. *Journal of Japanese Botany* 76: 174–175.
- Zhang M, Yahara T, Tagane S, Rueangruea S, Suddee S, Moritsuka E, Suyama Y (2020) *Cryptocarya kaengkrachanensis*, a new species of Lauraceae from Kaeng Krachan National Park, south-west Thailand. *PhytoKeys* 140: 139–157. <https://doi.org/10.3897/phytokeys.140.34574>

The rediscovery of *Carya poilanei* (Juglandaceae) after 63 years reveals a new record from China

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Abstract

Despite having been first published in 1941, *Carya poilanei* (A.Chev.) J.-F.Leroy is only known from three collections in Vietnam, Laos and Thailand. It has not been recollected since then and was long suspected to have become extinct through repeated deforestation events. Here, we report the rediscovery, and meanwhile the first new record in China, of this extremely rare gigantic hickory species at Yunnan province 63 years after its last collection in 1958. Three small patchy subpopulations were found with a total of about 50 adult trees having diameter at breast height (DBH) larger than 60 cm, together with some seedlings and saplings, but the fruit set was low. Based on new and fresh material, we present a revised morphological description of *C. poilanei*, and an updated distribution map for the species. In addition, we also provide a key for the hickories in China. Lastly, we suggest *C. poilanei* should be listed as a Critically Endangered (CR) species according to the latest IUCN Red List Categories and Criteria.

Keywords

Ailao Mountains, hickory, limestone, *Sinocarya*, Yunnan province

Introduction

Carya Nutt., consisting of ca. 17 currently accepted species (Manning 1978; Chang and Lu 1979; Lu et al. 1999; Zhang et al. 2013; Grauke et al. 2016), is the second largest genus in Juglandaceae DC. ex Perleb after *Juglans* L. (Kozłowski et al. 2018), with a discontinuous distribution in South-Eastern Asia and eastern North America

(Stone 1997; Lu et al. 1999). The genus *Carya* includes many internationally important and economically valuable nut crops such as pecan (*C. illinoensis* (Wangenh.) K.Koch) and Chinese hickory (*C. cathayensis* Sarg.). All *Carya* species are monoecious with male and female inflorescences being separate, dichogamous and anemophilous, and fruit maturation process is heterochronic (Grauke and Mendoza-Herrera 2012). Based on the presence, number and arrangement of bud scales, *Carya* was divided into three sections: sect. *Apocarya* C.DC., sect. *Carya* and sect. *Sinocarya* Cheng & R.H.Chang (de Candolle 1864; Chang and Lu 1979). The first two sections were established in eastern North America, while the last section was found in South-Eastern Asia.

So far, five hickory species have been recognized in South-Eastern Asia, specifically in southern China, northern Vietnam, northern Laos, northern Thailand and north-eastern India (Manning 1963; Chang and Lu 1979; Srisanga 2017). Among the five species, three (*C. cathayensis*, *C. hunanensis* W.C.Cheng & R.H.Chang and *C. kweichowensis* Kuang & A.M.Lu) are endemic to China; their distributions hardly overlap and are, in general, extremely rare (Chang and Lu 1979; Lu et al. 1999; Grauke and Mendoza-Herrera 2012). *Carya tonkinensis* Lecomte seems to be the most widespread species in South-Eastern Asia, distributed in southwest China, northern Vietnam, northern Thailand and north-eastern India (Manning 1963; Chang and Lu 1979; Srisanga 2017). *Carya poilanei* (A.Chev.) J.-F.Leroy was described in 1941 based on a single collection from northern Vietnam in 1937 (Chevalier 1941; Leroy 1950), and later, Manning (1963) added a record of specimen collected from Laos in 1932. Surprisingly, through the GBIF (Global Biodiversity Information Facility) network, we came across a specimen collected from Thailand in 1958 (Smitinand 4319, L0069301/L.1551797), which was identified as *C. poilanei* by Michael Vomborg in 2006 and has not yet been recorded in Flora of Thailand. Although botanists have made efforts to seek the surviving members in the area where the type tree was originally located (Grauke et al. 1991; Grauke et al. 2016), living trees of *C. poilanei* have not been found for over 63 years, and thus this species has been suspected to be extinct in the wild (Grauke and Mendoza-Herrera 2012; Grauke et al. 2016).

At the end of July 2021, during a scientific field trip in Jianshui County, southern Yunnan province of China, three fragmented subpopulations of *Carya* were discovered near the eastern edge of Ailao Mountains (Fig. 1). After morphological comparison to the images of type material and scrutiny of the brief description, we confirm that they belong to *C. poilanei* (Figs 2, 3). This finding allowed us to update its morphological description, discuss its geographic distribution, and assess its conservation status. Furthermore, it would be conducive to inferring its phylogenetic position within *Carya*, and valuable to exploit its genetic resources for breeding and crop development in future days.

Materials and methods

Specimens were collected in the field of Jianshui county, Yunnan province in July to October, 2021. Except for Fig. 1C and Fig. 3A which were taken by DJI Mavic 2 Pro, the rest of the photos were taken by Canon EOS 70D with Sigma 17–50 mm

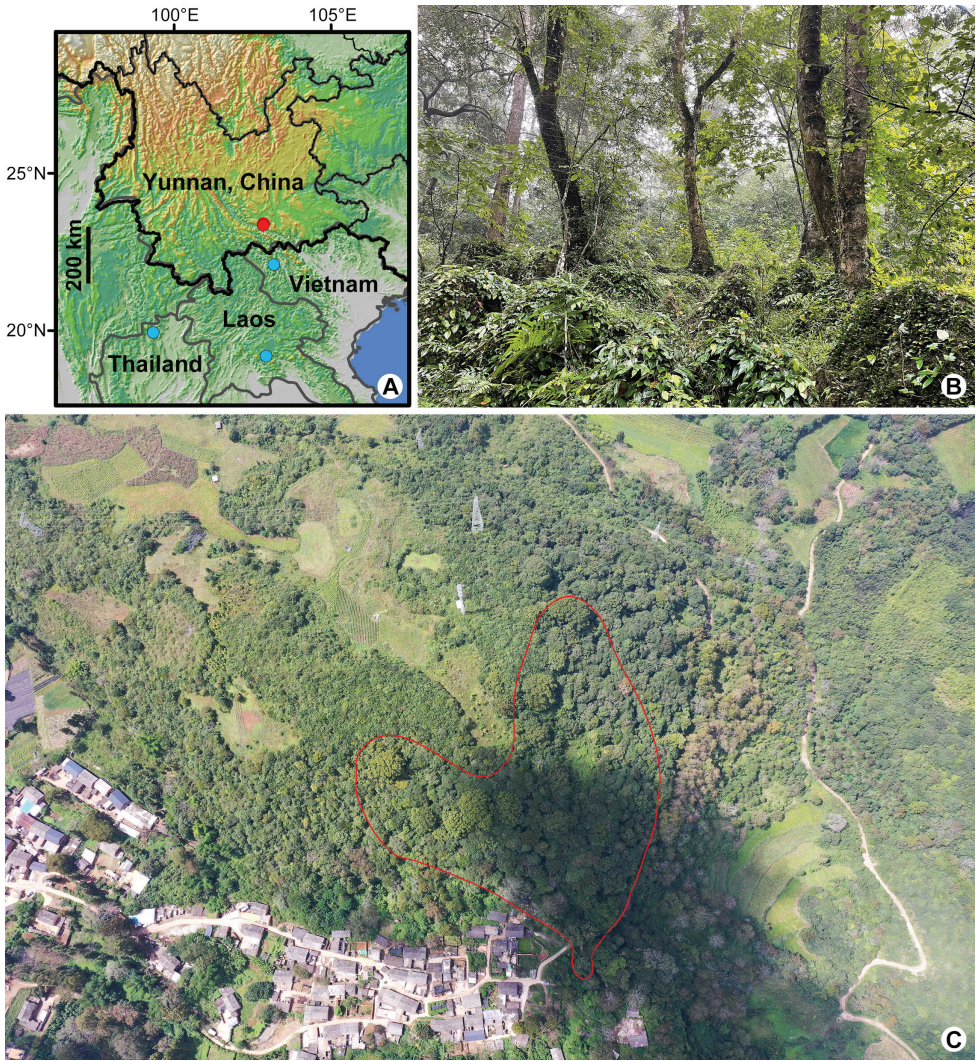


Figure 1. Distribution and habitat of *Carya poilanei* (A.Chev.) J.-F.Leroy **A** four distribution sites in Yunnan province of China, Vietnam, Laos and Thailand. Red circle indicates the localities taken from the new record areas, and blue circles indicate historical distribution localities where the trees have been presumably extirpated **B** the limestone mountain habitat **C** eastern edge of Ailao Mountains, with human habitation in plateau. Red lines mark the boundary of the *C. poilanei* distribution in Dajinglaozhai Village.

(f/2.8 EX DC OS HSM) and Canon EF 100 mm (f/2.8L IS USM) lens. Because these four months are the fruiting period, we were unable to investigate the flower phenology and characters. The morphology of the species was observed and measured based on living plants and dry specimens. Morphological measurements for more than six freshly differentiated samples from the adult trees were taken using both a ruler and a digital caliper. All herbarium voucher specimens collected by us are deposited in the Herbarium of College of Life Science, Beijing Normal University (BNU).

Taxonomy

Carya poilanei (A.Chev.) J.-F.Leroy, *Rev. Int. Bot. Appl. Agric. Trop.* 30: 428. 1950. Figures 2, 3

Juglans poilanei A.Chev., *Rev. Bot. Appl. Agric. Trop.* 21: 496. 1941.

Type. Vietnam. Lai-Chau province, within the great forest near the slopes of Pou-Nhou, in calcareous soil, at 1000 m. elev., 31 Dec. 1937, *Poilane* 26964, (Holotype: P [barcode P00605884, image!]; isotypes: P [barcode P00223582, P00605885, P00605886, image!]).

Revised description. Tree up to 15–40 m tall, deciduous, monoecious. Trunk to 0.5–2 m in diam.; bark smooth or somewhat rough, gray to whitish. Branches brown or gray-brown, initially with orange-yellow glandular and white pilose above, later almost glabrous and sparsely glandular, with roughish, scattered lenticels; pith solid in stem. Terminal buds 3–15 mm, both naked and with valvate scales, but the scales often drop easily, gray brown or brown. Leaf length 30–60 cm (incl. petiole), imparipinnate, soft green, papery; petiole 6–12 cm, enlarged at base, pubescent or glabrous; rachis pubescent or glabrous, sparsely glandular; leaflets (3 or) 5 (or 7), apical one shortly petiolulate, terminal petiolule 5–12 mm, lateral ones sessile or subsessile, broad obovate, occasionally obovate lanceolate or ovate-lanceolate, base skewed to nearly round, apex shortly obtuse or acuminate, margin serrate; adaxially smooth or finely scabrid, abaxially glabrous except for hairs along midvein and in axils of secondary veins, secondary vein 15–25 pairs, sometimes old leaflets blade densely covered with brown scales; apical and middle leaflets 25–40 × 12–20 cm, much larger than base leaflets. Flowers not seen. Fruits subglobose or compressed-globose, 2.8–3.2 × 3–3.5 cm, with peduncle, 1.5–6 cm length; husk wingless, sparsely orange-glandular, shortly pubescent, 3.6–5.6 mm thick, moderate keels extending to middle, cracks into 3 or 4 petals when dried; shell subglobose, closely white tomentose, with 2 longitudinal ridges, apex slightly convex, 2.8–3.5 mm thick, cracks into 2–4 sections when dried, equal or unequal; 3, 4 or 6 chambered at base, lacunae present in the wall near the secondary septa. Flower unclear. Fruit Sep. Germination hypogeal.

Distribution. China: Yunnan Province, Jianshui County; Vietnam: Lai-Chau Province, Pou-Nhou; Laos: Vientiane Province, Ban Mouang Cha (Muang Cha); Thailand: Chiang Mai (Chiangmai) Province, Fang District, Doi Pha Hom Pok Range (Fig. 1A).

Habitat and ecology. It grows on southeastern and southern slope of the limestone or calcareous mountain at an elevation of 1000–2050 m (Fig. 1B, C). The three sites we discovered are in the subtropical region, on the eastern edge of Ailao Mountain, and 15 kilometers to the south are hot dry valleys (elevation about 200 m). Of these three distribution points, the closest distance between the two points is about 2 km, while the farthest is just about 6 km. Among the three threatened relic forests, *C. poilanei* are dominant and impressive trees.



Figure 2. *Carya poilanei* (A.Chev.) J.-F.Leroy (representative specimen, BNU20210730-1, BNU).

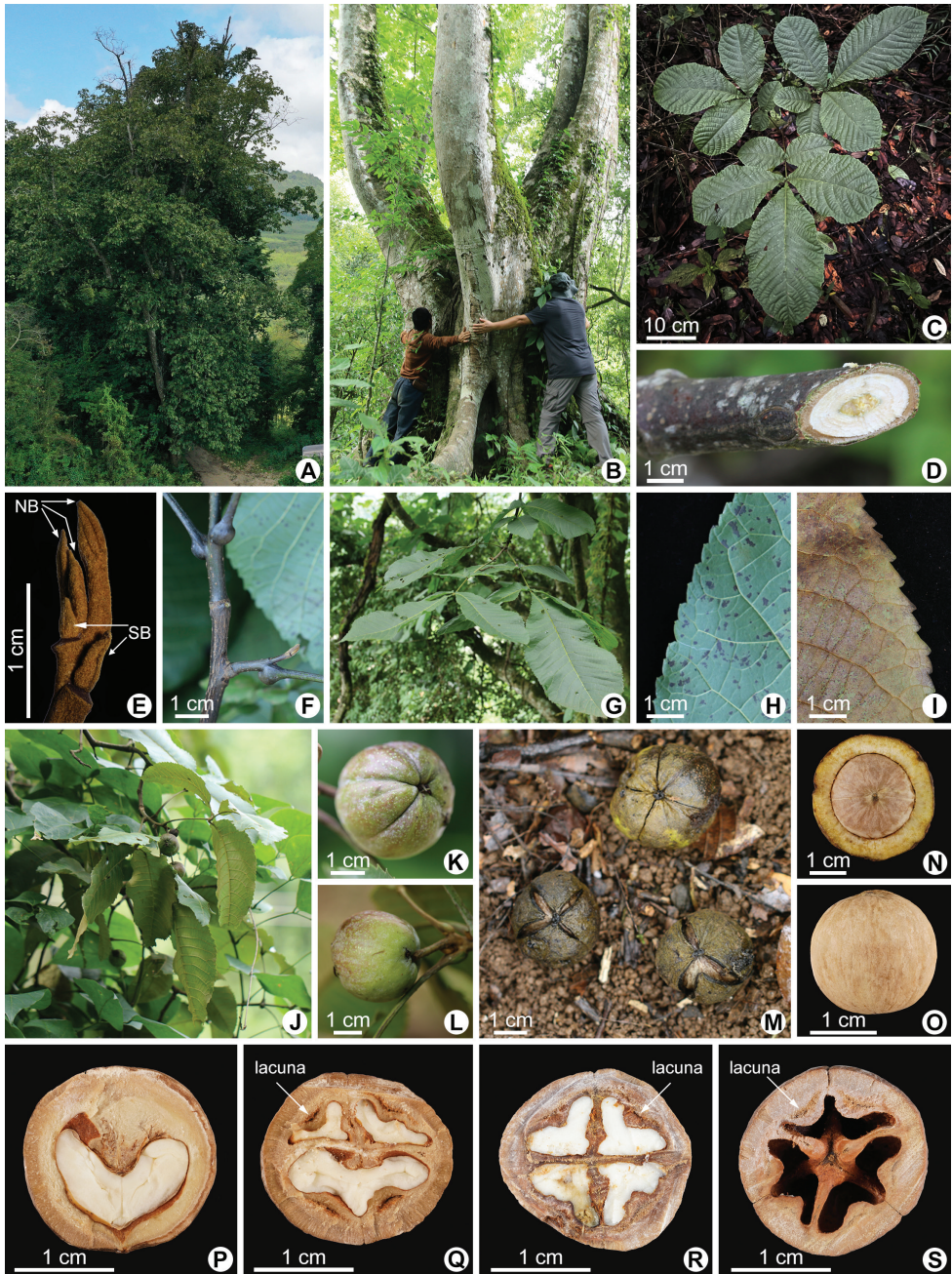


Figure 3. Living plants of *Carya poilanei* (A.Chev.) J.-F. Leroy **A** tree **B** trunk, with a maximum diameter at breast height (DBH) of 1.97 m **C** sapling **D** twig **E** terminal buds (NB: naked buds; SB: buds with valvate scales) **F** petiole enlarged at base **G** leaf, showing 5 leaflets **H** leaf abaxial glabrous **I** leaf abaxial densely brown scales **J** fruiting branch **K** husk, showing base **L** husk, showing peduncle **M** husk, irregularly dehiscent **N** shell, vertical view **O** shell, lateral view **P** longitudinal section of nut **Q–S** transversal section of nuts, with 3, 4 and 6 chambers, respectively; lacunae present.

Additional specimens examined. China: Yunnan Province, Jianshui County, Potou Town, Dajinglaozhai Village, on limestone, 23°23'40.42"N, 102°51'34.42"E, 1895 m, 30 Jul. 2021, *Zhang BNU20210730-1* (BNU, [barcode BNU0053602]) (Fig. 2), *ibid.*, 23°23'42.33"N, 102°51'36.65"E, 1889 m, 31 Jul. 2021, *Zhang 20210731-30* (BNU, KUN); Ximatang Village, on limestone, 23°24'5.68"N, 102°52'17.38"E, 2019 m, 31 Jul. 2021, *Zhang 20210731-3* (BNU), *ibid.*, 23°24'5.28"N, 102°52'11.25"E, 1980 m, 31 Jul. 2021, *Zhang 20210731-14* (BNU); Yuchu Village, on limestone, 23°20'47.75"N, 102°51'35.90"E, 1892 m, 31 Jul. 2021, *Zhang 20210731-17* (BNU, KUN), *ibid.*, 23°21'44.46"N, 102°51'27.65"E, 1859 m, 5 Oct. 2021, *Zhang 20211005-1* (BNU). **Laos:** Vientiane Province, Ban Mouang Cha (Muang Cha), on rocky limestone hill, c. 1500 m, 16 Apr. 1932, *Kerr 21092* (BM [barcode BM013822350, image!], K, P [barcode P06811763, image!]). **Thailand:** Chiang Mai (Chiengmai) Province, Fang District, Doi Pha Hom Pok Range, 19°55'0.80"N, 99°12'52.20"E, 1100–1180 m, 23 Feb. 1958, *Smitinand 4319* (L [barcode L0069301/L.1551797, image!]).

Key to the five native species of *Carya* Nutt. in China

- 1 Grows on limestone mountain; leaflets (3) 5 (7); nuts compressed-globose; husk smooth without longitudinal ridges, sparsely orange-glandular; lacunae present in nutshell **2**
- Grows on mountain slopes, valleys and riverbanks; leaflets 5–7 (9); nuts obovoid, ellipsoid or subglobose; husk wrinkled with longitudinal ridges; densely orange-glandular; lacunae absent in nutshell **3**
- 2 Leaflets mostly broad obovate, papery, rough, margin serrate ***C. poilanei***
- Leaflets elliptic to elliptic-lanceolate, more or less leathery, glossy, margin obtusely serrate ***C. kweichowensis***
- 3 Petiole tomentose; husk slightly winged ***C. tonkinensis***
- Petiole glabrescent; husk conspicuously winged **4**
- 4 Husk winged to middle ***C. hunanensis***
- Husk winged to base ***C. cathayensis***

Discussion

Initially, French botanist Auguste Chevalier placed *C. poilanei* into the genus *Juglans* after he observed the only specimen collected from Vietnam in 1941 (Chevalier 1941). The possible reason is that *C. poilanei* has the lacunae character (Fig. 3Q–R) which superficially resembles some species in the genus *Juglans*. Subsequently, Leroy (1950) placed the species to the genus *Carya*, based on the morphological features easy to distinguish from the *Juglans* genus such as unicular strands in the shell (Fig. 3O) and basal plexus, solid rather than chambered pith in the stem (Fig. 3D) (Leroy 1955). Although the presence of lacunae in the septum and/or shell walls are an atypical characteristic of relict hickory species, it has been recorded in seven fossil species from

Europe (Mai 1981), which may help to better understand the biogeographic histories of *Carya*. Besides, we also see evident lacunae in the shell walls of *C. kweichowensis*, which seems to be related to *C. poilanei* as it is also located in limestone mountain habitat (Fig. 1B, C). However, *C. poilanei* differs from *C. kweichowensis* in having broad obovate leaflets (Figs 2, 3C, G, J vs. elliptic to elliptic-lanceolate leaflets in *C. kweichowensis*) and gray brown or brown buds (Fig. 3E vs. black buds in *C. kweichowensis*) (Chang and Lu 1979). Significantly, the terminal buds of *C. poilanei* are not uniform, both naked and protected by valvate scales (Fig. 3E), but the scales are relatively small and easy to drop off. Molecular data would be necessary to explore its systematic status in the future work.

The hickory trees are not found in any nature reserve, but in the back hills of some aged village (Fig. 1C). Fortunately, these trees are close to villages and tall enough to be regarded as sacred trees by local villagers, and hence saved from being deforested. Based on our fieldwork in these areas, we found a total of three small and fragmented subpopulations, preserving about 50 adult trees with diameter at breast height (DBH) larger than 60 cm (Fig. 3B) as well as some understory seedlings (Fig. 3C) and juvenile trees; however, fruit sets were low. We evaluated the conservation status for the *C. poilanei* according to the latest IUCN Red List guidelines (IUCN Standards and Petitions Committee 2019) and suggested that the species should be ranked as critically endangered (CR). Meanwhile, we recommend that the species should be added to the new version of the List of National Key Protected Wild Plants and Plant Species with Extremely Small Populations, China. Given its rather limited number of individuals and narrow potential geographical range, this species clearly needs to be properly protected, even in the absence of known strategies of utilization. More efforts are required for strengthening its *in situ* and *ex situ* conservation, as well as studying its systematic position and genetic diversity. We propose that, in the future, protected areas should be established *in situ*, and a more exhaustive investigation could be launched into the nearby limestone mountains.

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Reference

- Chang RH, Lu AM (1979) A study of the genus *Carya* Nutt. in China. *Acta Phytotaxonomica Sinica* 17(2): 40–44. <https://www.jse.ac.cn/EN/Y1979/V17/I2/40> [In Chinese]
- Chevalier A (1941) Variabilité et Hybridité chez les Noyers. Notes sur des Juglans peu connus, sur l'Annamocarya et un Carya d'Indochine. *Revue de botanique appliquée et d'agriculture coloniale* 21(241): 477–509. <https://doi.org/10.3406/jatba.1941.1646>
- de Candolle ACP (1864) Juglandaceae. In: de Candolle A (Ed.) *Prodromus Systematis Naturalis Regni Vegetabili*. Sumptibus Victoris Masson et Filii, Paris, 134–146.
- Grauke LJ, Mendoza-Herrera MA (2012) Population structure in the genus *Carya*. *Acta Horticulturae* 948: 143–158. <https://doi.org/10.17660/ActaHortic.2012.948.16>
- Grauke LJ, Wood BW, Payne JA (1991) Genetic resources of *Carya* in Vietnam and China. *Annual report of the Northern Nut Growers Association* 82: 80–87.
- Grauke LJ, Wood BW, Harris MK (2016) Crop Vulnerability: *Carya*. *HortScience* 51(6): 653–663. <https://doi.org/10.21273/HORTSCI.51.6.653>
- IUCN Standards and Petitions Committee (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Kozłowski G, Bétrisey S, Song Y (2018) Wingnuts (*Pterocarya*) & walnut family. Relict trees: linking the past, present and future. Natural History Museum Fribourg, Switzerland, 127 pp.
- Leroy JF (1950) Note sur les noyers (*Carya* et *Annamocarya*) sauvages d'indochine. *Revue Internationale De Botanique Appliquée Et D'agriculture Tropicale* 30(333): 425–428. <https://doi.org/10.3406/jatba.1950.6726>
- Leroy JF (1955) Etude sur les Juglandaceae. A la recherche d'une conception morphologique de la fleur femelle et du fruit. *Bulletin du Museum national d'Histoire naturelle*, Paris, 1–11. <https://www.biodiversitylibrary.org/item/266009#page/1/mode/1up>
- Lu AM, Stone DE, Grauke LJ (1999) Juglandaceae. In: Wu ZY, Raven PH (Eds) *Flora of China*, Volume 4, Cycadaceae through Fagaceae. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 277–285.
- Mai DH (1981) Der Formenkreis der Vietnam-Nuß (*Carya poilanei* (Chev.) Leroy) in Europa. *Feddes Repertorium* 92(5–6): 339–385. <https://doi.org/10.1002/fedr.19810920502>
- Manning WE (1963) Hickories reported in India and Laos with other notes on *Carya* in Asia. *Brittonia* 15(2): 123–125. <https://doi.org/10.2307/2805397>
- Manning WE (1978) The classification within the Juglandaceae. *Annals of the Missouri Botanical Garden* 65(4): 1058–1087. <https://doi.org/10.2307/2398782>
- Srisanga P (2017) Juglandaceae. In: Santisuk T, Balslev H (Eds) *Flora of Thailand Volume Thirteen Part Three*. Niran Hetrakul, Prachachon Co. Ltd., Thailand, 512–519.
- Stone DE (1997) Juglandaceae. In: *Flora of North America Editorial Committee* (Eds) *Flora of North America north of Mexico*. Oxford University Press, New York, 416–428.
- Zhang JB, Li RQ, Xiang XG, Manchester SR, Lin L, Wang W, Wen J, Chen ZD (2013) Integrated fossil and molecular data reveal the biogeographic diversification of the eastern Asian-eastern North American disjunct hickory genus (*Carya* Nutt.). *PLoS ONE* 8(7): e70449. <https://doi.org/10.1371/journal.pone.0070449>

Supplementary material I

The rediscovery of *Carya poilanei* (Juglandaceae) after 63 years, a extremely gigantic hickory species new record from China

Authors: Wei-Ping Zhang

Data type: COL

Explanation note: Despite having been first published in 1941, *Carya poilanei* (A.Chev.) J.-F.Leroy is only known from three collections in Vietnam, Laos and Thailand. It has not been recollected since then and was long suspected to have become extinct through repeated deforestation events. Here, we report the rediscovery, and meanwhile the first new record in China, of this extremely rare gigantic hickory species at Yunnan province 63 years after its last collection in 1958. Three small patchy subpopulations were found with a total of about 50 adult trees having diameter at breast height (DBH) larger than 60 cm, together with some seedlings and saplings, but the fruit set was low. Based on new and fresh material, we present a revised morphological description of *C. poilanei*, and an updated distribution map for the species. In addition, we also provide a key for the hickories in China. Lastly, we suggest *C. poilanei* should be listed as a Critically Endangered (CR) species according to the latest IUCN Red List Categories and Criteria.

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Link: <https://doi.org/10.3897/phytokeys.188.77242.suppl1>

Nicotiana rupicola sp. nov. and *Nicotiana knightiana* (sect. *Paniculatae*, Solanaceae), a new endemic and a new record for the flora of Chile

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Abstract

Nicotiana knightiana is recorded for the first time for the flora of Chile. A new species of *Nicotiana*, endemic to the coast of the Coquimbo region is described and illustrated. Molecular analysis placed the new species within the *N.* sect. *Paniculatae*, as sister to *N. cordifolia*, an endemic to Juan Fernandez islands. The new species can be considered critically endangered (CR) according to the IUCN categories due to its restricted and fragmented distribution, small population number, and the threat that urbanization and mining activities represent for the conservation of the biodiversity of the area.

Keywords

Coquimbo, endemism, exotic species, Nicotianoidae, systematics, taxonomy

Introduction

Nicotiana L. is one of the largest genera in the Solanaceae with 75 recognised species (Clarkson et al. 2017) including the important crop plant *Nicotiana tabacum* L. *Nicotiana* is naturally distributed in America and Australia, with one species from south-west Africa (Hunziker 2001). Evidence of homoploidy and polyploidy showed that hybridization has been a major driver of speciation in the genus (Clarkson et al. 2010).

Since Reiche's (1903) treatment, no modern revision of *Nicotiana* in Chile has been published. According to the last catalogue of the flora of Chile (Rodriguez et al. 2018), the country is home to 12 native accepted species of *Nicotiana* distributed from Arica y Parinacota region to Magallanes region and Juan Fernandez Archipelago, and two introduced species, *Nicotiana tabacum*, in Juan Fernandez and Easter Island, and *Nicotiana glauca* Graham in North and Central Chile. In his monograph of the genus, Goodspeed (1954) provided a taxonomic classification in which he recognised three subgenera further divided into 14 sections. Most of his sections have been confirmed as monophyletic by molecular analyses (Aoki and Ito 2000; Chase et al. 2003; Clarkson et al. 2004; Mehmood et al. 2020). Two Chilean species are included in *Nicotiana* sect. *Paniculatae* Goodsp.: the endemics *Nicotiana cordifolia* Phil. found in Juan Fernandez Archipelago (Philippi 1856) and *Nicotiana solanifolia* Walp., that grows between Tarapacá and Coquimbo regions (Walpers 1844) and has been traditionally used in the economic and cultural life of the Atacama coastal communities (Ballester et al. 2016). According to Goodspeed (1954), *N.* sect. *Paniculatae* share cordate-ovate leaves, evenly spaced along the stem, a long and narrow cylindrical thyrse with thickened central axis, a calyx with regular, broadly triangular teeth, a short and distinct tube proper with a several times longer throat, a greenish to yellowish corolla, stamens of almost equal length, filaments curving immediately above their insertion (except *N. cordifolia* and *N. raimondii* J. F. Macbr.), and anthers bending towards the stigma.

The aim of the present work is to record *Nicotiana knightiana* Goodsp. for the first time for the flora of Chile and describe a new species of *Nicotiana* endemic to Chile, determining its phylogenetic position and conservation status.

Methods

Herbarium and fieldwork

Fieldwork was carried out during November 2020 in Fuerte Lambert and during March 2020 in the proximity of the rivermouth of Elqui river, within the city of La Serena, Coquimbo region (Fig. 1). Specimens were collected and deposited in SGO herbarium. Physical and digital specimens of *Nicotiana*, including types, were revised at SGO, EIF, CORD, L, E, SI, F, NYBG, UC and US to reach a confident identification and check for possible previous collections of the species found in the field (acronyms according to *Index herbariorum*; <http://sweetgum.nybg.org/science/ih/>). Additionally, the citizen science platform iNaturalist (www.inaturalist.org) was consulted to search for possible observations of any of the two species. Terminology of the descriptions followed Goodspeed (1954). Accordingly, the corolla will be differentiated in three parts: the narrowest basal part of the corolla tube (the tube proper), the part of the corolla between the portion where the corolla tube broadens and up to the limb (throat), and the limb. The corolla tube is defined as the tubular portion of the corolla, from the receptacle up to the limb.

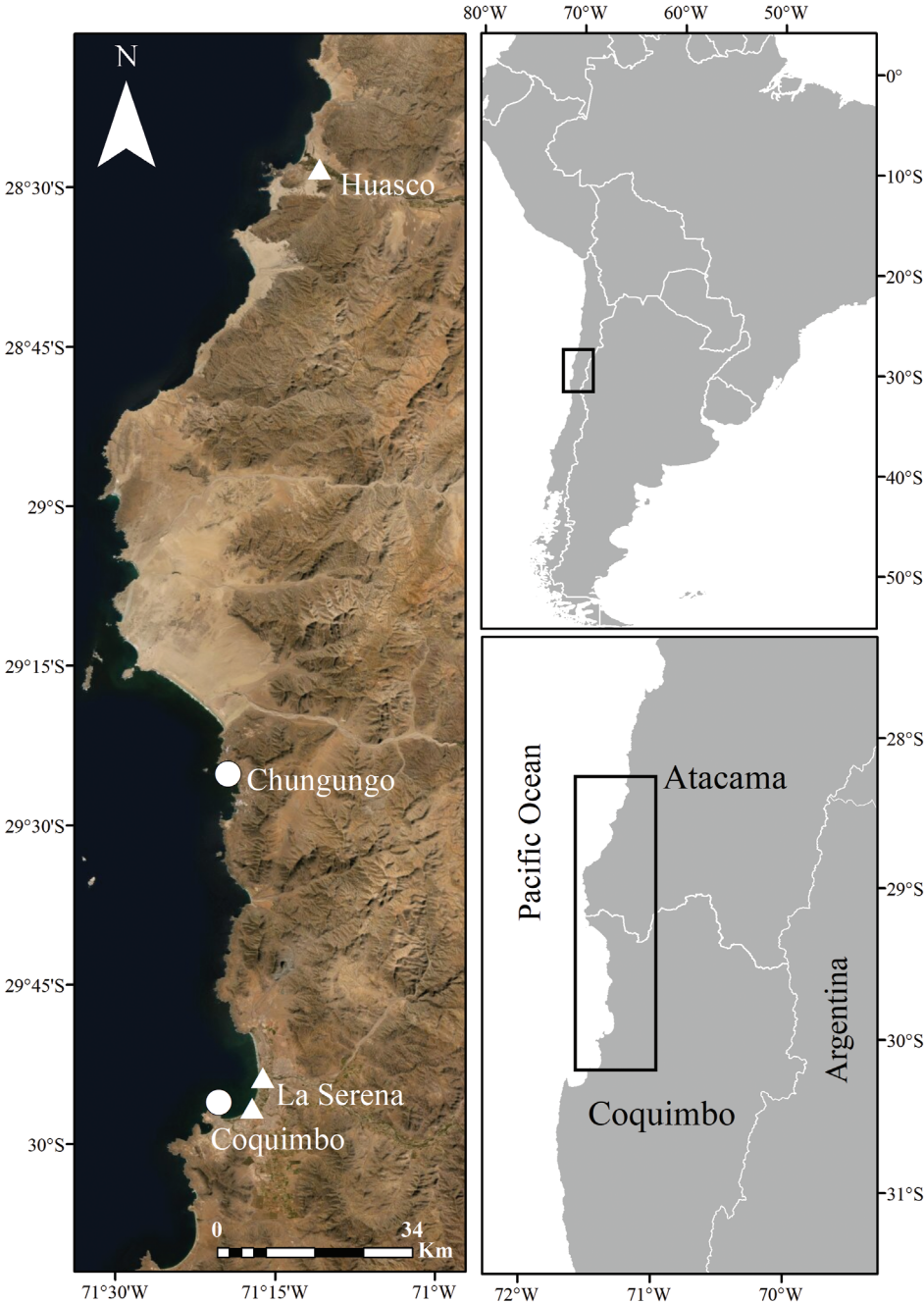


Figure 1. Distribution map of known locations of *Nicotiana knightiana* (triangles) and *Nicotiana rupicola* (circles) in Chile. Service Layer Credits: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

Taxon sampling for phylogenetic analysis

DNA sequences for cpDNA intergenic spacers *trnF-trnL*, *trnS-trnG*, and genes *ndhF* and *matK* were obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank) for all species of *Nicotiana* used in Clarkson et al. (2004). Sequences for the new species were generated in the present study. As outgroups we used *Symonanthus aromaticus* (C.A.Gardner) Haegi, and *Mandragora officinarum* L.

DNA extraction, amplification, sequencing, and phylogenetic analyses

Total genomic DNA was extracted from silica-dried material collected in the field using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Santiago, Chile), following the manufacturer's instructions. Genomic DNA was used to amplify by PCR the following chloroplast regions: *trnL-trnF* using the primers c and f (Taberlet et al. 1991), *trnS-trnG* using the primers *trnS*(GCU) and *3'trnG* (UUC) (Hamilton 1999; Shaw et al. 2005), *ndhF* using the primers 972F and 2110R (Olmstead and Sweere 1994), *matK* using the primers pairs *matK-TR* and *matK_4F*, *trnK-710F* and *matK-1848R*, and *matK_1F* and *matK_4R* (Johnson and Soltis 1995; Aoki and Ito 2000; Bremer et al. 2002). We amplified all regions in 25 µl PCR reactions using the following thermocycling conditions: initial denaturation of 95 °C for 5 min; 35 cycles at 95 °C for 1 min, a specific annealing temperature for 1 min at 50 °C (60 °C for *trnS-trnG*), 72 °C for 1 min; and a final elongation period of 72 °C for 15 min. Sanger sequencing was performed using the same primers used during amplification in the case of regions *matK* and *trnL-trnF*, the same primers used during amplification plus the primers *ndhF_1318* and *ndhF_1603R* (Olmstead and Sweere 1994) in the case of region *ndhF*, and the primers *trnS*(GCU) and *trnG*(UUC) (Hamilton 1999) for the *trnS-trnG* region. Sequencing was performed in the Plataforma de Secuenciación y Tecnologías Ómicas, Pontificia Universidad Católica de Chile, using the ABI PRISM 3500 xl Genetic Analyzer (Applied Biosystems). GenBank accession numbers for all DNA sequences are given in Suppl. material 1.

The assembled sequences were aligned using the MAFFT v7.450 algorithm (Katoh et al. 2002; Katoh and Standley 2013) in Geneious Prime 2021.1.1 (<https://www.geneious.com>). Phylogenetic analyses were run for both Maximum-likelihood (ML, Felsenstein 1981) and Bayesian inference (BI, Huelsenbeck and Ronquist 2001), using RAXML-AVX3 version (Stamatakis 2014) included in RAXMLGUI v.2.0 (Silvestro and Michalak 2012; Edler et al. 2020) and MrBayes x64 v3.2.7 (Ronquist et al. 2012), respectively. The best-supported model of nucleotide sequence evolution for each partition was determined based on the Akaike Information Criterion (AIC) using MrModeltest v2 (Nylander 2004). For the BI analysis, four partitions were used corresponding to each region, in which evolutionary models for each one were: GTR+G for *ndhF*, *trnL-trnF* and *trnS-trnG*, and GTR+I+G for *matK*. Maximum likelihood analyses were run using the GTRGAMMA approximation. The analysis included 1000 ML slow bootstrap replicates with 500 runs. Bayesian analyses were conducted under the

respective best fit models for each partition, with two independent runs for 4 million generations, sampling every 1000 generations. Time series plots and effective sample size (ESS) were analysed using TRACER v.1.7 (Rambaut et al. 2018) in order to check convergence for each run. The first 1 million generations were discarded as burn-in.

Conservation assessment

The assessment of the conservation status of the new species was made using the International Union for Conservation of Nature criteria (IUCN 2017). The extent of occurrence (**EOO**) and area of occupancy (**AOO**) were calculated using GeoCat (Bachman et al. 2011).

Results

We could not find any described species of *Nicotiana* for Chile that matched the morphology of the plants from Elqui river and Fuerte Lambert. A specimen matching the morphology of the species from Elqui river was found at SGO, collected in 2021 in the city of Huasco Bajo, Atacama, approx. 160 km north of La Serena. Plants from Elqui river are 2 m-long, somewhat ineffectively rooted, with branching short perennial shrubs, a pale yellow-green corolla, a dark green limb and eglandular indumentum (Fig. 2, Fig. 3C-D). This species was identified as *N. knightiana*, a member of *Nicotiana* sect. *Paniculatae* known from coastal southern Peru. Two geo-referenced and misidentified observations of *N. knightiana* in Chile are available on iNaturalist, one from November 2018 in the proximity of our collection site (<https://www.inaturalist.org/observations/19777218>) and one from May 2021 at approx. 6 km southwards (<https://www.inaturalist.org/observations/80390445>) (Fig. 1). A specimen matching the morphology of the species from Fuerte Lambert was found at EIF, collected in 2006 in the whereabouts of Chungungo, Coquimbo, aprox 50 km north of Fuerte Lambert. Plants from Fuerte Lambert are perennial, rupicolous shrubs, with glandular indumentum, a characteristic congested inflorescence, and small, yellow flowers with an almost glabrous corolla (Fig. 3A-B, Fig. 4). Both species have capsules that produce a large number of seeds but the ones of *N. knightiana* are more rounded in shape compared to those of the new species (Fig. 5).

Molecular phylogenetic analyses

The concatenated DNA matrix contained 4427 nucleotide characters (1554 *matK*, 1074 *ndhF*, 932 *trnL-trnF* and 867 *trnS-trnG*), representing 60 ingroup and 2 outgroup accessions. Both BI and ML analyses yielded congruent topologies. The topology of the phylogenetic tree constructed in this study is congruent with the clades found by Clarkson et al. (2004) (Fig.6). Overall, the support given by Bayesian posterior probabilities are higher than bootstrap values given by ML analyses. *Nicotiana* sections *Tomentosae*

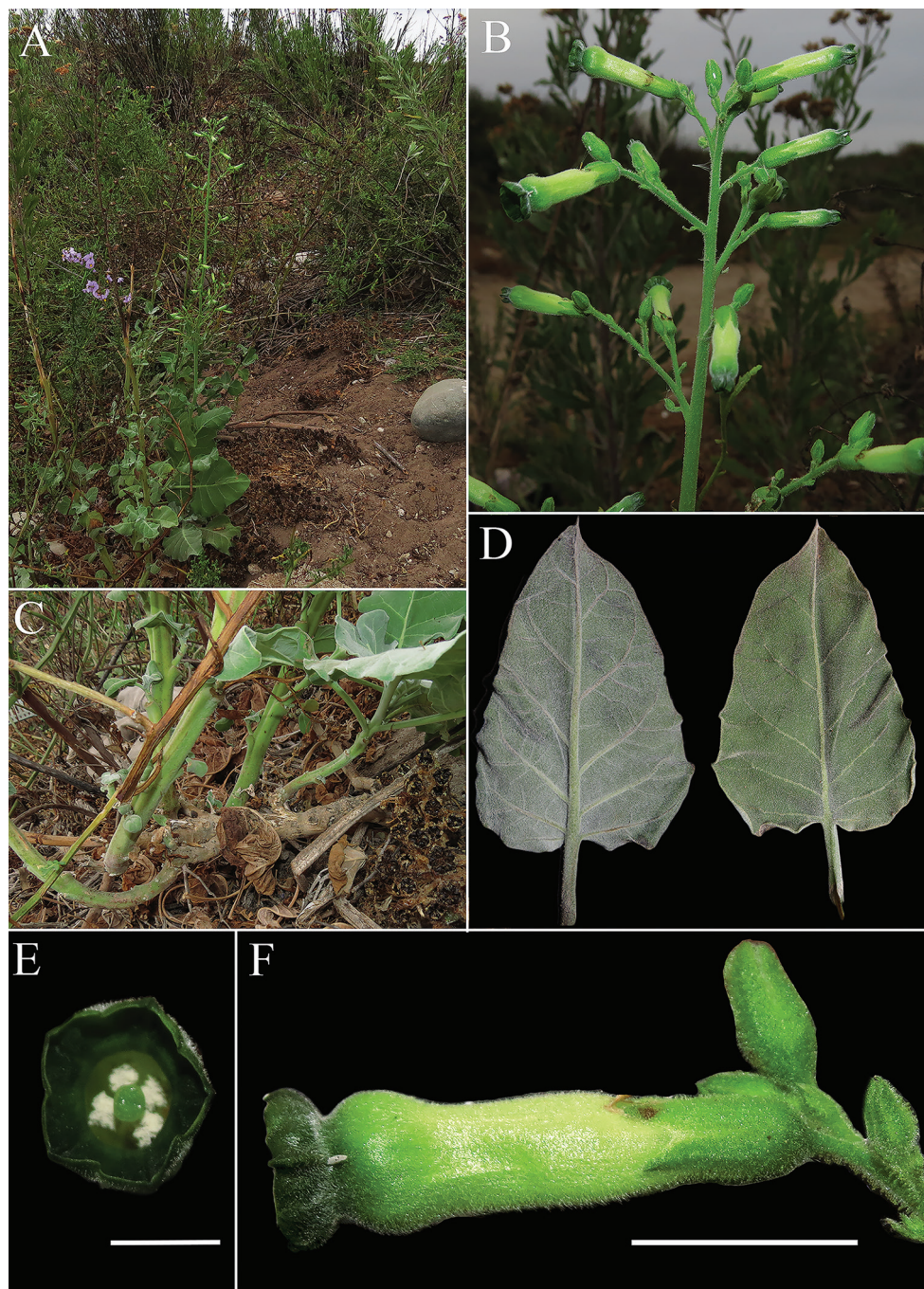


Figure 2. *Nicotiana knightiana* (L. Santilli 210323) **A** habit **B** inflorescence **C** detail of lignified horizontal stem **D** adaxial and abaxial side of a leaf **E** frontal view of a flower showing limb area **F** lateral view of a flower. Scale bars: 1 cm.

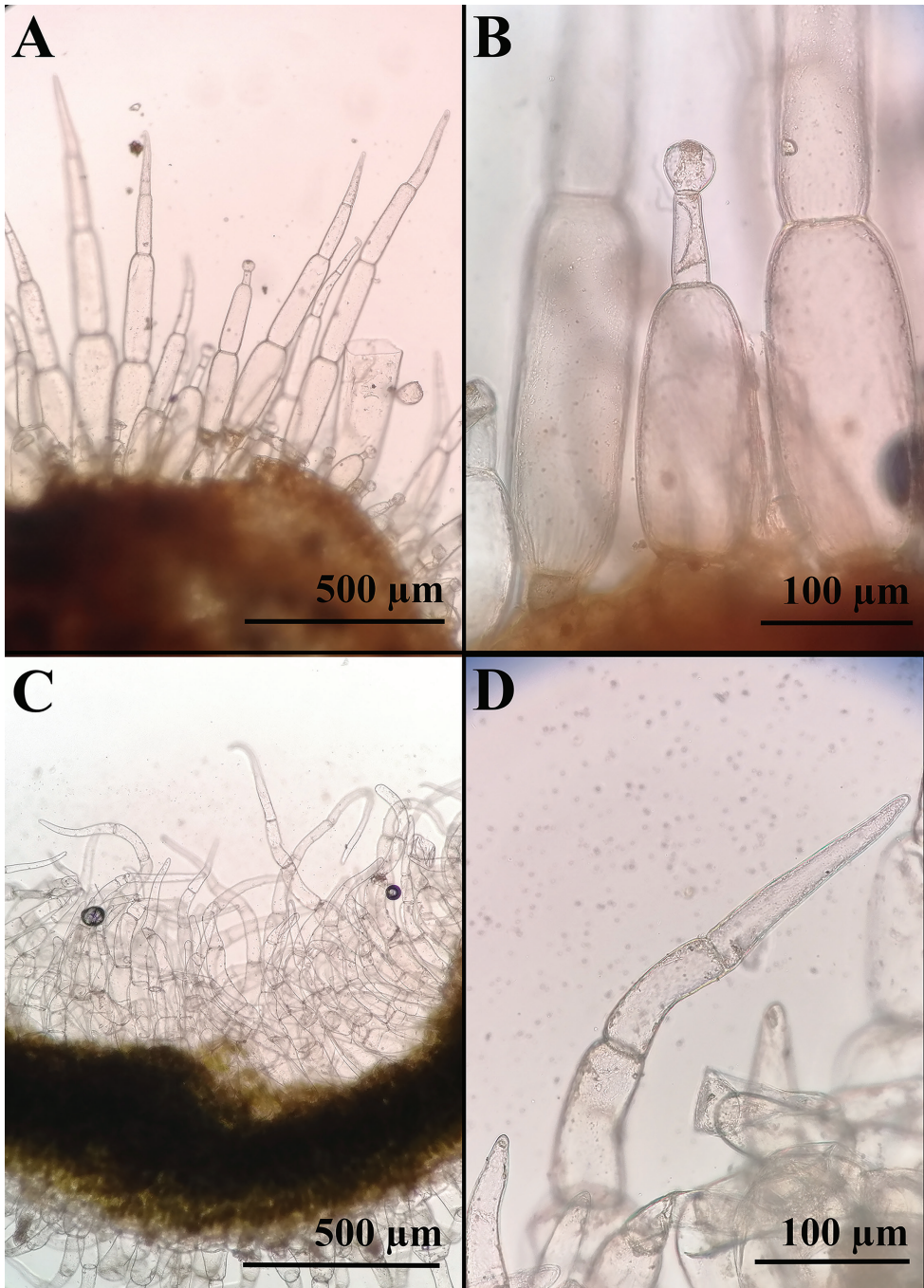


Figure 3. Indumentum of the leaves **A, B** *Nicotiana rupicola* with glandular hairs (*N. Lavandero* 1011) **C, D** *Nicotiana knightiana* with eglandular hairs (*L. Santilli* 210323).

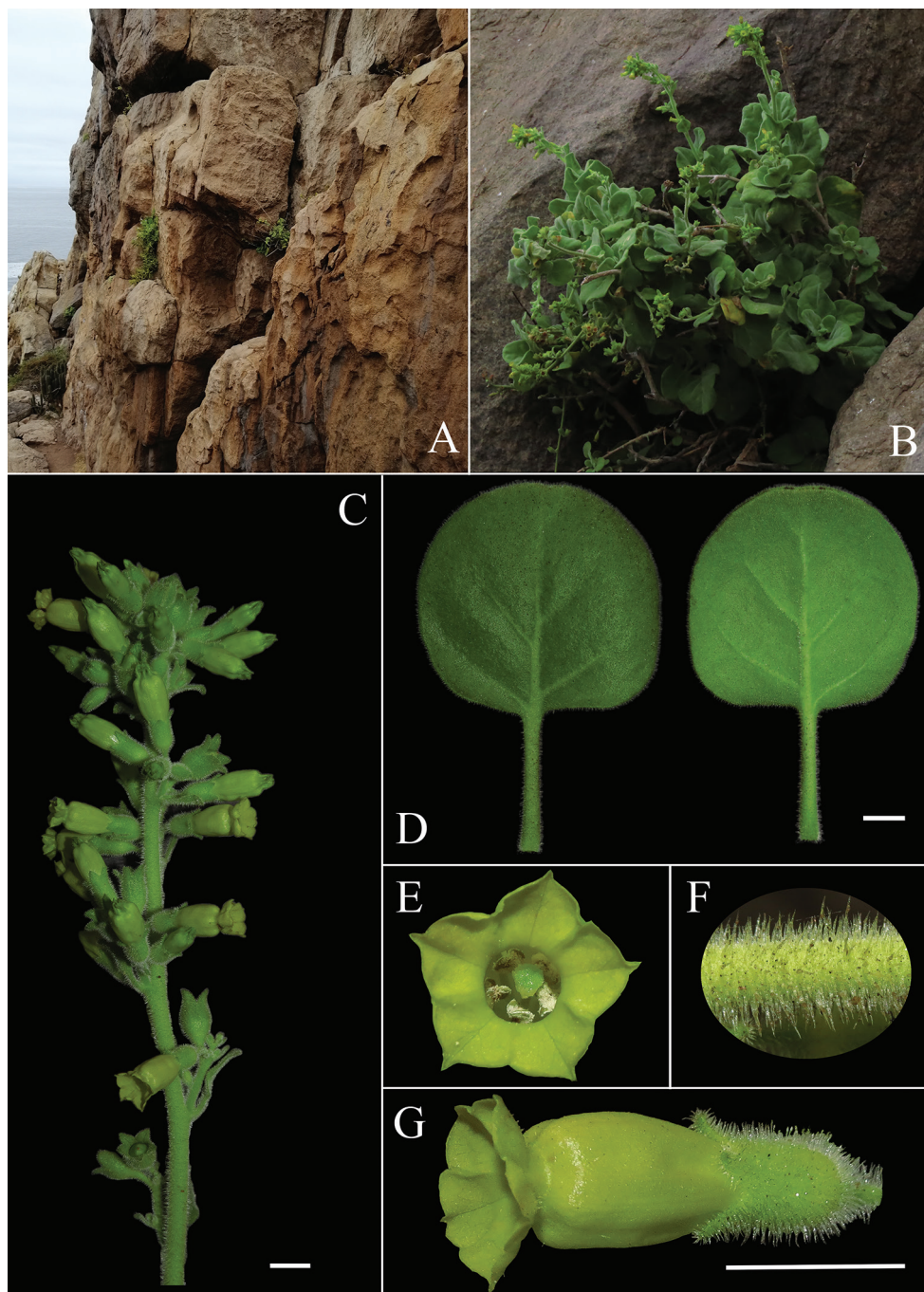


Figure 4. *Nicotiana rupicola* (*N. Lavandero* 1011) **A** habitat **B** habit **C** inflorescence **D** adaxial and abaxial side of a leaf **E** frontal view of a flower showing limb area **F** detail of indumentum **G** lateral view of a flower. Scale bars: 1 cm.

Goodsp. (PP = 1, BS = 88), *Undulatae* Goodsp. (PP = 1, BS = 95), *Paniculatae* (PP = 1, BS = 87), *Trigonophyllae* Goodsp. (PP = 1, BS = 100), Petunioides G.Don (PP = 1, BS = 100), *Alatae* Goodsp. (PP = 1, BS = 96), *Repandae* Goodsp. (PP = 1, BS = 100), *Noctiflorae* Goodsp. (PP = 1, BS = 100), and *Suaveolentes* Goodsp. (PP = 0.94, BS = 60) all form monophyletic groups with moderate to high supporting values.

Section *Paniculatae*, including *N. rustica*, forms a well-supported clade (PP = 1.0, BS = 87). Relationships among the clades largely reflect Clarkson et al. (2004), including the position of the section *Paniculatae* as sister to section *Undulatae* and together as sister to the rest of the genus excluding section *Tomentosae*. The new species falls within section *Paniculatae* as sister to *N. cordifolia* (PP = 0.99, BS = 89), and together they form a clade with *N. solanifolia* (PP = 1, BS = 100). This clade is sister to a clade including the rest of section *Paniculatae* (PP = 0.92, BS = 66). *Nicotiana knightiana* and *N. paniculata* are closely related species (PP = 0.98, BS = 63) and form a clade with *N. rustica* (PP = 1, BS = 100). The species collected in the mouth of Río Elqui, and identified as *N. knightiana*, falls as sister to *N. knightiana* sequenced in Clarkson et al. (2004) with moderate support (PP < 0.5, BS = 62). A base-by-base comparison of the sequences of *N. knightiana* from Clarkson et al. (2004) and *N. knightiana* from this study, show that they are identical, while they present differences at 6 nucleotide positions (3 from *trnS-trnG* and 3 from *matK* regions) with *N. paniculata*.

Taxonomic treatment

***Nicotiana knightiana* Goodsp., Univ. Calif. Publ. Bot. 18: 139, pls. 11, 12b (1938).**
Figures 2, 3C–D, 5A–C, 7

Type. Peru. Dept. Arequipa, Prov. Islay, near Mollendo, 40 m, 16 November 1935 (fl, fr), *Y. Mexia 04161* (holotype: UC [UC448735 photo!]; isotypes: MO, NA, NY).

Description. Robust annual or short-lived shrub up to 3 m with many new stems at different stages of development arising from a lignified horizontal stem poorly anchored to the soil. **Stems** herbaceous, green, tomentose. **Leaves** ovate, undulate, base rounded to subcordate, apex obtuse to acute; bigger leaves 13 × 10 cm, indumentum similar to the stem but much denser on the abaxial side which confers a whitish colour, hairs simple, eglandular, pluricellular, brochidodromous venation, petioles a third or half as long as the leaves. **Inflorescence** a broad thyrses or lax panicle, 40 cm. Pedicels 0.5–1 cm in mature fruits, covered in glandular hairs. **Calyx** up to 7 mm, cylindric, tomentose, teeth up to 2 mm, triangular. **Corolla** 20–23 mm excluding the limb (tubular part), tube proper 4 mm, throat 16 mm, pale yellow-green, covered in short, eglandular, hairs, limb bottom 3 mm wide, dark green, same indumentum as tube proper, notched into 5 lobes shorter than 1 mm. **Stamens** extending below the limb, 19 mm except one slightly shorter, filaments adnate to the tube proper, then free, pubescent in the proximal 6 mm, then glabrous and slightly curved, with stamens bending toward the stigma. **Capsule** 6–8 mm, ovoid. **Seeds** mainly subrotund, 0.5–0.7 mm, brown, surface reticulate. **Embryo** straight.

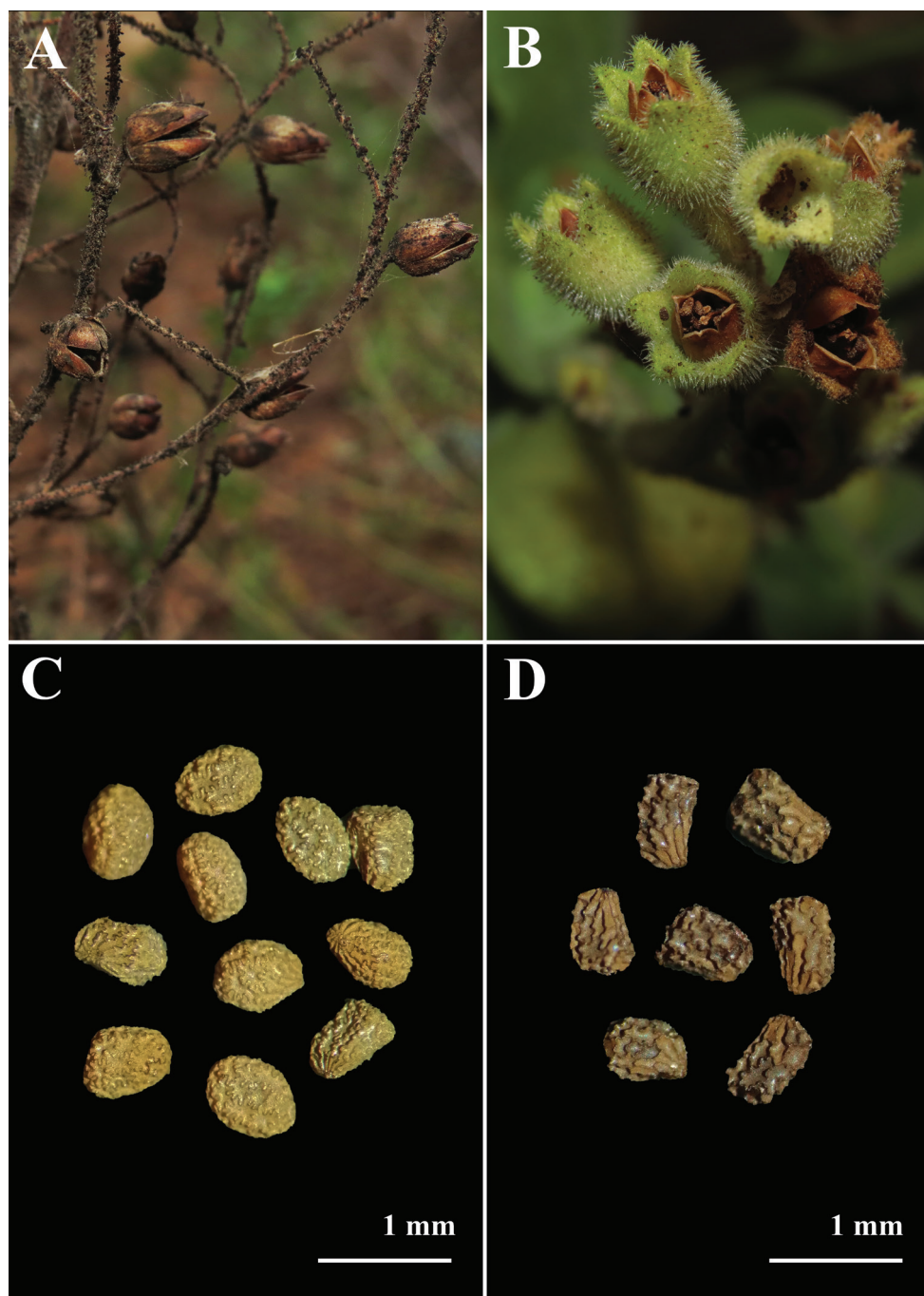


Figure 5. Fruits and seeds **A, C** *Nicotiana knightiana* (L. Santilli 210323) **B, D** *Nicotiana rupicola* (N. Lavandero 1011).

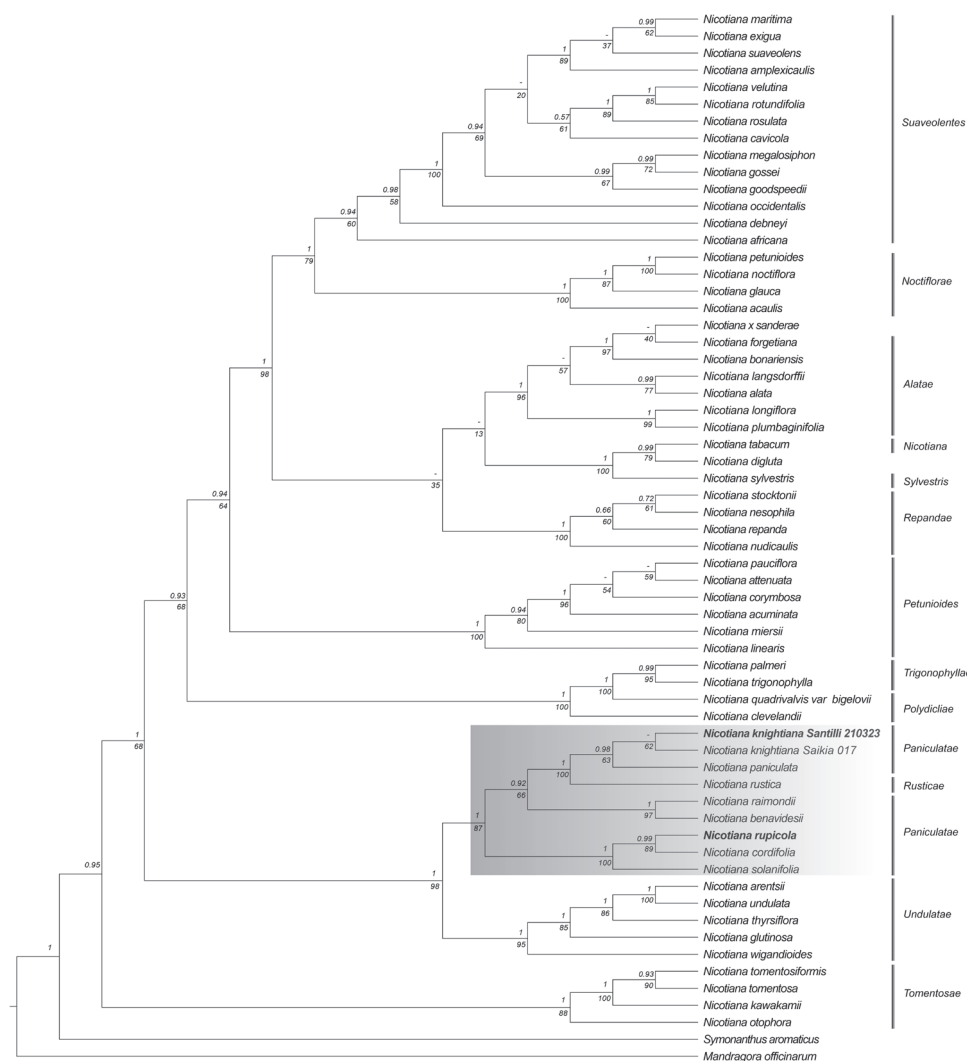


Figure 6. Phylogeny of *Nicotiana* resulting from Maximum Likelihood analysis of the plastid regions *matK*, *rps16*, *trnS-trnG* and *trnL-trnF*. Numbers above and below the branches represent the Posterior probabilities from the BI analysis and bootstrap values from the ML analysis, respectively. The species whose sequences were obtained in the present study are highlighted in bold, while section *Paniculatae* including *N. rustica* is highlighted in grey.

Distribution and habitat. *Nicotiana knightiana* grows naturally in the coast of southern Perú in roadsides, pastures and rocky ravines bottoms. It was found in Chile, within the city of La Serena, Coquimbo region, in the proximity of the rivermouth of Rio Elqui, and in the proximity of Avenida Los Pescadores. It was also found growing in the city of Huasco Bajo, Atacama region (Fig. 1). It grows in a dense *Tessaria absinthioides* (Hook. & Arn.) DC. scrub, associated with *Myoporum laetum* G. Forst.,

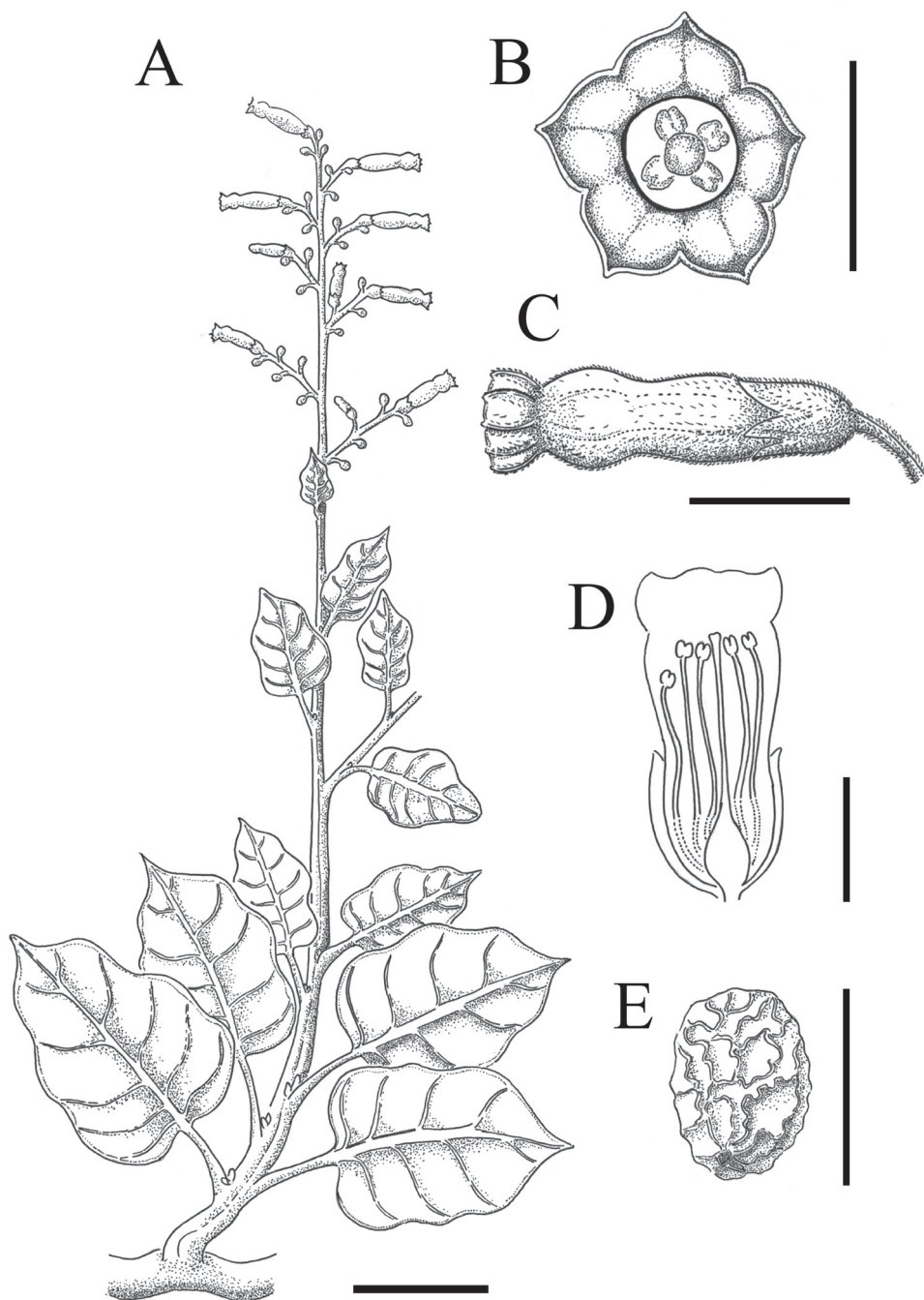


Figure 7. Illustration of *Nicotiana knightiana* **A** entire branch **B** frontal view of a flower **C** lateral view of a flower **D** schematic drawing of a longitudinal section of a flower **E** seed. Scales: 5 cm (**A**); 0.5 cm (**B**); 1 cm (**C, D**); 0.5 mm (**E**). *Santilli 210323* (SGO).

Phyla nodiflora (L.) Greene, *Schoenoplectus californicus* (C.A. Mey.) Soják, *Solanum pinnatum* Cav., *Lycium chilense* Miers ex Bertero, *Sarcocornia neei* (Lag.) M.A. Alonso & M.B. Crespo, *Distichlis spicata* (L.) Greene, *Thypha angustifolia* L., *Ambrosia chamissonis* (Less.) Greene, *Nicotiana glauca* and *Stemodia durantifolia* (L.) Sw. var. *chilensis* (Benth.) C.C. Cowan. It thrives in sandy soils with water table very close to the surface.

Phenology. *Nicotiana knightiana* is found flowering and fruiting between November and May.

Specimens examined. PERÚ. Arequipa: Prov. Islay, Quebrada Canyon, 5–6 km north of Mollendo, 300 m, 29 September 1938 (fl, fr) *C.R. Worth & J.L. Morrison 15742* (US); 12 km southeast of Islay, 250–300 m, 28 September 1938 (fl, fr), *C.R. Worth & J.L. Morrison 15724* (US). CHILE. Atacama: Huasco bajo, 28 m, 16 October 2021 (fl), *J.H. Macaya 1782* (SGO). Coquimbo: Prov. del Elqui, La Serena, ribera sur del Río Elqui a ca. 200 m de la desembocadura, 2 m, 23 March 2021 (fl, fr), *L. Santilli 210323* (SGO); La Serena, ribera sur del Río Elqui a ca. 200 m de la desembocadura, 1 m, 24 Nov 2018 (fl, fr), *A. Ryan* (iNaturalist); La Serena, avenida Los Pescadores con Canto del Agua, 3 m, 26 May 2021 (fl), *B.L. Bedard* (iNaturalist).

***Nicotiana rupicola* Santilli, De Schrevel, Lavandero & Dandois, sp. nov.**

urn:lsid:ipni.org:names:77248643-1

Type. Chile. Región de Coquimbo: Prov. Elqui, Comuna de Coquimbo, Fuerte Lambert, 29°56'2.52"S, 71°20'16.46"W, 29 m, 12 November 2020 (fl, fr), *N. Lavandero 1011* (holotype: SGO!; isotypes: EIF!, CONC!).

Diagnosis. *Nicotiana rupicola* is most similar to *N. solanifolia*, from which it differs by its congested panicle (vs. lax panicle), its short and glabrous corolla up to 18 mm (vs. corolla of 35–50 mm, pubescent), non-retroflexed corolla limb (vs. retroflexed corolla limb), mature capsule included or slightly exserted from calyx, 6–10 mm (vs. more than half the length excluded from calyx at maturity, 12–18 mm).

Description. Perennial shrub up to 2 m with many stems arising from a lignified horizontal stem. **Stems** lignified, light brown, glabrous. **Leaves** orbicular to ovate, flat to slightly undulate, margins slightly revolute, base rounded to cordate, apex retuse to obtuse; bigger leaves 10 × 8.5 cm, reducing their size towards the apex, densely covered in two types of hairs in both sides: simple, straight, pluricellular, up to 1 mm long, and glandular (capitate), straight, pluricellular, 50–600 µm long, brochidodromous venation, petioles a quarter to half as long as the leaves, 0.5–2.5(–5.0) cm long. **Inflorescence** a compact panicle, up to 35 cm; pedicels up to 0.5 cm, same indumentum as the leaves. **Calyx** up to 10 mm, cylindric, same indumentum as the leaves, teeth up to 3.5 mm, triangular. **Corolla** 17–18 mm excluding the limb (tubular part), tube proper up 5.5–6 mm, throat up to 12 mm, yellow, glabrous, limb 4 mm wide, yellow, glabrous or with scattered hairs, notched into 5 lobes. **Stamens** extending below the limb, similar length; filaments adnate for the first 5 mm to the tube proper, then free, pubescent at the base, then glabrous and slightly curved, with the distal portion bending toward the stigma. **Capsule** 6–10 mm long, ovoid.

Seeds mainly angular, laterally compressed, 0.4–0.6 mm long, dark brown, surface reticulate. **Embryo** unknown. **Chromosome number** unknown. (Fig. 3; Fig. 4B–D; Fig. 8)

Distribution and habitat. *Nicotiana rupicola* is endemic to Chile where it is currently known from two locations, Fuerte Lambert and Chungungo, both in the region of Coquimbo (Fig. 1). In Fuerte Lambert it grows among the rocks in a cliff near the ocean together with *Alstroemeria magnifica* Herb., *Cistanthe grandiflora* (Lindl.) Schtdl., *Diplolepis boerhaviifolia* (Hook. & Arn.) Liede & Rapini, *Eulychnia breviflora* Phil., *Loasa elongata* Hook. & Arn., *Myrcianthes coquimbensis* (Barnéoud) Landrum & Grifo, *Nolana acuminata* (Miers) Miers ex Dunal, *N. rupicola* Gaudich., *N. sedifolia* Poepp., *Ophryosporus triangularis* Meyen, *Plumbago caerulea* Kunth, *Polyachyrus poeppigii* Kuntze ex Less., *Puya venusta* Phil., *Sicyos baderoa* Hook. & Arn. var. *baderoa*, *Solanum pinnatum* Cav., *Stachys pannosa* Phil., In Chungungo it grows on a rocky cliff facing the ocean, together with *Nolana crassulifolia* Poepp. and *N. sedifolia* Poepp and surrounded by a scrub of *Balbisia peduncularis* (Lindl.) D. Don, *Heliotropium stenophyllum* Hook. & Arn., and *Oxalis virgosa* Molina.

Phenology. *Nicotiana rupicola* was found flowering and fruiting in November.

Etymology. The specific epithet derives from the Latin *rupes* (rock), and *cola* (dweller), alluding to rocky habitat of this species.

Additional specimens examined (paratypes). Chile. Región de Coquimbo. Prov. Elqui, Comuna La Higuera, costa al Norte de Chungungo, 7 November 2006 (fl), N. García 3085 (EIF).

Conservation status. *Nicotiana rupicola* can be considered as Critically Endangered (CR) under the IUCN categories and criteria B1ab(iii); D. The criterion B1 was selected because its extent of occurrence is < 100 km² (8 km²). The criterion “a” was selected because the distribution is highly fragmented. The criterion “b(iii)” was selected because there is a projected decline in the area, extent and quality of habitat. This area is constantly threatened by the expansion of urbanization that is affecting central-north coastal Chile. One of the locations is currently found at less than 300 m from the residential area of Coquimbo and the habitat is being altered by numerous and increasing amounts of formal and informal paths and human activity (camping, garbage, etc.). Moreover, mining activities within the extent of occurrence, especially Minera Dominga, which pretends to settle between the two known localities, will more likely affect possible unknown populations and the quality of the habitat. The criterion D was selected because we observed less than 50 individuals around the two known localities.

Key to the species of *Nicotiana* sect. *Paniculatae* found in Chile

- 1 Inflorescence a congested panicle, corolla tube glabrous except for sparse hairs on the limb *N. rupicola*
- Inflorescence a lax panicle, corolla tube entirely covered by trichomes..... 2
- 2 Corolla tube 3–5 cm, indumentum of corolla made of glandular trichomes.. *N. solanifolia*
- Corolla tube 2–2.3 cm, indumentum of corolla made of eglandular trichomes 3

- 3 Corolla tube 2 cm, limb dark green, continental Chile *N. knightiana*
 — Corolla tube 2–3 cm, limb yellow or purple, Juan Fernandez Islands 4
 4 Corolla tube purple, Alejandro Selkirk Island
 *N. cordifolia* subsp. *cordifolia*
 — Corolla tube yellow, Santa Clara Island *N. cordifolia* subsp. *sanctaeclarae*

The characters that proved to be useful to differentiate species of *Nicotiana* sect. *Paniculatae* are the type of inflorescence, the types of trichomes and distribution of the indumentum, as well as the size and colour of flowers. *N. knightiana* resembles most *N. paniculata* from which it differs on account of its shorter flowers with dark green limbs (vs. yellow) (Fig. 2E-F). The large undulate leaves, the narrow panicles and the long throat of the flowers of *N. knightiana* also resemble *N. solanifolia* from which it can be distinguished for its smaller flowers (2–2.3 cm vs 3–5 cm respectively), eglandular indumentum (vs. glandulous) and dark green limb (vs. yellow) (Figs 2E, F, 3C, D). *Nicotiana rupicola* is morphologically similar to *N. solanifolia* from which it can be distinguished by its congested panicle (vs. lax), glabrous corolla tube (vs. hairy), its smaller flowers and no-retroflexed corolla (Fig. 4).

It is worth mentioning that *N. solanifolia* has been erroneously reported for the Coquimbo region based on a collection held at K (Cuming 860), which is the type of *Nicotiana breviloba* Jeffr., considered by Goodspeed (1954) as a synonym of *N. solanifolia*. Johnston (1936) considered that Cuming's collections with collection number between 853 and 911 were obtained north of Huasco, Atacama region. This is consistent with the collection localities of the specimens of *N. solanifolia* revised in the present work which show that the distribution of *N. solanifolia* and of *N. rupicola* do not overlap. The southernmost collection of *N. solanifolia* is found at approx. 100 km north of the northernmost collection locality of *N. rupicola*.

Molecular analyses showed that plants from Elqui river are likely correctly identified as *N. knightiana* and that our initial conjectures about the phylogenetic position of *N. rupicola* as part of the sect. *Paniculatae* were confirmed (Fig. 6). Our topology retrieves Clarkson's et al. (2004) results of two separate clades within the sect. *Paniculatae* that reflect geography, one including species from Peru and one including the two endemics *N. solanifolia* from the North of Chile and *N. cordifolia* from Juan Fernández Archipelago (Fig. 6). Species of Chilean sect. *Paniculatae* share long tubular flowers and long-petiolate leaves. Surprisingly, *N. rupicola* results in being more closely related to the Juan Fernández species *N. cordifolia* than to the continental species *Nicotiana solanifolia*, despite their morphological affinity.

An important question regards whether *N. knightiana* has to be considered native or introduced to Chile. Either the species was never noticed or collected during the last two centuries of botanical expeditions, and presents naturally disjunct populations, being almost 1500 km apart from the closest population found in Peru, or it was recently introduced in Chile by anthropogenic means. The earliest evidence of its

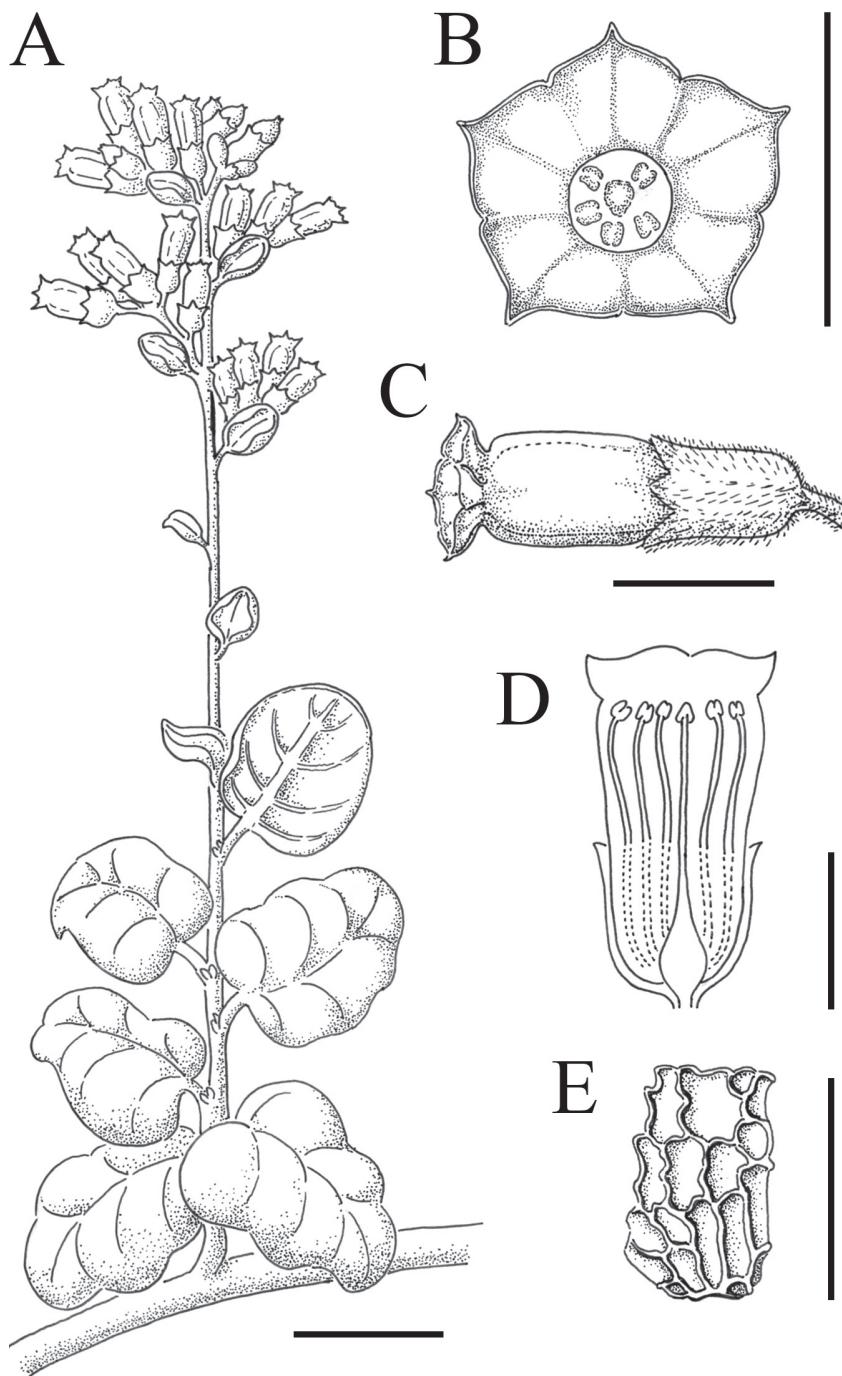


Figure 8. Illustration of *Nicotiana rupicola* **A** entire branch **B** frontal view of a flower **C** lateral view of a flower **D** schematic drawing of a longitudinal section of a flower **E** seed. Scales: 2 cm (**A**); 1 cm (**C**, **D**); 0.5 mm (**E**). *N. Lavandero* 1011 (SGO, EIF, CONC).

presence dates back to 2018 (iNaturalist) and it seems to have been established and possibly expanded to the surrounding area in sites with similar ecological conditions to the river mouth of Elqui river, such as Huasco Bajo. The production of abundant and small seeds, together with the ability of some species to grow in a broad range of open and disturbed habitats, is considered as a common adaptation that ensures high probability of dispersal and establishment. Such is the case for *Nicotiana paniculata*, *N. glauca*, and *N. plumbaginifolia* Viv. that are considered invasive species (Gallo et al. 2008; Gairola et al. 2016; Rodríguez-Caballero et al. 2020; Alharthi et al. 2021)

Nicotiana rupicola presents a restricted distribution limited to a small portion of the coastal area of northern Chile, where it grows on two locations on easily accessible coastal rocky cliffs at less than 300 m from urbanization. The population from Fuerte Lambert is situated in an area where urban expansion has caused major damage to the vegetation. The coastal area between Tongoy and Coquimbo is catalogued as a site of interest for the conservation of woody and succulent species due to its high diversity and rates of endemism (Squeo 2000). The area is home to various threatened species such as: *Carica chilensis* (Planch. ex A. DC.) Solms, *Myrcianthes coquimbensis* (Barnéoud) Landrum & Grifo, *Porlieria chilensis* I.M. Johnst. Controversially, it is an area particularly affected by intense expanding urbanization that seriously threatens the conservation of the local biodiversity. Additionally, a new mining facility and a discharge port (Minera Dominga) will be constructed within its extent of occurrence, close to its northernmost known locality. It is likely that several more individuals of *N. rupicola* are present in the area, since the abiotic and biotic conditions are similar to the currently known localities. None of these individuals were considered during the environmental impact assessment of the project. It is of great importance for the conservation of the species to search for more individuals and localities, and to raise the attention to much needed conservation measures for the species and the unique ecosystem where it is found.

Additional specimens examined

Nicotiana solanifolia. **CHILE. Antofagasta:** [Antofagasta Province]. Lomas de Taltal, near road from Taltal to the panamericana, 430 m, 25 October 2002 (fl), *M. Ackermann* 500 (SGO); bei Hueso Parado, nahe von Taltal, 400 m, 9 July 1972 (fl, fr), *O. Zöllner* 5942 (L); Quebrada de Taltal, 410 m, 17 September 1992 (fl, fr), *S. Teillier*, *P. Rundel* & *P. García* 2850 (F); Hueso Parado, *s.d.*, *s.c.* (SGO); Ravine ca. 16 km north from Paposo, 207 m, 21 November 2008 (fl), *R. Baines*, *M. Gardner*, *P. Hechenleitner*, *C. Morter* & *D. Rae* 38. (E); Mirador above the Thermoelectric plant below Quebrada Paposo, 680 m, 1 December 2004 (fl, fr), :*M. Dillon* & *M. Finger* 8670 (SGO); Paposo, Peralito, 15 November 1959 (fl), *A. Torres s.n.* (SGO); Paposo, borde quebrada, 24 October 2009, *A. Moreira* & *F. Luebert* 1205 (SGO); Camino Paposo-Caleta Blanco Encalada, Queb. Miguel Diaz, 160 m, 15 November 1996, *R. Rodríguez* 3131 (SGO); El Rincón, al N de Paposo, 17 September 1941 (fl, fr), *C. Muñoz* & *G. Johnson* 2877 (SGO); Quebrada el Des poblado, 25–26 August 1992 (fl), *J.C. Torres s.n.* (SGO);

Taltal-Paposo, September 1909 (fl, fr), *K. Reiche s.n.* (SGO); Paposo, entrada a la Qda. Los Peralitos, 30 September 2005 (fl, fr), *M. Muñoz 4607* (SGO); 10 km al sur de Caleta Blanco Encalado, 200–800 m, 11 December 1940 (fl), *W. Biese 3209* (SGO). Taltal, Quebrada de Taltal, 410 m, 17 September 1992 (fl, fr), *S. Teillier, P. Rundel, P. García 2837* (SGO); 6 Km east of Taltal, 300–600 m, 14 October 1938 (fl, fr), *C.R. Worth & J.L. Morrison 16122* (US); Cerro Perales, ca. 5 km E of Taltal, 550–960 m, 27 September 1988 (fl, fr), *M.O. Dillon, D. Dillon & V. Poblete 5536* (F); Quebrada Rinconada, ca. 5 Km N of Caleta Paposo, 250 m, 25 October 1988 (fl), *M.O. Dillon, D. Dillon & B. Tay 5741* (F);

Atacama: [Chañaral Province]. Parque Nacional Pan de Azúcar, Quebrada de Coquimbo, 10 November 1987, *Paez s.n.* (SGO); Chañaral, 24 October 1985 (fl), *G. Nieuwenhuizen 132–27* (SGO); Camino Chañaral a Flamenco, 3.5 km al interior camino izquierdo desde Portofino, quebrada y cono de deyección, 14 October 1992 (fl, fr), *M. Muñoz 3095* (SGO). [Copiapó Province]. Caldera, Quebrada León, 20 m, October 1924 (fl), *E. Werdermann. 437* (E, SI, F); Quebrada de los leones, Caldera, 1888 (fl), *W. Geisse s.n.* (Type of *Nicotiana cardiophylla* Phil.) (SGO); Caldera, September 1900, *K. Reiche s.n.* (SGO). [Huasco Province]. Camino Carrizal Bajo – Huasco, 30 m, 13 October 1991 (fl, fr), *S. Teillier, L. Villaroel & R. Torres 2579* (SGO); sector Aguada Tongoy, road to Los Bronces near Corral El Sauce – road junction, 276 m, 6 December 2004 (fl, fr), *P. Baxter, M. Gardner, P. Hechenleitner, P.I. Thomas & C. Zamorano 1877* (E, SGO); Carrizal Bajo, September 1885 (fr), *F. Philippi s.n.* (SGO); Camino Carrizal Bajo, km 50, 2 November 1991 (fl, fr), *M. Muñoz, S. Teillier & I. Meza 2944* (SGO); Camino de vuelta Carrizal a Canto de Agua en Qda. Exposición sur, 23 September 1977 (fl, fr), *M. Muñoz 1119* (SGO); Carrizal, September 1885, *F. Philippi s.n.* (SGO).

Nicotiana cordifolia. **CHILE. Valparaíso:** [Valparaíso Province]. Archipiélago de Juan Fernandez, Isla Santa Clara, Bahía Matriz, 12 December 1998 (fl, fr), *P. Danton s.n.* (SGO); Isla Masafuera, s.d. (fl), *R. A. Philippi 730* (F), Isla Masafuera, October 1854 (fl, fr), *P. Germain s.n.* (SGO); Isla Masatierra, San Juan Bautista, Conaf Garden, 56 m, 13 December 2003 (fl, fr), *M. Gardner, P. Hechenleitner & M. Tobar* (E)

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References

- Alharthi AS, Abd-ElGawad AM, Assaeeda AM (2021) Influence of the invasive shrub *Nicotiana glauca* Graham on the plant seed bank in various locations in Taif region, western of Saudi Arabia. *Saudi Journal of Biological Sciences* 28(1): 360–370. <https://doi.org/10.1016/j.sjbs.2020.10.014>
- Aoki S, Ito M (2000) Molecular phylogeny of *Nicotiana* (Solanaceae) based on the nucleotide sequence of the matK gene. *Plant Biology* 2(3): 316–324. <https://doi.org/10.1055/s-2000-3710>
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity science. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Ballester B, Carrasco C, Del Desierto C (2016) *Nicotianas* litorales del desierto de Atacama: Historia de registro y consumo de tabaco cimarrón (*Nicotiana solanifolia* Warp.). *Taltalia* (9): 69–87.
- Bremer B, Bremer K, Heidari N, Erixon P, Anderberg AA, Olmstead RG, Källersjö M, Barkhordarian E (2002) Phylogenetics of asterids based on three coding and three non-coding chloroplast DNA markers and the utility of noncoding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24(2): 274–301. [https://doi.org/10.1016/S1055-7903\(02\)00240-3](https://doi.org/10.1016/S1055-7903(02)00240-3)
- Chase MW, Knapp S, Cox AV, Clarkson JJ, Butsko Y, Joseph J, Parokonny AS (2003) Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). *Annals of Botany* 92(1): 107–127. <https://doi.org/10.1093/aob/mcg087>
- Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase MW (2004) Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. *Molecular Phylogenetics and Evolution* 33(1): 75–90. <https://doi.org/10.1016/j.ympev.2004.05.002>
- Clarkson JJ, Kelly LJ, Leitch AR, Knapp S, Chase MW (2010) Nuclear glutamine synthetase evolution in *Nicotiana*: Phylogenetics and the origins of allotetraploid and homoploid (diploid) hybrids. *Molecular Phylogenetics and Evolution* 55(1): 99–112. <https://doi.org/10.1016/j.ympev.2009.10.003>
- Clarkson JJ, Dodsworth S, Chase MW (2017) Time-calibrated phylogenetic trees establish a lag between polyploidisation and diversification in *Nicotiana* (Solanaceae). *Plant Systematics and Evolution* 303(8): 1001–1012. <https://doi.org/10.1007/s00606-017-1416-9>
- Edler D, Klein J, Antonelli A, Silvestro D (2020) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12(2): 1–5. <https://doi.org/10.1111/2041-210X.13512>
- Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* 17(6): 368–376. <https://doi.org/10.1007/BF01734359>
- Gairola S, El-Keblawy A, Mahmoud T (2016) A note on the current distribution of *Nicotiana plumbaginifolia* (Solanaceae) in the United Arab Emirates. *National Academy Science Letters* 39(6): 461–464. <https://doi.org/10.1007/s40009-016-0490-9>
- Gallo AG, de la Torre WW, Rodríguez VM (2008) Especies vegetales consideradas invasoras de hábitats, en la Historia Natural de Canarias. *Lazaroa* 29: e49.
- Goodspeed TH (1954) The genus *Nicotiana*. *Chronica Botanica Company*, Waltham, Mass, USA, V. 16.

- Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8(3): 521–523.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* (Oxford, England) 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Hunziker AT (2001) *Genera Solanacearum*. The genera of Solanaceae illustrated, arranged according to a new system. Gantner ARG, Verlag K & Ruggell G, 500 pp.
- IUCN (2017) Guidelines for using the IUCN red list categories and criteria, version 13. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Johnson LA, Soltis DE (1995) Phylogenetic inference in Saxifragaceae sensu stricto and Gilia (Polemoniaceae) using matK sequences. *Annals of the Missouri Botanical Garden* 82(2): 149–175. <https://doi.org/10.2307/2399875>
- Johnston IM (1936) A study of the Nolanaceae. *Proceedings of the American Academy of Arts and Sciences* 71(1): 1–87. <https://doi.org/10.2307/20023213>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Mehmood F, Ubaid Z, Shahzadi I, Ahmed I, Waheed MT, Poczaï P, Mirza B (2020) Plastid genomics of *Nicotiana* (Solanaceae): Insights into molecular evolution, positive selection and the origin of the maternal genome of Aztec tobacco (*Nicotiana rustica*). *PeerJ* 8: e9552. <https://doi.org/10.7717/peerj.9552>
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author.
- Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43(4): 467–481. <https://doi.org/10.1093/sysbio/43.4.467>
- Philippi RA (1856) Bemerkungen über die Flora der Insel Juan Fernández. *Botanische Zeitung, Berlin* 14: 641–650.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67(5): 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Reiche K (1903) Estudio crítico sobre la Flora de Chile. Solanaceae. *Anales de la Universidad de Chile* 5: 378–390.
- Rodríguez R, Marticorena C, Alarcón D, Baeza C, Cavieres L, Finot VL, Fuentes N, Kiessling A, Michoc M, Pauchard A, Ruiz E, Sanchez P, Marticorena A (2018) Catálogo de las plantas vasculares de Chile. *Gayana. Botánica* 75(1): 1–430. <https://doi.org/10.4067/S0717-66432018000100001>
- Rodríguez-Caballero G, Roldán A, Caravaca F (2020) Invasive *Nicotiana glauca* shifts the soil microbial community composition and functioning of harsh and disturbed semi-arid Mediterranean environments. *Biological Invasions* 22(10): 2923–2940. <https://doi.org/10.1007/s10530-020-02299-1>

- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92(1): 142–166. <https://doi.org/10.3732/ajb.92.1.142>
- Silvestro D, Michalak I (2012) raxmlGUI: A graphical front-end for RAxML. *Organisms, Diversity & Evolution* 12(4): 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Squeo FA (2000) Libro rojo de la flora nativa y de los sitios prioritarios para su conservación: Región de Coquimbo. Ediciones de la Universidad de La Serena, La Serena, Chile.
- Stamatakis A (2014) Raxml version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* (Oxford, England) 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17(5): 1105–1109. <https://doi.org/10.1007/BF00037152>
- Walpers WG (1844) Synopsis solanacearum, scrophularinarum, orobancheorum et labiatarum in Repertorium botanices systematicae. The New York Botanical Garden 3: e12.

Supplementary material I

GenBank accession numbers

Authors: Ludovica Santilli, Fernanda Pérez, Claire De Schrevel, Philippe Dandois, Hector Mondaca, Nicolás Lavandero

Data type: Xlsx file.

Explanation note: GenBank accession numbers for the trnL-trnF, trnS-trn-G, matK and ndhF sequences used in this study. GenBank accessions in bold are new to this study.

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Link: <https://doi.org/10.3897/phytokeys.188.73370.suppl1>

Thismia latiffiana (Thismiaceae), an unusual new species from Terengganu, Peninsular Malaysia

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Abstract

Thismia latiffiana Siti-Munirah & Dome, a new species from Terengganu, Peninsular Malaysia, is described and illustrated. The new species differs from all other species of *Thismia*, described so far, in having golden trichomes that are present on the outer surface of its floral tube and mitre, as well as pyramidal protuberances on the inner surface of the floral tube. Additionally, it is remarkable in its supraconnective apically bearing two long trichomes. *Thismia latiffiana* is assigned a preliminary conservation status as Critically Endangered (CR) according to IUCN Criteria.

Keywords

Hulu Nerus Forest Reserve, lowland dipterocarp forest, mycoheterotrophic, taxonomy

Introduction

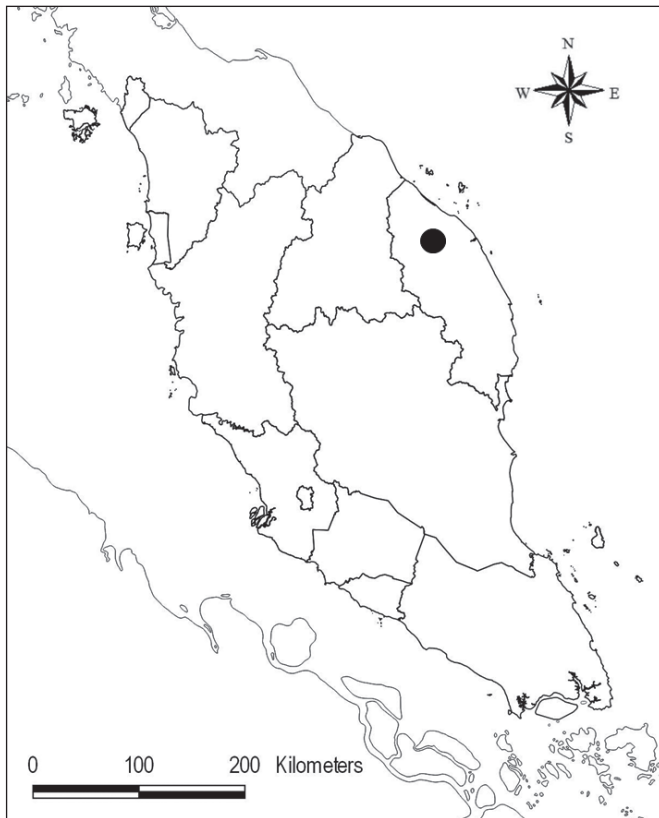
Thismia Griffith (1844) is a genus of small mycoheterotrophic herbs found on the forest floor that usually go unnoticed due to their size and habit. *Thismia* belongs to the family Thismiaceae, which consists of 95 accepted species, of which 16 species have been recognised in Peninsular Malaysia. In particular, the State of Terengganu currently has eight reported species of *Thismia*: *T. alba* Holttum ex Jonker, *T. arachnites* Ridl., *T. aseroe* Becc. (all three Siti-Munirah and Dome 2021), *T. domei* Siti-Munirah (Siti-Munirah and Dome 2019), *T. javanica* J.J.Sm. (Siti-Munirah and Dome 2019), *T. ornata* Dančák, Hroneš & Sochor (Siti-Munirah and Dome 2021), *T. sitimeriamiae*

Siti-Munirah, Dome & Thorogood (Siti-Munirah et al. 2021) and *T. terengganuensis* Siti-Munirah (Siti-Munirah and Dome 2019).

In December 2019, the second author discovered an odd-looking *Thismia* at Hulu Nerus Forest Reserve (FR) located in Setiu District, State of Terengganu, eastern Peninsular Malaysia. The specimens have been deposited to the Kepong Herbarium (KEP). After comparing it with all the species of *Thismia* known to date, we concluded that this plant does not match any of them. Herein, we describe it as a new species, *Thismia latiffiana* Siti-Munirah & Dome.

Materials and methods

This study is based on material collected in December 2019 in Hulu Nerus FR, Setiu District, Terengganu (Map 1). Morphological characteristics were studied using a stereomicroscope and high-resolution macrophotography. Measurements were taken from both fresh and liquid-preserved materials. The specimen details were compared with original drawings and descriptions in the protologues of *Thismia* species around the world.



Map 1. Map of Malay Peninsula with Hulu Nerus Forest Reserve (●), the type locality of *Thismia latiffiana*.

Taxonomic account

Thismia latiffiana Siti-Munirah & Dome, sp. nov.

urn:lsid:ipni.org:names:77248767-1

Figures 1–3

Diagnosis. *Thismia latiffiana* differs from all its congeners by the following combination of traits: the presence of golden trichomes on the outer surface of floral tube and mitre, outer tepals absent, inner tepals form a mitre without appendages, an inner surface of floral tube covered by pyramidal protuberances and supraconnective bilobed with each lobe terminated by a long, needle-like trichome.

Type. MALAYSIA. Peninsular Malaysia: Terengganu: Setiu District, Hulu Nerus Forest Reserve, approximately 220 m elev., 4 February 2020, *Siti-Munirah & Dome Nikong FRI94686* (holotype KEP!, spirit collection, barcode no. 280004).

Herb achlorophyllous, approximately 12 mm tall. **Roots** loosely coralliform, compressed, light brown, surface covered with trichomes. **Stem** very short or absent, obscured by trichomes during flowering. **Leaves** spirally arranged, crowded, scale-like, triangular, apex acute to acuminate, margin entire, 2–8 mm long and 1.5–2 mm wide, brown. **Bract** similar to leaves. **Flowers** actinomorphic, solitary; floral tube 1.2 cm long, ellipsoid to ovoid, widest in middle part (7–7.3 mm in diameter), in upper part ca. 6.4 mm wide and at base ca. 6 mm wide, black or dark brown, whitish in upper part with round to oblong sectors (opposite each anther thecae), separated by blackish-brown stripes; surface partially covered with individual unbranched trichomes, stellate trichomes or unbranched trichomes crowded on warts (resembling spines on areoles of cacti); inner surface covered with very thick pyramidal protuberances arranged longitudinally in each section continuously, brown to black at middle towards base, upper part reticulate, light brown. **Annulus** at apical part of floral tube, dark brown, broadly funnel-shaped, ca. 2.7 mm wide, margin 6-lobed, glabrous, smooth, and thick. **Outer tepals** absent. **Inner tepals** 3, dark brown to blackish, erect and turning inwards, connate at the top forming a mitre without any appendages; **mitre** black or dark brown, on outer surface partially covered in a patchy manner with individual unbranched trichomes, stellate trichomes or unbranched trichomes crowded on warts (resembling spines on areoles of cacti); glabrous, smooth, blackish-brown on inner surface; mitre openings 3, ca. 3.5 mm × 5.2 mm. **Stamens** 6, pendent from the apical margin of the floral tube, dark brown; filaments free, short; connectives broad, flattened, laterally fused together and skirt-like, trigonous, ca. 2.4–2.7 mm × 1–1.4 mm, somewhat raised below thecae, flat on the side pointing to the centre of flower (towards apex); supraconnective apex 2-lobed, each lobe with solitary transparent needle-like trichomes ca. 0.5–0.6 mm long; **thecae** yellowish, surrounded by tufts of hairs at middle part; **lateral appendage** protruding towards floral tube, quadrangular, equalling the apex of supraconnective, with a horn-like projection arising from each side, margin shallowly dentate and sparsely hairy; **interstaminal glands** inserted on the lines of fusion between connectives. **Ovary** inferior, bowl-shaped, whitish-brown, warty, enveloped

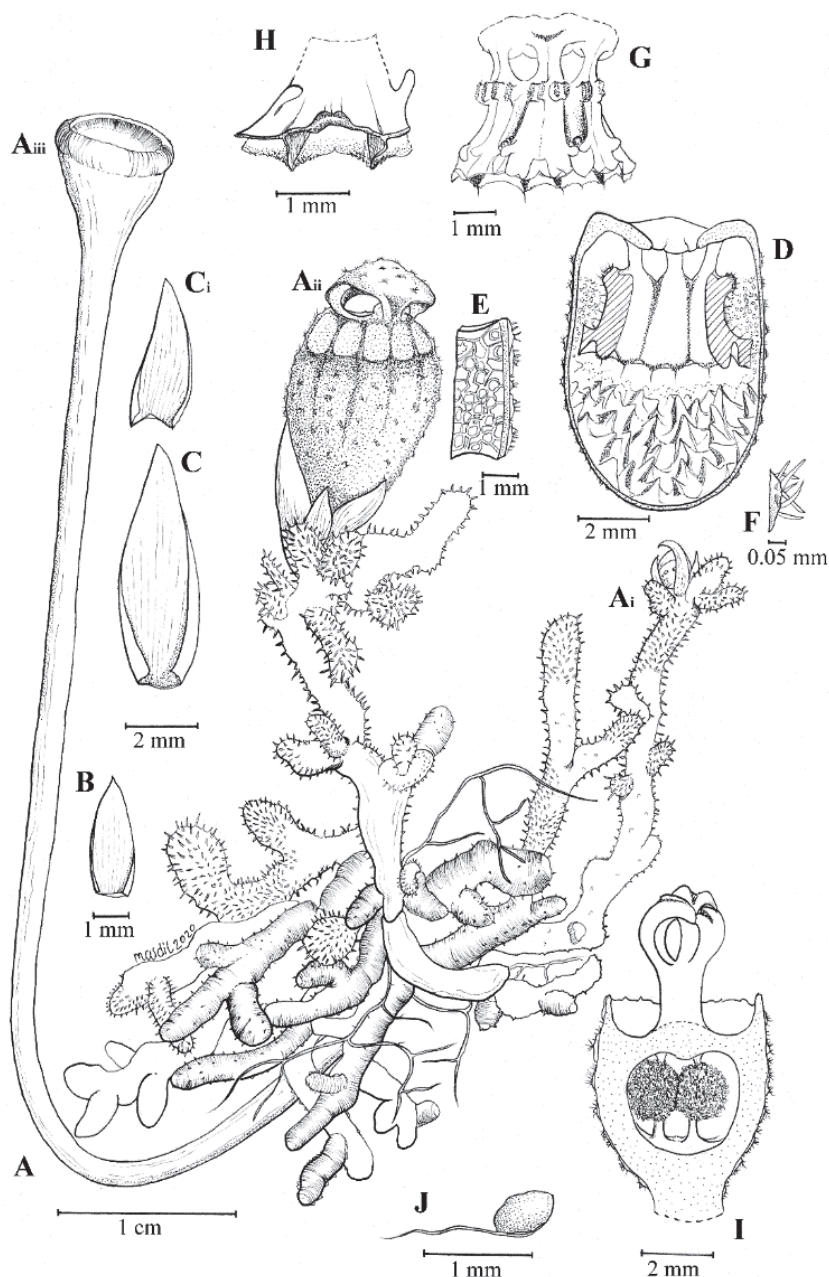


Figure 1. Illustration of *Thismia latiffiana* **A** habit, showing roots, young bud (Ai; note stems covered with trichomes), mature flower (Aii) and fruit (Aiii; note glabrescent stem with trichomes detached) **B** Leaf (adaxial) **C** bract (adaxial), smaller bract (Ci) **D** longitudinally dissected floral tube showing inner (abaxial) view of stamens and apical parts of connectives **E** portion of inner surface of floral tube (upper part) **F** trichomes on outer surface of floral tube; **G** outer (adaxial) view of stamens showing lateral appendages **H** stamen, view from below **I** gynoecium, longitudinal section, showing pistil with trilobed stigma and ovary **J** seed. All from FRI94686 (spirit material). Drawings by Mohamad Aidil Noordin.

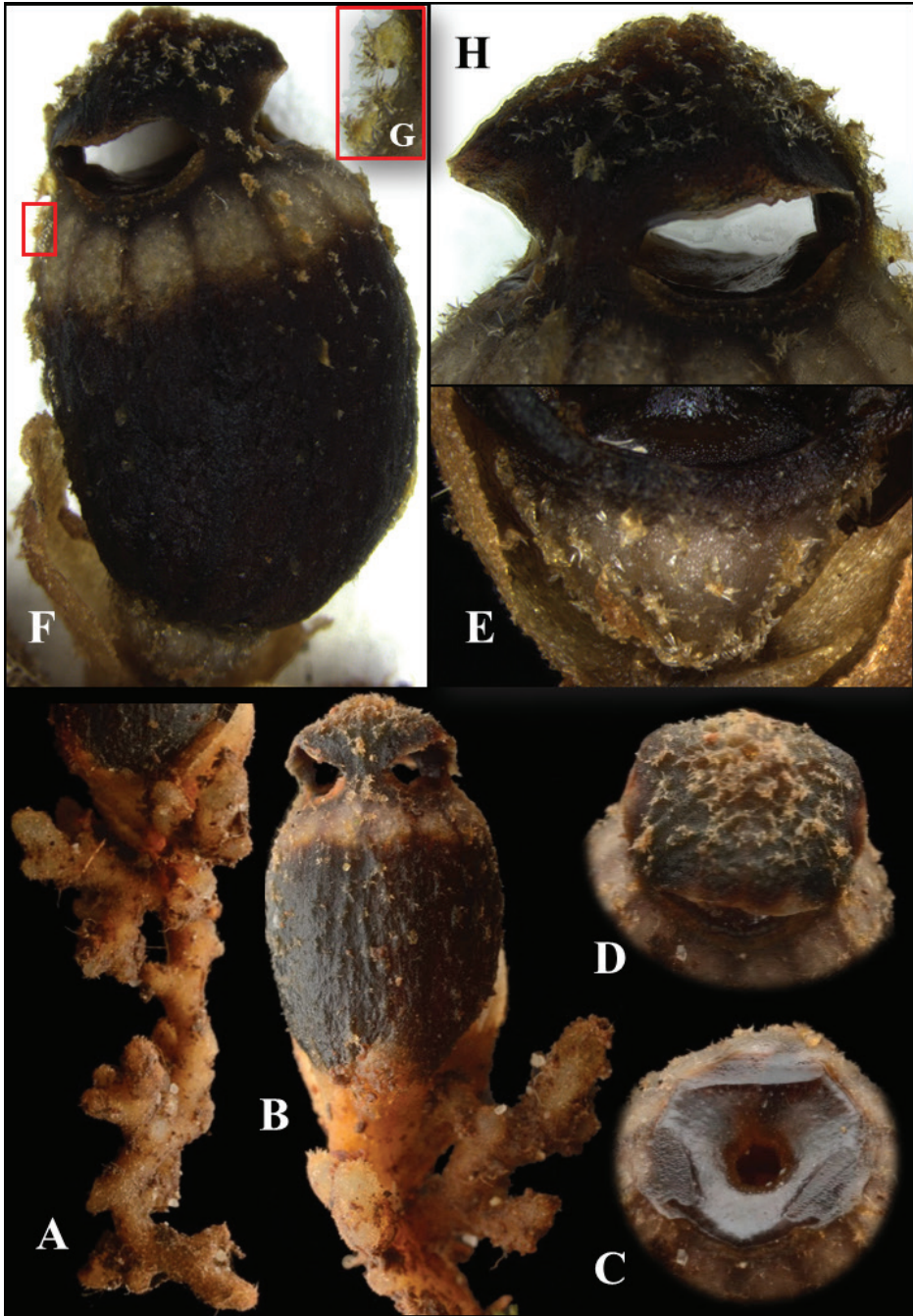


Figure 2. *Thismia latiffiana*, external morphology **A** roots **B** mature flower **C** apical part of floral tube with opening aperture **D** apex of mitre **E** ovary, side view **F** mature flower **G** trichomes resembling spines on an areole of cacti **H** mitre, side view. All from *FRI91117*: **A, B, C, D** *FRI94686*: **E, F, G, H**. Photos by Dome Nikong (**A–D**) and Siti-Munirah MY (**E–H**). Images not to scale (see dimensions in description and Figure 1).

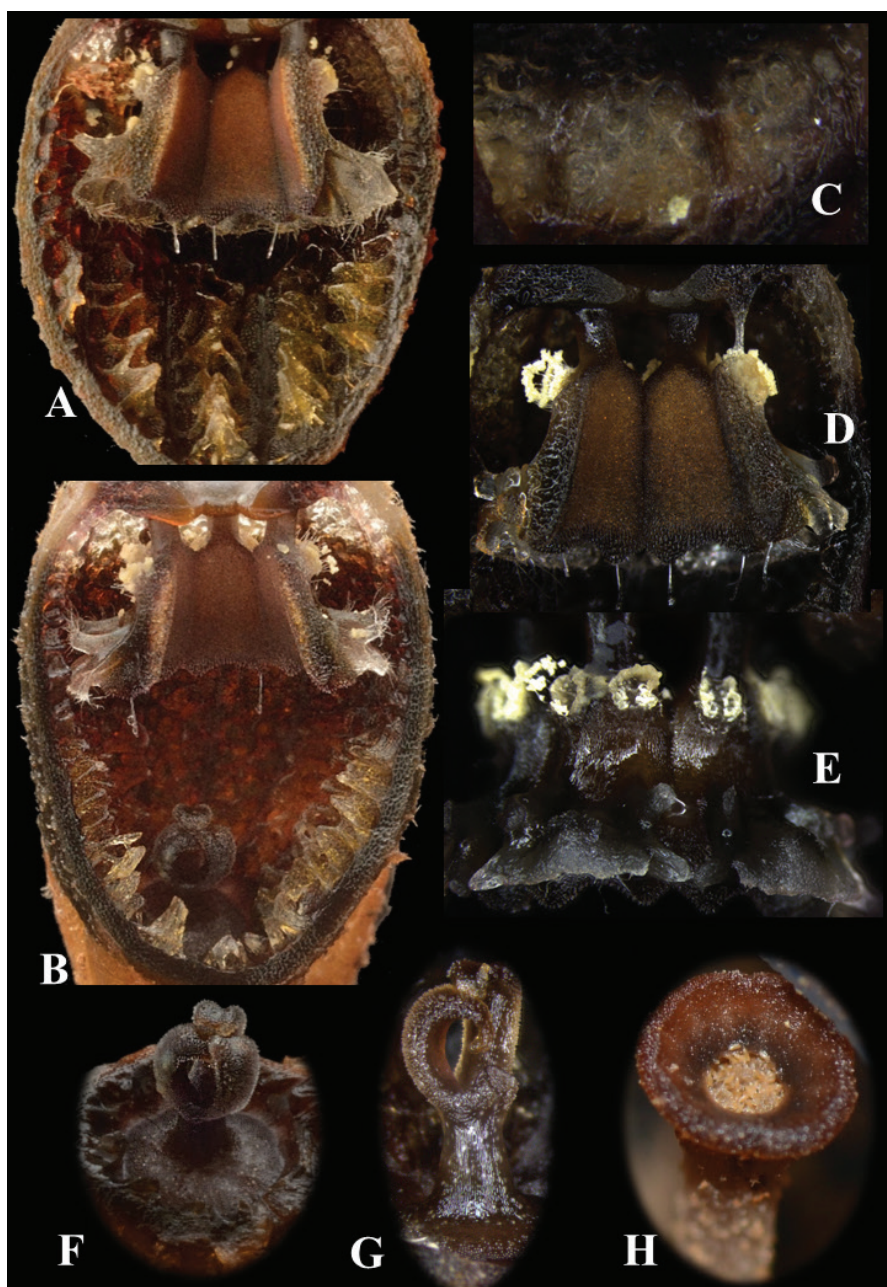


Figure 3. *Thysmia latiffiana*: inner floral structure **A, B** floral tube, longitudinal section **B** shows pistil and inner surface of floral tube covered with pyramidal protuberances arranged longitudinally **C** floral tube, upper portion of longitudinal section **D** inner (abaxial) view of stamens showing supraconnectives **E** outer (adaxial) view of stamens showing lateral appendages **F** pistil, top view, showing stigma **G** pistil, side view **H** fruit, top view. All from *FRI91117*: **A, B, F** *FRI94686*: **C, D, E, G, H**. Photos by Dome Nikong (**A, B, F**) and Siti-Munirah MY (**C, D, E, G, H**). Images not to scale (see dimensions in description and Figure 1).

by bracts and leaves. **Style** ca. 0.8–0.9 mm long \times 0.5–0.8 mm wide; **stigma** deeply trilobed; lobes curved inwards, ca. 2.2 mm long, surface papillose, dark brown. **Fruit** a cup- or bowl-shaped or pyxidium capsule, 2.6 mm in diameter, dark brown to blackish-brown, borne on an elongated pedicel up to 7–10 cm long, distally covered with old dusty trichomes, proximally glabrous.

Additional specimen examined (paratype). Peninsular Malaysia. Terengganu: Setiu, Hulu Nerus Forest Reserve, ca. 220 m elev., 26 December 2019, *Dome Nikong FRI91117* (KEP, spirit collection, No. barcode 280003).

Distribution. Endemic to Peninsular Malaysia, Terengganu. Currently known only from the type locality.

Ecology. In a lowland dipterocarp forest, on moist soil, under shade, near an open place (walking trail) (Figure 4) at elevation of 220 m. Flowering from December to February.

Etymology. *Thismia latiffiana* is named in honour of Emeritus Professor Dato' Dr. Abdul Latiff Mohamad, a prominent botanist and conservationist in Malaysia.

Vernacular name. We suggest a local name as 'Thismia burung hantu' in Malay, due to its appearance resembling an owl ('burung hantu' = owl).

Preliminary conservation status. Critically Endangered (CR B2ab(ii,iii)). Following the IUCN Red List Categories and Criteria (IUCN 2019), this species is assessed as Critically Endangered because it is currently known only from a single locality, where only two individuals were observed. The locality is within the Forest Reserve, but it is exposed to tourism activities within the Lata Payung Recreational Forest and Gunung Sarut. The habitat of the species is near the main trail from the entrance of Lata Payung to the 'blue pool' towards Gunung Sarut. Efforts to trace this species in the surrounding area were unsuccessful. Considering its small population and the threats to its microhabitat, *T. latiffiana* is assessed as Critically Endangered.

Notes. As follows from its morphology, *T. latiffiana* belongs to the section *Sarcosiphon* (Blume) Jonker (Jonker 1938). In addition, following the identification key in Kumar et al. (2017), *T. latiffiana* is falling within the subgen. *Thismia* sect. *Sarcosiphon* due to the arrangement of inner tepals into a mitre, the absence of outer tepals and a mitre lacking a filiform appendage. Additionally, based on the phylogeny in Shepeleva et al. (2020), *T. latiffiana* should belong to or near to clade 3 for its coralliform roots, inner tepals fused into a mitre, absence of mitre foveae and absence of outer tepals.

In the section *Sarcosiphon*, the gross morphology of *T. latiffiana* is similar to that of several other species, such as *T. brunneomitroides* Suetsugu & Tsukaya (Suetsugu et al. 2017), *T. brunneomitra* Hroneš, Koblířová & Dančák (Hroneš et al. 2015), *T. crocea* (Becc.) J.J.Sm. (Beccari 1878) and *T. cladeatina* (Blume) Miq. (Chantanaorrapint et al. 2015). These species share with *T. latiffiana* a brown flower colour and an erect mitre with three lateral holes. However, all these abovementioned species have a long stem during flowering and densely clustered coralliform roots and *T. latiffiana* differs from them in having a very short (almost lacking) stem during flowering and rather loose coralliform roots. The new species is unique amongst the known *Sarcosiphon* species in having several unparalleled traits. *Thismia latiffiana* is recognisably different from all its



Figure 4. *Thismia latiffiana* Siti-Munirah & Dome **A** walking trail near the habitat **B** plant in its habitat **C** plant ex-situ. Photos by Siti-Munirah MY (**A, C**) and Dome Nikong (**B**).

congeners by the presence of golden trichomes on the outer surface of the floral tube, the pyramidal protuberances on the inner side of the floral tube and the supraconnective terminating into two long trichomes.

In Peninsular Malaysia, the most similar species is *T. sitimeriamiae* as it also has a very short stem during flowering and the presence of simple or occasionally apically stellate trichome structure on the outer side of the floral tube. However, it has minute, but distinctly developed, outer tepals and inner tepals forming a flattish, umbrella-like mitre. Therefore, the overall morphology of both species is completely different. For the record, *T. latiffiana* have been discovered in the same Forest Reserve as *T. sitimeriamiae*. Further investigation should be carried to better understand their relationship.

Interestingly, another species of sect. *Sarcosiphon* has been reported from Peninsular Malaysia (Perak, Gunung Hijau) by Ridley who described it as *Bagnisia crocea* var. *brunnea* Ridl. (Ridley 1907). Jonker (1938, 1948) pointed out that it is highly unlikely that the specimen from Perak can be attributed to *T. crocea* from New Guinea although he did not see any specimen. A possible holotype of *Bagnisia crocea* var. *brunnea* (Ridley s.n., SING 0052732!) contains an illustration and single dried plant that has coralliform roots and almost lacks stem during flowering and it is, in general appearance, similar to *T. latiffiana*. Therefore, it is possible that both taxa are conspecific. However, the illustration differs from *T. latiffiana* by small processes on the top of the mitre. Additional fieldwork and research is needed to resolve whether these two taxa are conspecific or not.

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References

- Beccari O (1878) Burmanniaceae. Malesia 1: 240–254.
- Chantanaorrapint S, Tetsana N, Sridith K (2015) Notes on *Thismia clandestina* (Thismiaceae), a little known mycoheterotrophic species. Polish Botanical Journal 60(1): 71–74. <https://doi.org/10.1515/pbj-2015-0013>

- Griffith W (1844) On the root parasites referred by authors to Rhizanthae and their allies. *Proceedings of the Linnean Society of London* 1: 216–221.
- Hroneš M, Koblířová L, Taraška V, Popelka O, Hédli R, Sukri RS, Metali F, Dančák M (2015) *Thismia brunneomitra*, another new species of *Thismia* (Thismiaceae) from Ulu Temburong, Brunei Darussalam. *Phytotaxa* 234(2): 172–178. <https://doi.org/10.11646/phytotaxa.234.2.7>
- IUCN (2019) Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Jonker FP (1938) A monograph of the Burmanniaceae. *Mededeelingen van het Botanisch Museum en Herbarium van de Rijks Universiteit Utrecht* 51: 1–279.
- Jonker FP (1948) Burmanniaceae. In: van Steenis CGGJ (Ed.) *Flora Malesiana*. Leiden, Noordhoff-Kolf, series 1(4): 13–26.
- Kumar P, Gale SW, Li JH, Bouamanivong S, Fischer GA (2017) *Thismia nigricoronata*, a new species of Burmanniaceae (Thismieae, Dioscoreales) from Vang Vieng, Vientiane Province, Laos, and a key to subgeneric classification. *Phytotaxa* 319(3): 225–240. <https://doi.org/10.11646/phytotaxa.319.3.2>
- Ridley HN (1907) *Materials for a Flora of the Malayan Peninsula*. Part II. Methodist Publishing House, Singapore, 235 pp. <https://doi.org/10.5962/bhl.title.10758>
- Shepeleva EA, Schelkunov MI, Hroneš M, Sochor M, Dančák M, Merckx VSFT, Kikuchi IABS, Chantanaorrapint S, Suetsugu K, Tsukaya H, Mar SS, Luu HT, Li H-Q, Logacheva MD, Nuraliev MS (2020) Phylogenetics of the mycoheterotrophic genus *Thismia* (Thismiaceae: Dioscoreales) with a focus on the Old World taxa: delineation of novel natural groups and insights into the evolution of morphological traits. *Botanical Journal of the Linnean Society* 193(3): 287–315. <https://doi.org/10.1093/botlinnean/boaa017>
- Siti-Munirah MY, Dome N (2019) *Thismia domei* and *T. terengganuensis* (Thismiaceae), two new species, and *T. javanica*, a new record from Terengganu, Peninsular Malaysia. *PhytoKeys* 124: 123–137. <https://doi.org/10.3897/phytokeys.124.34728>
- Siti-Munirah MY, Dome N (2021) *Thismia* in Nerus Forest Reserve. *Conservation Malaysia Bulletin* 33: 8–9.
- Siti-Munirah MY, Dome N, Thorogood CJ (2021) *Thismia sitimeriamiae* (Thismiaceae), an extraordinary new species from Terengganu, Peninsular Malaysia. *PhytoKeys* 179: 75–89. <https://doi.org/10.3897/phytokeys.179.68300>
- Suetsugu K, Tsukaya H, Tagane S, Suddee S, Rueangruea S, Yahara T (2017) *Thismia brunneomitroides* (Thismiaceae), a new mycoheterotrophic species from southern Thailand. *Phytotaxa* 314(1): 103–109. <https://doi.org/10.11646/phytotaxa.314.1.9>

Phylogenetic relationships in *Coryphantha* and implications on *Pelecyphora* and *Escobaria* (Cactaceae, Cactoideae, Cactaceae)

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Abstract

The genus *Coryphantha* includes plants with globose to cylindrical stems bearing furrowed tubercles, flowers arising at the apex, and seeds with flattened testa cells. *Coryphantha* is the second richest genus in the tribe Cactaceae. Nevertheless, the genus lacks a phylogenetic framework. The limits of *Coryphantha* with its sister genus *Escobaria* and the infrageneric classification of *Coryphantha* have not been evaluated in a phylogenetic study. In this study we analyzed five chloroplast regions (*matK*, *rbcL*, *psbA-trnH*, *rpl16*, and *trnL-F*) using Bayesian phylogenetic analysis. We included 44 species of *Coryphantha* and 43 additional species of the tribe Cactaceae. Our results support the monophyly of *Coryphantha* by excluding *C. macromeris*. *Escobaria* + *Pelecyphora* + *C. macromeris* are corroborated as the sister group of *Coryphantha*. Within *Coryphantha* our phylogenetic analyses recovered two main clades containing seven subclades, and we propose to recognize those as two subgenera and seven sections, respectively. Also, a new delimitation of *Pelecyphora* including *C. macromeris* and all species previously included in *Escobaria* is proposed. To accommodate this new delimitation 25 new combinations are proposed. The seven subclades recovered within *Coryphantha* are morphologically and geographically congruent, and partially agree with the traditional classification of this genus.

Keywords

Coryphantha macromeris, extrafloral glands, groove on tubercule, infrageneric classification, taxonomy

Introduction

Coryphantha (Engelm.) Lem. was described by Engelmann (1856) as a subgenus of *Mammillaria* Haw. Later, Lemaire (1868) raised it to generic level. Hunt and Benson (1976) proposed *Coryphantha sulcata* (Engelm.) Britton & Rose as the type of this genus. *Coryphantha* is morphologically characterized by adult plants with globose to cylindrical stems, covered with numerous spirally-arranged tubercles, flowers that arise at the apex of the stem, stem tubercles with a groove in maturity, and seeds with flattened testa cells (Anderson 2001; Dicht and Lüthy 2005; Hunt et al. 2006). Species of *Coryphantha* mainly inhabit the Mexican highlands in xerophytic shrublands and grasslands, although some prefer tropical deciduous forests and coniferous forests (Dicht and Lüthy 2005).

The taxonomy of *Coryphantha* has been complicated. Attributes such as the shape and size of the stem, the number, color, and orientation of the spines change according to the development state of the specimen, causing confusion with members of other genera such as *Escobaria* Britton & Rose, *Mammillaria*, and *Neolloydia* Britton & Rose (Vázquez-Benítez et al. 2016). For instance, Benson (1969, 1982) recognized *Escobaria* as a subgroup of *Coryphantha* because of the tubercle grooves, an opinion that persists to this day (Zimmerman and Parfitt 2004).

Species number in *Coryphantha* (excluding *Escobaria*) has varied over time, Lemaire (1868) recognized 25 species, Bravo-Hollis and Sánchez-Mejorada (1991) 59 species, Dicht and Lüthy (2001) and Hunt et al. (2006) 43 species, and Vázquez-Benítez et al. (2016) 45 species. This last study was based on a broad and inclusive morphometric analysis (Vázquez-Benítez et al. 2016). Regardless of the differences in species number, *Coryphantha* is the second richest genus in the tribe Cacteeae (Vázquez-Benítez et al. 2016).

Current infrageneric classifications in *Coryphantha* have been entirely based on morphology, which has been evaluated according to different criteria, generating artificial classifications. Bravo-Hollis and Sánchez-Mejorada (1991) recognized three series within the genus: *Macromeres* Britton & Rose, *Aulacothelae* Lem., and *Glanduliferae* Salm-Dyck. Dicht and Lüthy (2001, 2005) recognized two subgenera: *Coryphantha* and *Neocoryphantha* Backeb., divided into sections and series. Finally, Hunt et al. (2006) proposed an artificial classification in which three subgenera and three informal groups were recognized. Those proposals have been based on the presence/absence of extrafloral glands at the areole, the type of development and position of the areole on the tubercles, growth form and shape of the tubercle. None of these proposals has been evaluated within a phylogenetic framework.

A previous molecular phylogenetic study of the tribe Cacteeae included a few species of the genus *Coryphantha* (Butterworth et al. 2002). This study suggested that *Coryphantha* is part of the *Mammillaria* (=mammilloid) clade, a group that includes other genera such as *Escobaria*, *Neolloydia*, *Ortegocactus* Alexander, and *Pelecyphora* Ehrenb. The position of *Coryphantha* within mammilloid clade was further supported by other studies with better sampling and larger molecular data set (Butterworth and Wallace 2004; Crozier 2005; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Vázquez-Sánchez et al. 2013). Overall, these phylogenetic studies suggest that *Coryphantha* is not monophyletic (Bárcenas et al. 2011; Vázquez-Sánchez et al. 2013). Recently, Breslin et al. (2021) proposed the recircumscription of the mammilloid clade by recognizing three genera, *Mammillaria*, *Cochemiea* (K.Brandegee), and *Coryphantha* (including *Escobaria*). However, sampling in the *Coryphantha* clade was poor. In this study, we performed phylogenetic analyses focusing on the tribe Cacteeae to test for the monophyly of *Coryphantha* and to better understand its relationship to *Escobaria*. With the phylogenetic hypothesis obtained we evaluated the infrageneric classification proposed by Dicht and Lüthy (2005), and propose the set of morphological characters that define the genus *Coryphantha*.

Materials and methods

The monophyly of the tribe Cacteeae has been largely corroborated by phylogenetic studies (Butterworth et al. 2002; Vázquez-Sánchez et al. 2013). The most comprehensive phylogenetic hypothesis of the tribe recovers three grades and the clade named “core Cacteeae”, which is in turn composed by two subclades, the “Ferocactus clade” and the clade B (henceforth “mammilloid clade”) (Vázquez-Sánchez et al. 2013). The present comprehensive study included 44 species of *Coryphantha* (95.6%), eight species of *Escobaria* (44%), 30 additional taxa belonging to the “mammilloid clade”, four taxa of the “Ferocactus clade”, 10 taxa of the “Sclerocactus clade”, and *Echinocactus platyacanthus* Link & Otto as the functional outgroup (Appendix 1). For the genus *Coryphantha*, we followed the species accepted by Dicht and Lüthy (2005) and those accepted by Arias et al. (2012). Our analyses included mostly new sequences for *Coryphantha* and complementary sequences previously published (Butterworth et al. 2002; Butterworth and Wallace 2004; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Fehlberg et al. 2013; Schwabe et al. 2015; Kuzmina et al. 2017; Aquino et al. 2019, and Vázquez-Sánchez et al. 2013, 2019) (Appendix 1).

Samples of plant tissue from the epidermis and hypodermis of the stem were dried, frozen, and pulverized. Total DNA extraction was achieved by using the DNeasy plant mini kit (Qiagen, California). We amplified chloroplast markers widely used in phylogenetic reconstruction in Cacteeae (Vázquez-Sánchez et al. 2013, 2019). Specifically, we amplified the chloroplast coding regions *matK* and *rbcL*, and the intergenic spacers *psbA-trnH* and the *trnL-trnF* (including part of the *trnL*), and the *rp16* intron. Primers and profiles of thermal cycles followed Vázquez-Sánchez et al. (2013). The PCR products

were sequenced at the High Throughput Genomics Unit at the University of Washington (now unavailable). Appendix 1 shows in detail the GenBank accessions for each taxon.

The sequences for each marker were assembled using SEQUENCHER (v. 4.8, Gene Codes Corporation 2007). The matrices were aligned manually with MESQUITE (v. 2.75, Maddison and Maddison 2015). Table 1 shows some numeric records about the taxa and the aligned sequences included in the subsequent analyses. Insertion-deletion events in aligned sequences (indels) were coded using the simple coding method (Simmons and Ochoterena 2000) (Appendix 2). Additionally, eight morphological characters, proposed as diagnostic for *Coryphantha* and related genera were coded and used in a combined phylogenetic analysis. It has been suggested that in Cactaceae the inclusion of indels and a set of morphological characters in phylogenetics analysis results in more accurate hypotheses (Sánchez et al. 2019; Martínez-Quezada et al. 2020). Character states were extracted from the descriptions of the species (Bravo Hollis and Sánchez-Mejorada 1991; Barthlott and Hunt 2000; Dicht and Lüthy 2005; Hunt et al. 2006) and corroborated in the field, in living collections (Jardín Botánico, Instituto de Biología, UNAM), and with herbarium specimens (MEXU). Characters and character states are listed in Table 2. DNA evolution models for each sequence were estimated using the corrected Akaike information criterion (AICc) in JMODELTEST2 (Darriba et al. 2012) on the CIPRES Science Gateway (v. 3.3 Miller et al. 2010) (Table 1). The Mkv model (Lewis 2001) was assigned for the indels and the morphological partitions. The first matrix was concatenated by including the five DNA regions. The second matrix included the five DNA regions and the indels partition. Finally, the third matrix included the five DNA regions, the indels and morphological characters. A partitioned Bayesian inference (BI) analysis was performed in MRBAYES (v. 3.2.1, Ronquist et al. 2012). The BI analysis for those matrices consisted of two runs of four chains for

Table 1. Data of the aligned sequences used in the phylogenetic analysis.

	<i>matK</i>	<i>psbA-trnH</i>	<i>rebL</i>	<i>rpl16</i>	<i>trnL-F</i>	Combo
Taxa	95/99	91/99	83/99	86/99	85/99	–
Length (aligned)	817	391	538	1349	1218	4313
Non-informative sites	730	313	509	1100	1048	3700
Informative sites	87	78	29	249	170	613
% informative sites	10.6	19.9	5.4	18.4	13.9	14.2
Informative indels	1	11	0	8	14	34
Substitution model	TPM1uf+I+G	TPM1uf+I+G	K80+I	TIM1+I+G	TVM+G	–

Table 2. Characters and character states for the ancestral states reconstruction.

1. Growth form: (0) globose, (1) short cylindrical, (2) cylindrical, (3) depressed-globose.
2. Groove on tubercle in mature plant: (0) absence, (1) complete, (2) incomplete.
3. Extrafloral glands at or near the axil: (0) absence, (1) turgid throughout the year, (2) turgid only at flowering season.
4. Position of the flowers: (0) apical or nearly apical, (1) in a ring distant from the apex.
5. Margin of the outer tepals: (0) fimbriate, (1) entire.
6. Color of the mature fruit: (0) red-pink, (1) green, (2) yellow.
7. Type of cortex: (0) watery, (1) mucilaginous, (2) laticiferous.
8. Multicellular sculpture of the lateral side of the seed: (0) flat, (1) tuberculate, (2) pitted.

20 million iterations, saving one tree every 1000 generations, and beginning with one random tree. The burn-in parameter was fixed as 25%.

The ancestral states of the eight morphological characters were traced in the selected phylogeny to test them as potential synapomorphies of clades. The tracing of characters was performed in MESQUITE (v.2.75, Maddison and Maddison 2015) using the parsimony ancestral state method on the majority consensus tree from the combined BI analysis.

Results

Phylogenetic analyses including DNA sequences only (Appendix 3: Fig. A1) and DNA sequences + indels partition (henceforth “molecular analysis”) showed identical topologies (Fig. 1). The phylogenetic analysis with morphological data (henceforth “combined analysis”) recovered a more resolved phylogeny (Fig. 2) with minor changes in the main clades, except for the position of one clade. In the molecular analysis, *Mammillaria sphacelata* and *M. beneckeii* were recovered as the sister clade to *Coryphantha* s.s. (PP = 0.96, Fig. 1). This clade formed a polytomy with *Cochemiea* and *Escobaria* (including *Pelecyphora*) clades (Fig. 1). In the combined analysis, *M. sphacelata* and *M. beneckeii* were included in the *Mammillaria* clade PP = 0.98, Fig. 2). Each clade; *Cumarinia*, *Mammillaria*, *Cochemiea*, *Escobaria*, and *Coryphantha* s.s. showed resolved relationships between them with moderate to low support (Fig. 2).

In all analyses the *Cochemiea* clade included *Mammillaria mazatlanensis* (PP = 1.0) *Ortegocactus macdougalii* (PP = 0.7, Fig. 1; PP = 0.79, Fig. 2), and *Neolloydia* (PP = 0.8, Fig. 1; PP = 0.52, Fig. 2). Phylogenetic relationships in both analyses indicate that *Coryphantha* is not a monophyletic group, because *C. macromeris* was recovered in the *Escobaria* clade (Figs 1, 2). *Coryphantha* s.s. is divided into two main clades, with 33 species grouped in clade I (PP = 0.99, Fig. 1; P = 1, Fig. 2), and 13 species grouped in clade II (PP = 0.91; Fig. 1; PP = 0.99, Fig. 2). Clade I is composed by five subclades (A, B, C, D, E), and Clade II by two subclades (F, G) (Figs 1, 2), all of them with high supports. The *Escobaria* clade (PP = 0.98, Fig. 1; PP = 0.97, Fig. 2) is divided into two subclades, the first one includes *Coryphantha macromeris*, *Escobaria cubensis*, *E. dasyacantha*, *E. missourensis*, *E. vivipara*, and *E. zilziana* (PP = 1, Figs 1, 2); while the second subclade includes *E. laredoi*, *Pelecyphora asselliformis*, *P. strobiliformis*, *E. tuberculosa*, and *E. chihuahuensis* (PP = 1.0; Figs 1, 2).

The ancestral state reconstruction (Appendix 3: Fig. A1) showed that the presence of a complete groove on the tubercle (Appendix 3: Fig. A1B), the apical origin of the flowers (Appendix 3: Fig. A1D), the entire margin of the outer tepals (Appendix 3: Fig. A1E), the green color of the fruit (Appendix 3: Fig. A1F), and the flat multicellular sculpture of the lateral side of the seed (Appendix 3: Fig. A1H) were ancestral states to *Coryphantha* s.s., few or null changes on these characters states occurred inside the clade. In contrast, in the *Escobaria* clade, the fimbriate margin of the outer tepals (Appendix 3: Fig. A1E), the red color of the mature fruit (Appendix 3: Fig. A1F), and the pitted multicellular sculpture of the seed were ancestral character states (Appendix 3:

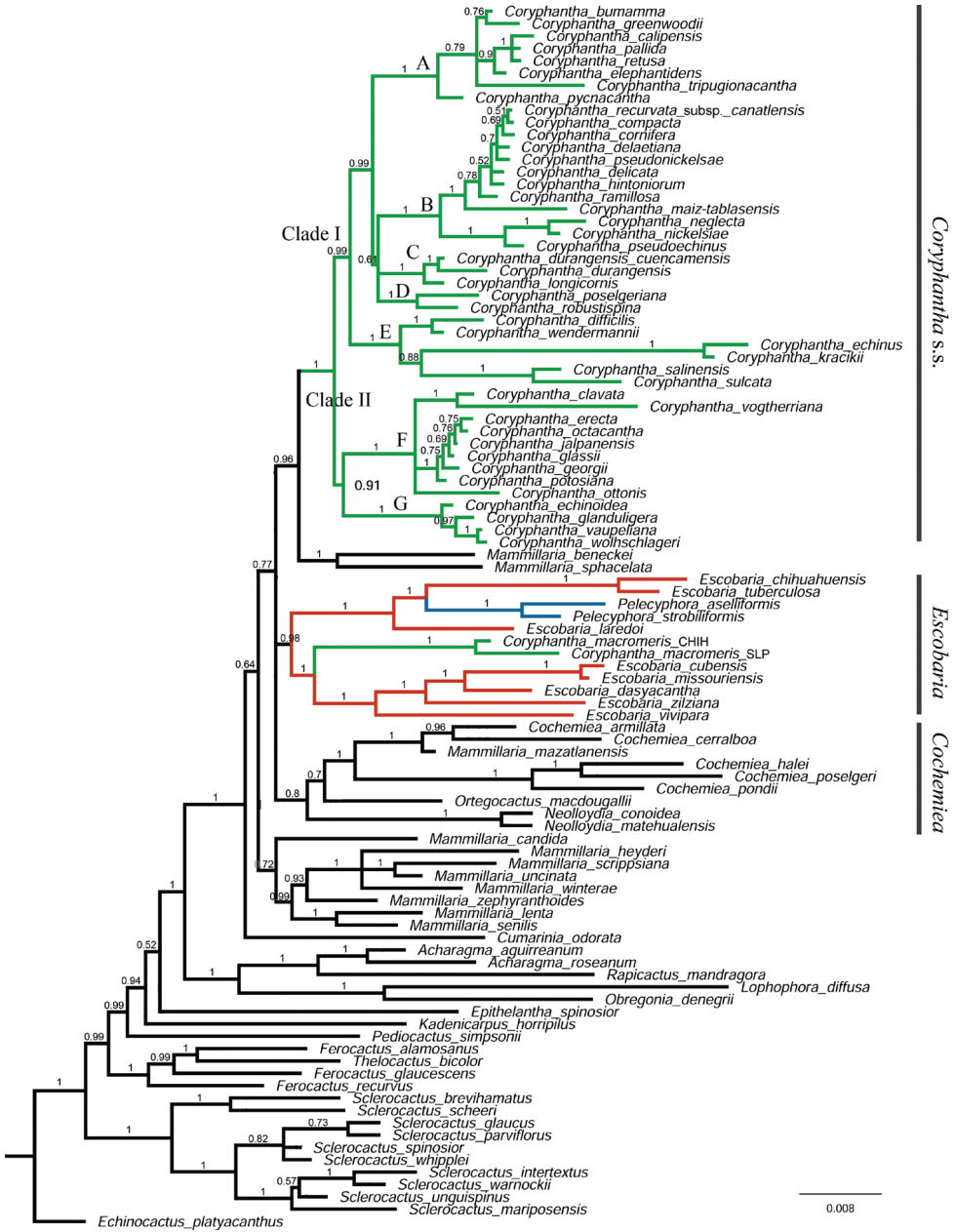


Figure 1. Phylogenetic relationships of *Coryphantha* and close related genera. Majority rule phylogram, from the BI analysis using cpDNA sequences and indels partitions (molecular analysis). Numbers in nodes indicate posterior probabilities. Labels indicate the main recovered clades and subclades.

Fig. A1H). Additionally, growth form was ambiguous in *Coryphantha* s.s. and *Escobaria* clade. The absence of glands near the axil of the tubercles was ancestral to *Coryphantha* s.s., and the presence of those glands evolved independently in two subclades of *Coryphantha* (Appendix 3: Fig. A1C). In clade II, turgid glands present all year-long

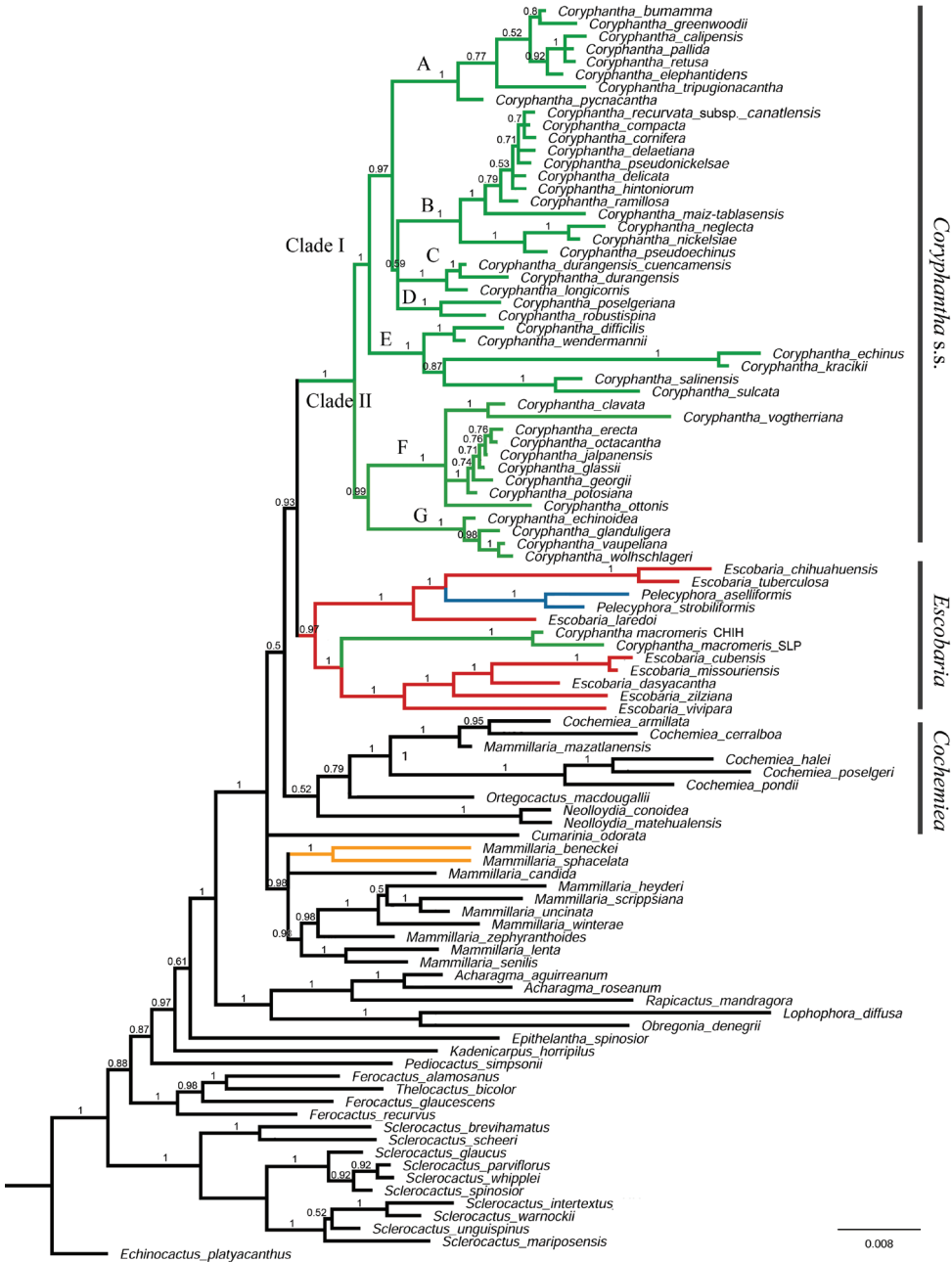


Figure 2. Phylogenetic relationships of *Coryphantha* and close related genera. Majority rule phylogram, from the BI analysis using cpDNA sequences, indels, and morphological partitions (combined analysis). Numbers in nodes indicate posterior probabilities. Labels indicate the main recovered clades and subclades.

were ancestral, while glands present only during flowering season evolved once in sub-clade D (Appendix 3: Fig. A1C. Finally, watery cortex was ancestral in *Coryphantha* s.s., but it changed into mucilaginous cortex in the subclade F (Appendix 3: Fig. A1G).

Discussion

The close relationships among *Cochemiea*, *Coryphantha*, *Cumarinia*, *Escobaria*, and *Mammillaria* have been recognized by several studies (Butterworth and Wallace 2004; Crozier 2005; Vázquez-Sánchez et al. 2013; Breslin et al. 2021). Breslin et al. (2021) recovered them as closely related lineages and redefined their limits. These authors proposed to expand the limits of *Cochemiea* to include 37 species of *Mammillaria*, *Neolloydia*, and *Ortegocactus*. Our results (Figs 1, 2) recovered, with moderate to low support, the same phylogenetic position of *Ortegocactus* and *Neolloydia*. Additionally, *Mammillaria mazatlanensis* was nested within *Cochemiea*. Morphological (Hunt 1985) and molecular evidence (Butterworth and Wallace 2004) suggest that *M. mazatlanensis* is closely related to other taxa now classified within *Cochemiea*, so it should be transferred (see Taxonomic summary).

In the molecular analysis, *Mammillaria sphacelata* and *M. benecki* were recovered, with low support, as the sister group to *Coryphantha* s.s. In contrast, Breslin et al. (2021) found *M. sphacelata* to be the sister to *Escobaria* + *Coryphantha*. The addition of eight morphological characters in the combined analysis recovered *M. sphacelata* and *M. beneckeii* within the clade *Mammillaria*, and supported *Coryphantha* s.s. and *Escobaria* as sister lineages. We argue that the low sampling of this early diverged lineage of *Mammillaria* (Butterworth and Wallace 2004) and the few sequences included do not allow us to conclude about their relationships.

Finally, Breslin et al. (2021) proposed *Escobaria* and *Coryphantha* to be a single genus, as traditionally treated by North American botanists (Benson 1982; Zimmerman and Parfitt 2004). However, sampling in Mexican *Coryphantha* was not representative. Molecular and combined analyses recovered *Coryphantha* and *Escobaria* as independent lineages and the ancestral state reconstruction (Appendix 3: Fig. A1) showed that each genus has a unique combination of morphological characters. Our results support the traditional recognition of *Coryphantha* and *Escobaria* as separate genera (Taylor 1979; Bravo-Hollis and Sánchez Mejorada 1991; Dicht and Lüthy 2005; Hunt et al. 2006; Korotkova et al. 2021).

Escobaria clade

The eight sampled species of *Escobaria*, together with *Coryphantha macromeris*, *Pelecypora aselliformis*, and *P. strobiliformis* form a monophyletic group with high support values (Figs 1, 2). This clade is diagnosed by the tubercles with complete grooves, external tepals with fimbriate margins, and seeds with pitted multicellular sculpture on the lateral side (except in *C. macromeris*, and *Escobaria chihuahuensis*) (Appendix 3: Fig. A1, Fig. 3).

Although previous molecular analyses recovered *C. macromeris* outside the core *Coryphantha* clade, phylogenetic relationships of *C. macromeris* were not clear due to lack of resolution (Bárcenas et al. 2011) and insufficient sampling of *Coryphantha* (Vázquez-Sánchez et al. 2013; Crozier 2005). Our analyses, including 46 taxa of *Coryphantha*, recovered two different samples of *C. macromeris* in the *Escobaria* clade (PP = 1.0, Figs 1, 2), contrasting with the traditional classification in the monotypic section *Lepidocoryphantha* (Backeberg) Moran, or subgenus *Neocoryphantha* Backeb.

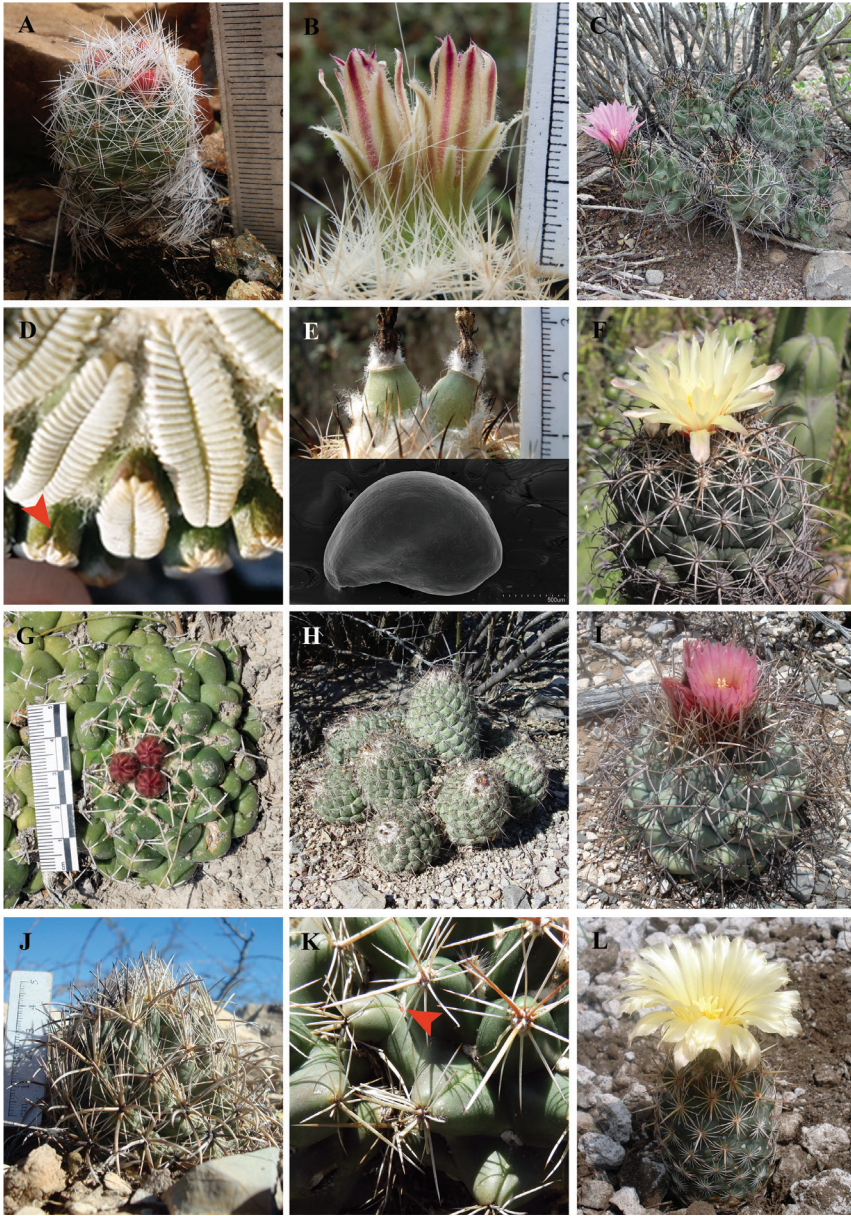


Figure 3. Representative species and morphology of *Coryphantha* and *Escobaria* **A** *Escobaria dasyacantha* bearing red fruits (S. Arias 2090, MEXU) **B** flower of *Escobaria emskoetteriana* (Quehl) Borg with fimbriate outer tepals (D. Aquino 322, MEXU) **C** *Coryphantha macromeris* bearing flowers with fimbriate outer tepals (S. Arias 1788, MEXU) **D** close-up of the furrow on the tubercles (arrow) in *Pelecyphora aselliformis* (H. Sánchez-Mejorada 3616, MEXU) **E** green fruits (top) and flat multicellular sculpture of the lateral side of the seed (bottom) in *Coryphantha calipensis* (B. Vázquez 2555, MEXU) **F** *Coryphantha maiz-tablasensis* (D. Aquino 400, MEXU) **G** *Coryphantha cornifera* (SA 2212, MEXU) **H** *Coryphantha durangensis* (B. Vázquez 2625, MEXU) **I** *Coryphantha poselgeriana* (S. Arias 2109, MEXU) **J** *Coryphantha kracikii* (B. Vázquez 2618, MEXU) **K** glands at the axil (arrow) in *Coryphantha ottonis* (D. Sánchez s.n., IBUG) **L** *Coryphantha glanduligera* (S. Arias 2129, MEXU).

(sensu Dicht and Lüthy 2005). Previous morphological analysis of *Coryphantha* concluded that *C. macromeris* was the most dissimilar taxon of the genus *Coryphantha* (Vázquez-Benítez et al. 2016). The main character that differentiates this species from the rest of the species in the *Coryphantha* clade is the presence of an incomplete groove in the tubercles and fimbriate outer tepals.

Coryphantha macromeris shares the fimbriate outer tepals with the other species of the genus *Escobaria* (Fig. 3B, C). Interestingly, *C. macromeris* and *Escobaria vivipara* show identical flower morphology (Zimmerman and Parfitt 2004). Additionally, *E. chihuahuensis* shows a shallowly pitted lateral seed coat (Barthlott and Hunt 2000, plate 73.3–4), similar to the flat cells observed in *Coryphantha*. Probably, the flat sculpture of the lateral side of the seed in *C. macromeris* is the result of the same development observed in *E. chihuahuensis*. As observed in *Ferocactus* (Taylor and Clark 1983) the change of pitted to flat relief of periclinal walls of the seed testa has evolved independently in several lineages of the tribe Cacteeae (Appendix 3: Fig. A1H). Given our results, we propose the recognition of *C. macromeris* as a member within the new rearrangement of *Escobaria* and *Pelecyphora* described in the following paragraphs (see Taxonomic summary).

As in previous analysis our phylogenetic hypothesis recovered the two species of *Pelecyphora* in the *Escobaria* clade (Butterworth and Wallace 2004; Bárcenas et al. 2011; Vázquez-Sánchez et al. 2013). Traditionally, *Pelecyphora* is recognized (Boke 1959; Anderson and Boke 1969) by the presence of a rudimentary groove on the tubercles and the “reticulate or striate” seed structure (“par-concave” sensu Barthlott and Hunt 2000). However, *Pelecyphora* also falls into Taylor’s (1979) concept of *Escobaria*, which is circumscribed by seeds with intracellular pits (par-concave) and grooved tubercles. Following Boke (1959), the rudimentary groove in *Pelecyphora* (Fig. 3D) is morphologically equivalent to the groove found on the tubercles of *Coryphantha* and *Escobaria*. Regarding seed morphology, the pitted appearance of the seed coat in *Escobaria* happens because only the central portion of the outer wall of the testa cell is thinner and collapses, while in *Pelecyphora* the entire outer wall of the testa cell is thin and collapses (Barthlott and Hunt 2000). Therefore, *Escobaria* and *Pelecyphora* show a pitted lateral seed coat, differing in cell shape and pit diameter.

Finally, the margin of the outer tepals in *P. aselliformis* may be entire or fimbriate, while in *P. strobiliformis* is always fimbriate (Anderson and Boke 1969); this character is also observed in all species of *Escobaria* (Zimmerman and Parfitt 2004; Hunt et al. 2006). We hypothesized that *Pelecyphora* represents a derived lineage in *Escobaria* that has changed radically its growth form and the shape of its tubercles to occupy specific niches in the Sierra Madre Oriental. A similar trend is observed in species of the genus *Turbinicarpus* (Backeb.) Buxb. & Backeb., in which some species have evolved into a globose-depressed growth form with cylindrical and flattened distally (hatchet-shaped) tubercles (e.g., *Turbinicarpus pseudopectinatus* (Backeb.) Glass & R.A.Foster) or pyramidal and dorsiventrally flattened (scale-like) tubercles (e.g., *Turbinicarpus schmidickeanus* (Boed.) Buxb. & Backeb.) (Vázquez-Sánchez et al. 2019).

Several studies recovered with high support the alliance of *Pelecyphora* and a clade including *Escobaria tuberculosa*, the type species of *Escobaria*. A diagnostic character of

Escobaria and *Pelecyphora* is the outerperianth-segments with ciliated margins as shown in *E. emskoetteriana* (Fig. 3B), *E. abdita* Řepka & Vaško (Řepka and Vaško 2011) and *E. sneedi* Britton & Rose (Benson 1982) not included in this analysis. The genus *Pelecyphora* was published in 1843 by Ehrenberg, while *Escobaria* was published 80 years later, in 1923, by Britton and Rose. In this context, we propose to merge *Escobaria* members, including *C. macromeris* into *Pelecyphora* (see Taxonomic summary) following priority of publication as dictated by the principle III of the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018).

Coryphantha clade

Coryphantha can be recognized as a natural group by excluding *C. macromeris*. *Coryphantha* s.s. (henceforth *Coryphantha*) conformed a robust clade (PP = 1, Figs 1, 2) and can be diagnosed by tubercles with a complete groove, flowers with apical origin, outer tepals with entire margin, green fruits, and seed with flat multicellular sculpture on the lateral side (Appendix 3: Fig. A1, Fig. 3).

Although subgenera *Neocoryphantha* and *Coryphantha* recognized by Dicht and Lüthy (2005) are partially recovered, our phylogenetic analyses showed that most of the infrageneric sections and series proposed by these authors do not represent natural entities. All sampled members of subgenus *Coryphantha* were recovered in clade I, including taxa without turgid glands near the axil throughout the year (Appendix 3: Fig. A1C). However, this clade also included two of the species assigned to section *Robustispina* Dicht & A. Lüthy in the subgenus *Neocoryphantha* (Table 3), making *Coryphantha* subgenus *Coryphantha* (sensu Dicht and Lüthy 2005) a paraphyletic group. Clade II grouped all the members of the subgenus *Neocoryphantha* section *Neocoryphantha*, but the members of the sections *Lepidocoryphantha* and *Robustispina* (Fig. 1) were recovered in the clade *Escobaria* and the clade I, respectively. Therefore, *Coryphantha* subgenus *Neocoryphantha* (sensu Dicht and Lüthy 2005) represents a polyphyletic group. All members of clade II show turgid glands at or near the axil throughout the year (Fig. 3K), which is recognized as a consistent diagnostic feature and a potential synapomorphy for this lineage (Appendix 3: Fig. A1C).

In order to reflect the relationships found in our phylogenetic hypothesis and to provide a natural infrageneric classification of the genus, we re-circumscribe the two subgenera in *Coryphantha*. One for clade I, the subgenus *Coryphantha*, and another one for clade II, the subgenus *Neocoryphantha* (see Taxonomic summary). We further propose to recognize the recovered subclades as sections (see Taxonomic summary). The species belonging to each section, their morphological similarities, and their distribution (biogeographic provinces) are discussed below.

Coryphantha subgenus *Coryphantha* (clade I) emerged in five subclades that partially represent some taxonomic groups proposed by Dicht and Lüthy (2005). However, series and subseries suggested by these authors do not represent monophyletic groups. Clade A included species from series *Retusae* Dicht & A. Lüthy, *Pycnacanthae* Dicht & A. Lüthy and *Salinenses* Dicht & Lüthy (Table 3). In this case, members of

clade A present most of the radial spines with subulate shape (Fig. 3F) (Bravo-Hollis and Sánchez-Mejorada 1991; Dicht and Lüthy 2005). Our results found that the species complexes *C. elephantidens* and *C. pallida* do not represent monophyletic groups. This result corroborates that *C. bumamma* and *C. greenwoodii* are different species from *C. elephantidens* as proposed by Vázquez-Benítez et al. (2016). Additionally, our results support the proposal of Arias et al. (2012) to recognize *C. calipensis* and *C. pallida* as two distinct species. The distinction of *C. pseudoradians* Bravo from *C. pallida* Britton & Rose, remains unresolved, since the former was not included in our analysis.

As documented by Dicht and Lüthy (2005), there was a historical confusion between *C. pycnantha* and *C. pallida*, since they are morphologically similar (Arias et al. 2012). This affinity is now justified since they belong to the same clade. Dicht and Lüthy (2005) classified *C. pallida* within series *Salinensis* along with northern species. This species emerged in Clade A, which is recognized here as section *Retusae* (see Taxonomic summary). This is distributed in central Mexico, encompassing the southern portion of the piedmont of Sierra Madre Occidental, the Mexican High Plateau, the plains and piedmonts of the Mexican Transvolcanic Belt, the southern portion of Sierra Madre Oriental, and the Balsas Basin.

Clade B included members of the series *Coryphantha* and *Corniferae* Dicht & A. Lüthy (Table 3). Members of this clade show upright or radiate tubercles (Fig. 3G). This lineage is recognized in the present work as the section *Corniferae*. This clade presents a wide distribution and occupies several northern ecoregions. An eastern group of species inhabits the Chihuahuan Desert, the Sierra Madre Oriental, and the Tamaulipas-Texas Semiarid Plain, and a western group occupies the Chihuahuan Desert, the piedmont of the Sierra Madre Occidental, and the Sierra Madre Occidental.

Coryphantha gracilis is classified into the monotypic section *Gracilicoryphantha* Dicht & Lüthy by the presence of globose seed and broad basal hylum (Dicht and Lüthy 2005). Although *C. gracilis* was not included in our analysis, we suggest that it belongs to clade B, because of its morphological affinity to *C. compacta* and *C. recurvata* (Vázquez-Benítez et al. 2016), and also the similar geographic distribution. *Coryphantha pulleineana* (Backeb.) Glass was not included in our analysis. Dicht and Lüthy (2005) mention some morphological affinities to *C. ramillosa*. In addition, *C. pulleineana* and *C. pseudoechinus* shared the presence of glands in the spiniferous areole. For now, we propose *C. pulleineana* as a member of this group because of its morphological and geographical congruence to other species of this clade (Dicht and Lüthy 2005).

Subclade C included two members of the series *Salinenses* (Table 3). These taxa can be distinguished by the presence of appressed tubercles and woolly stem tips (Fig. 3H) (Bravo-Hollis and Sánchez-Mejorada 1991; Dicht and Lüthy 2005). Our study included *C. durangensis* subsp. *durangensis* and *C. durangensis* subsp. *cuencamensis*, which formed a monophyletic group. However, they showed different branch lengths, which suggests that its recognition as different species, as proposed by Vázquez-Benítez et al. (2016), must be considered. This small group is recognized in the present work as the

section *Durangenses* (see Taxonomic summary). This group presents a narrow distribution in the state of Durango, inhabiting the Chihuahuan Desert and the piedmont of the Sierra Madre Occidental.

Subclade D corresponds to *Coryphantha* section *Robustispina* (Table 3, Taxonomic summary). This clade is supported by the presence of turgid glands near the axil only during the flowering season (Fig. 3I; Appendix 3: Fig. A1C). Although those species have been grouped in the past with other taxa with glands (Dicht and Lüthy 2005; Vázquez-Benítez et al. 2016), our results suggested that this character state emerged independently from an ancestral with absent glands. This species occurs in the Chihuahuan Desert and in the northern piedmont of the Sierra Madre Occidental.

Subclade E was formed by six taxa classified into the series *Coryphanta*, series *Salinenses*, and series *Corniferae* (Table 3). There are no evident morphological characters that define clade C. Affinities such as the red filaments have been observed in *C. echinus*, *C. kracikii*, *C. salinensis*, and *C. sulcata*. Particularly, *C. salinensis* and *C. sulcata* share a yellow flower with a brilliant red flower throat (Dicht and Lüthy 2005). Also, *C. difficilis*, *C. kracikii*, *C. salinensis* show tubercles appressed, and slightly appressed in *C. werdermannii* (Fig. 3J). Members of subclade E are proposed here as the *Coryphantha* section *Coryphantha*, which is distributed in the Chihuahuan Desert, the Sierra Madre Oriental, and the Tamaulipas-Texas Semiarid Plain.

We propose the division of subgenus *Neocoryphantha* (clade II) into two sections. The first one is section *Clavatae* (see Taxonomic summary), which corresponds to subclade F (Table 3). This section presents mucilaginous cortex (Dicht and Lüthy 2005), a character recovered as ancestral to the group in our analyses (Fig. 3K, Appendix 3: Fig. A1G). Section *Clavatae* occurs mainly in the southern part of the Chihuahuan Desert and in the Mexican High Plateau, with *C. ottonis* ranging to the interior plains and piedmonts of the Sierra Madre Occidental and the Mexican Transvolcanic Belt. The second is sec-

Table 3. Species memberships of the main clades obtained in this study and their previous infrageneric classification by Dicht and Lüthy (2005).

Clade I	Subgenus <i>Coryphantha</i> and subgenus <i>Neocoryphantha</i> section <i>Robustispina</i>
Subclade A	Series <i>Retusae</i> : <i>Coryphantha elephantidens</i> complex and <i>C. retusa</i> .
Subclade A	Series <i>Pycnanthae</i> : <i>C. pycnantha</i> and <i>C. tripugionacantha</i>
Subclade A	Series <i>Salinenses</i> (in part): <i>C. pallida</i> complex
Subclade B	Series <i>Coryphantha</i> (in part): <i>Coryphantha hintoni</i> and <i>C. maiz-tablasensis</i>
Subclade B	Series <i>Corniferae</i> (in part): <i>C. compacta</i> , <i>C. cornifera</i> , <i>C. delaetiana</i> , <i>C. delicata</i> , <i>C. echinus</i> , <i>C. neglecta</i> , <i>C. nickelsiae</i> , <i>C. pseudoechinus</i> , <i>C. pseudonickelsiae</i> , <i>C. ramillosa</i> , and <i>C. recurvata</i> subsp. <i>canatlanensis</i>
Subclade C	Series <i>Salinenses</i> (in part): <i>Coryphantha durangensis</i> , <i>C. durangensis</i> subsp. <i>cuencamensis</i> , and <i>C. longicornis</i>
Subclade D	Section <i>Robustispina</i> : <i>Coryphantha poselgeriana</i> and <i>C. robustispina</i>
Subclade E	Series <i>Coryphanta</i> (in part): <i>C. sulcata</i>
Subclade E	Series <i>Salinenses</i> (in part): <i>C. difficilis</i> , <i>C. kracikii</i> , and <i>C. salinensis</i>
Subclade E	Series <i>Corniferae</i> (in part): <i>C. werdermannii</i> and <i>C. echinus</i>
Clade II	Subgenus <i>Neocoryphantha</i> except section <i>Robustispina</i>
Subclade F	Series <i>Clavatae</i> : <i>C. octacantha</i> , <i>C. jalpanensis</i> , <i>C. clavata</i> , <i>C. clavata</i> , <i>C. glassii</i> , <i>C. erecta</i> , and <i>C. potosiana</i>
Subclade F	Series <i>Otonis</i> : <i>C. ottonis</i> , <i>C. vogtherriana</i> , and <i>C. georgii</i>
Subclade G	Series <i>Echinoideae</i> : <i>C. woblschlageri</i> , <i>C. vaupeliana</i> , <i>C. glanduligera</i> , and <i>C. echinoidea</i>

tion *Echinoideae*, which corresponds to subclade G (Fig. 3L, Table 3). This section can be recognized by the presence of watery cortex (Appendix 3: Fig. A1G). Members of the section are distributed in the Chihuahuan Desert and the Sierra Madre Oriental.

Taxonomic summary

Cochemiea

Phylogenetic analyses support the addition of *Mammillaria mazatlanensis* within *Cochemiea*. Three lectotypes are proposed.

***Cochemiea* (K.Brandegee) Walton. Cact. J. (London) 2: 50. 1899.**

***Cochemiea mazatlanensis* (K.Schum.) D.Aquino & Dan.Sanchez, comb. nov.**

urn:lsid:ipni.org:names:77248940-1

- ≡ *Mammillaria mazatlanensis* K.Schum., Monatsschr. Kakteenk. 11: 154. 1901. *Neomammillaria mazatlanensis* (K.Schum.) Britton & Rose, Cactaceae 4: 138. 1923. *Chilita mazatlanensis* (K.Schum.) Orcutt, Cactography 2. 1926. *Ebnerella mazatlanensis* (K.Schum.) Buxb., Oesterr. Bot. Z. 98: 89. 1951. *Escobariopsis mazatlanensis* (K.Schum.) Doweld, Sukkulenty 3: 40. 2000. Type: México, Sinaloa, Matzatlán [Mazatlán], W. Mundt s.n. (not preserved, lectotype, designated here, Monatsschr. Kakteenk. 15: 154. 1905: Illustration “*Ma[m]millaria mazatlanensis* K.Schum. Nach einer von Herrn Mundt für die “Monatsschrift für Kakteenkunde” hergestellten Photographie”). **Notes.** Both the original description of *Mammillaria* (= *Cochemiea*) *mazatlanensis* (Schumann 1901), and the later extension of the description by Gurke (1905) do not indicate that a type specimen has been preserved. Hunt (1985) confirms that a type specimen was not formally designated.
- = *Mammillaria littoralis* K.Brandegee, Bull. Misc. Inform. Kew 1908: App. 91. 1908. Type: Not designed.
- = *Neomammillaria occidentalis* Britton & Rose, Cactaceae 4: 161–162, f. 179. 1923. *Chilita occidentalis* (Britton & Rose) Orcutt, Cactography 2. 1926. *Mammillaria occidentalis* (Britton & Rose) Boed., Mammillarien-Vergleichs-Schlüssel: 36. 1933. *Ebnerella occidentalis* (Britton & Rose) Buxb., Oesterr. Bot. Z. 98: 90. 1951. *Mammillaria mazatlanensis* var. *occidentalis* (Britton & Rose) Neutel., Succulenta (Netherlands) 65: 119. 1986. Type: México, Colima, near Manzanillo, Dec 1890, E. Palmer 1053 (holotype: US [208544 image!]).
- = *Neomammillaria sinaloensis* Rose, Fl. Indig. Sinaloa Cact.: 3. 1929. Nom. Inval.
- = *Neomammillaria patonii* Bravo, Anales Inst. Biol. Univ. Nac. Mexico 2: 129. 1931. *Mammillaria patonii* (Bravo) Werderm., Backeberg, Neue Kakteen: 97. 1931. *Mammillaria occidentalis* var. *patonii* (Bravo) R.T.Craig, Mammill. Handb.: 169. 1945. *Mammillaria mazatlanensis* f. *patonii* (Bravo) Neutel., Succulenta

- (Netherlands) 65: 119. 1986. *Mammillaria mazatlanensis* subsp. *patonii* (Bravo) D.R.Hunt, *Mammillaria* Postscripts 7: 3. 1998. *Escobariopsis mazatlanensis* subsp. *patonii* (Bravo) Doweld, *Sukkulenty* 3: 41. 2000. Type: México, Sinaloa [Nayarit], Isla Tres Marias 1930, *Heilfurth s.n.* (MEXU).
- = *Mammillaria occidentalis* var. *sinalensis* R.T.Craig, *Mammill. Handb.*: 169. 1945. *Mammillaria patonii* var. *sinalensis* (R.T.Craig) Backeb., *Cactaceae* 5: 3291. 1961. *Mammillaria mazatlanensis* f. *sinalensis* (R.T.Craig) Neutel., *Succulenta* (Netherlands) 65: 119. 1986. Type: México, Sinaloa, Arroyo de Ibarra, near Rosario 1940, *E. Baxter s.n.* (lectotype, designated here, *Mammill. Handb.*: 169. 1945: Illustration “f. 151 *Mammillaria occidentalis* var. *sinalensis* X 1”). **Notes.** The protologue indicates that the type specimen was collected, however, it is not mentioned in which herbarium it was deposited. Some specimens collected by Craig (1945) were deposited in the UC herbarium, currently fused with the CAS herbarium. A search was made in the CAS database (<https://www.calacademy.org/scientists/botany-collections>) and it was not possible to locate the material, on the other hand, type specimens deposited in CAS from UC apparently were lost (Breslin et al. 2021).
- = *Mammillaria mazatlanensis* var. *monocentra* R.T.Craig, *Mammillaria Handb.*: 242, 1945. Type: México, Sonora, Yaqui Valley, in the lower delta of the Río Yaqui 1936, *J. Hilton & R. T. Craig s.n.* (lectotype, designated here, *Mammill. Handb.*: 242. 1945: Illustration “f. 219 *Mammillaria mazatlanensis* var. *monocentra* X 1”). **Notes.** See *Mammillaria occidentalis* var. *sinalensis*

Pelecyphora

Phylogenetic evidence supports the transference of *Escobaria* to *Pelecyphora* (see discussion) which results in 25 new combinations. Also, nine lectotypes, and three isolectotypes are proposed. Twenty species and 14 subspecies of *Pelecyphora*, are recognized.

Pelecyphora Ehrenb., *Bot. Zeitung* (Berlin) 1: 737. 1843.

- = *Cochiseia* W.Earle, *Saguaroland Bull.* 30: 65. 1976. Type: *Cochiseia robbinsorum* W.Earle.
- = *Encephalocarpus* A.Berger, *Kakteen* 331. 1929. Type: *Encephalocarpus strobiliformis* (Werderm.) A.Berger.
- = *Escocoryphantha* Doweld, *Sukkulenty* 1: 10. 1999. Type: *Escocoryphantha chihuahuensis* (Britton & Rose) Doweld.
- = *Escobaria* Britton & Rose, *Cactaceae* 4: 53. 1923. Type: *Escobaria tuberculosa* (Engelm.) Britton & Rose.
- = *Escobeseya* Hester, *Desert Pl. Life* 17: 23. 1945. Type: *Escobeseya dasyacantha* (Engelm.) Hester, *Desert Pl. Life* 17: 25. 1945.
- = *Fobea* Frič ex Boed., *Kakteenkunde* 1933: 155. 1933. Type: *Fobea viridiflora* Frič ex Boed.

- = *Lepidocoryphantha* Backeb., Blätt. Kakteenf. 1938: 22. 1938. Type: *Lepidocoryphantha macromeris* (Engelm.) Bakeb.
 = *Neobesseyia* Britton & Rose, Cactaceae 4: 51. 1923. Type: *Neobesseyia missouriensis* (Sweet) Britton & Rose.

Type. *Pelecyphora aselliformis* Ehrenb.

***Pelecyphora abdita* (Řepka & Vaško) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248941-1

- ≡ *Escobaria abdita* Řepka & Vaško, Cact. Succ. J. (Los Angeles) 83: 265. 2012. *Neobesseyia abdita* (Řepka & Vaško) Lodé, Cact.-Avent. Int. 98(Suppl.): 6. 2013. Type: México, Coahuila, basin east of the settlement El Oro, 1100 m, Oct 2011, *M. K. Hernández s.n.* (holotype: IZTA).

***Pelecyphora abdita* subsp. *tenuispina* (Pérez-Badillo, Delladdio & Raya-Sánchez) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248942-1

- ≡ *Escobaria abdita* Řepka & Vaško subsp. *tenuispina* Pérez-Badillo, Delladdio & Raya-Sánchez, Piante Grasse 36: 9. 2016. Type: México, Coahuila, Parras de la Fuente, *G. B. Hinton 29727* (holotype: GBH).

***Pelecyphora alversonii* (J.M.Coult.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248943-1

- ≡ *Cactus radiosus* var. *alversonii* J.M.Coult. Contr. U.S. Natl. Herb. 3: 122. 1894. *Mammillaria alversonii* (J.M.Coult.) Zeiss., Monatsschr. Kakteenk. 5: 70. 1895. *Mammillaria radiosa* var. *alversonii* (J.M.Coult.) K.Schum., Gesamtbeschr. Kakt.: 481. 1898. *Coryphantha alversonii* (J.M.Coult.) Orcutt, Cactography: 3. 1926. *Mammillaria vivipara* var. *alversonii* (J.M.Coult.) L.D.Benson, Cacti Ariz.: 118. 1950. *Coryphantha vivipara* var. *alversonii* (J.M.Coult.) L.D.Benson, Cacti Ariz. ed. 3: 26. 1969. *Escobaria vivipara* var. *alversonii* (J.M.Coult.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Escobaria alversonii* (J.M.Coult.) N.P.Taylor, Cactaceae Consensus Init. 3: 10. 1997. Type: United States, California, Mohave desert Calif., *A. H. Alverson s.n.* (lectotype, designated by Benson Cacti Ariz. 3 ed.: 200. 1969: UC [205017 image!]; isolecotype: F [260000 image!]). **Notes.** The isolecotype label also indicates the date of collection in 1892.

***Pelecyphora aselliformis* Ehrenb., Bot. Zeitung 1: 737. 1843.**

= *Ariocarpus aselliformis* (Ehrenb.) F.A.C. Weber, Dict. Hort. 2: 931. 1898. *Anhalonium aselliforme* (Ehrenb.) F.A.C. Weber, Dict. Hort. 2: 931. 1898. Type: México, San Luis Potosí, 18 miles [28.96 km] north of San Luis Potosí, 31 Jul 1959, *E. F. Anderson 1206* (neotype, designated by Anderson & Boke, Amer. J. Bot.: 325. 1969: POM [298106]).

***Pelecyphora chihuahuensis* (Britton & Rose) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248944-1

≡ *Escobaria chihuahuensis* Britton & Rose, Cactaceae 4: 55. 1923. *Coryphantha chihuahuensis* (Britton & Rose) A.Berger, Kakteen: 339. 1929. *Escocoryphantha chihuahuensis* (Britton & Rose) Doweld, Sukkulenty 2: 10. 1999. Type: México, Chihuahua, Vicinity of Chihuahua, 08 Apr 1908, *E. Palmer 72* (holotype: US [573550]; isotype: K [250731 image!]).

= *Mammillaria strobiliformis* Scheer ex Salm-Dyck, Cact. Hort. Dyck. 104–105. 1850, not *Mammillaria strobiliformis* Engelm., Mem. Tour N. Mexico: 113. 1848, not *Mammillaria strobiliformis* Muehlenpf., Allg. Gartenzeitung 16: 19. 1848. *Echinocactus strobiliformis* Poselg., Allg. Gartenzeitung 21: 107. 1853. *Cactus strobiliformis* (Sheer) Kuntze, Revis. Gen. Pl. 1: 261. 1891. *Escobaria strobiliformis* (Poselg.) F.Boedeker, Mammillarien-Vergleichs-Schlüssel 16. 1933. *Coryphantha strobiliformis* (Poselg.) Moran, Gentes Herbarium 8: 318. 1953. Type: [Fragments from] Potts's original specimen, cult. 1857, hort., Jan 1857, *J.M.E.A.H.I.Salm-Reifferscheid-Dyck s.n.* (lectotype, designated by Benson, Cact. Succ. J. (Los Angeles): 189. 1969: MO). **Notes.** Britton and Rose (1923) chose Engelmann's epithet *tuberculosa* over *strobiliformis*, because the last represents a homonym. However, Benson (1969) suggested that the epithet *strobiliformis* should be preferred over the epithet *tuberculosa*. Zimmerman and Parfitt (1993+) mention that *Escobaria tuberculosa* and *E. strobiliformis* represent two independent entities and the name *E. chihuahuensis* Britton & Rose should be considered a synonym of *E. strobiliformis*. Given the difference in opinions, Hunt et al. (2006) explained that the name *Escobaria strobiliformis* has been incorrectly applied to *E. tuberculosa* and should be rejected. Hunt (2016) concludes that *E. strobiliformis* is an inadmissible name or with indeterminate application. In order to maintain the stability of the names listed in this treatment, the name *Mammillaria strobiliformis* is considered a homonym and should not be applied (Turland et al. 2018). In turn, this decision makes it possible to retain the epithet *strobiliformis* for the name *Pelecyphora strobiliformis* (Werderm.) Fric. & Schelle (basonym *Ariocarpus strobiliformis* Werderm.). Finally, original description is not complete and lacks data on floral characters, so it is not feasible to decide on the correct interpretation.

- = *Mammillaria strobiliformis* var. *caespititia* Quehl, Monatsschr. Kakteenk. 19: 173. 1909. *Mammillaria strobiliformis* f. *caespititia* (Quehl) Schelle, Kakteen: 285. 1926. *Escobaria tuberculosa* var. *caespititia* (Quehl) Borg, Cacti 304. 1937. Type: Probably Mexico. (Not preserved).

***Pelecyphora chihuahuensis* subsp. *henricksonii* (Glass & R.A.Foster) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248945-1

- ≡ *Escobaria henricksonii* Glass & R.A.Foster, Cact. Succ. J. (Los Angeles) 49: 195. 1977. *Coryphantha henricksonii* (Glass & R.A.Foster) Glass & R.A.Foster, Cact. Succ. J. (Los Angeles) 51: 125. 1979. *Escobaria chihuahuensis* subsp. *henricksonii* (Glass & R.A.Foster) N.P.Taylor, Cactaceae Consensus Init. 5: 13. 1998. *Escocoryphantha henricksonii* (Glass & R.A.Foster) Doweld, Sukkulenty 2: 10. 1999. Type: México, Chihuahua, c. 16 mi. [25.74 km] E of Escalón, Sep 1972, J. S. Henrickson 7744 (holotype: POM [325439 image, two sheets!]).

***Pelecyphora cubensis* (Britton & Rose) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248946-1

- ≡ *Coryphantha cubensis* Britton & Rose, Torreya 12: 15. 1912. *Neobesseyia cubensis* (Britton & Rose) Hester, Desert Pl. Life 13: 192. 1941. *Escobaria cubensis* (Britton & Rose) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: Cuba, Holguín, Oriente, 1909, J. A. Shafer 2946 (lectotype, designated here: NY [120678 image!]; islectotype: US [1821121 image!]). **Notes.** According to Britton and Rose (1912), the original specimen of *Coryphantha cubensis* was kept in cultivation at the New York Botanical Garden. A specimen deposited in NY (120678!) whose data on the label coincide with those referred to in the protologue. Elements such as collector and number (J. A. Shafer 2946) and date of collection (1909) coincide with the label of the specimen referred to here, which is why we designate it as lectotype, while the specimen deposited in the US herbarium (1821121 image!) corresponds to the islectotype.

***Pelecyphora dasyacantha* (Engelm.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248947-1

- ≡ *Mammillaria dasyacantha* Engelm., Proc. Amer. Acad. Arts 3: 268. 1856. *Cactus dasyacanthus* (Engelm.) Kuntze, Revis. Gen. Pl. 1: 259. 1891. *Coryphantha dasyacantha* (Engelm.) Orcutt, Circular to Cactus Fanciers: 1. 1922. *Escobaria dasyacantha* (Engelm.) Britton & Rose, Cactaceae 4: 55. 1923. *Escobesseyia dasyacantha*

(Engelm.) Hester, Desert Pl. Life 17: 25. 1945. *Neobesseyia dasyacantha* (Engelm.) Lodé, Cact.-Avent. Int. 98(Suppl.): 6. 2013. Type: United States, Texas, El Paso, 1852, *C. Wright* s.n. (lectotype, designated by Benson, Cacti U. S. Canada: 964. 1982: MO [106919 image!]).

- = *Mammillaria chlorantha* Engelm., Rep. U.S. Geogr. Surv., Wheeler 6: 127. 1878. *Cactus radiosus* var. *chloranthus* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 121. 1894. *Mammillaria radiosa* f. *chlorantha* (Engelm.) Schelle, Handb. Kakteenkult.: 235. 1907. *Coryphantha chlorantha* (Engelm.) Britton & Rose, Cactaceae 4: 43. 1923. *Mammillaria vivipara* var. *chlorantha* (Engelm.) L.D.Benson, Cacti Ariz., ed. 2 117. 1950. *Escobaria chlorantha* (Engelm.) Buxb., Oesterr. Bot. Z. 98: 78. 1951. Type. United States, St George, May 1874, *C. C. Parry* s.n. (lectotype, designated by Benson, Cacti U. S. Canada: 961. 1982: MO).

***Pelecyphora dasyacantha* subsp. *chaffeyi* (Britton & Rose) D.Aquino & Dan. Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248948-1

- ≡ *Escobaria chaffeyi* Britton & Rose, Cactaceae 4: 56. 1923. *Coryphantha chaffeyi* (Britton & Rose) Fosberg, Bull. S. Calif. Acad. Sci. 30: 58. 1931. *Mammillaria chaffeyi* (Britton & Rose) Backeb., Neue Kakteen: 16. 1931. *Escobaria dasyacantha* var. *chaffeyi* (Britton & Rose) N.P.Taylor, Kakteen And. Sukk. 34: 157. 1983. *Escobaria dasyacantha* subsp. *chaffeyi* (Britton & Rose) N.P.Taylor, Cactaceae Consensus Init. 5: 13. 1998. *Neobesseyia dasyacantha* subsp. *chaffeyi* (Britton & Rose) Lodé, Cact.-Avent. Int. 98(Suppl.): 6. 2013. Type. México, Zacatecas, near Cedros, Jun 1910, *E. Chaffey* 5 (lectotype, designated here: US [1821124 image!]; isolectotype: NY [image 271944!]). **Notes.** A label attached to the specimen deposited in the US (1821124!) indicates that this specimen was designated as a lectotype. However, the typification was not published, so it cannot be accepted (Turland et al. 2018). The sample is part of the original collection since it was collected in the type locality by E. Chaffey, coinciding with the data stipulated in the protologue. Therefore, we formalize the typification of the US specimen.

- = *Escobaria fobei* Frič ex A.Berger, Kakteen: 280. 1929. Type. not designated.
- = *Fobea viridiflora* Frič ex Boed., Kakteenkunde 1933: 155. 1933. *Escobaria chaffeyi* f. *viridiflora* (Frič) Řiha, Kaktusy (Brno) 22: 25. 1986. Type: not designated.

***Pelecyphora duncanii* (Hester) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248949-1

- ≡ *Escobesseyia duncanii* Hester, Desert Pl. Life 13: 192. 1941. *Escobaria duncanii* (Hester) Buxb. Kakteen, Lief. 14, 108c. 1960. *Escobaria duncanii* (Hester) Backeb., Cactaceae 5: 2966. 1961. *Coryphantha duncanii* (Hester) L.D.Benson,

Cact. Succ. J. (Los Angeles) 41: 189. 1969. *Escobaria dasyacantha* var. *duncanii* (Hester) N.P.Taylor, Kakteen And. Sukk. 34: 157. 1983. *Neobesseyia duncanii* (Hester) Lodé, Cact.-Avent. Int. 98(Suppl.): 6. 2013. Type: United States, Texas, Brewster County, in the Edwards limestone of mountains a few mi NW of Terlingua in the Edwards limestone, 3400 ft [1036 m], 1937, *F. Duncan* s.n. (holotype: DS [271944]).

***Pelecyphora emskoetteriana* (Quehl) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248950-1

- ≡ *Mammillaria emskoetteriana* Quehl, Monatsschr. Kakteenk. 20: 139. 1910. *Coryphantha emskoetteriana* (Quehl) A.Berger, Kakteen: 339. 1929. *Escobaria emskoetteriana* (Quehl) Borg, Cacti: 304. 1937. *Neobesseyia emskoetteriana* (Quehl) Lodé, Cact.-Avent. Int. 98(Suppl.): 6. 2013. Type: México, San Luis Potosí, raised in Germany from seed received from San Luis Potosí, *R. Emsköttter* s. n. (lectotype, designated here: US [2767373 image! = Monatsschr. Kakteenk.: 139. 1910. Illustration “Mamillaria Emsköttteriana Quehl. Nach einer von Herrn Emil Weddy in Halle aufgenommenen Photographie]). **Notes.** The photograph published in the protologue is considered part of the original material and is designated here as a lectotype (Quehl 1910). A specimen deposited in the US herbarium (2767373!) consists of a duplicate of the original photograph mounted on the sheet. The label indicates it was designated as a lectotype by A. Zimmerman, but it was not published. Here, we formalize this proposal.
- = *Escobaria bella* Britton & Rose, Cactaceae 4: 56. 1923. *Coryphantha bella* (Britton & Rose) Fosberg, Bull. S. Calif. Acad. Sci. 30: 58. 1931. Type. United States, Texas, on hills of Devil’s River, 16 Oct 1913, *J. N. Rose & W. Ficht 17991* (lectotype, designated by Benson, Cacti U. S. Canada: 963. 1982: US [1821125 image!]).
- = *Escobaria runyonii* Britton & Rose, Cactaceae 4: 55. 1923. *Mammillaria escobaria* Cory, Rhodora 38: 405. 1936. nom. nov. Type. United States, Texas, Rio Grande city, 10 Aug 1921, *R. Runyon* s.n. (lectotype, designated by Benson, Cacti U. S. Canada: 964. 1982: US [not numbered]).
- = *Coryphantha roberti* A.Berger, Kakteen: 280. 1929. Type. United States, Texas, vom Río Grande, Type: not preserved.
- = *Coryphantha muehlbaueriana* Boed., Monatsschr. Deutsch. Kakteen-Ges. 2: 18. 1930. *Escobaria muehlbaueriana* (Boed.) F.M.Knuth, Kaktus-ABC: 380. 1936. *Neobesseyia muehlbaueriana* (Boed.) Boed., Mammill.-Vergl.-Schluessel: 15. 1933. Type. México, Tamaulipas, bei Jaumave, 1929, *F. Viereck* s.n. (lectotype, designated here, Monatsschr. Deutsch. Kakteen-Ges.: 18. 1930a: Illustration “*Coryphantha muehlbaueriana* Boed. sp. nov. Nat. Gr.”).

***Pelecyphora hesteri* (Y.Wright) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248951-1

- ≡ *Coryphantha hesteri* Y.Wright, Cact. Succ. J. (Los Angeles) 4: 274. 1932. *Escobaria hesteri* (Y.Wright) Buxb., Oesterr. Bot. Z. 98: 78. 1951. Type: United States, Hill on U.S. 385, 3.5 miles south of U.S. 90 east of Marathon. South side of gap and road cut. Crest of hill. Drainage Area Rio Grande, 06 Apr 1965, *L. D. Benson & B. H. Warnock*, 16500 (neotype, designated by Benson, Cacti U. S. Canada: 961. 1982: POM [315706 image!]).

***Pelecyphora hesteri* subsp. *grata* (Kaplan, Kunte & Snicer) D.Aquino & Dan. Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248952-1

- ≡ *Escobaria grata* Kaplan, Kunte & Snicer, Kaktusy (Brno) 37: 37. 2001. *Escobaria hesteri* subsp. *grata* (Kaplan, Kunte & Snicer) Lüthy & Dicht, Cact. World 25: 175. 2007. Type: México, Coahuila, collibus calcareis montibus Sierra el Burro ca 150 km situ septentrio-occidentali ab oppido Monclova, *J. Snicer et al. s.n.* (holotype: PR).

***Pelecyphora laredoi* (Glass & R.A.Foster) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248953-1

- ≡ *Coryphantha laredoi* Glass & R.A.Foster, Cact. Succ. J. (Los Angeles) 50: 235. 1978. *Escobaria laredoi* (Glass & R.A.Foster) N.P.Taylor, Cact. Succ. J. Gr. Brit. 41: 20. 1979. Type: México, Coahuila, SE Coahuila, about 2 km N of El Cinco, SE of General Cepeda, near top of a mountain pass, Sierra de Parras, Feb 1972, *C. Glass & R. Foster* 3761 (holotype: POM; isotype: ASU [0018460 image!]).

***Pelecyphora lloydii* (Britton & Rose) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248954-1

- ≡ *Escobaria lloydii* Britton & Rose, Cactaceae 4: 57. 1923. *Coryphantha lloydii* (Britton & Rose) Fosberg, Bull. S. Calif. Acad. Sci. 30: 58. 1931. *Neobesseya lloydii* (Britton & Rose) Lodé, Cact.-Avent. Int. 98(Suppl.): 7. 2013. Type: México, Zacatecas, Foothills of Sra. Zuluago [Sierra de Zuloaga], 29 Mar 1908, *F.E. Lloyd* 5 (holotype: US [535108 image!]).

***Pelecyphora macromeris* (Engelm.) D. Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248955-1

- ≡ *Mammillaria macromeris* Engelm. Mem. Tour N. Mexico [Wislizenus] 97. 1848. *Echinocactus macromeris* (Engelm.) Poselg. Allg. Gartenzeitung 21: 102. 1853. *Coryphantha macromeris* (Engelm.) Lem., Cactées 35. 1868. *Lepidocoryphantha macromeris* (Engelm.) Backeb. Cactaceae (Berlin) 1941: 61. 1942. Type: United States, New México, sandy soil near Doñana [Dona Ana], 08 May 1846, *A. Wislizenius s.n.* (lectotype, designated by Benson, Cacti U. S. Canada: 959. 1982: MO [2017406 image!, 2017407 image!, two sheets]).
- = *Mammillaria dactylothele* Labour., Monogr. Cact.: 146. 1853. Type. Not designated.

***Pelecyphora macromeris* subsp. *runyonii* (Britton & Rose) D.Aquino & Dan. Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248956-1

- ≡ *Coryphantha runyonii* Britton & Rose, Cactaceae (Britton & Rose) 4: 26. 1923. *Lepidocoryphantha runyonii* (Britton & Rose) Backeb., Cactaceae (Backeberg) 5: 2975. 1961. *Coryphantha macromeris* var. *runyonii* (Britton & Rose) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 188. 1969. *Coryphantha macromeris* subsp. *runyonii* (Britton & Rose) N.P.Taylor, Cactaceae Consensus Init. 6: 15. 1998. *Lepidocoryphantha macromeris* subsp. *runyonii* (Britton & Rose) Doweld, Sukkulenty 2: 28. 1999. Type: United States, Texas, to Rio Grande [City], 10 Aug 1921, *R. Runyon 15* (lectotype, designated by Benson, Cact. Succ. J. (Los Angeles): 188. 1969: US [2761309 image!]).
- = *Coryphantha pirtlei* Werderm. Notizbl. Bot. Gart. Berlin-Dahlem 12: 226. 1934. Type: United States, Texas, Starr County, 1931. *W. A. Pirtle s.n.* **Notes.** Benson (1982) indicates that the material type of *Coryphantha pirtlei* was preserved. However, there is no certainty about the herbarium where it was deposited.

***Pelecyphora minima* (Baird) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248957-1

- ≡ *Coryphantha minima* Baird, Amer. Bot. (Binghamton) 37: 150. 1931. *Escobaria minima* (Baird) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 30. 1978. *Neobesseyia minima* (Baird) Lodé, Cact.-Avent. Int. 98(Suppl.): 7. 2013. Type: United States, Texas, near Marathon, Mar 1931, *A. R. Davis s.n* (lectotype, designated by Benson, Cacti U. S. Canada: 959. 1982: US [1530466 image!]).
- = *Coryphantha nellieae* Croizat, Torreya 34: 15. 1934. *Escobaria nellieae* (Croizat) Backeb., Cactaceae 5: 2967. 1961. *Mammillaria nellieae* (Croizat) Croizat, Cact. Succ. J. (Los Angeles) 14: 34. 1942. Type. United States, Texas, about 4 miles south of Marathon, in limestone formations, *Davis s.n.* (holotype: NY).

***Pelecyphora missouriensis* (Sweet) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248958-1

- ≡ *Mammillaria missouriensis* Sweet, Hort. Brit.: 171. 1826. *Cactus missouriensis* (Sweet) Kuntze, Revis. Gen. Pl. 1: 259. 1891. *Mammillaria missouriensis* Sweet ex K.Schum., Gesamtbeschr. Kakt.: 498. 1898. *Coryphantha missouriensis* (Sweet) Britton & Rose, Ill. Fl. N. U.S. 2: 570. 1913. *Neobesseyia missouriensis* (Sweet) Britton & Rose, Cactaceae 4: 53. 1923. *Neomammillaria missouriensis* (Sweet) Britton & Rose ex Rydb., Fl. Plains N. Amer. 561. 1932. *Escobaria missouriensis* (Sweet) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, North Dakota, Burleigh County, 3 mi [4.8 km] W of Baldwin turnoff, 1.3 m [2.09 km] E of Missouri River, Jun 1970, *L. Mitich s.n.* (neotype, designated by Mitich & Benson, Cact. Succ. J. (Los Angeles): 8. 1977: POM [317949]; isoneotype NDA).
- = *Mammillaria similis* Engelm. & A.Gray, Boston J. Nat. Hist. 5: 246. 1845. *Echinocactus similis* (Engelm.) Poselg., Allg. Gartenzeitung 21: 107. 1853. *Cactus missouriensis* var. *similis* (Engelm.) J.M.Coult. in Contr. U.S. Natl. Herb. 3: 111. 1894. *Cactus similis* (Engelm.) Small, Fl. S.E. U.S.: 812. 1903. *Coryphantha similis* (Engelm.) Britton & Rose, Ill. Fl. N. U.S.: 571. 1913. *Neobesseyia similis* (Engelm.) Britton & Rose, Cactaceae 4: 52. 1923. *Escobaria missouriensis* var. *similis* (Engelm.) N.P.Taylor, Kakteen And. Sukk. 34: 184. 1983. *Neobesseyia missouriensis* subsp. *similis* (Engelm.) Doweld, Sukkulenty 3: 37. 2000. Type: United States, Sandstone rocks, near Industry (not preserved).
- = *Mammillaria nuttallii* Engelm., Mem. Amer. Acad. Arts n.s., 4: 49. 1849. *Coryphantha nuttallii* Engelm. ex C.F.Först., Handb. Cacteenk.: 407. 1885. *Mammillaria missouriensis* var. *nuttallii* (Engelm.) Schelle, Handb. Kakteenkult.: 241. 1907. *Neobesseyia nuttallii* Boed., Mammill.-Vergl.-Schluessel: 15. 1933. *Neobesseyia nuttallii* (Engelm.) Borg, Cacti: 303. 1937. Type: United States, South Dakota, Ft. Pierre, on the Upper Missouri, 1847, *F. V. Hayden s.n.* (lectotype, designated by Benson, Cacti U. S. Canada: 964. 1982: MO [899104 image!, 899105 image!, two sheets]).
- = *Mammillaria similis* var. *robustior* Engelm., Boston J. Nat. Hist. 6: 200. 1850. *Mammillaria nuttallii* var. *robustior* (Engelm.) Engelm. & J.M.Bigelow, Pacif. Railr. Rep. 4: 28. 1856. *Mammillaria missouriensis* var. *robustior* (Engelm.) S.Watson, Bibl. Index N. Amer. Bot.: 440. 1878. *Cactus missouriensis* var. *robustior* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 111. 1894. *Coryphantha missouriensis* var. *robustior* (Engelm.) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 190. 1969. *Escobaria missouriensis* var. *robustior* (Engelm.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, Texas, Piedernales [Perdenales] [River, Texas], May 1846, *F. Lindheimer s.n.* (lectotype, designated by Benson, Cact. Succ. J. (Los Angeles): 190. 1969: MO [2017430 image!]).
- = *Mammillaria similis* var. *caespitosa* Engelm., Boston J. Nat. Hist. 6: 200. 1850. *Mammillaria nuttallii* var. *caespitosa* Engelm., Proc. Amer. Acad. Arts 3: 265. 1856. *Mammillaria missouriensis* var. *caespitosa* (Engelm.) S.Watson, Smithsonian Misc.

- Collect. 258: 403. 1878. *Mammillaria wissemani* Hildm. ex K.Schum., Gesamtbeschr. Kakt.: 498. 1898. nom. nov. *Neobesseyia wissemani* (Hildm. ex K.Schum.) Britton & Rose, Cactaceae 4: 52. 1923. *Coryphantha wissemani* (Hildm. ex K.Schum.) A.Berger, Kakteen: 278. 1929. *Coryphantha missouriensis* var. *caespitosa* (Engelm.) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 189. 1969. *Escobaria missouriensis* var. *caespitosa* (Engelm.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, Cult. In hort. Göbels, St Louis from Texas near Industry, May 1846, *F. Lindheimer s.n.* (lectotype, designated by Benson, Cact. Succ. J. (Los Angeles): 190. 1969: MO).
- = *Mammillaria nuttallii* var. *borealis* Engelm., Proc. Amer. Acad. Arts 3: 264. 1856. Type: United States, on the Upper Missouri. Not preserved.
- = *Mammillaria notesteinii* Britton, Bull. Torrey Bot. Club 18: 367. 1891. *Cactus notesteinii* (Britton) Rydb., Mem. New York Bot. Gard. 1: 272. 1900. *Neobesseyia notesteinii* (Britton) Britton & Rose, Cactaceae 4: 53. 1923. Type: United States, Deer Lodge, Mont., 01 Jun 1891, *F. N. Notestein s.n.* (lectotype, designated here: NY [385874 image, four sheets!]; isolectotype: US [1821122 image!]).
- = *Coryphantha marstonii* Clover, Bull. Torrey Bot. Club 65: 412. 1938. *Coryphantha missouriensis* var. *marstonii* (Clover) L.D.Benson, Cacti Ariz. ed. 3: 26. 1969. *Escobaria missouriensis* var. *marstonii* (Clover) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type. United States, Utah, Kane County, east side of Buckskin Mountains, 5200 ft [1584 m], 08 Aug 1953, *L. D. Benson & R. Benson 15205* (neotype, designated by Benson, Cacti Ariz. ed. 3: 26. 1969: POM [285320, 296309, two sheets]).
- = *Escobaria missouriensis* subsp. *navajoensis* Hochstätter, Succulenta (Netherlands) 75: 257. 1996. Type. United States, Arizona, Navajoa, 1600–1800, *F. Hochstätter 1000* (holotype: HBG).

***Pelecyphora missouriensis* subsp. *asperispina* (Boed.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248959-1

- ≡ *Coryphantha asperispina* Boed., Monatsschr. Deutsch. Kakteen-Ges. 1: 192. 1929. *Neobesseyia asperispina* (Boed.) Boed., Mammill.-Vergl.-Schlüssel: 14. 1933. *Neobesseyia asperispina* (Boed.) Boed. ex Backeb. & F.M.Knuth, Kaktus-ABC: 379. 1936. *Escobaria asperispina* (Boed.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Escobaria missouriensis* var. *asperispina* (Boed.) N.P.Taylor, Kakteen And. Sukk. 34: 185. 1983. *Escobaria missouriensis* subsp. *asperispina* (Boed.) N.P.Taylor, Cactaceae Consensus Init. 5: 13. 1998. *Neobesseyia missouriensis* subsp. *asperispina* (Boed.) Lodé, Cact.-Avent. Int. 100: 30. 2013. Type: Mexico, Coahuila, südlich von Saltillo, und dort in grasigen, 2500 m, *F. Ritter s.n.* (lectotype, designated

here, Monatsschr. Deutsch. Kakteen-Ges.: 192. 1929: Illustration “*Coryphantha asperispina* Boed. sp. nov. natür. Größe”).

***Pelecyphora robbinsorum* (W.H.Earle) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248960-1

- ≡ *Cochiseia robbinsorum* W.H.Earle, Saguaroland Bull. 30: 65. 1976. *Coryphantha robbinsorum* (W.H.Earle) A.D.Zimmerman, Cact. Succ. J. (Los Angeles) 50: 294. 1978. *Escobaria robbinsorum* (W.H.Earle) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Neobesseyia robbinsorum* (W.H.Earle) Doweld, Sukkulenty 3: 37. 2000. Type: United States, Arizona, SE Cochise County, rocky hills, 4250 ft [1280 m], 1976, *J. Robbins et al. s.n.* (holotype: ASU [18455]).

***Pelecyphora sneedii* (Britton & Rose) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248961-1

- ≡ *Escobaria sneedii* Britton & Rose, Cactaceae 4: 56. 1923. *Coryphantha sneedii* (Britton & Rose) A.Berger, Kakteen: 280. 1929. *Mammillaria sneedii* (Britton & Rose) Cory, Rhodora 38: 407. 1936. Type: United States, Texas, 8 km N of El Paso, McKelligan Canyon, Mt. Franklin., 22 Feb 1921, *S. L. Pattinson s.n.* (lectotype, designated by Benson, Cacti U. S. Canada: 963. 1969: US [image 2767376!]).
- = *Escobaria albicolumnaria* Hester, Desert Pl. Life 13: 129. 1941. *Coryphantha albicolumnaria* (Hester) Zimmerman, Cact. Succ. J. (Los Angeles) 44: 157. 1972. *Escobaria sneedii* subsp. *albicolumnaria* (Hester) Lüthy, Kakteen And. Sukk. 50: 278. 1999. *Coryphantha sneedii* var. *albicolumnaria* (Hester) A.D.Zimmerman, Cacti Trans-Pecos: 424. 2004. Type: United States, Texas, mountainous limestone area W.N.W. of Terlingua and N.E. of Lajitas, in the southern part of Brewster County, 01 Apr 1940, *J. P. Hester s.n.* (holotype: DS [271855 image!, two sheets]).
- = *Escobaria guadalupensis* S.Brack & K.D.Heil, Cact. Succ. J. (Los Angeles) 58: 165. 1986. *Coryphantha sneedii* var. *guadalupensis* (S.Brack & K.D.Heil) A.D.Zimmerman, Cacti Trans-Pecos: 420. 2004. Type: United States, Texas, Culberson County, Guadalupe Mountains National Park, 2000–2600 m, *Heil et al. s.n.* (holotype: SJNM).
- = *Escobaria leei* Rose ex Boed., Mammillarien-Vergleichs-Schlüssel: 17. 1933. *Coryphantha sneedii* var. *leei* (Rose) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 189. 1969. *Escobaria sneedii* var. *leei* (Rose ex Boed.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 30. 1978. *Escobaria sneedii* subsp. *leei* (Rose ex Boed.) D.R.Hunt, Cactaceae Consensus Init. 4: 5. 1997. Type: United States, New México, Rattlesnake Canyon, 30 mi SW of Carlsbad, 5500 ft [1676 m] 1924, *W.T. Lee s.n.* (lectotype, designated by Castteter & Pierce, Madroño: 138. 1966: US [72134 image!]).

***Pelecyphora sneedii* subsp. *orcuttii* (Boed.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248962-1

- ≡ *Escobaria orcuttii* Boed., Mammillarien-Vergleichs-Schlüssel: 17. 1933. *Escobaria sneedii* subsp. *orcuttii* (Boed.) Lüthy, Kakteen And. Sukk. 50: 278. 1999. *Coryphantha sneedii* var. *orcuttii* (Boed.) Gorelick, J. Bot. Res. Inst. Texas 9: 28. 2015. *Escobaria sneedii* var. *orcuttii* (Boed.) Gorelick, J. Bot. Res. Inst. Texas 9: 28. 2015. Type: United States, New Mexico, Granite Pass, Mar 1926, C. R. Orcutt s.n. (lectotype, designated by Benson, Cacti Ariz. ed. 3: 26. 1969: DS [307410 image!]).
- = *Escobaria orcuttii* Rose ex Orcutt, Cactography 5, 1926. Nom. inval. *Coryphantha orcuttii* (Rose ex Orcutt) Zimmerman, Cact. Succ. J. (Los Angeles) 44: 156. 1972. Nom. inval. *Coryphantha strobiliformis* var. *orcuttii* (Rose ex Orcutt) L.D.Benson, Cacti Ariz. ed. 3: 156. 1972. nom. inval.
- = *Escobaria orcuttii* var. *koenigii* Castetter, P.Pierce & K.H.Schwer., Cact. Succ. J. (Los Angeles) 47: 68. 1975. Type: United States, New México, Luna County, Florida Mts., Central Valley on east slope of the Koenig Ranch on black limestone, E & NE slopes of hill (el.5200 [152.4 m]) which is 500 ft [152.4 m] above plains, 5200 ft [1584 m] 07 May 1962, E. F. Castetter 961 (holotype: UNM [38768 image!]).
- = *Escobaria orcuttii* var. *macraxina* Castetter, P.Pierce & K.H.Schwer., Cact. Succ. J. (Los Angeles) 47: 66. 1975. Type: United States, New México, Hidalgo County, Big Hatchet Mountains, west slope, 21 Dec 1973, K. D. Heil 4287 (holotype: UNM [54141 image!]; isotypes: UNM [54138 image!, 54142 image!, 54143 image!]).
- = *Coryphantha organensis* Zimmerman, Cact. Succ. J. (Los Angeles) 44: 114. 1972. *Escobaria organensis* (Zimmerman) Castetter, P.Pierce & K.H.Schwer., Cact. Succ. J. (Los Angeles) 47: 60. 1975. *Escobaria sneedii* subsp. *organensis* (Zimmerman) Lüthy, Kakteen And. Sukk. 50: 278. 1999. Type: United States, New México, Dona Ana County, c. 15 mi E of Las Cruces, Organ Mountains, 17 Jan 1971, D. A. Zimmerman & A. D. Zimmerman, 1535 (holotype: SNM; isotype: DS [642362 image!], MICH [1123478 image!]).
- = *Escobaria sandbergii* Castetter, P.Pierce & K.H.Schwer., Cact. Succ. J. (Los Angeles) 47: 62. 1975. *Escobaria sneedii* subsp. *sandbergii* (Castetter, P.Pierce & K.H.Schwer.) Lüthy, Kakteen And. Sukk. 50: 278. 1999. Type: United States, New México, Sierra County, at Rope Springs, west slope of the San Andres Mts., 01 Apr 1967, P. Pierce 3409 (holotype: UNM [38739 image!]).
- = *Escobaria villardii* Castetter, P.Pierce & K.H.Schwer., Cact. Succ. J. (Los Angeles) 47: 64. 1975. *Escobaria sneedii* subsp. *villardii* (Castetter, P.Pierce & K.H.Schwer.) Lüthy, Kakteen And. Sukk. 50: 278. 1999. Type: United States, New México, Otero County, Alamo Canyon, near Alamagordo, 18 Mar 1972, R. Reeves 3984 (holotype: UNM [50789 image!]).

***Pelecyphora strobiliformis* (Werderm.) Fric. & Schelle, Verzeichniss 9, 1935.**

- ≡ *Ariocarpus strobiliformis* Werderm., Z. Sukkulentenk. 3: 126. 1927. *Encephalocarpus strobiliformis* (Werderm.) A.Berger, Kakteen: 332. 1929. Type: México, Tamaulipas, near Miquihuana, 22 Jan 1961 (neotype, designated by Anderson & Boke, Amer. J. Bot.: 325. 1969: POM [298105]).

***Pelecyphora tuberculosa* (Engelm.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248963-1

- ≡ *Mammillaria tuberculosa* Engelm., Proc. Amer. Acad. Arts 3: 268. 1856. *Coryphantha tuberculosa* (Engelm.) Orcutt, Circular to Cactus Fanciers: i. 1922. *Escobaria tuberculosa* (Engelm.) Britton & Rose, Cactaceae 4: 54. 1923. *Coryphantha tuberculosa* (Engelm.) A.Berger, Kakteen: 280. 1929. Type: [México], Flounce mountains below El Paso, Below San Elisario on the Río Grande, Jun 1852, *J. Bigelow s.n.* (lectotype, designated by Benson, Cacti U. S. Canada: 962. 1982: MO [2017442 image!]).
- = *Mammillaria tuberculata* Engelm., Syn. Cact. U.S.: 12. 1856. *Cactus tuberculosus* (Engelm.) Kuntze, Revis. Gen. Pl. 1: 261. 1891. **Notes.** When comparing the original description *Mammillaria tuberculata* (<https://www.biodiversitylibrary.org/page/32558530#page/12/mode/1up>), it clearly corresponds a duplicate of the description of *M. tuberculosa* Engelm. Therefore, in the absense of diagnosis and designation of a type, it should be considered as *nomen nudum* (Turland et al. 2018).
- = *Mammillaria strobiliformis* var. *rufispina* Quehl, Monatsschr. Kakteenk. 17: 87. 1907. *Mammillaria strobiliformis* f. *rufispina* (Quehl) Schelle, Kakteen: 285. 1926. Type: Mexico (Not preserved).
- = *Mammillaria strobiliformis* var. *pubescens* Quehl, Monatsschr. Kakteenk. 17: 87. 1907. *Mammillaria strobiliformis* f. *pubescens* (Quehl) Schelle, Kakteen: 285. 1926. *Escobaria tuberculosa* var. *pubescens* (Quehl) Y.Itô, Cacti 1952: 113. 1952. Type: Mexico (Not preserved).
- = *Mammillaria strobiliformis* var. *durispina* Quehl, Monatsschr. Kakteenk. 17: 87. 1907. *Mammillaria strobiliformis* f. *durispina* (Quehl) Schelle, Kakteen: 285. 1926. *Escobaria tuberculosa* var. *durispina* (Quehl) Børgesen, Borg, J., Cacti 304. 1937. *Coryphantha strobiliformis* var. *durispina* (Quehl) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 189. 1969. *Escobaria strobiliformis* var. *durispina* (Quehl) Bravo, Cact. Suc. Mex. 27: 17. 1982. Type. United States, Texas, Brewster County, Terlingua H. Kuenzler s.n. (neotype, designated by Benson, Cact. Succ. J. (Los Angeles): 189. 1969: POM [311333 image!]).
- = *Coryphantha varicolor* Tiegel, Monatsschr. Deutsch. Kakteen-Ges. 3: 278. 1932. *Coryphantha dasyacantha* var. *varicolor* (Tiegel) L.D.Benson, Cact. Succ. J. (Los Angeles) 41: 189. 1969. *Escobaria dasyacantha* var. *varicolor* (Tiegel) D.R.Hunt,

- Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Escobaria tuberculosa* var. *varicolor* (Tiegel) S.Brack & K.D.Heil, Cact. Succ. J. (Los Angeles) 60: 17. 1988. *Escobaria tuberculosa* subsp. *varicolor* (Tiegel) Lüthy, Kakteen And. Sukk. 50: 257. 1999. *Coryphantha tuberculosa* var. *varicolor* (Tiegel) A.D.Zimmerman, Cacti Trans-Pecos: 436. 2004. Type. United States, Texas, Brewster County, hills south of Marathon, 3800 ft [1158 m], 03 Apr 1947, B. H. Warnock 47–467 (neotype, designated by Benson, Cact. Succ. J. (Los Angeles): 189. 1969: SRSC).
- = *Escobaria strobiliformis* subsp. *sisperai* Halda & Sladk. Acta Mus. Richnov. Sect. Nat. 7: 35. 2000. Type: México, Nuevo León, via bitumine constrata inter-Monterrey et Tampico, non procul a via publica prope compitum Marin, 07 Apr 1985, J.J. Halda & J. Sladkovský 85040073 (holotype PR).

***Pelecyphora vivipara* (Nutt.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248964-1

- ≡ *Cactus viviparus* Nutt. Nutt., Cat. Pl. Upper Louisiana no. 22. 1813. *Mammillaria vivipara* (Nutt.) Haw., Suppl. Pl. Succ.: 72. 1819. *Echinocactus viviparus* Poselg., Allg. Gartenzeitung 21: 107. 1853. *Mammillaria radiosa* var. *vivipara* (Nutt.) Schelle, Handb. Kakteenkult.: 236. 1907. *Coryphantha vivipara* (Nutt.) Britton & Rose, Ill. Fl. N. U.S.: 571. 1913. *Escobaria vivipara* (Nutt.) Buxb., Oesterr. Bot. Z. 98: 78. 1951. *Coryphantha neovivipara* Y.Itô, Cactaceae: 556. 1981. comb. inval. Type: United States, North Dakota, McClean County, 12 mi [19.31 km] E of Fort Mandan, E of Missouri River, Jun 1971, L. Mitich s.n. (neotype, designated by Mitich & Benson, Cact. Succ. J. (Los Angeles): 8. 1977: POM [317948]; iso-neotype NDA).
- = *Mammillaria radiosa* Engelm., Boston J. Nat. Hist. 6: 196. 1850. *Echinocactus radiosus* Poselg., Allg. Gartenzeitung 21: 107. 1853. *Mammillaria vivipara* var. *radiosa* Engelm. Proc. Amer. Acad. Arts 3: 269. 1856. *Mammillaria vivipara* subsp. *radiosa* Engelm., Rep. U.S. Mex. Bound. Cact.: 15. 1858. *Cactus radiosus* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 120. 1894. *Coryphantha radiosa* (Engelm.) Rydb., Fl. Rocky Mts.: 581. 1917. *Neomammillaria radiosa* (Engelm.) Rydb., Fl. Plains N. Amer.: 562. 1932. *Coryphantha vivipara* var. *radiosa* (Engelm.) Backeb., Cactaceae 5: 2998. 1961. *Escobaria vivipara* var. *radiosa* (Engelm.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Escobaria radiosa* (Engelm.) G.Frank place of publication unknown, nom. inval. *Coryphantha neovivipara* var. *radiosa* (Engelm.) Y.Itô, Cactaceae: 556. 1981. Type: United States, Texas, sterile soils on the Pedernales [Pedernales], and cult. In St Louis, Jun 1846, F. Lindheimer s.n. (lectotype, designated by Benson, Cacti U. S. Canada: 960. 1982: MO [2017377 image!, 2017376 image!]).
- = *Mammillaria vivipara* var. *vera* Engelm., Proc. Amer. Acad. Arts 3: 269. 1856. Type: United States. On the upper Missouri and Yellowstone rivers (Not preserved).

- = *Mammillaria vivipara* var. *radiosa* subvar. *neomexicana* Engelm., Proc. Amer. Acad. Arts 3: 269. 1856. *Cactus radiosus* var. *neomexicanus* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 120. 1894. *Cactus neomexicanus* (Engelm.) Small, Fl. S.E. U.S.: 812. 1903. *Mammillaria neomexicana* (Engelm.) A.Nelson, New Man. Bot. Centr. Rocky Mt.: 327. 1909. *Coryphantha neomexicana* (Engelm.) Britton & Rose, Cactaceae 4: 45. 1923. *Escobaria neomexicana* (Engelm.) Buxb., Oesterr. Bot. Z. 98: 78. 1951. *Coryphantha vivipara* var. *neomexicana* (Engelm.) Backeb., Cactaceae 5: 2999. 1961. *Coryphantha neovivipara* var. *neomexicana* (Engelm.) Y.Itô, Cactaceae: 556. 1981. nom. inval. *Escobaria vivipara* var. *neomexicana* (Engelm.) Buxb., Kakteen (H. Krainz) 108c, 1973. Type: United States, South New Mexico, 1849, *C. Wright* s. n. (lectotype, designated by Benson, Cacti U. S. Canada: 960. 1982: MO [2019650 image!]).
- = *Mammillaria arizonica* Engelm., Bot. California 1: 244. 1876. *Cactus radiosus* var. *arizonicus* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 121. 1894. *Mammillaria radiosa* var. *arizonica* (Engelm.) K.Schum., Gesamtbeschr. Kakt.: 481. 1898. *Mammillaria radiosa* f. *arizonica* (Engelm.) Schelle, Handb. Kakteenkult.: 235. 1907. *Coryphantha arizonica* (Engelm.) Britton & Rose, Cactaceae 4: 45. 1923. *Mammillaria vivipara* var. *arizonica* (Engelm.) L.D.Benson, Proc. Calif. Acad. Sci., ser. 4, 25: 263. 1944. *Coryphantha vivipara* var. *arizonica* (Engelm.) W.T.Marshall, Desert. Bot. Gard. Arizona, Sci. Bull. 1: 94. 1950. *Escobaria arizonica* (Engelm.) Buxb. in Oesterr. Bot. Z. 98: 78. 1951. *Escobaria vivipara* var. *arizonica* (Engelm.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. *Coryphantha neovivipara* var. *arizonica* (Engelm.) Y.Itô, Cactaceae: 556. 1981. Nom. inval. Type: United States, Arizona, *E. Coues* & *E. Palmer* s. n. (lectotype, designated by Benson, Cacti U. S. Canada: 961. 1982: MO [2017352 image!]).
- = *Mammillaria deserti* Engelm., Bot. California 2: 449. 1880. *Cactus radiosus* var. *deserti* (Engelm.) J.M.Coult., Contr. U.S. Natl. Herb. 3: 121. 1894. *Mammillaria radiosa* var. *deserti* (Engelm.) K.Schum., Gesamtbeschr. Kakt.: 481. 1898. *Mammillaria radiosa* f. *deserti* (Engelm.) Schelle, Handb. Kakteenkult.: 236. 1907. *Coryphantha deserti* (Engelm.) Britton & Rose, Cactaceae 4: 46. 1923. *Mammillaria vivipara* var. *deserti* (Engelm.) L.D.Benson in Proc. Calif. Acad. Sci., ser. 4, 25: 263. 1944. *Coryphantha vivipara* var. *deserti* (Engelm.) W.T.Marshall, Desert. Bot. Gard. Arizona, Sci. Bull. 1: 94. 1950. *Escobaria deserti* (Engelm.) Buxb., Oesterr. Bot. Z. 98: 78. 1951. *Coryphantha chlorantha* var. *deserti* (Engelm.) Backeb., Cactaceae 5: 3003. 1961. *Escobaria vivipara* var. *deserti* (Engelm.) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, California, at Ivapah, 30 miles northeast of San Bernardino, in one mountain range stretching into the desert, *S. B. Parish* 455 (lectotype, designated by Benson, Cacti U. S. Canada: 961. 1982: MO [2267169 image!]).
- = *Mammillaria hirschtiana* F.Haage, Monatsschr. Kakteenk. 6: 127. 1896. Type: No designated.
- = *Mammillaria radiosa* var. *texensis* Schelle, Handb. Kakteenkult.: 236. 1907. Type: No designated.

- = *Mamillaria ramosissima* Quehl, Monatsschr. Kakteenk. 18: 127. 1908. Type: United States, California, *R. C. Orcutt s. n.* (lectotype, designated here, Monatsschr. Kakteenk.: 127. 1908: Illustration “*Mamillaria ramosissima* Quehl Nach einer von Herr De Laet aufgenommenen Photographie”).
- = *Coryphantha bisbeeana* Orcutt, Cactography: 3. 1926. *Escobaria bisbeeana* (Orcutt) Borg, Cacti: 305. 1937. *Coryphantha vivipara* var. *bisbeeana* (Orcutt) L.D.Benson, Cacti Ariz. ed. 3: 25. 1969. *Escobaria vivipara* var. *bisbeeana* (Orcutt) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, Arizona, *J. N. Rose 11958* (lectotype, designated by Benson, Cacti Ariz. ed 3: 25. 1969: US [3050430 image!]).
- = *Coryphantha columnaris* Lahman, Cact. Succ. J. (Los Angeles) 6: 27. 1934. Type: United States, Oklahoma, Jackson County, near Altus, 600 ft [182 m], 1926, *M. S. Lahman s.n.* (holotype: MO).
- = *Coryphantha fragrans* Hester, Desert Pl. Life 13: 152. 1941. Type: United States, Texas, in a fertile, sandy loam valley, along the railroad right-of-way and Highway 90, a few miles west of Sanderson, 03 May 1940, *J. P. Hester s.n.* (holotype: DS [278622 image!]).
- = *Coryphantha rosea* Clokey, Madroño 7: 75. 1943. *Coryphantha vivipara* var. *rosea* (Clokey) L.D.Benson, Cacti Ariz. ed. 3: 26. 1969. *Escobaria vivipara* var. *rosea* (Clokey) D.R.Hunt, Cact. Succ. J. Gr. Brit. 40: 13. 1978. Type: United States, Nevada, Clark County, between Kyle Canyon and Deer Creek. 24 Jun 1938, *I. W. Clokey 8038* (holotype: UC [905407 image!]; isotypes: F [52864 image!]; MEXU [86081 image!]; MICH [1127565 image!]; NY [120673 image!, 120672 image!], TEX [255617]).
- = *Coryphantha oklahomensis* Lahman, Cact. Succ. J. (Los Angeles) 21: 165. 1949. *Escobaria oklahomensis* (Lahman) Buxb., Oesterr. Bot. Z. 98: 78. 1951. Type: United States, Oklahoma, Caddo County, Range throughout western Oklahoma, *collector not mentioned* (lectotype, designated here Cact. Succ. J. (Los Angeles): 165. 1949: Illustration “fig. 107. *Coryphantha oklahomensis* sp. nov. Photo by Jim Slack”).
- = *Coryphantha alversonii* var. *exaltissima* Wiegand & Backeb., Cactaceae 5: 3001. 1961. Type: United States, California, ohne nähere Standortsangabe, *E. F. Wiegand s. n.* (lectotype, designated here Cactaceae (Backeberg): 3001. 1961: Illustration “Abb. 2817. Links: *Coryphantha alversonii* (Coult.) Orc.; rechts: deren v. *exaltissima* Wieg & Backbg. (photo: E. F. Wiegand.)”).
- = *Coryphantha vivipara* var. *kaibabensis* P.C.Fisch., Cact. Succ. J. (Los Angeles) 51: 287. 1979. *Escobaria vivipara* var. *kaibabensis* (P.C.Fisch.) N.P.Taylor, Kakteen And. Sukk. 34: 139. 1983. Type: United States, Arizona, *P. C. Fischer 4094*. (holotype: UC).
- = *Coryphantha vivipara* var. *buoflama* P.C.Fisch., Cact. Succ. J. (Los Angeles) 52: 28. 1980. *Escobaria vivipara* var. *buoflama* (P.C.Fisch.) N.P.Taylor, Kakteen And. Sukk. 34: 140. 1983. Type: United States, Arizona, Yavapai County, 05 May 1979, *P. C. Fischer 6582*. (holotype: ARIZ; isotype: ASU [image 018464!]).
- = *Coryphantha vivipara* var. *bisbeeana* f. *sonorensis* P.C.Fisch., Cact. Succ. J. (Los Angeles) 52: 191. 1980. Type: México, Sonora, 84 km north of Nacozari, on the road to U.S. border, 1430 m, 27 Apr 1971, *P. C. Fischer 4364*. (holotype: UC).

***Pelecyphora zilziana* (Boed.) D.Aquino & Dan.Sánchez, comb. nov.**

urn:lsid:ipni.org:names:77248965-1

- ≡ *Coryphantha zilziana* Boed., Monatsschr. Deutsch. Kakteen-Ges. 2: 233. 1930. *Neobesseyia zilziana* (Boed.) Boed., Mammill.-Vergl.-Schluessel: 14. 1933. *Neobesseyia zilziana* (Boed.) Boed. ex Backeb. & F.M.Knuth, Kaktus-ABC: 379. 1936. *Escobaria zilziana* (Boed.) Backeb., Cactaceae 5: 2957. 1961. Type: Mexico, Coahuila, nördlich des Paila-Gebirges auf felsigen Hügeln von dunklem Eruptivgestein und auf Kalkhügeln sehr vereinzelt, 1928, *F. Ritter s.n.* (lectotype, designated here, Monatsschr. Deutsch. Kakteen-Ges.: 233. 1930b: Illustration “*Coryphantha Zilziana* Boed. sp. nov. natür. Grösse”).
- = *Escobaria zilziana* subsp. *fricii* Halda & Sladk. in Acta Mus. Richnov., Sect. Nat. 7: 35. 2000. Type. México, Coahuila, Sierra de la Paila, in the vicinity of Castanos [Castaños], 2000 m, 13 Apr 1985, *J. J. Halda, J. Sladkovsky* 8504013520 (holotype: PR).

Coryphantha

Phylogenetic analyses obtained here support the recognition of two subgenera in *Coryphantha* (clade C1 and clade C2), which are composed by two section (subclade A and subclade B) and five sections (subclades C to G), respectively. Also, 46 species and 12 subspecies of *Coryphantha*, are recognized. Asterisk (*) indicates species that were not included in the phylogenetic analyses. A taxonomic synthesis is presented.

***Coryphantha* (Engelm.) Lem., Cactées 32. 1868.**

Mammillaria subgen. *Coryphantha* Engelm., Proc. Amer. Acad. Arts 3: 264. 1856.
Mammillaria subsect. *Glanduliferae* Salm-Dyck, Cact. Hort. Dyck. 1844: 13. 1845. *Glandulifera* (Salm-Dyck) Frič, Českoslov. Zahradn. Listy 1924: 122. 1924. nom. illeg.
Escobrittonia Doweld, Sukkulenty 3: 17. 2000. Type: *Escobrittonia gracilis* (L.Bremer & A.B.Lau) Doweld. Sukkulenty 3: 17. 2000.

Type. *Coryphantha sulcata* (Engelm.) Britton & Rose

Coryphantha* subgenus *Coryphantha***Coryphantha* section *Corniferae* (Dicht & A.Lüthy) Dan.Sánchez & D.Aquino, stat. nov.**

urn:lsid:ipni.org:names:77248966-1

- ≡ *Coryphantha* ser. *Corniferae* Dicht & A.Lüthy, *Coryphantha*. Kakteen Nordamer. 91. 2003. ≡ *Coryphantha* subser. *Corniferae* Dicht & A.Lüthy, Cactaceae Syst. Init. 11: 19. 2001. Type: *Coryphantha cornifera* (DC.) Lem., Cactées 35. 1868.

***Coryphantha* section *Gracilicoryphantha* Dicht & A.Lüthy, Cactaceae Syst. Init. 11: 21, 2001.**

Type. *Coryphantha gracilis* Bremer & A.B.Lau, Cact. Succ. J. (Los Angeles) 49: 72. 1977.

***Coryphantha* subser. *Delaetianae* Dicht & A.Lüthy, Cactaceae Syst. Init. 11: 20. 2001.**

Type. *Coryphantha delaetiana* (Quehl) A.Berger, Kakteen: 270, 339. 1929.

***Coryphantha* subser. *Neglectae* Dicht & A.Lüthy, Cactaceae Syst. Init. 11: 20. 2001.**

Type. *Coryphantha neglecta* L.Bremer, Cact. Suc. Mex. 24: 3. 1979.

Species included (*inserta sedis). *Coryphantha compacta* (Engelm.) Orcutt, *C. cornifera* (DC.) Lem., *C. delaetiana* (Quehl) A.Berger, *C. delicata* L.Bremer, **C. gracilis* L. Bremer & A.B.Lau, *C. hintoniorum* Dicht & A.Lüthy, *C. hintoniorum* subsp. *geofreyii* Dicht & A.Lüthy, *C. maiz-tablasensis* Backeb., *C. neglecta* L.Bremer, *C. nickelsiae* (K.Brandegee) Britton & Rose, *C. pseudoechinus* Boed., *C. pseudonickelsiae* Backeb., **C. pulleineana* (Backeb.) Glass, *C. ramillosa* Cutak, *C. ramillosa* subsp. *santarosae* Dicht & A.Lüthy, *C. recurvata* (Engelm.) Britton & Rose and *C. recurvata* subsp. *canatlanensis* Dicht & A.Lüthy.

Coryphantha* section *Coryphantha

Coryphantha ser. *Salinenses* Dicht & A.Lüthy, Cactaceae Syst. Init. 11: 15. 2001.

Type: *Coryphantha salinensis* (Poselg.) Dicht & A.Lüthy, Kakteen And. Sukk. 49: 257.

Type. *Coryphantha sulcata* (Engelm.) Britton & Rose, Cactaceae 4: 48. 1923.

Species included. *Coryphantha difficilis* (Quehl) Orcutt, *C. echinus* (Engelm.) Britton & Rose, *C. kracikii* Halda, Chalupa & Kupčák, *C. salinensis* (Poselg.) Dicht & A.Lüthy, *C. sulcata* (Engelm.) Britton & Rose, and *C. werdermannii* Boed.

***Coryphantha* section *Durangenses* Dan.Sánchez & D.Aquino, sect. nov.**

urn:lsid:ipni.org:names:77248967-1

Type. *Coryphantha durangensis* Britton & Rose, Cactaceae (Britton & Rose) 4: 42. 1923.

Species included. *Coryphantha durangensis* (Runge ex K.Schum.) Britton & Rose, *C. durangensis* subsp. *cuencamensis* (L.Bremer) Dicht & A.Lüthy, and *C. longicornis* Boed.

***Coryphantha* section *Pycnacanthae* (Dicht & A.Lüthy) Dan.Sánchez & D.Aquino, stat. nov.**

urn:lsid:ipni.org:names:77248968-1

Coryphantha ser. *Retusae* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 14. 2001. Type: *Coryphantha retusa* (Pfeiff.) Britton & Rose, *Cactaceae* 4: 38. 1923.

Basionym. *Coryphantha* ser. *Pycnacanthae* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 15. 2001.

Type. *Coryphantha pycnantha* (Mart.) Lem., *Cactées*: 35. 1868.

Species included (*inserta sedis): *Coryphantha bumamma* (C.Ehrenb.) Britton & Rose, *C. calipensis* Bravo ex S.Arias, U.Guzmán & S.Gama, *C. elephantidens* (Lem.) Lem., *C. greenwoodii* Bravo, *C. pallida* Britton & Rose, **C. pseudoradians* Bravo, *C. pycnantha* (Mart.) Lem., *C. retusa* (Pfeiff.) Britton & Rose, and *C. tripugionacantha* A.B. Lau.

***Coryphantha* section *Robustispina* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 9. 2001.**

Type. *Coryphantha robustispina* (Ant.Schott ex Engelm.) Britton & Rose, *Cactaceae* 4: 33. 1923.

Species included. *Coryphantha robustispina* (Ant.Schott ex Engelm.) Britton & Rose, *C. robustispina* subsp. *scheeri* (Lem.) N.P. Taylor, and *C. poselgeriana* (A.Dietr.) Britton & Rose.

***Coryphantha* subgenus *Neocoryphantha* Backeb. ex Dicht & A. Lüthy, *Cactaceae Syst. Init.* 11: 8, 2001.**

Type. *Coryphantha clavata* (Scheidw.) Backeb., *Jahrb. Deutsch. Kakt. Ges.* 1941: 61. 1942.

***Coryphantha* section *Clavatae* (Dicht & A. Lüthy) Dan.Sánchez & D.Aquino, stat. nov.**

urn:lsid:ipni.org:names:77248969-1

≡ *Coryphantha* Ser. *Clavatae* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 11. 2001. Type: *Coryphantha clavata* (Scheidw.) Backeb., *Jahrb. Deutsch. Kakt. Ges.* 1941: 61. 1942. *Coryphantha* sect. *Ottonis* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 13. 2001. Type: *Coryphantha ottonis* (Pfeiff.) Lem., *Cactées* 34. 1868.

Species included. *Coryphantha clavata* (Scheidw.) Backeb., *C. clavata* subsp. *stipitata* (Scheidw.) Dicht & A.Lüthy, *C. erecta* (Lem.) Lem., *C. georgii* Boed., *C. glassii* Dicht & A.Lüthy, *C. jalpanensis* Buchenau, *C. octacantha* (DC.) Britton & Rose, *C. ottonis* (Pfeiff.) Lem., *C. potosiana* (Jacobi) Glass & R.A.Foster, and *C. vogterriana* Werderm. & Boed.

***Coryphantha* section *Echinoideae* (Dicht & A. Lüthy) Dan.Sánchez & D.Aquino, stat. nov.**

urn:lsid:ipni.org:names:77248971-1

≡ *Coryphantha* Ser. *Echinoideae* Dicht & A.Lüthy, *Cactaceae Syst. Init.* 11: 10. 2001. Type: *Coryphantha echinoidea* Britton & Rose, *Cactaceae* (Britton & Rose) 4: 30. 1923.

Species included. *Coryphantha echinoidea* (Quehl) Britton & Rose, *C. glanduligera* (Otto & A.Dietr.) Lem., *C. vaupeliana* Boed., and *C. wolfschlagerei* Holzeis.

New neotypes and lectotypes

Furthermore, two neotypes and three lectotypes are proposed. For a more extensive review of the accepted names in *Coryphantha*, see Dicht and Lüthy (2005).

***Coryphantha potosiana* (Jacobi) Glass & R.A.Foster, *Cact. Succ. J. (Los Angeles)* 43: 7. 1971.**

≡ *Mammillaria potosiana* Jacobi, *Allg. Gartenzeitung* (Otto & Dietrich) 24: 92. 1856. *Coryphantha potosiana* (Jacobi) Glass & R.A.Foster ex Rowley, *Rep. Pl. Succ.* 21: 8. 1972. Type: México, San Luís Potosí, 1847, *Jacobi & Galeottii s.n.* (not preserved). Neotype designated here: México, San Luís Potosí, Villa de Arriaga, Rincón de Silva, 2200 m, 23 Jun 1983, *R. Hernández s.n.* (MEXU: 363520!).

***Coryphantha ottonis* (Pfeiff.) Lem., *Cactées*: 34. 1868.**

- ≡ *Mammillaria ottonis* Pfeiff., *Allg. Gartenzeitung* 6: 274. 1838. *Cactus ottonis* (Pfeiff.) Kuntze, *Revis. Gen. Pl.* 1: 261. 1891. Type: Not designed. Neotype designated here: México, Estado Mex., Polotitlán, Colonia Doctores, a unos 2 km al E de la Carretera de Cuota México Querétaro, a la altura del km 130, 2000 m, 27 May 1977, *H. Sánchez-Mejorada* 2728 (MEXU: 204376!).
- = *Mammillaria asterias* Cels ex Salm-Dyck, *Cact. Hort. Dyck.*: 129. 1850. *Coryphantha asterias* (Cels) Hübner, *Kakteenfreund* (Beil) 2: 8. 1933. Type: Not designated.
- = *Echinocactus ottonianus* Poselg., *Allg. Gartenzeitung* 21: 102. 1853. *Coryphantha ottonianus* (Poselg.) Y.Itô, *Cactaceae*: 553. 1981. Type: Not designated.
- = *Mammillaria bussleri* Mundt ex K.Schum., *Monatsschr. Kakteenk.* 12: 47. 1902. *Coryphantha bussleri* (Mundt) Scheinvar, *Phytologia* 49: 313. 1981. Type: México, *Anonymous s.n.* (lectotype, designated here, *Monatsschr. Kakteenk.*: 47. 1902: Illustration “Mamillatia Bussleri Mundt. Nach einer von dem Herrn Autor angefertigten Photographie”).
- = *Mammillaria golziana* F.Haage ex R.E.Kunze, *Monatsschr. Kakteenk.* 19: 100. 1909. Type: México, Zacatecas, *Anonymous s.n.* (lectotype, designated here, *Monatsschr.*

Kakteenk.: 100. 1909: Illustration “Mamillatia Golziana” Ferd. Haage jun. Nach einer von Herrn Dr R. E. Kunze in Phoenix (Arizona) aufgenommenen Photographie”). = *Mamillaria guerkeana* Boed., Monatsschr. Kakteenk. 24: 52. 1914. *Coryphantha guerkeana* (Boed.) Britton & Rose, Cactaceae 4: 29. 1923. Type: México, Durango, 1911, *F. De Laet s.n.* (lectotype, designated here: US [2975102 image!]).

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References

- Anderson EF (2001) The Cactus Family. Timber Press, Oregon, 776 pp.
- Anderson EF, Boke N (1969) The genus *Pelecypora* (Cactaceae): Resolution of a controversy. American Journal of Botany 56(3): 314–326. <https://doi.org/10.1002/j.1537-2197.1969.tb07538.x>
- Aquino D, Cervantes RC, Gernandt DS, Arias S (2019) Species Delimitation and Phylogeny of *Epithelantha* (Cactaceae). Systematic Botany 44(3): 600–615. <https://doi.org/10.1600/036364419X15620113920635>
- Arias S, Guzmán-López L, Guzmán-Cruz U, Vázquez-Benítez B (2012) Cactaceae. In: Medina R, Sánchez G, García A, Arias S (Eds) Flora del Valle de Tehuacán-Cuicatlán. Universidad Nacional Autónoma de México 95: 1–235.
- Bárcenas RT, Yesson C, Hawkins JA (2011) Molecular systematics of the Cactaceae. Cladistics 27(5): 470–489. <https://doi.org/10.1111/j.1096-0031.2011.00350.x>
- Barthlott W, Hunt DR (2000) Seed diversity in the Cactaceae subfamily Cactoideae. Succulent Plant Research. Milborne Port 5, 173 pp.
- Benson LD (1969) The cacti of the United States and Canada. New names and nomenclatural combinations. Cactus and Succulent Journal (Los Angeles) 41(4): 185–190.
- Benson LD (1982) The Cacti of the United States and Canada. Stanford University Press, California, 1044 pp.
- Boedeker F (1929) *Coryphantha asperispina* Böd., sp. n. Monatsschrift der Deutschen Kakteen-Gesellschaft, 11: 192–194.
- Boedeker F (1930a) *Coryphantha Mühlbaueriana* Böd., spec. nov. (Mit Abb.). Monatsschrift der Deutschen Kakteen-Gesellschaft 2: 18–20.
- Boedeker F (1930b) *Coryphantha Zilziana* Böd., sp. n. Monatsschrift der Deutschen Kakteen-Gesellschaft 9: 233–234.

- Boke NH (1959) Endomorphic and ectomorphic characters in *Pelecypora* and *Encephalocarpus*. American Journal of Botany 46(3): 197–209. <https://doi.org/10.1002/j.1537-2197.1959.tb07004.x>
- Bravo-Hollis H, Sánchez-Mejorada H (1991) Las Cactáceas de México (Vol. 3). Universidad Nacional Autónoma de México, 643 pp.
- Breslin P, Wojciechowski MF, Majure LM (2021) Molecular phylogeny of the Mammilloid clade (Cactaceae) resolves the monophyly of *Mammillaria*. Taxon 70(2): 308–323. <https://doi.org/10.1002/tax.12451>
- Britton NL, Rose JN (1912) Undescribed species of cuban cacti. Torreya 12: 13–16.
- Britton NL, Rose JN (1923) The *Cactaceae*: Descriptions and Illustrations of Plants of the Cactus Family, vol. 4. The Carnegie Institution, Washington DC, 318 pp.
- Butterworth C, Wallace RS (2004) Phylogenetic studies of *Mammillaria* (Cactaceae) insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. American Journal of Botany 91(7): 1086–1098. <https://doi.org/10.3732/ajb.91.7.1086>
- Butterworth C, Cota-Sánchez H, Wallace RS (2002) Molecular systematics of tribe Cacteae (Cactaceae: Cactaceae): A phylogeny based on *rpl16* intron sequence variation. Systematic Botany 27(2): 257–270. <https://doi.org/10.1043/0363-6445-27.2.257>
- Craig RT (1945) The *Mammillaria* Handbook. Abbey Garden Press, Pasadena.
- Crozier BS (2005) Systematics of *Cactaceae* Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw. PhD Thesis, The University of Texas at Austin, United States.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Dicht R, Lüthy A (2001) The genus *Coryphantha* (Engelm.) Lem. Cactaceae Systematics Initiatives 11: 7–21.
- Dicht R, Lüthy A 2005: *Coryphantha*: Cacti of Mexico and southern USA. Springer Science & Business Media, Berlin, 200 pp.
- Engelmann G (1856) Synopsis of the Cactaceae of the territory of the United States and adjacent regions. Proceedings of the American Academy of Arts and Sciences 3: 259–346. <https://doi.org/10.5962/bhl.title.46890>
- Fehlberg SD, Allen JM, Church K (2013) A novel method of genomic DNA extraction for Cactaceae. Applications in Plant Sciences 1(3): e1200013. <https://doi.org/10.3732/apps.1200013>
- Gene Codes Corporation (2007) Sequencher 4.8. Gene Codes Corporation, Ann Arbor.
- Gurke M (1905) *Ma[m]millaria mazatlanensis* K. Schum. Monatsschrift für Kakteenkunde. 15: 154–156.
- Hernández-Hernández T, Hernández HM, De Nova JA, Puente R, Eguiarte LE, Magallón S (2011) Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae). American Journal of Botany 98(1): 44–61. <https://doi.org/10.3732/ajb.1000129>
- Hunt DR (1985) A new review of *Mammillaria* names. Bradleya 3(3): 53–66. <https://doi.org/10.25223/brad.n3.1985.a3>
- Hunt DR (2016) CITES Cactaceae checklist (3rd edn.). Royal Botanical Gardens, Kew, 170 pp.
- Hunt DR, Benson LD (1976) The lectotype of *Coryphantha*. Cactus and Succulent Journal (Los Angeles) 48: e72.

- Hunt DR, Taylor NP, Glass H (2006) The new Cactus Lexicon: Descriptions and Illustrations of the Cactus Family (Vol. 1). DH Books, Milborne Port, 375 pp.
- Korotkova N, Aquino D, Arias S, Eggli U, Franck A, Gómez-Hinostrosa C, Guerrero PC, Hernández HM, Kohlbecker A, Köhler M, Luther K, Majure LC, Müller A, Metzinger D, Nyffeler R, Sánchez D, Schulumberg B, Berendsohn WG (2021) Cactaceae at Caryophyllales.org a dynamic online species-level taxonomic backbone for the family. *Willdenowia* 51(2): 251–270. <https://doi.org/10.3372/wi.51.51208>
- Kunze RE (1909) *Mammillaria Golziana* Ferd. Haage jun. *Monatsschrift für Kakteenkunde*. 19: 100–101.
- Kuzmina M, Braukmann TW, Fazekas AJ, Graham SW, Dewaar SL, Rodrigues A, Bennett BA, Dickinson TA, Saarela JM, Catling PM, Newmaster SG (2017) Using herbarium derived DNAs to assemble a large scale DNA barcode library for the vascular plants of Canada. *Applications in Plant Sciences* 5(12): e1700079. <https://doi.org/10.3732/apps.1700079>
- Lahman MS (1949) A new species of *Coryphantha*. *Cactus and Succulent Journal* (Los Angeles) 21(6): e165.
- Lemaire C (1868) *Les Cactées Histoire, Patrie Organes de Vegetation, Inflorescence Culture, etc.*, Librairie Agricole de la Maison Rustique, Paris, 140 pp.
- Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50(6): 913–925. <https://doi.org/10.1080/106351501753462876>
- Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. Version 2.75. <http://www.mesquiteproject.org>
- Martínez-Quezada DM, Arias S, Korotkova N, Terrazas T (2020) The phylogenetic significance of the stem morpho-anatomy in the Hylocereeae (Cactoideae, Cactaceae). *Plant Systematics and Evolution* 306(1): 1–4. <https://doi.org/10.1007/s00606-020-01639-x>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*. IEEE Press, New Orleans, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Quehl L (1908) *Mammillaria ramosissima* Quehl n. sp. *Monatsschrift für Kakteenkunde*. 18: e127.
- Quehl L (1910) *Mammillaria Emskötteriana* Quehl. n. sp. (Mit einer Abbildung.). *Monatsschrift für Kakteenkunde*. 20: 139–140.
- Řepka R, Vaško Z (2011) *Escobaria abdita*—A new species from northern Mexico. *Cactus and Succulent Journal* (Los Angeles) 83(6): 264–269. <https://doi.org/10.2985/0007-9367-83.6.264>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sánchez D, Arias S, Vázquez-Sánchez M, Terrazas T (2019) Combined phylogenetic analysis in *Echinocereus* (Cactaceae), the use of morphology, and taxonomic implications. In: Ramawat K (Ed.) *Biodiversity and Chemotaxonomy. Sustainable Development and Biodiversity*. Springer, Cham 24: 239–269. https://doi.org/10.1007/978-3-030-30746-2_11
- Schumann K (1902) *Mammillaria Bussleri* Mundt. *Monatsschrift für Kakteenkunde* 12: e47.

- Schumann K (1901) Beschreibungen unbekannter oder wenig bekannter Kakteenblüten. Monatsschrift für Kakteenkunde 11: 154–157.
- Schwabe AL, Neale JR, McGlaughlin ME (2015) Examining the genetic integrity of a rare endemic Colorado cactus (*Sclerocactus glaucus*) in the face of hybridization threats from a close and widespread congener (*Sclerocactus parviflorus*). Conservation Genetics 16(2): 443–457. <https://doi.org/10.1007/s10592-014-0671-3>
- Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49(2): 369–381. <https://doi.org/10.1093/sysbio/49.2.369>
- Taylor NP (1979) Further notes on *Escobaria* B. & R. Cactus and Succulent Journal of Great Britain 41(1): 17–20.
- Taylor NP, Clark JY (1983) Seed morphology and classification in *Ferocactus* subg. *Ferocactus*. Bradleya 1983(1): 3–16. <https://doi.org/10.25223/brad.n1.1983.a2>
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF [Eds] (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>
- Vázquez-Benítez B, Arias S, Cervantes-Sandoval A (2016) Variación morfológica de *Coryphantha* (Cactaceae): Un enfoque multivariado. Acta Botánica Mexicana 116(116): 21–47. <https://doi.org/10.21829/abm116.2016.1117>
- Vázquez-Sánchez M, Terrazas T, Arias S, Ochoterena H (2013) Molecular phylogeny, origin and taxonomic implications of the tribe Cacteae (Cactaceae). Systematics and Biodiversity 11(1): 103–116. <https://doi.org/10.1080/14772000.2013.775191>
- Vázquez-Sánchez M, Sánchez D, Terrazas T, De La Rosa-Tilapa A, Arias S (2019) Polyphyly of the iconic cactus genus *Turbinicarpus* (Cactaceae) and its generic circumscription. Botanical Journal of the Linnean Society 190(4): 405–420. <https://doi.org/10.1093/botlinnean/boz027>
- Zimmerman AD, Parfitt BD (1993+) Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 4. http://beta.floranorthamerica.org/Coryphantha_tuberculosa [Accessed 27.07.2021]
- Zimmerman AD, Parfitt BD (2004) *Coryphantha* (Engelmann) Lemaire, (Cactaceae). In: Flora of North America Editorial Committee (Eds) Flora of North America – volume 4. Oxford University Press, New York, 220–237.

Appendix I

Accessions included in this study, presented in alphabetical order, and following this format: taxon name in bold, country, estate, collector, collecting number (HERBARIUM ACRONYM) and Gen Bank accession as follow: *matK*/*rbcL*/*psbA-trnH*/*rpl16*/*trnL-F*. A dash (–) indicates that the locus was not sequenced for that speci-

men. Living voucher specimens are identified by their specimen number in cultivation at Desert Botanical Garden (DES), Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México (JB, UNAM), and El Charco del Ingenio, A.C. ND: no data.

Acharagma aguirreanum (Glass & R.A.Foster) Glass. Mexico, Coahuila, S. Arias 1459 (MEXU): MK449027/ MK449085/ MK449274/ AF267915/ MK449212; *Acharagma roseanum* (Boed.) E.F.Anderson. Mexico, Coahuila, C. Glass 6443 (MEXU): MK449028/ MK449086/ MK449275/ MK449151/ MK449213; *Cochemiea armillata* (K.Brandegee) P.B.Breslin & Majure, cult.: FN997315/ –/ AY545949/AY545240/–; *Cochemiea cerralboa* (Britton & Rose) P.B.Breslin & Majure, cult.: FN997003/ –/ AY545364/ –/ –; *Cochemiea halei* (K.Brandegee) Walton, Mexico, S. Arias 1287 (MEXU): OL513239/ OL513243/ OL513236/ OL513246/ –; *Cochemiea pondii* (Greene) Walton, Mexico, S. Arias 1862 (MEXU): OL513240/ OL513244/ OL513237/ OL513247/ –, V. Alvarado s.n.: –/ –/ –/ –/ HM041244; *Cochemiea poselgeri* (Hildm.) Britton & Rose, Mexico, Baja California, S. Arias 1824 (MEXU): OL513241/ OL513245/ OL513238/ OL513248/ –, T. Hernández p106: –/ –/ –/ –/ HM041245; *Coryphantha bumamma* Britton & Rose. Mexico, Guerrero, B. Vázquez 2628 (MEXU): OK340224/ OK340287/ OK340349/ OK340410/ OK340462; *Coryphantha calipensis* Bravo ex S.Arias, Gama & U. Guzman. Mexico, Oaxaca, B. Vázquez 2555 (MEXU): OK340225/ OK340288/ OK340350/ OK340411/ OK340463; *Coryphantha clavata* (Scheidw.) Backeb. Mexico, San Luis Potosí, T. Terrazas 886 (MEXU): OK340227/ OK340290/ OK340352/ –/ OK340465; *Coryphantha compacta* (Engelm.) Orcutt. Mexico, Chihuahua, B. Vázquez 2608 (MEXU): OK340228/ OK340291/ OK340353/ OK340413/ OK340466; *Coryphantha cornifera* Lem. Mexico, Querétaro, S. Arias 1700 (MEXU): OK340229/ OK340292/ OK340354/ OK340414/ OK340467; *Coryphantha delaetiana* A.Berger. Mexico, Durango, S. Arias 1901 (MEXU): OK340231/ OK340294/ OK340356/ OK340416/ –; *Coryphantha delicata* L.Bremer. Mexico, San Luis Potosí, B. Vázquez 2546 (MEXU), OK340232/ OK340295/ OK340357/ OK340417/ OK340469; *Coryphantha difficilis* Orcutt. Mexico, Coahuila, B. Vázquez 2541 (MEXU): OK340233/ OK340296/ OK340358/ –/ OK340470; *Coryphantha durangensis* Britton & Rose. Mexico, Durango, B. Vázquez 2626 (MEXU): OK340234/ OK340297/ OK340359/ OK340418/ OK340471; *Coryphantha durangensis* subsp. *cuencamensis* (L.Bremer) Dicht & A.Lüthy. Mexico, Durango, B. Vázquez 2627 (MEXU): OK340230/ OK340293/ OK340355/ OK340415/ OK340468; *Coryphantha echinoidea* Britton & Rose. Mexico, San Luis Potosí, B. Vázquez 2514 (MEXU): OK340235/ OK340298/ OK340360/ –/ OK340472; *Coryphantha echinus* (Engelm.) Orcutt. Mexico, Chihuahua, S. Arias 2072 (MEXU): OK340236/ OK340299/ OK340361/ OK340419/ OK340473; *Coryphantha elephantidens* Lem. Mexico, Guerrero, B. Vázquez 2629 (MEXU): OK340237/ OK340300/ OK340362/ OK340420/ OK340474; *Cory-*

phantha erecta Lem. Mexico, Querétaro, S. Arias 1684 (MEXU): OK340238/ OK340301/ OK340363/ OK340421/ OK340475; *Coryphantha georgii* Boed. Mexico, San Luis Potosí, B. Vázquez 2517 (MEXU): OK340239/ OK340302/ OK340364/ OK340422/ OK340476; *Coryphantha glanduligera* (Otto & A.Dietr.) Lem. Mexico, San Luis Potosí, B. Vázquez 2547 (MEXU): OK340240/ OK340303/ OK340365/ OK340423/ OK340477; *Coryphantha glassii* Dicht & A.Lüthy. Mexico, San Luis Potosí, B. Vázquez 2525 (MEXU): OK340241 / OK340304/ OK340366/ OK340424/ OK340478; *Coryphantha greenwoodii* Bravo. Mexico, Veracruz, B. Vázquez 2630 (MEXU): OK340242/ OK340305/ OK340367/ OK340425/ OK340479; *Coryphantha hintoniorum* Dicht & A. Lüthy. Mexico, Nuevo León, B. Vázquez 2539 (MEXU): OK340243/ OK340306/ OK340368/ OK340426/ OK340480; *Coryphantha jalpanensis* Franc.G.Buchenau. Mexico, Querétaro, B. Vázquez 2586 (MEXU): OK340244/ OK340307/ OK340369/ OK340420/ OK340481; *Coryphantha kracikii* Halda, Chalupa & Kupčák. Mexico, Durango, B. Vázquez 2618 (MEXU): OK340245/ OK340308/ OK340370/ –/ OK340482; *Coryphantha longicornis* Boed. Mexico, Durango, B. Vázquez 2623 (MEXU): OK340246/ OK340309/ OK340371/ OK340428/ OK340483; *Coryphantha macromeris* (Engelm.) Lem. Mexico, San Luis Potosí, B. Goettsch 169 (MEXU): FN997086 / –/ –/ –. Mexico, Chihuahua, S. Arias 1788 (MEXU): OK340247/ OK340310/ OK340372/ OK340429/ –; *Coryphantha maiz-tabla-sensis* Fritz Schwarz. JE280502 (cult. JB, UNAM), ND: OK340248/ OK340311/ OK340373/ OK340430/ OK340484; *Coryphantha neglecta* L.Bremer. Mexico, Coahuila, S. Arias 2116 (MEXU): OK340249/ OK340312/ OK340374/ OK340431/ OK340485; *Coryphantha nickelsiae* (K.Brandege) Britton & Rose. Mexico, Nuevo León, B. Vázquez 2565 (MEXU): OK340250/ OK340313/ OK340375/ OK340432/ OK340486; *Coryphantha octacantha* Britton & Rose. Mexico, Hidalgo, B. Vázquez 2531 (MEXU): OK340251/ OK340314/ OK340376/ OK340433/ OK340487; *Coryphantha ottonis* Lem. Mexico, Estado de México, B. Vázquez 2588 (MEXU): OK340252/ OK340315/ OK340377/ –/ OK340488; *Coryphantha pallida* Britton & Rose. Mexico, Puebla, B. Vázquez 2552 (MEXU): OK340253/ OK340316/ OK340378/ OK340434/ OK340489; *Coryphantha poselgeriana* Britton & Rose. Mexico, Coahuila, B. Vázquez 2544 (MEXU): OK340254/ OK340317/ OK340379/ OK340435/ OK340490; *Coryphantha potosiana* (Jac.) Glass & R.A.Foster ex G.D.Rowley. Mexico, San Luis Potosí, U. Guzmán 2771 (MEXU): OK340255/ OK340318/ OK340380/ OK340436/ OK340491; *Coryphantha pseudoechinus* Boed. Mexico, Coahuila, B. Vázquez 2542 (MEXU): OK340256/ OK340319/ OK340381/ OK340438/ OK340492; *Coryphantha pseudonickelsiae* Backeb. Mexico, Durango, B. Vázquez 2620 (MEXU): OK340257/ OK340320/ OK340382/ OK340438/ OK340493; *Coryphantha pycnacantha* (Mart.) Lem. Mexico, Estado de México, B. Vázquez 2589 (MEXU): OK340258/ OK340321/ OK340383/ OK340439/ OK340494; *Coryphantha ramillosa* Cutak. Mexico, Chihuahua, S. Arias 2070 (MEXU): OK340259/ OK340322/ OK340384/ –/ OK340495; *Cory-*

phantha recurvata subsp. *canatlanensis* Dicht & A.Lüthy. Mexico, Durango, S. Arias 1893 (MEXU): OK340226/ OK340289/ OK340351/ OK340412/ OK340464; *Coryphantha retusa* Britton & Rose. Mexico, Oaxaca, B. Vázquez 2558 (MEXU): OK340260/ OK340323/ OK340385/ OK340440/ OK340496; *Coryphantha robustispina* (A.Schott ex Engelm.) Britton & Rose. Mexico, Chihuahua, B. Vázquez 2581 (MEXU): OK340261/ OK340324/ OK340386/ OK340441/ OK340497; *Coryphantha salinensis* (Poselg.) Dicht & A.Lüthy. Mexico, Nuevo León, B. Vázquez 2566 (MEXU): OK340262/ OK340325/ OK340387/ –/ OK340498; *Coryphantha sulcata* (Engelm.) Britton & Rose. Mexico, Nuevo León, S. Arias 2162 (MEXU): OK340263/ OK340326/ OK340388/ OK340442/ OK34049; *Coryphantha tripugionacantha* A.B.Lau. cult. (JB El Charco del Ingenio, AC), ND: FN997162/ –/ –/ –/ –; *Coryphantha vaupeliana* Boed. Mexico, Tamaulipas, B. Vázquez 2564 (MEXU): OK340264/ OK340327/ OK340389/ –/ OK340500; *Coryphantha vogtherriana* Werderm. & Boed. Mexico, San Luis Potosí, B. Vázquez 2538 (MEXU): OK340265/ OK340328/ OK340390/ OK340443/ OK340501; *Coryphantha wedermannii* Boed. Mexico, Coahuila, S. Arias 2104 (MEXU): OK340266/ OK340329/ OK340391/ –/ OK340502; *Coryphantha wohlshlageri* Holzeis. Mexico, San Luis Potosí, B. Vázquez 2587 (MEXU): OK340267/ OK340330/ OK340392/ OK340444/ OK340503; *Cumarinia odorata* (Boed.) Buxb. Mexico, San Luis Potosí, J. Reyes 5940 (cult. JB, UNAM): MK449037/ MK449094/ MK449284/ MK449160/ MK449222; *Echinocactus platyacanthus* Link & Otto. Mexico, Querétaro, S. Arias 1679 (MEXU): OK340223/ OK340286/ –/ OK340409/ –; *Epithelantha spinosior* C. Schmoll. Mexico, Coahuila, S. Arias 1507 (MEXU): MK449039/ MK449096/ MK449286/ MK449162/ MK449224; *Escobaria chihuahuensis* Britton & Rose. Mexico, Chihuahua, S. Arias 1908 (MEXU): OK340271/ OK340334/ OK340395/ OK340448/ OK340506; *Escobaria cubensis* (Britton & Rose) D.R.Hunt. Cuba, Holguin, D. Barrios 24 (HAJB): OL513242/ –/ MK284092/ OL513249/ MK284152; *Escobaria dasyacantha* (Engelm.) Britton & Rose. Mexico, Coahuila, S. Arias 1955 (MEXU): OK340272/ OK340335/ OK340396/ OK340449/ OK340507; *Escobaria laredoi* (Glass & R.A.Foster) N.P.Taylor. Mexico, Coahuila, S. Arias 1951 (MEXU): MK449040/ MK449097/ MK449287/ MK449163/ MK449225; *Escobaria missouriensis* (Sweet) D.R.Hunt. Mexico, Nuevo León, S. Arias 1945 (MEXU): MK449041/ MK449098/ MK449288/ MK449164/ MK449226; *Escobaria tuberculosa* (Engelm.) Britton & Rose. cult. (JB El Charco del Ingenio, AC), ND: FN997185/ –/ –/ –/ –. cult. DES 1986-0619-0101 (ISC), ND: –/ –/ AY545343/ AY545235/ –; *Escobaria vivipara* (Nutt.) Buxb. United States, Nevada, Andrew Salywon 1885 (DES): –/ –/ KC196847/ KC196809/ –. CCDB-23325-H02 (CAN), Canada, Saskatchewan: –/ MG246257/ –/ –/ –. McElroy s.n. (MEXU), ND: FN997563/ –/ –/ –/ –; *Escobaria zilziana* (Boed.) Backeb. cult. s.n. (JB El Charco del Ingenio, AC), ND: –/ –/ AY545344/ AY545236/ –; *Escobaria zilziana* (Boed.) Backeb. cult. s.n. (JB El Charco del Ingenio, AC), ND: FN997193/ –/ –/ –/ –; *Ferocactus alamosanus* (Britton & Rose) Britton &

Rose. Mexico, Sonora, *S. Arias* 1846 (MEXU): OK340273/ OK340336/ OK340397/ OK340450/ OK340508; ***Ferocactus glaucescens*** Britton & Rose. Mexico, Querétaro, *S. Arias* 1701 (MEXU): OK340274/ OK340337/ OK340398/ OK340451/ OK340509; ***Ferocactus recurvus*** (Mill.) Borg. Mexico, Puebla, *S. Arias* 1794 (MEXU): OK340275/ OK340338/ OK340399/ OK340452/ OK340510; ***Kadenicarpus horripilus*** (Lem.) Vázquez-Sánchez. Mexico, Hidalgo, *J.M Chalet* 204 (cult. JB, UNAM): MK449042/ MK449121/ MK449311/ MK449185/ MK449247; ***Lophophora diffusa*** (Croizat) Bravo. Mexico, Querétaro, *S. Arias* 35 (MEXU): MK449046/ MK449100/ MK449290/ MK449166/ MK449228; ***Mammillaria beneckeii*** Ehrenb. cult.: FN997206/ –/ AY545353/ AF267944/ AJ583216; ***Mammillaria heyderi*** Muehlenpf. Mexico, San Luis Potosí, *T. Terrazas* 829 (MEXU): OK340276/ OK340339/ OK340400/ OK340453/ OK340511; ***Mammillaria lenta*** K.Brandege. Mexico, Coahuila, MX *T. Terrazas* 907 (MEXU): MK449047/ MK449102/ MK449292/ MK449167/ MK449230; ***Mammillaria mazatlanensis*** K.Schum.: FN997141/ –/ AY545407/ AY545287/ AJ583226; ***Mammillaria scrippsiana*** (Britton & Rose) Orcutt. Mexico, Nayarit, *S. Arias* 1886 (MEXU): OK340277/ OK340340/ OK340401/ OK340454/ OK340512; ***Mammillaria senilis*** Lodd. ex Salm-Dyck. Mexico, Durango, *S. Arias* 1890 (MEXU): OK340278/ OK340341/ OK340402/ OK340455/ OK340513; ***Mammillaria sphacelata*** Mart.: FN997483/ –/ AY545442/ AY545320/ –; ***Mammillaria uncinata*** Zucc. ex Pfeiff. Mexico, Guanajuato, *S. Arias* 1687 (MEXU): OK340279/ OK340342/ OK340403/ OK340456/ OK340514; ***Mammillaria winterae*** Boed. Mexico, Nuevo León, *S. Arias* 1870 (MEXU): OK340280/ OK340343/ OK340404/ OK340457/ OK340515; ***Mammillaria zephyranthoides*** Scheidw. Mexico, San Luis Potosí, *T. Terrazas* 887 (MEXU): OK340281/ OK340344/ OK340405/ OK340458/ OK340516; ***Mamilloydia candida*** (Scheidw.) Buxb. Mexico, San Luis Potosí, *T. Terrazas* 885 (MEXU): OK340282/ OK340345/ OK340406/ OK340459/ OK340517; ***Neolloydia conoidea*** (DC.) Britton & Rose. Mexico, Nuevo León, *T. Terrazas* 843 (MEXU): MK449048/ MK449103/ MK449293/ MK449168/ MK449231; ***Neolloydia matehualensis*** Backeb. Mexico, San Luis Potosí, *B. Vázquez* 2551 (MEXU): OK340283/ OK340346/ OK340407/ –/ OK340518; ***Obregonia denegrii*** Frič, México, Tamaulipas, *H. Sánchez-Mejorada* 3670 (MEXU): MK449049/ MK449104/ MK449294/ MK449169/ MK449232; ***Ortegocactus macdougalii*** Alexander. Mexico, Oaxaca, *S. Arias* 483 (MEXU): MK449050/ MK449105/ MK449295/ MK449170/ MK449233; ***Pediocactus simpsonii*** (Engelm.) Britton & Rose. cult. s.n. (JB Instituto de Biología, UNAM), ND: MK449019/ MK449106/ MK449296/ MK449171/ MK449234; ***Pelecyphora aselliformis*** Ehrenb. Mexico, San Luis Potosí, *H. Sánchez-Mejorada* 3610 (MEXU): MK449051/ MK449107/ MK449297/ MK449172/ MK449235; ***Pelecyphora strobiliformis*** (Werderm.) Frič & Schelle ex Kreuz. Mexico, Nuevo León, *H. Sánchez-Mejorada* 3844 (MEXU): OK340284 / OK340347/ MK284097/ OK340460/ MK284157; ***Rapicactus mandragora*** (Frič ex A.Berger) Buxb. & Oehme. Mexico, Coahuila, *U. Guzmán* 1445 (MEXU):

MK449052/ MK449126/ MK449316/ MK449190/ MK449252; *Sclerocactus breviamatus* (Engelm.) D.R.Hunt. DES 1989-0315-0101 (DES), ND: -/-/-/ AF267964/-. Mexico, Nuevo León, *T. Hernández* 68 (MEXU): HM041770/ -/-/-/ HM041351; *Sclerocactus glaucus* (K.Schum.) L.D.Benson. Schwabe & al. (2015) (ND), United States Colorado: -/-/-/-/ KJ958760; *Sclerocactus intertextus* (Engelm.) N.P.Taylor. cult. DES 1993-0823-1001 (DES), ND: HM041683/ -/-/-/ HM041417/ HM041263; *Sclerocactus mariposensis* (Hester) N.P. Taylor. Mexico, Coahuila, *D. Aquino* 343 (MEXU): OK340268/ OK340331/ MK284098/ OK340445/ MK284158; *Sclerocactus parviflorus* Clover & Jotter. Schwabe & al. (2015) (ND), United States Colorado: -/-/-/-/ KJ958785; *Sclerocactus scheeri* (Salm-Dyck) N.P.Taylor. Mexico, Coahuila, *T. Terrazas* 903 (MEXU): MK449053/ MK449108/ MK449298/ MK449173/ MK449236; *Sclerocactus spinosior* (Engelm.) D.Woodruff & L.D.Benson. *Hughes* 2 (ISC), ND: -/-/-/ AF267965/ -; *Sclerocactus unguispinus* (Engelm.) N.P.Taylor. Mexico, Durango, *S. Arias* 1902 (MEXU): OK340269/ OK340332/ OK340393/ OK340446/ OK340504; *Sclerocactus warnockii* (L.D.Benson) N.P.Taylor. Mexico, Chihuahua, *S. Arias* 2089 (MEXU): OK340270/ OK340333/ OK340394/ OK340447/ OK340505; *Sclerocactus whipplei* (Engelm. & J.M.Bigelow) Britton & Rose. DES 1993-0925-0103 (DES), ND: -/-/-/ AF267966/ -; *Thelocactus bicolor* (Galeotti ex Pfeiff.) Britton & Rose. Mexico, Coahuila, *T. Terrazas* 895 (MEXU): OK340285/ OK340348/ OK340408/ OK340461/ OK340519.

Appendix 2

Table A1. Insertion-deletion events coded in the alignment for each sequence. Deletion=DEL, insertion=INS, simple sequence repetition (SSR).

Sequence	Event	Sites	Sequence	Event	Sites
<i>matk</i>	INS	675-677	<i>rpl16</i>	DEL	939-957
<i>psbA-trnH</i>	DEL	96-109	<i>rpl16</i>	INS	1079-1082
<i>psbA-trnH</i>	Del	110-154	<i>rpl16</i>	SSR	1148-1150
<i>psbA-trnH</i>	Del	127-138	<i>trnL-F</i>	INS	365-385
<i>psbA-trnH</i>	Del	132-138	<i>trnL-F</i>	DEL	390-609
<i>psbA-trnH</i>	Del	170-179	<i>trnL-F</i>	DEL	345-592
<i>psbA-trnH</i>	INS	383-389	<i>trnL-F</i>	INS	438-442
<i>psbA-trnH</i>	SSR	214-217	<i>trnL-F</i>	DEL	453-521
<i>psbA-trnH</i>	INS	222	<i>trnL-F</i>	SSR	483-484
<i>psbA-trnH</i>	DEL	272-364	<i>trnL-F</i>	SSR	540-553
<i>psbA-trnH</i>	INS	343	<i>trnL-F</i>	DEL	848-855
<i>psbA-trnH</i>	DEL	362-371	<i>trnL-F</i>	Del	853-890
<i>rpl16</i>	DEL	30-44	<i>trnL-F</i>	DEL	871-1117
<i>rpl16</i>	DEL	210-213	<i>trnL-F</i>	INS	894-897
<i>rpl16</i>	DEL	278-280	<i>trnL-F</i>	DEL	1049-1057
<i>rpl16</i>	INS	550-567	<i>trnL-F</i>	SSR	1151-1152
<i>rpl16</i>	SSR	733-738	<i>trnL-F</i>	SSR	1205-1209

Appendix 3

A

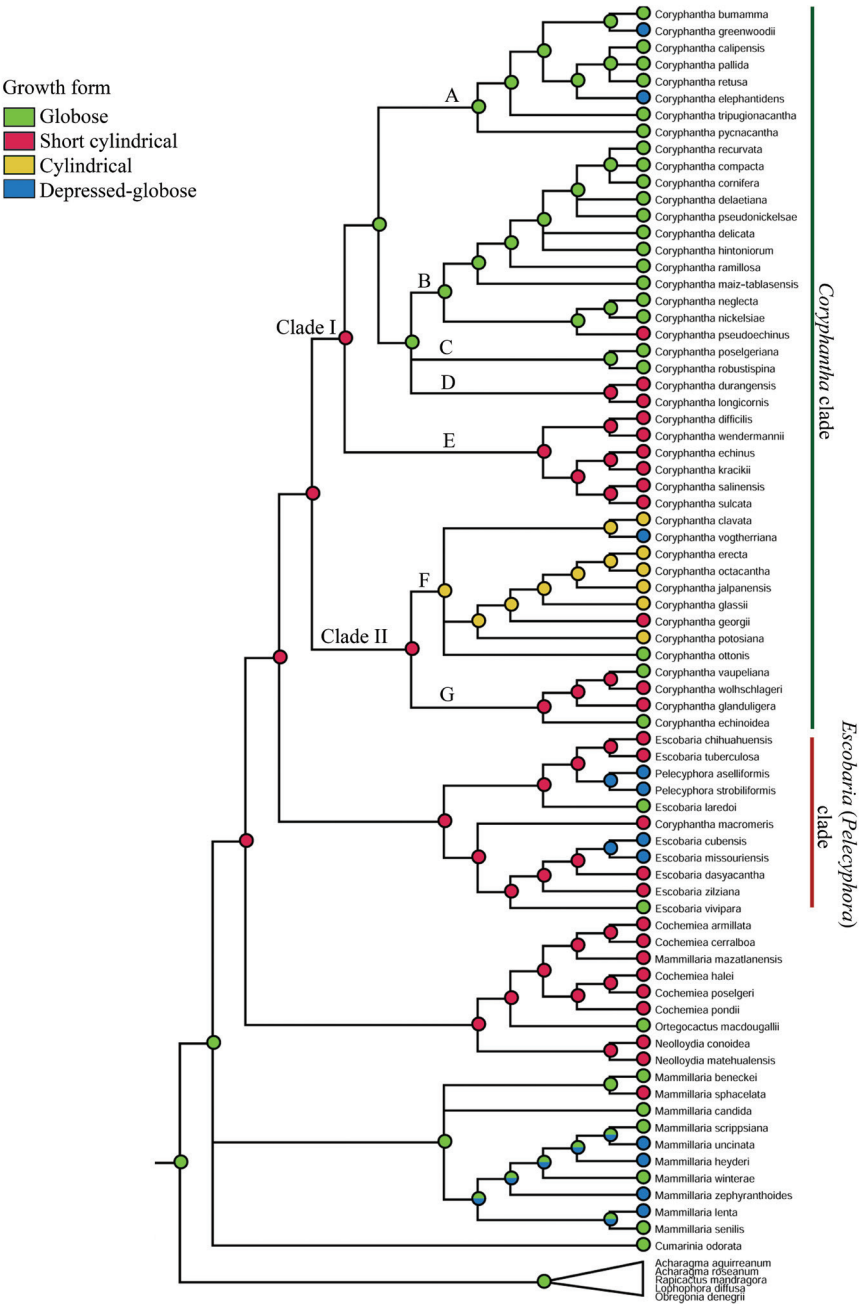


Figure A1. Ancestral states reconstruction in *Coryphantha* and related genera **A** growth form **B** groove on the tubercle in mature plant **C** extrafloral glands at or near the axil **D** position of the flowers **E** margin of the outer tepals **F** color of the mature fruit **G** type of cortex **H** multicellular sculpture of the lateral side of the seed.

B

Groove on the tubercle in mature plant

Absence

Complete

Incomplete

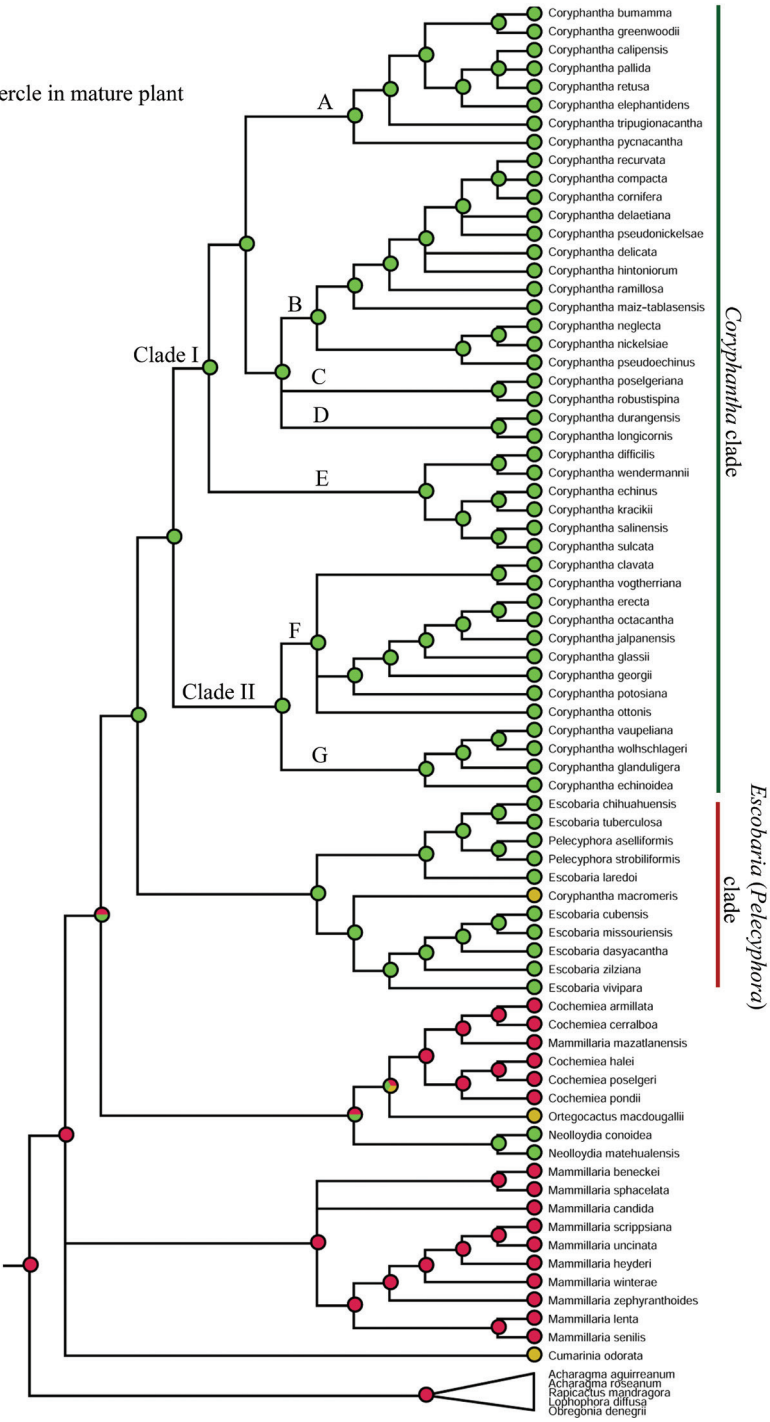


Figure A1. Continued.

C

Extrafloral glands at or near the axil

- Absence
- Turgid throughout the year
- Turgid only at flowering season

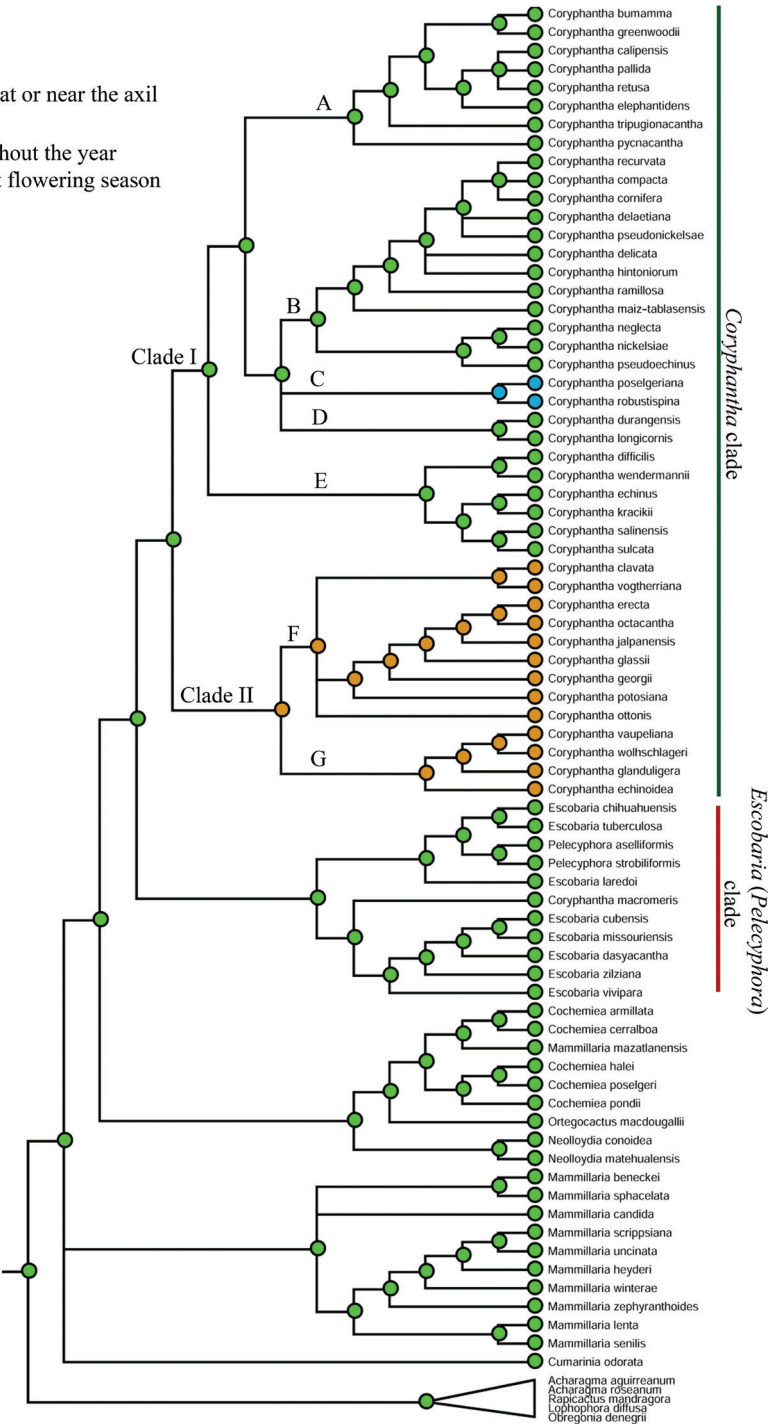


Figure A1. Continued.

D

Position of the flowers
■ Apical
■ Around the apex

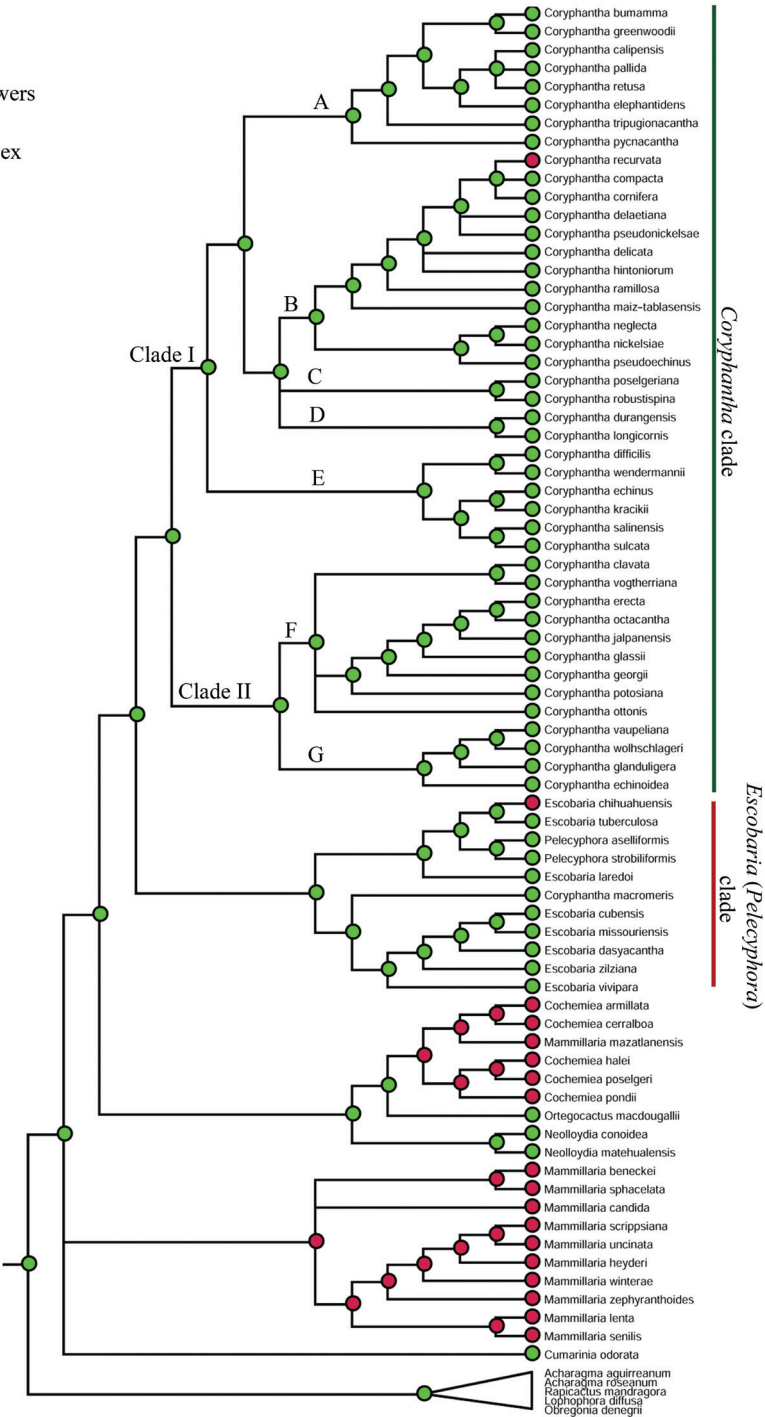


Figure A1. Continued.

E

Margin of the outer tepals
Fimbriate
Entire

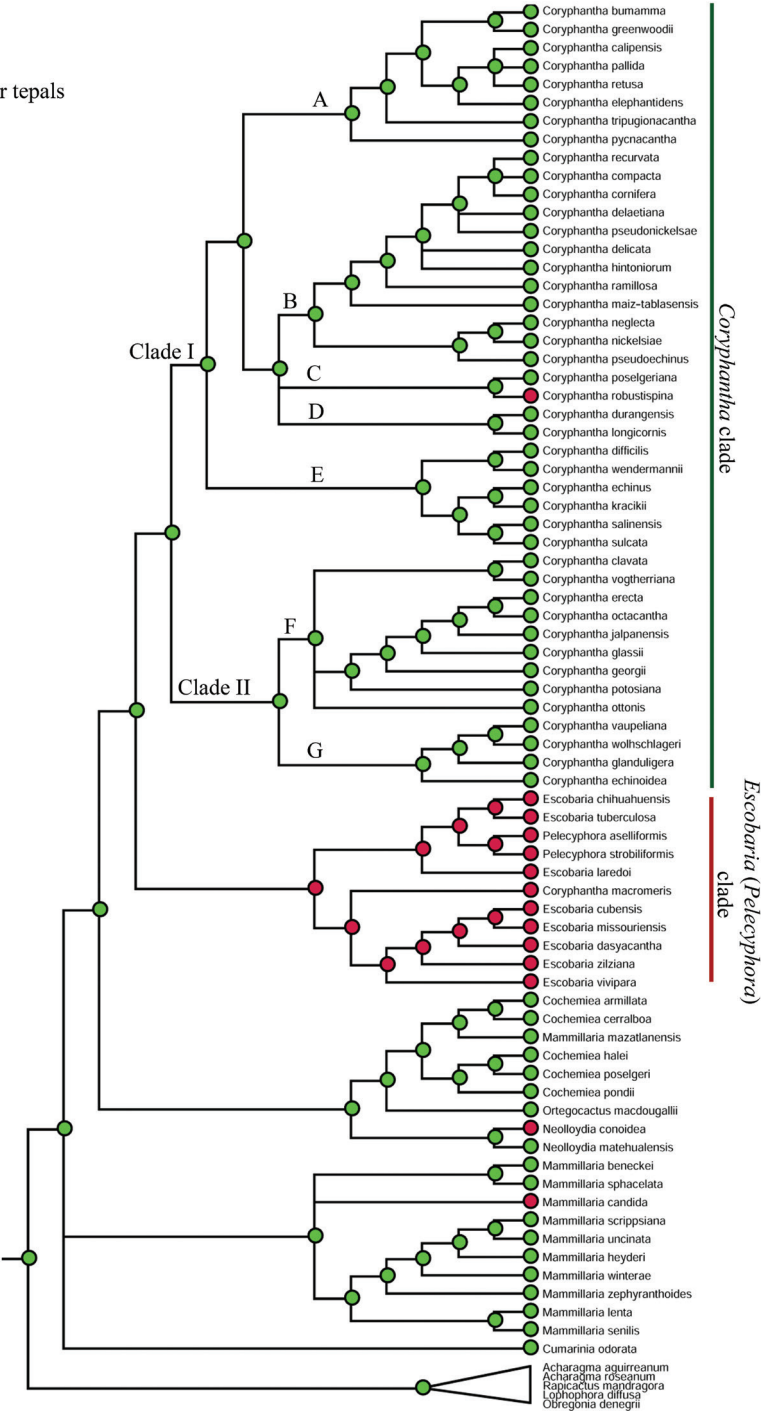





Figure A1. Continued.

Color of the mature fruit

	Red-pink
	Green
	Yellow

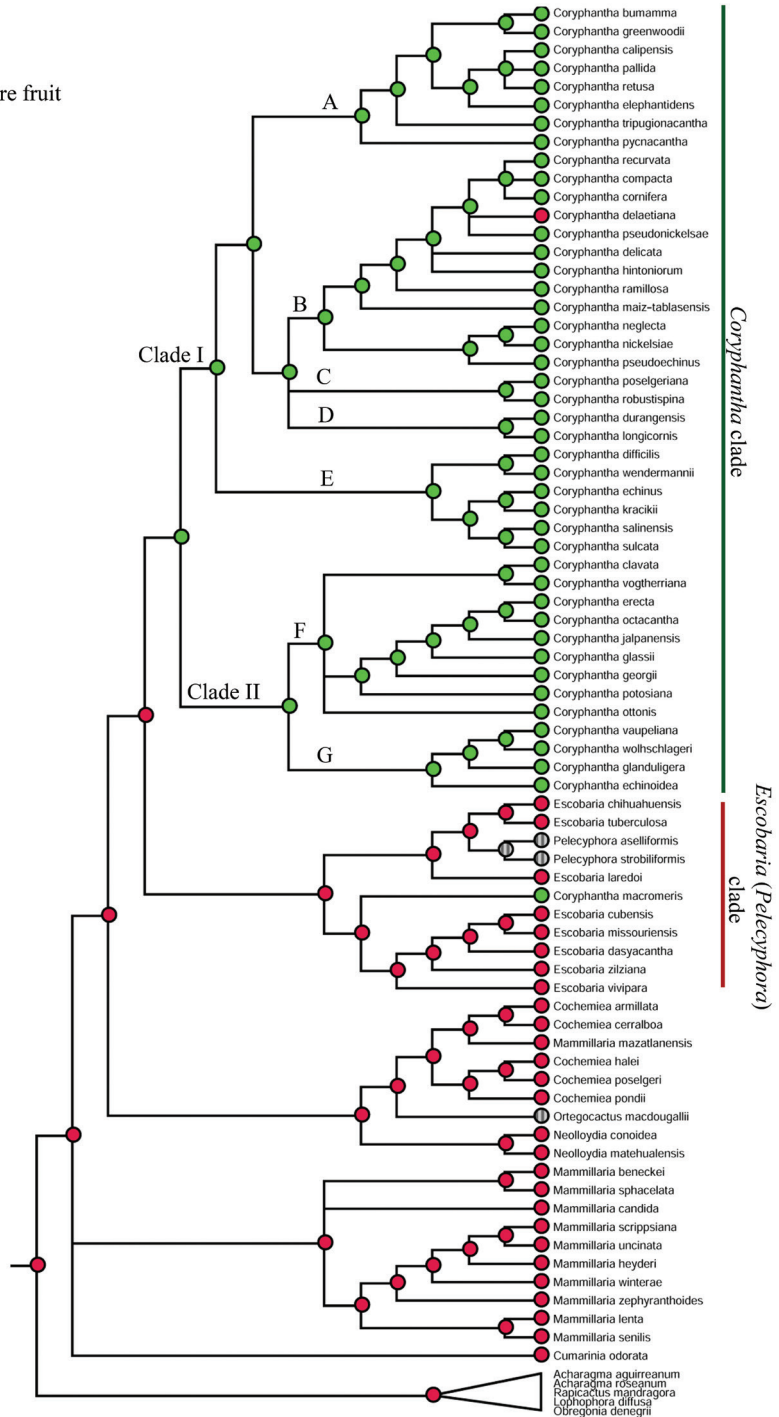


Figure A1. Continued.

G

Type of cortex

- Watery
- Mucilaginous
- Laticiferous

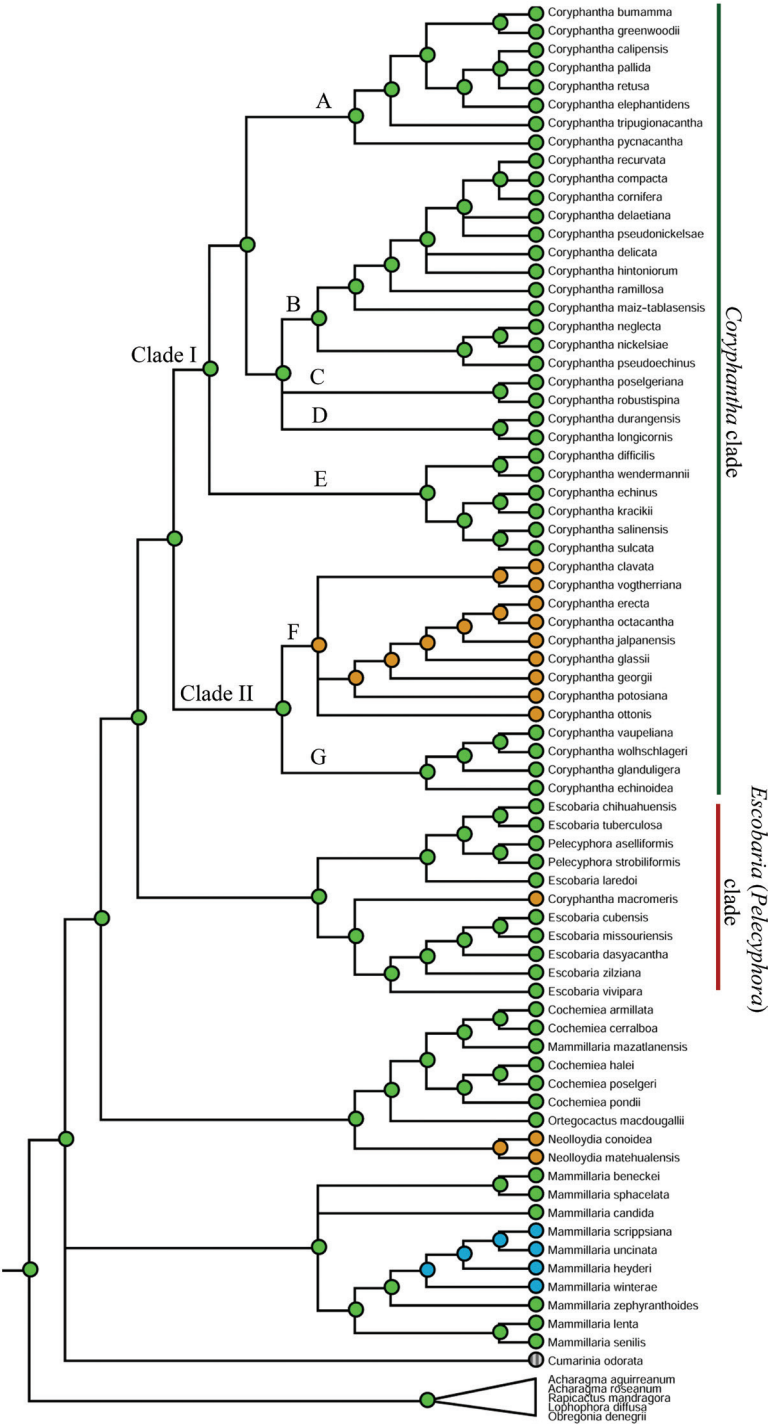





Figure A1. Continued.

 Flat
 Tuberculated
 Pitted

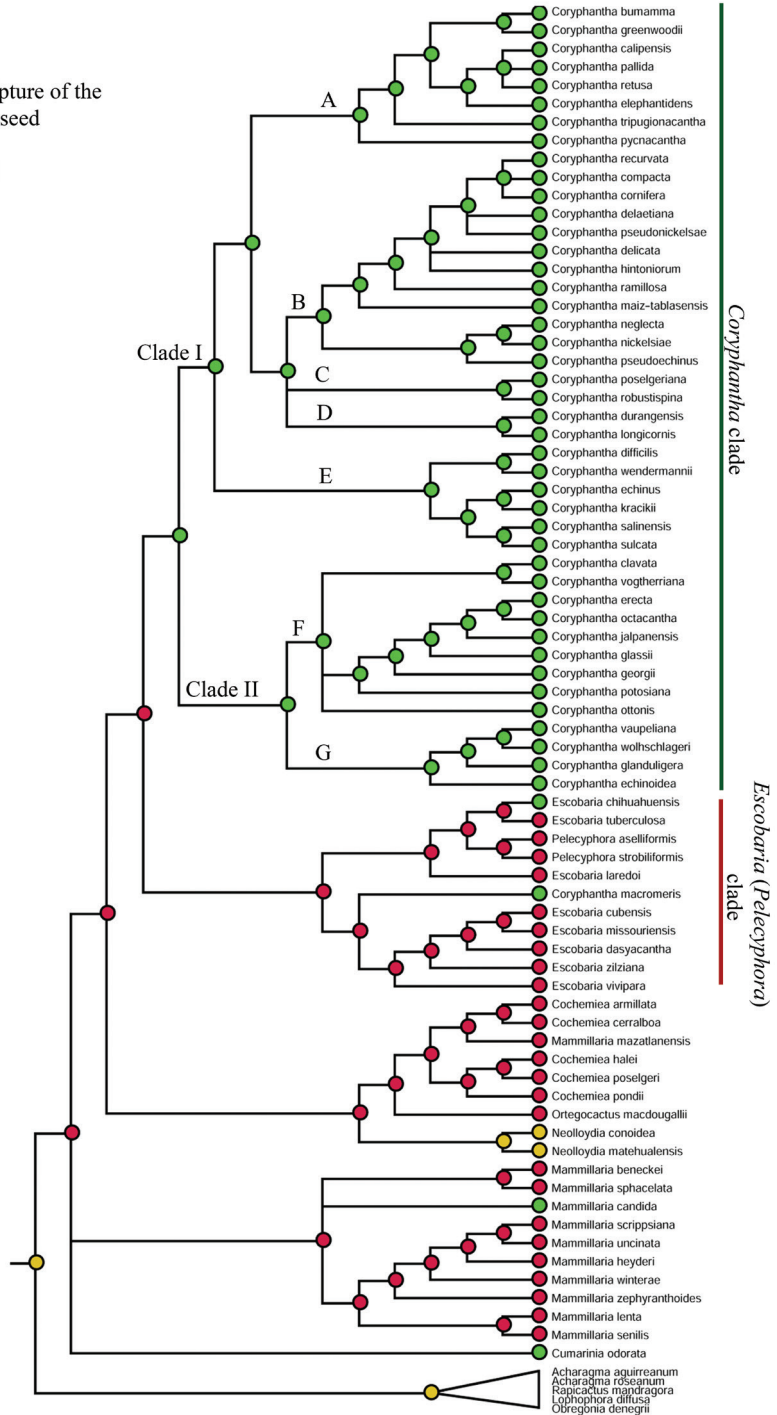


Figure A1. Continued.

Achnantheidium bratanense sp. nov. (Bacillariophyceae, Achnanthidiaceae), a new diatom from the Lake Bratan (Bali, Indonesia)

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Abstract

A new species, *Achnantheidium bratanense*, is described from Lake Bratan, located on the island of Bali (Indonesia). The morphology of this species was analyzed with light (LM) and scanning electron microscopy (SEM). *A. bratanense* is characterized by linear-elliptic to nearly elliptic valves with convex margins and rounded, broadly subcapitate apices. The striae of this species are hardly discernable under LM; they are weakly radiate throughout the valve and composed of one to four large transapically elongated areolae of different length and shape. The most similar taxon to *A. bratanense* is *A. macrocephalum*, a species described from Sumatra, another Indonesian island. The differences of *A. bratanense* from similar taxa are discussed.

Keywords

Indonesia, monoraphid diatoms, morphology, new species

Introduction

The genus *Achnantheidium* Kützing, 1844 is one of the largest genus among monoraphid diatoms. Although it had been described as a separate genus it was considered as a subgenus of *Achnanthes* Bory, from 1822 until the 90s (Round et al. 1990; Round and Bukhtiyarova 1996). Currently, *Achnantheidium* includes, according to different estimates, between 139 (Guiry and Guiry 2017) and nearly 200 species

(Kociolek et al. 2021a). Revision of the genus continues up to now. Recently, two genera, namely *Gogorevia* (Kulikovskiy et al. 2020b) and *Gomphothidium* (Kociolek et al. 2021b) have been segregated from *Achnantheidium*.

The *Achnantheidium* taxa are common in different climatic zones all over the world (e.g., Ponader and Potapova 2007; Wojtal et al. 2011; Novais et al. 2015; Karthick et al. 2017; Marquardt et al. 2017; Krahm et al. 2018; Yu et al. 2019). However, their identification is challenging because of the small size of these diatoms, often requiring examination using electron microscopy, and significant variability of diagnostic features (Ponader and Potapova 2007; Hlúbíková et al. 2011).

The number of publications dealing with freshwater diatoms from Indonesia is still rather low. The most comprehensive treatment was made by Hustedt (1937, 1942). Some of his new taxa have been re-examined (e.g., Hamsher et al. 2014; Kapustin et al. 2017, 2020; Kapustin and Kulikovskiy 2018; Wetzal et al. 2019; Kulikovskiy et al. 2020a). Also, a lot of new diatom species were described from Indonesian freshwaters over the last two decades (Bramburger et al. 2006; Kociolek et al. 2018; Kapustin et al. 2019, 2021; Kulikovskiy et al. 2019; Rybak et al. 2019), including two new *Achnantheidium* species (Tseplik et al. 2021a,b). The aim of this paper is to describe a new monoraphid species, *Achnantheidium bratanense* sp. nov., from Lake Bratan located on the island of Bali, Indonesia.

Materials and methods

A benthic sample containing *Achnantheidium* was collected from a volcanic Lake Bratan on 14 November 2010 (08°16.579'S, 115°09.985'E). For general characteristics of this lake see Green et al. (1978). Environmental variables were measured with a Hanna multiparameter probe meter (HANNA HI98128).

The sample was boiled in concentrated hydrogen peroxide (~37%) to dissolve the organic matter. It was then washed with deionized water four times at 12 h intervals. After decanting and filling with deionized water up to 100 ml, the suspension was spread on to coverslips and left to dry at room temperature. Permanent diatom slides were mounted in Naphrax. Light microscopic (LM) observations were performed with a Zeiss Scope A1 microscope equipped with an oil immersion objective (100×/n.a.1.4, differential interference contrast [DIC]) and Zeiss Axio-Cam ERc 5s camera. Valve ultrastructure was examined with a JSM-6510LV scanning electron microscope (Papanin Institute for Biology of Inland Waters RAS, Borok, Russia), operated at 10 kV and 11 mm distance. For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminum stubs after air-drying. The stubs were sputter coated with 50 nm of gold.

The original sample preserved with Lugol's solution, as well as cleaned material preserved with 96% ethanol, are housed at the Laboratory of Molecular Systematics of Aquatic Plants, K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences (Moscow, Russia).

Results

Achnanthidium bratanense Kapustin, Glushchenko & Kulikovskiy, sp. nov.

Figures 1, 2

Description. *LM* (Fig. 1A–T). Valves linear-elliptic to nearly elliptic with convex margins and rounded, broadly subcapitate apices. Frustules rectangular in girdle view and not bent (Fig. 1T). Length 5.0–8.7 μm , breadth 2.7–3.2 μm ($n=32$). In raphe valves axial area narrow, linear, slightly widening at center. Central area very small in raphe valves, outlined by shortened striae; central area in rapheless valves rhomboid (Fig. 1J). Raphe straight, filiform. In rapheless valves axial area expanded widening towards rhombic central area (Fig. 1E). Striae hardly discernable in LM, weakly radiate (Fig. 1A, H, K, M). Areolae indistinct in LM.

SEM (Fig. 2). Externally, raphe straight, filiform with drop-shaped proximal and distal raphe endings (Fig. 2A, B). Internally, proximal raphe endings deflected in opposite directions, distal raphe endings terminating in helictoglossae (Fig. 2E, F). Striae weakly radiate throughout the valve, 41–44 in 10 μm , and composed of one to four large transapically elongated areolae of different length and shape (from slit-like to irregularly rectangular). Areolae absent along valve margins; mantle with a single row of slit-like to almost rectangular areolae. Internally, areolae occluded by a hymen (Fig. 2E, F).

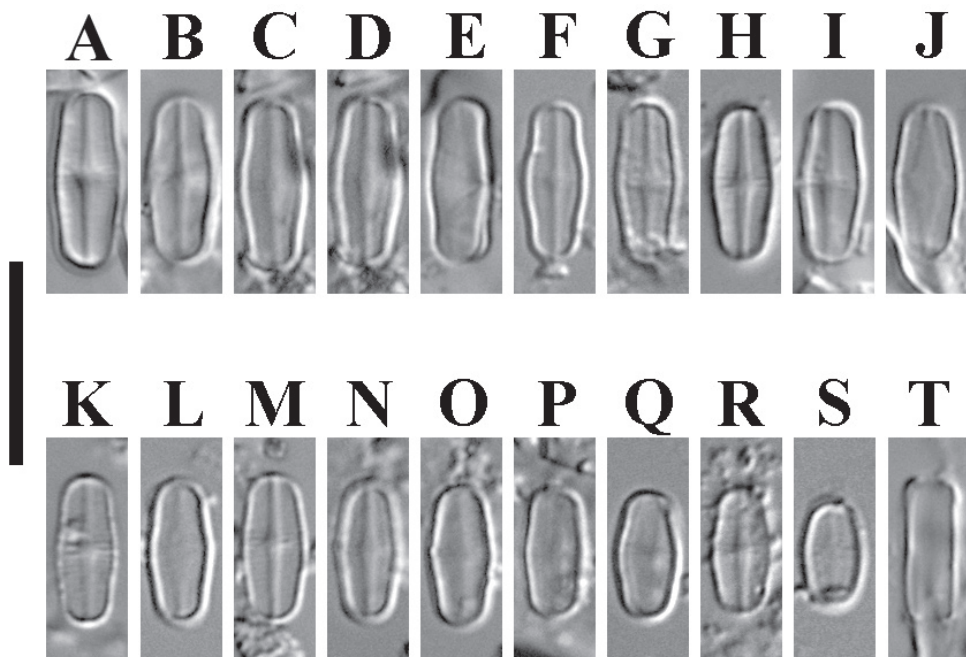


Figure 1. A–T *Achnanthidium bratanense* sp. nov. (LM). A–S size diminution series showing variation in valve outline A holotype specimen A–D, F–I, K, M, N, R raphe valves E, J, L, O–Q, S rapheless valves T frustule in girdle view. Scale bar: 10 μm .

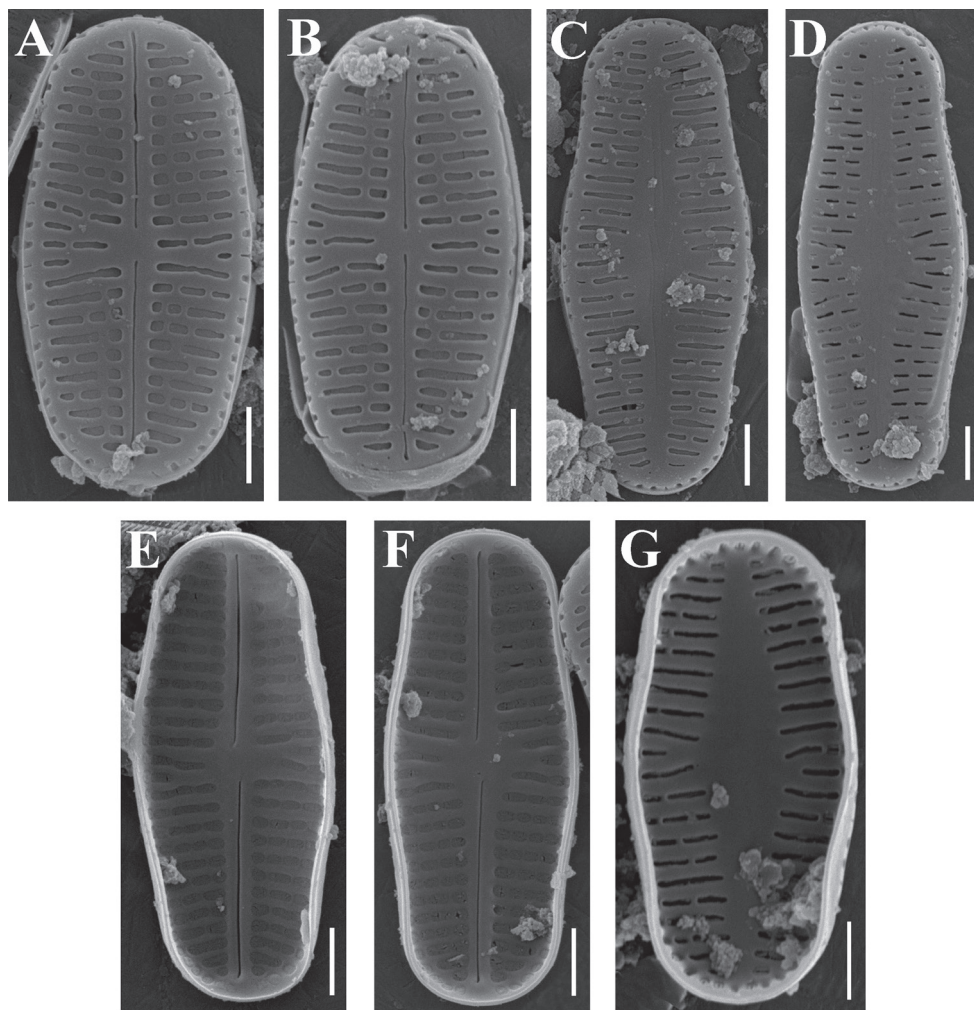


Figure 2. A–G *Achmanthidium bratanense* sp. nov. (SEM). A, B raphe valve, external view C, D rapheless valve, external view E, F raphe valve, internal view G rapheless valve, internal view. Scale bar: 1 μ m.

Holotype (here designated): permanent slide No. MHA 01125, deposited at the Main Botanical Garden, Russian Academy of Sciences (MHA). Fig. 1A illustrates the holotype.

Isotype (here designated): permanent slide No. 01125a, deposited in collection of Maxim Kulikovskiy, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences.

Type locality. Indonesia, Island of Bali, Lake Bratan, 08°16.579'S, 115°09.985'E, leg. I.I. Ivanov on 14 November 2010.

Etymology. The specific epithet refers to the type locality, Lake Bratan.

Ecology. *Achmanthidium bratanense* together with *Gogorevia rinatii* were the most abundant species in the sample. Rarely single frustules of *Planothidium* sp.,

Stauroneis sp., *Cymbella* sp. and other diatoms were encountered. During sampling the temperature was recorded as 25.7 °C, pH as 7.82, and conductivity as 22 $\mu\text{S}\cdot\text{cm}^{-1}$.

Distribution. So far, this species is known from its type locality only.

Discussion

Our new species is closely related to *Achnanthidium macrocephalum* (Hustedt) Round & Bukhtiyarova, 1996. This taxon was described by Hustedt (1937) as *Achnanthes minutissima* var. *macrocephala* from Sumatra. Recently, Wetzel et al. (2019) have re-examined Hustedt's type material using both LM and SEM. Although the length and breadth of both species overlapped, *A. macrocephalum* is generally larger than *A. bratanense* (Table 1). However, the larger valves of *A. macrocephalum* have distinctly capitate apices. Despite the high abundance of *A. bratanense* we could not find such valves with capitate apices. Additionally, both taxa differ in striae density: *A. macrocephalum* has ca. 38 striae in 10 μm (Wetzel et al. 2019), whereas *A. bratanense* has 41–44 striae in 10 μm (see Table 1). In contrast to *A. macrocephalum*, the striae of *A. bratanense* is composed of 1–4 transapically elongated areolae. In *A. macrocephalum* the striae composed of 1 (smaller valves) or two (rarely 3) areolae (Wetzel et al. 2019). Also *A. bratanense* has weakly radiate striae throughout the valve whereas in *A. macrocephalum* the striae become parallel towards the valve ends.

It should be noted that Hustedt (1942) reported *Achnanthes minutissima* var. *macrocephala* from Lake Bratan on Bali and suggested that it might be widely distributed in the Indo-Malayan region. Unfortunately, he gave neither description nor images to support the written statement. It is very likely he actually observed *A. bratanense* instead of *A. macrocephalum*. Wetzel et al. (2019) supposed that Hustedt (1937) included in his description of *A. macrocephalum* two morphotypes.

Also *A. bratanense* is similar to several other taxa including *A. rosenstockii* (Lange-Bertalot) Lange-Bertalot var. *rosenstockii*, 2004, *A. rosenstockii* var. *inareolatum* Lange-

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

	<i>A. bratanense</i>	<i>A. macrocephalum</i>	<i>A. rosenstockii</i> var. <i>rosenstockii</i>	<i>A. rosenstockii</i> var. <i>inareolatum</i>	<i>Kolbesia sichuanensis</i>
Valve length, μm	5.0–8.7	7–12	6–14	9.6–15.1	10.8–14.1
Valve width, μm	2.7–3.2	2.5–3.2	3–4	4.2–5.1	3.2–3.7
Valve outline	linear-elliptic	linear-elliptic with convex margins	linear-lanceolate	linear-lanceolate	linear-lanceolate
Valve apices	subcapitate	rounded, broadly capitate	subcapitate	subcapitate	broadly capitate
Striae density	41–44	38	27–32	20	22–26
Number of areolae per stria	1–4	1–2(3)	2–4	1	1
Reference	This study	Wetzel et al. 2019	Krammer and Lange-Bertalot 2004	Krammer and Lange-Bertalot 2004; Yu et al. 2019	Yu et al. 2019

Bertalot, 2004, and *Kolbesia sichuanensis* P. Yu, Q-M. You & Q-X Wang, 2019 (Table 1). *A. rosenstockii* var. *rosenstockii* is slightly wider than *A. bratanense* and the stria density is lesser (27–32 in 10 μm vs. 41–44 in 10 μm). *A. rosenstockii* var. *inareolatum* differs from the type variety in having striae composed of a single macroareola. Probably, this taxon will be better to place in the genus *Karayevia* Round & Bukhtiyarova emend. Bukhtiyarova, 2006. From both *A. rosenstockii* var. *inareolatum* and *Kolbesia sichuanensis*, *A. bratanense* differs in stria structure (number of areolae per stria) and stria density. Also, these taxa are significantly larger than *A. bratanense* (see Table 1).

Traditionally, three morphological groups are recognized within *Achnantheidium* (e.g. Novais et al. 2015; Karthick et al. 2017; Krahm et al. 2018; Yu et al. 2019): 1) *A. minutissimum* complex which is characterized by having straight external distal raphe ends, and striae density that increase towards the apex; 2) *A. pyrenaicum* complex which is characterized by having external distal raphe ends that deflect or hook to one side of the valve, and 3) *A. exiguum* complex have external distal raphe ends curved in opposite directions. Recently, the latter complex has been segregated into a new genus, *Gogorevia* (Kulikovskiy et al. 2020a). Although, *A. bratanense*, *A. macrocephalum* and *A. rosenstockii* can be placed in *A. minutissimum* complex based on the raphe structure, they have completely different striae structure and represent a separate morphological group. Interestingly, Pinseel et al. (2017) revealed 12 distinct lineages within *A. minutissimum* complex and one of them was described as the new species, *A. digitatum* Pinseel et al., 2017. Recently, Tseplik et al. (2021b) described from the ancient Lake Matano (island of Sulawesi, Indonesia) the new species, *A. gladius* Tseplik et al., 2021b, which was closely related to the latter taxon. Thus, further detailed study of the pore apparatus ultrastructure as well as molecular studies of *A. bratanense* and allied taxa will help to better understand the taxonomic status and phylogenetic placement of this morphological group.

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References

- Bramburger AJ, Haffner GD, Hamilton PB, Hinz F, Hehanussa PE (2006) An examination of species within the genus *Surirella* from the Malili lakes, Sulawesi Island, Indonesia, with descriptions of 11 new taxa. *Diatom Research* 21(1): 1–56. <https://doi.org/10.1080/0269249X.2006.9705650>

- Bukhtiyarova LN (2006) Additional data on the diatom genus *Karayevia* and a proposal to reject the genus *Kolbesia*. Nova Hedwigia. Beiheft 130: 85–96.
- Green J, Corbet SA, Watts E, Lan OB (1978) Ecological studies on Indonesian lakes. The montane lakes of Bali. Journal of Zoology 186(1): 15–38. <https://doi.org/10.1111/j.1469-7998.1978.tb03354.x>
- Guiry MD, Guiry GM (2017) *AlgaeBase*. https://www.algaebase.org/search/genus/detail/?genus_id=43670 [Accessed 2022–01–03]
- Hamsher SE, Graeff CL, Stepanek JG, Kociolek JP (2014) Variation in valve and girdle band morphology in freshwater *Denticula* (Bacillariophyceae) species: Implications for the systematic position of the genus including the description of *Tetralunata* gen. nov. (Epithemiaceae, Rhopalodiales). Plant Ecology and Evolution 147: 346–365. <https://doi.org/10.5091/plecevo.2014.990>
- Hlúbíková D, Ector L, Hoffmann L (2011) Examination of the type material of some diatom species related to *Achnanthidium minutissimum* (Kütz.) Czarn. (Bacillariophyceae). Algological Studies 136/137: 19–43. <https://doi.org/10.1127/1864-1318/2011/0136-0019>
- Hustedt F (1937) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. Systematischer Teil I. Archiv für Hydrobiologie (Supplement 15): 131–177.
- Hustedt F (1942) Süßwasser-Diatomeen des indomalayischen Archipels und der Hawaii – Inseln. Internationale Revue der Gesamten Hydrobiologie und Hydrographie 42(1–3): 1–252. <https://doi.org/10.1002/iroh.19420420102>
- Kapustin DA, Kulikovskiy MS (2018) Transfer of *Stenopterobia* and *Surirella* taxa (Bacillariophyceae) described from the insular Southeast Asia to the genus *Iconella*. Nova Hedwigia. Beiheft 147: 237–245. <https://doi.org/10.1127/nova-suppl/2018/019>
- Kapustin DA, Kulikovskiy MS, Kociolek JP (2017) *Celebesia* gen. nov., a new cymbelloid diatom genus from the ancient Lake Matano (Sulawesi Island, Indonesia). Nova Hedwigia. Beiheft 146: 147–155. <https://doi.org/10.1127/1438-9134/2017/147>
- Kapustin DA, Kociolek JP, Glushchenko AM, Kulikovskiy MS (2019) Four new species of *Cymbella* (Bacillariophyta) from the ancient Malili Lakes (Sulawesi Island, Indonesia). Botanicheskii Zhurnal 104(5): 766–780. <https://doi.org/10.1134/S0006813619050065>
- Kapustin DA, Kociolek JP, Glushchenko AM, Kulikovskiy MS (2020) A rediscovery of *Cymbella mirabilis* Hustedt, a rare endemic diatom, and description of *Alveocymba* gen. nov. Diatom Research 35(3): 281–287. <https://doi.org/10.1080/0269249X.2020.1772888>
- Kapustin DA, Glushchenko AM, Kociolek JP, Kulikovskiy MS (2021) *Encyonopsis indonesica* sp. nov. (Bacillariophyceae, Cymbellales), a new diatom from the ancient lake Matano (Sulawesi, Indonesia). PhytoKeys 175: 1–11. <https://doi.org/10.3897/phytokeys.175.61044>
- Karthick B, Taylor JC, Hamilton PB (2017) Two new species of *Achnanthidium* Kützting (Bacillariophyceae) from Kolli Hills, Eastern Ghats, India. Fottea 17(1): 65–77. <https://doi.org/10.5507/fot.2016.020>
- Kociolek JP, Kapustin DA, Kulikovskiy MS (2018) A new, large species of *Gomphonema* Ehrenberg from ancient Lake Matano, Indonesia. Diatom Research 33(2): 241–250. <https://doi.org/10.1080/0269249X.2018.1513868>
- Kociolek JP, Blanco S, Coste M, Ector L, Liu Y, Karthick B, Kulikovskiy M, Lundholm N, Ludwig T, Potapova M, Rimet F, Sabbe K, Sala S, Sar E, Taylor J, Van de Vijver B,

- Wetzel CE, Williams DM, Witkowski A, Witkowski J (2021a) DiatomBase. *Achnantheidium* F.T. Kützing, 1844. <http://www.diatombase.org/aphia.php?p=taxdetails&cid=163531> [Accessed on 2021–10–09]
- Kociolek JP, You Q, Yu P, Li Y, Wang Y, Lowe R, Wang Q (2021b) Description of *Gomphothidium* gen. nov., with light and scanning electron microscopy: A new freshwater monoraphid diatom genus from Asia. *Fottea* 21(1): 1–7. <https://doi.org/10.5507/fot.2020.011>
- Krahn KJ, Wetzel CE, Ector L, Schwalb A (2018) *Achnantheidium neotropicum* sp. nov., a new freshwater diatom from Lake Apastepeque in El Salvador (Central America). *Phytotaxa* 382(1): 89–101. <https://doi.org/10.11646/phytotaxa.382.1.4>
- Krammer K, Lange-Bertalot H (2004) Bacillariophyceae 4. Teil: Achnanthaceae. Kritische Ergänzungen zu *Achnanthes* s. l., *Navicula* s. str., *Gomphonema*. Gesamtliteraturverzeichnis Teil 1–4. In: Ettl H, Gärtner G, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa Band 2/4. Heidelberg, Elsevier GmbH, Spektrum Akademischer Verlag, 1–468.
- Kulikovskiy M, Maltsev Y, Andreeva S, Glushchenko A, Gusev E, Podunay A, Ludwig TV, Tusset E, Kociolek JP (2019) Description of a new diatom genus *Dorofeyukea* gen. nov. with remarks on phylogeny of the family Stauroneidaceae. *Journal of Phycology* 55(1): 173–185. <https://doi.org/10.1111/jpy.12810>
- Kulikovskiy M, Kapustin D, Glushchenko A, Sidelev S, Maltsev Y, Gusev E, Kezlya E, Shkurina N, Kuznetsova I, Kociolek P (2020a) Morphological and molecular investigation of *Gomphonema longissimum* and related taxa from Malili lakes (Indonesia) with comments on diatom evolution in ancient lakes. *European Journal of Phycology* 55(2): 147–161. <https://doi.org/10.1080/09670262.2019.1664771>
- Kulikovskiy M, Maltsev Y, Glushchenko A, Kuznetsova I, Kapustin D, Gusev E, Lange-Bertalot H, Genkal S, Kociolek JP (2020b) *Gogorevia*, a new monoraphid diatom genus for *Achnanthes exigua* and allied taxa (Achnanthidiaceae) described on the basis of an integrated molecular and morphological approach. *Journal of Phycology* 56(6): 1601–1613. <https://doi.org/10.1111/jpy.13064>
- Marquardt GC, Costa LF, Bicudo DC, Bicudo CEM, Blanco S, Wetzel CE, Ector L (2017) Type analysis of *Achnantheidium minutissimum* and *A. catenatum* and description of *A. tropicocatenatum* sp. nov. (Bacillariophyta), a common species in Brazilian reservoirs. *Plant Ecology and Evolution* 150(3): 313–330. <https://doi.org/10.5091/plecevo.2017.1325>
- Novais MH, Jüttner I, Van de Vijver B, Morais MM, Hoffmann L, Ector L (2015) Morphological variability within the *Achnantheidium minutissimum* species complex (Bacillariophyta): Comparison between the type material of *Achnanthes minutissima* and related taxa, and new freshwater *Achnantheidium* species from Portugal. *Phytotaxa* 224(2): 101–139. <https://doi.org/10.11646/phytotaxa.224.2.1>
- Pinseel E, Vanormelingen P, Hamilton PB, Vyverman W, Van de Vijver B, Kopalova K (2017) Molecular and morphological characterization of the *Achnantheidium minutissimum* complex (Bacillariophyta) in Petuniabukta (Spitsbergen, High Arctic) including the description of *A. digitatum* sp. nov. *European Journal of Phycology* 52(3): 264–280. <https://doi.org/10.1080/09670262.2017.1283540>

- Ponader KC, Potapova MG (2007) Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): Ecology, distribution and taxonomic notes. *Limnologica* 37(3): 227–241. <https://doi.org/10.1016/j.limno.2007.01.004>
- Round FE, Bukhtiyarova L (1996) Four new genera based on *Achnanthes* (*Achnanthidium*) together with a re-definition of *Achnanthidium*. *Diatom Research* 11(2): 345–361. <https://doi.org/10.1080/0269249X.1996.9705389>
- Round FE, Crawford RM, Mann DG (1990) The diatoms: biology and morphology of the genera. Cambridge University Press, 1–747.
- Rybak M, Solak CN, Noga T, Glushchenko A, Williams DM, Kulikovskiy M (2019) *Nupela brevistriata* sp. nov. – a new, terrestrial diatom species from Southeast Asia. *Diatom Research* 34(4): 251–258. <https://doi.org/10.1080/0269249X.2019.1698467>
- Tsepplik ND, Maltsev YI, Glushchenko AM, Kuznetsova IV, Genkal SI, Kociolek JP, Kulikovskiy MS (2021a) *Achnanthidium tineae* sp. nov. – a new monoraphid diatom (Bacillariophyceae) species, described on the basis of molecular and morphological approaches. *PhytoKeys* 174: 147–163. <https://doi.org/10.3897/phytokeys.174.60337>
- Tsepplik ND, Maltsev YI, Glushchenko AM, Kuznetsova IV, Genkal SI, Gusev ES, Kulikovskiy MS (2021b) *Achnanthidium gladius* sp. nov. (Bacillariophyceae) – a new monoraphid diatom species from Indonesia. *PhytoKeys* 187: 129–140. <https://doi.org/10.3897/phytokeys.187.73913>
- Wetzel CE, Jüttner I, Gurung S, Ector L (2019) Analysis of the type material of *Achnanthes minutissima* var. *macrocephala* (Bacillariophyta) and description of two new small capitate *Achnanthidium* species from Europe and the Himalaya. *Plant Ecology and Evolution* 152(2): 340–350. <https://doi.org/10.5091/plecevo.2019.1628>
- Wojtal AZ, Ector L, Van de Vyver B, Morales EA, Blanco S, Piatek J, Smieja A (2011) The *Achnanthidium minutissimum* complex (Bacillariophyceae) in southern Poland. *Algological Studies* 136/137: 211–238. <https://doi.org/10.1127/1864-1318/2011/0136-0211>
- Yu P, You Q-M, Pang W-T, Cao Y, Wang Q-X (2019) Five new Achnanthidiaceae species (Bacillariophyta) from Jiuzhai Valley, Sichuan Province, Southwestern China. *Phytotaxa* 405(3): 147–170. <https://doi.org/10.11646/phytotaxa.405.3.5>

Molecular and morphological evidence for a new species of *Pogostemon* (Lamiaceae) from Hainan Island, China

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Abstract

Pogostemon hainanensis, a new species of Lamiaceae from Hainan Island, China, is described. The phylogenetic position of the new species within *Pogostemon* was investigated based on analyses of the nuclear ribosomal internal transcribed spacer (nrITS) and five plastid markers (viz. *matK*, *psbA-trnH*, *rbcL*, *rsp16*, *trnL-F*). The results show that *P. hainanensis* is supported to be a member of subgenus *Pogostemon* and is sister to *P. parviflorus*, a species widely distributed from Eastern Himalaya, through the Indo-China peninsula to China. Morphologically, the new species can be distinguished from all the other taxa of subgenus *Pogostemon* in having long petioles usually 4.5–11.5 cm in length, and the calyx teeth 2/3 to subequal as long as the calyx tube. The new species differs from *P. parviflorus* further by its obviously double serrate leaf margin, spikes of inflorescence usually 2.5–8.0 cm long, calyx 4–5 mm long and corolla 6–7 mm long.

Keywords

China, Lamiaceae, *Pogostemon*, Pogostemoneae, taxonomy

Introduction

Pogostemon Desf. is the largest genus in Pogostemoneae, Lamioideae, Lamiaceae (Zhao et al. 2021), and it is circumscribed recently in its broad sense that includes *Pogostemon* s.s. and *Dysophylla* Bl. (Yao et al. 2016; Zhao et al. 2021). The genus consists of more than 80 species distributed mainly in the tropical and subtropical Asia, and with five species endemic to Africa (Bhatti and Ingrouille 1997; Yao et al. 2015). The highest species diversity of the genus is found in the Indian subcontinent (Bhatti and Ingrouille 1997). Morphologically, the genus can be easily distinguished from other Pogostemoneae members by the presence of exerted stamens bearing moniliform hairs (Bhatti and Ingrouille 1997; Yao et al. 2015). Based on morphological and molecular evidence, the genus was divided into two subgenera, viz. subgenus *Pogostemon* and subgenus *Dysophyllus* (Bl.) Bhatti & Ingr. ex. G. Yao, Y.F. Deng & X.J. Ge (Yao et al. 2016). The former subgenus is characterized as perennial subshrubs, shrubs or rarely perennial herbs, spikes of inflorescence with more than two lateral branches, bracts and bracteoles large and usually broad-ovate, ovate or rarely lanceolate; while the latter subgenus is characterized as annual herbs or rarely perennial herbs or subshrubs, inflorescence a single terminal spike or rarely with two lateral branches, bracts and bracteoles small and narrow, and usually lanceolate, linear or filiform (Yao et al. 2016).

Based on results from extensive field investigations conducted recently, multiple new species of *Pogostemon* were reported, such as *P. nudus* Bongcheewin & Pramali from Thailand (Bongcheewin et al. 2017), *P. guamensis* Lorence & W.L. Wagner from Guam, Mariana Islands (Lorence et al. 2020), and *P. monticola* from Taiwan, China (Liu et al. 2021). In addition, the rare species *P. dielsianus* Dunn, which is endemic to southwestern China and known previously only from its type collected in 1905 (*G. Forrest* 875, E00087126, K000249619), was also rediscovered in a recent scientific field trip (Hu et al. 2021). Thus, extensive field investigations should be conducted and more new discoveries might be revealed, enabling better understanding of the biodiversity of the genus *Pogostemon* as well as for other biological groups.

In our taxonomic revision of Chinese *Pogostemon* (Yao et al. 2015), a specimen (*Z. Huang* 36483, IBSC-0585902) of *Pogostemon* collected in 1934 from Lingshui Hsien of Hainan Province, China, seemed to be very different from all the other congeneric taxa, especially in its large ratio of the length of calyx teeth and calyx tube (2/3 to ca. 1.0). However, due to the unavailability of intact leaves and flowers for measurements, the specimen was not further studied and the species that it represented was not included in our previous study (Yao et al. 2015). Recently, during a field investigation in Yinggeling Nature Reserve, Hainan Province, China, one of the authors (L.X. Yuan) collected a *Pogostemon* specimen that is very similar to the specimen *Z. Huang* 36483 in plant morphology. Later, another two wild populations of the same species were discovered in Jiayi Nature Reserve and Qixianling, Hainan Province, China. Detailed morphological comparison and specimen examination confirmed that the newly collected specimens are conspecific with *Z. Huang* 36483 and the species is different from all the other *Pogostemon* taxa, thus it is formally described here. The phylogenetic posi-

tion of the new species within *Pogostemon* is also studied here based on analyses of the nuclear ribosomal internal transcribed spacer (nrITS) and five plastid regions (*matK*, *psbA-trnH*, *rbcL*, *rps16*, *trnL-F*).

Materials and methods

Morphological study

Specimens of *Pogostemon* deposited in the herbaria BM, E, HITBC, IBK, IBSC, K, KUN, L, PE, US, NAS, TAI and NY were studied carefully in the present study. Herbarium abbreviations cited in the present study follow the Index Herbariorum (Thiers 2013 onwards). Extensive field investigations of Chinese *Pogostemon* were conducted over the last decade. Morphological characters of stems, leaves, inflorescences, flowers and nutlets were photographed and measured.

Phylogenetic study

To study the phylogenetic position of the new species within the genus *Pogostemon*, a phylogenetic study of the genus was performed, based on analyses of six DNA markers (nrITS, *matK*, *psbA-trnH*, *rbcL*, *rps16*, *trnL-F*), following Yao et al. (2016). Total genomic DNA of the new species was extracted from silica gel-dried leaves (voucher specimens: *L.X. Yuan 20210206001* & *20210207001*; IBSC) using a Plant Genomic DNA Kit (Biomed, Shenzhen, China). Detailed information of primers of relevant DNA markers used in Polymerase Chain Reaction (PCR) amplification and sequencing, as well as the procedures of PCR, can be found in our previous study (Yao et al. 2016). All the DNA sequences used in Yao et al. (2016) and some *Pogostemon* sequences provided by other authors (Bendiksby et al. 2011; Hu et al. 2021) were downloaded from NCBI (www.ncbi.nlm.nih.gov). In total, 57 accessions representing 35 species of *Pogostemon* were sampled, in which 34 accessions representing 16 species of subgenus *Pogostemon* and 23 accessions representing 19 species of subgenus *Dysophyllus* were included. Other nine genera of Pogostemoneae (viz., *Achyrospermum* Bl., *Anisomeles* R. Br., *Colebrookea* Sm., *Comanthosphace* S. Moore, *Craniotome* Rchb., *Eurysolen* Prain, *Leucosceptrum* Sm., *Rostrinucula* Kudô., *Microtoena* Prain), the genus *Gomphostemma* Wall. ex Benth. of Gomphostemmateae and the genus *Colquhounia* Wall. of Colquhounieae were selected here as outgroups based on phylogenetic framework provided by Yao et al. (2016). Detailed information of all species sampled and sequences used are available in Appendix I.

All the DNA sequences were aligned using MAFFT 7.221 (Katoh and Standley 2013), and then three different datasets were constructed: the cpDNA dataset (including *matK*, *psbA-trnH*, *rbcL*, *rps16*, *trnL-F*), the nrITS dataset, and the combined dataset (including all the six DNA markers). All the three datasets were analyzed using two approaches: Bayesian Inference (BI) and Maximum Likelihood (ML) were conducted

using MrBayes v. 3.2.7a (Ronquist and Huelsenbeck 2003) and RAxML (Stamatakis 2006) on the CIPRES cluster (Miller et al. 2010), respectively. The models of nucleotide substitution of the six DNA markers used were selected independently under the Akaike Information Criterion (AIC) using jModelTest v. 3.7 (Posada 2008): GTR+I+ Γ for nrITS, GTR+ Γ for *matK*, TrN+ Γ for *psbA-trnH*, GTR+I for *rbcL*, TVM+ Γ for *rps16* and GTR+I for *trnL-F*. Detailed methods for BI and ML analyses could refer to the phylogenetic study conducted in Yao et al. (2021), except that each of Markov Chain Monte Carlo (MCMC) analysis was run for 10,000,000 generations and sampling every 500 generations in BI analysis. Number of generations in BI analysis was sufficient, because the effective sample size (ESS) of all parameters were over 200 as evaluated in Tracer v. 1.6 (Rambaut et al. 2014), and the average standard deviations (SD) of split frequencies for the dataset was below 0.01. The first 25% of the trees obtained in BI analysis were discarded as burn-in and then posterior probabilities (PP) were determined from the posterior distribution. A rapid bootstrap (BS) analysis using the model GTR+ Γ with 1000 pseudoreplicates was conducted to obtain the support values in ML analysis.

Results

Phylogenetic analyses

The cpDNA dataset, nrITS dataset and combined dataset alignments contained 3872 bp, 707 bp and 4,579 bp, respectively. The topology of *Pogostemon* and its relatives derived from the nrITS dataset was largely consistent with that derived from the cpDNA dataset, except several nodes that were lowly supported (defined here as BS < 80% or PP < 0.80) (Suppl. material 1 and 2). Phylogenetic relationships derived from the combined dataset (Figure 1) were much better resolved compared with those obtained from both the cpDNA dataset and nrITS dataset (Suppl. material 1 and 2), thus we focus on describing phylogenetic relationships based on the result derived from the combined dataset.

Results from analyses of the combined dataset recovered a highly supported (defined here as BS \geq 90% or PP \geq 0.99) sister relationship between the two genera *Anisomeles* and *Pogostemon* (BS = 100%, PP = 1.00). Phylogenetic relationships of the genus *Pogostemon* obtained here are also consistent with those reported in Yao et al. (2016). In *Pogostemon*, two major clades were highly supported: Clade I representing the subgenus *Pogostemon* (BS = 100%, PP = 1.00) and clade II representing the subgenus *Dysophyllus* (BS = 98%, PP = 1.00) (Fig. 1). The monophyly of the new species is well-supported (BS = 100%, PP = 1.00) and it is nested deeply within the first clade. Furthermore, a sister relationship between the new species and *P. parviflorus* Benth. is moderately supported (defined here as 80% \leq BS < 90% or 0.80 \leq PP < 0.99) in ML analysis (BS = 86%) and highly supported in BI analysis (PP = 1.00). Detailed information about the phylogenetic relationships of other nodes can be referred to Figure 1.

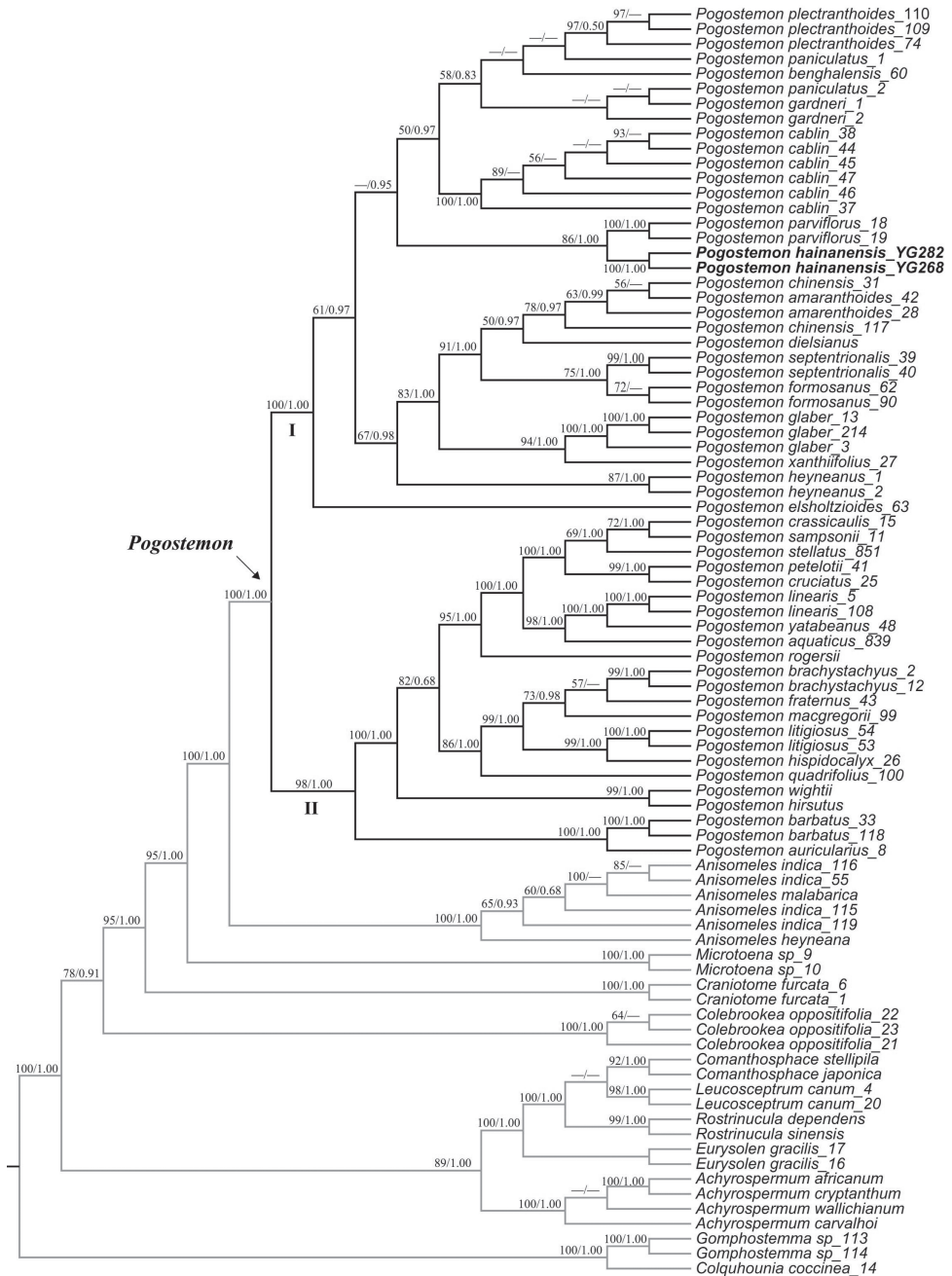


Figure 1. Maximum likelihood (ML) tree of *Pogostemon* and its relatives inferred from the combined dataset (including nrITS, *matK*, *psbA-trnH*, *rbcL*, *rps16*, *trnL-F*). Bootstrap (BS) value in ML analysis and posterior probability (PP) in Bayesian inference (BI) is indicated on the left and right of slanting bar associated with phylogenetic node, respectively. Dashes denote that the phylogenetic node associated was not supported or the BS value is < 50% in ML analysis or PP < 0.50 in BI. The crown node of *Pogostemon* is shown by the arrowhead.

Morphological comparison

A detailed morphological comparison between the new species and other species of *Pogostemon* was conducted. A series of morphological characters of the new species, such as the obviously double serrated leaf margin (Figs 2, 3A–D), long petioles (usually 4.5–11.5 cm long; Fig. 3B–D), and the large ratio of the length of calyx teeth and calyx tube ($2/3$ – 1.0 ; Fig. 3J–K), can be used to distinguish the new species from all the other members of subgenus *Pogostemon* easily. In habit, the new species is similar to *P. parviflorus* Benth. and *P. septentrionalis* C.Y. Wu & Y.C. Huang, which also have wild populations discovered in South China (Yao et al. 2015). However, the new species further differs from *P. parviflorus* in having large spikes of inflorescence (usually 2.5–8 cm long and 9–12 mm wide; Fig. 3A, E), larger calyx (4–5 mm long; Figure 3J) and corolla (6–7 mm long), besides above-mentioned three traits. In contrast, *P. parviflorus* has obscurely or shallowly double crenated to double serrated leaves margin, shorter petioles (1–4.5 cm long), smaller spikes of inflorescence (1–4.5 cm long and 8–10 mm wide), calyx (4–4.2 mm long) and corolla (4–4.5 mm long), as well as the ratio of the length of calyx teeth and calyx tube (less than $1/2$) (Yao et al. 2015). While *P. septentrionalis* has shorter petioles (0.5–5.5 cm long), narrow spikes of inflorescence (7–9 mm in diameter), smaller calyx (3–4 mm long) and corolla (4–4.5 mm long), the ratio of the length of calyx teeth and calyx tube ($1/3$ – $1/2$), and larger nutlets (0.9–1.0 mm long) (Yao et al. 2015).

Only two species of subgenus *Pogostemon* [viz., *P. cablin* (Blanco) Benth. and *P. esquirolii* (H. Lévêillé) C. Y. Wu & Y. C. Huang] were recorded previously in Hainan Island, China (Guangdong Institute of Botany 1977; Wu and Huang 1977). While the specific name *P. esquirolii* had been reduced previously to be a synonym of *P. glaber* Benth. by Rehder (1935), and this treatment was accepted by Yao et al. (2015) in their taxonomic revision of Chinese *Pogostemon*. The new species can be distinguished from *P. cablin* (a cultivated species in China) (Yao et al. 2015) by a series of morphological traits, such as its spikes of inflorescences are 9–12 mm in diameter (Fig. 3A, E) (vs. 13–18 mm in diameter), calyx 4–5 mm long (Fig. 3J–K) (vs. 6–8 mm long), the ratio of the length of calyx teeth and calyx tube is $2/3$ – 1.0 (Figure 3J–K) (vs. ca. $1/4$). While the new species differs from *P. glaber* by its leaves margin obviously double serrate (Figs 2, 3A–D) (vs. usually shallowly double serrate or double crenate), spikes of inflorescences usually 2.5–8.0 cm long (Fig. 3A, E) (vs. 3.0–15.0 cm long), calyx 4–5 mm long (Figure 3J–K) (vs. 3–4.5 mm long), the ratio of the length of calyx teeth and calyx tube is $2/3$ – 1.0 (Fig. 3J–K) (vs. ca. $1/3$), corolla 6–7 mm long (vs. 3–5.5 mm long).

A detailed morphological comparison among the new species and its relatives or morphologically similar species also can be referred to Table 1.

Discussion

Morphologically, the new species has spikes of inflorescence with more than two later branches (Figs 2, 3A), bracts and bracteoles large and broad-ovate, ovate or lanceolate

Table 1. Morphological comparison among *Pogostemon glaber* Bentham, *P. hainanensis* L.X. Yuan & Gang Yao, *P. parviflorus* Bentham and *P. septentrionalis* Wu & Huang.

Morphology	<i>P. glaber</i>	<i>P. hainanensis</i>	<i>P. parviflorus</i>	<i>P. septentrionalis</i>
Leaf blade	Margin shallowly double serrate or double crenate	Margin obviously double serrate	Margin obscurely double crenate to double serrate	Margin double serrate
Petiole	Usually 3–5 cm long	Usually 4.5–11.5 cm long	Usually 1–4.5 cm long	Usually 0.5–5.5 cm long
Inflorescence	Usually 3.0–15.0 cm long and 6–10 mm wide	Usually 2.5–8.0 cm long and 9–12 mm wide	Usually 0.7–3.5 cm long and 8–10 mm wide	Usually 3–13 cm long and 7–9 mm wide
Calyx	3–4.5 mm long; the ratio of the length of calyx teeth and calyx tube is ca. 1/3	4–5 mm long; the ratio of the length of calyx teeth and calyx tube is 2/3–1.0	4–4.2 mm long; the ratio of the length of calyx teeth and calyx tube is less than 1/2	3–4 mm long; the ratio of the length of calyx teeth and calyx tube is 1/3–1/2
Corolla	3–5.5 mm long	6–7 mm long	4–4.5 mm long	4–4.5 mm long

in shape (Fig. 3H), indicating that the species is a member of subgenus *Pogostemon*, which is further confirmed in phylogenetic analyses (Figure 1). According to current circumscription, about 28 species (including the new species) are accepted in subgenus *Pogostemon* (Bhatti and Ingrouille 1997; Yao et al. 2015; Liu et al. 2021), among which 16 species were sampled in the present phylogenetic analyses (Figure 1). The species *Pogostemon monticola* T.C. Hsu, S.W. Chung, S.H. Liu & W.J. Huang described recently from Taiwan, China, was not sampled in the present phylogenetic study due to the unavailability of DNA material or DNA sequences, but its phylogenetic position within subgenus *Pogostemon* was resolved and it was closely related to *P. formosanus* Oliver and *P. septentrionalis* Wu & Huang (Liu et al. 2021). While for the other 11 species of subgenus *Pogostemon* not sampled in the present phylogenetic analyses, viz. *P. cristatus* Hassk., *P. griffithii* Prain, *P. hispidus* Prain, *P. latifolius* (C.Y. Wu & Y.C. Huang) Gang Yao, *P. nelsonii* Doan, *P. nepetoides* Stapf, *P. pubescens* Benth., *P. purpurascens* Dalzell, *P. tuberculosus* Benth., *P. villosus* Benth., and *P. wattii* C.B. Clarke, they also have a series of morphological characters that can be distinguished from the new species from Hainan, China, especially in terms of the margin of leaves, the length of petioles, the size of calyx and the ratio of the length of calyx teeth and calyx tube. Detailed information about the morphological characters of these 11 species can be referred in Bhatti and Ingrouille (1997) and Yao et al. (2015). Thus, as mentioned above, the combined evidence from morphological and phylogenetic analyses well supported the independently taxonomic status of the new species in *Pogostemon*.

Taxonomic treatment

Pogostemon hainanensis L.X. Yuan & Gang Yao, sp. nov.

urn:lsid:ipni.org:names:77248974-1

Figures 2–3

Diagnosis. The species is similar to *Pogostemon parviflorus* Benth. in general morphology, but differs from the latter by its leaves margin obviously double serrate, petioles usually 4.5–11.5 cm long, spikes of inflorescence up to 8 cm long, calyx 4–5 mm long, corolla 6–7 mm long, and the calyx teeth is 2/3 to subequal as long as the calyx tube.



Figure 2. Habit of *Pogostemon hainanensis*.

Type. CHINA. Hainan province, Yinggeling Nature Reserve, Nanleshan, Fanyang, Wuzhishan, 18°54'38.25"N, 109°22'26.58"E, at an elevation of about 570 m, 6 February 2021, *L.X. Yuan 20210206001* (holotype, IBSC; isotypes: IBSC, KUN).



Figure 3. *Pogostemon hainanensis* **A** branch **B** leaf **C, D** leaf **E** spike of inflorescence **F** stem **G** cross section of stem **H** bract (the left one) and bracteoles (the right two) **I, J** flower **K** calyx **L** nutlets.

Description. Perennial herbs or shrubs, 0.8–2 m tall. Stem erect, 6–8 mm in diameter, 4-angular, slightly dilated at nodes, a few branched, strigose, or villous at the upper part. Leaves opposite; blade ovate, rarely ovate-lanceolate, (5.5–) 9–13.5 × (2.5) 6.5–10.5 cm, papery or membranous, strigose on both surfaces, base broadly cuneate, margin obviously double serrate, entire at base, apex acuminate.

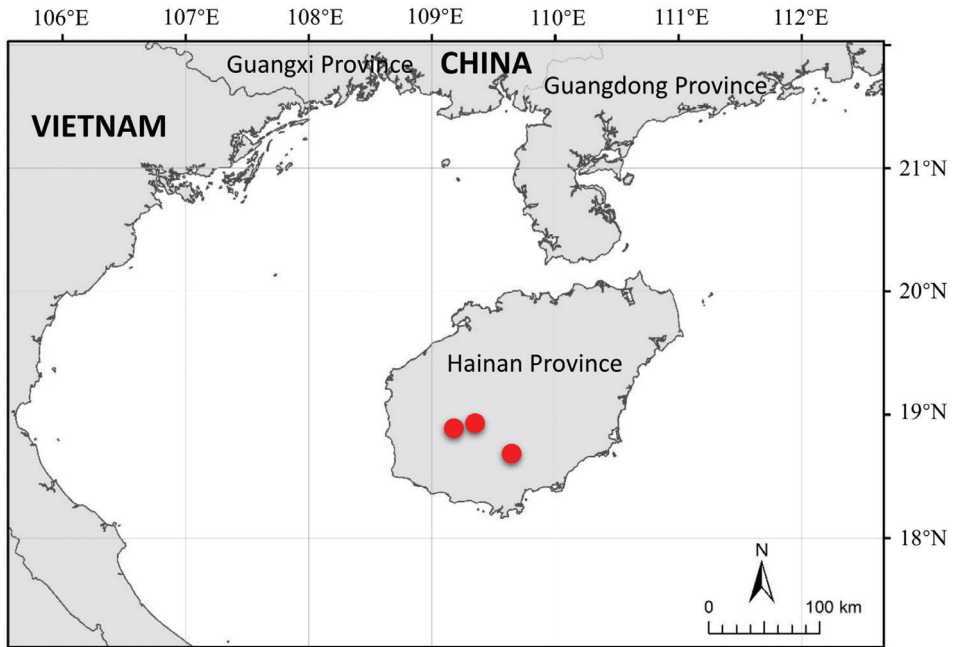


Figure 4. Distribution of *Pogostemon hainanense* (red circular).

nate; midvein elevated abaxially, lateral vein 3–4 (rarely 5) pairs on each side of the midvein, slightly elevated abaxially; petioles (1.5–) 4–11.5 cm long, ca. 1 mm in diameter, strigose. Spikes of inflorescence (1.0–) 2.5–8.0(–10.5) cm long, 9–12 mm in diameter, terminal and axillary, interrupted basally in long spikes, usually with more than two lateral branches; peduncle (1–) 2–4 cm long, densely villous; verticillasters many-flowered, flowers sessile. Bracts oblong, 5–13 × 2.5–5 mm, strigose, midvein elevated abaxially, lateral vein 1–2 pairs on each side of the midvein or sometimes obscure; bracteoles ovate-lanceolate to narrowly lanceolate, 2.5–5 × 0.7–1.8 mm, strigose. Calyx tubular-inflated, 4–5 mm long, 5-veined, strigose and sparsely golden glandular outside, sometimes sparsely strigillose inside at the upper part of tube; teeth 5, narrowly triangular, equal, 1.8–2 mm long, 0.6–0.8 mm wide at base, 2/3 to subequal as long as the calyx tube, subglabrous or strigillose inside. Corolla white, 6–7 mm long, exserted evidently from calyx, 2-lipped, upper lip 3-lobed, lower lip entire. Stamens 4, erect, much exserted from corolla; filaments 7–7.5 mm long, all inserted at a height of ca. 2 mm in the tube, bearded at middle, bearded portion exserted; anther 1-locular, cell apex dehiscent. Style 7–7.5 mm long; stigma bifid, lobes subequal, 1.2–1.7 mm long. Disc ca. 0.6 mm long. Nutlets 4, ca. 0.7 × ca. 0.6 mm long, ellipsoid or slightly depressed globose, abaxially slightly flat, adaxially ribbed, black or dark brown.

Etymology. *Pogostemon hainanensis* is named after its type locality, Hainan province, China.

Phenology. Flowering from December to the next February and fruiting from January to April.

Paratype. CHINA. Hainan Province, Lingshui Hsien, 12 January 1934, near river, Z. Huang 36483 (IBSC-0585902!); Ledong Hsien, Jiaxi Nature Reserve, 18°52'35.85"N, 109°10'36.99"E, at an elevation of about 800 m, 7 February 2021, L.X. Yuan 20210207001 (IBSC); Baoting Hsien, near the Tiantan waterfall, 18°42'16.17"N, 109°41'50.55"E, at an elevation of about 560 m, 25 April 2021, L.X. Yuan 20210425001 (IBSC).

Distribution and habit. The new species is endemic to Hainan Province, China (Figure 4). It grows under forests, usually near ravines, at an elevation of 550–800 m.

Chinese name. Hai Nan Ci Rui Cao (海南刺蕊草).

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References

- Bendiksby M, Thorbek L, Scheen AC, Lindqvist C, Ryding O (2011) An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. *Taxon* 60(2): 471–484. <https://doi.org/10.1002/tax.602015>
- Bhatti GR, Ingrouille M (1997) Systematics of *Pogostemon* (Labiatae). *Bulletin of the Natural History Museum. London (Botany)* 27: 77–147.
- Bongcheewin B, Pramali K, Traiperm P, Chantaranonthai P, Paton A (2017) *Pogostemon nudus* sp. nov. (Lamiaceae) from Thailand. *Nordic Journal of Botany* 35(3): 289–299. <https://doi.org/10.1111/njb.01439>
- Guangdong Institute of Botany (1977) *Flora Hainanica* 4. Science Press, Beijing, 644 pp.
- Hu GX, Su T, An MT, Wan XY (2021) Rediscovery of *Pogostemon dielsianus* (Lamiaceae, Lamioideae), a rare endemic species from southwestern China, after one century. *PhytoKeys* 171: 61–73. <https://doi.org/10.3897/phytokeys.171.60389>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Liu SH, Hsu TC, Huang WJ, Chung SW (2021) *Pogostemon monticola* (Lamiaceae: Lamioideae), a new species from Taiwan. *Phytotaxa* 507(1): 51–66. <https://doi.org/10.11646/phytotaxa.507.1.2>

- Lorence DH, Wagner WL, Wood KR, Johnson G (2020) *Pogostemon guamensis* Lorence & W.L. Wagner (Lamiaceae), a new species from Guam, Mariana Islands. *PhytoKeys* 169: 61–73. <https://doi.org/10.3897/phytokeys.169.58107>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetics trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Posada D (2008) JModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut A, Suchard MA, Drummond AJ (2014) Tracer v1.6. <http://beast.bio.ed.ac.uk/Tracer>
- Rehder A (1935) Notes on the Iligneous plants described by Leveille from Eastern Asia. *Journal of the Arnold Arboretum* 16(3): 311–340. <https://doi.org/10.5962/p.324579>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* (Oxford, England) 19(12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Stamatakis A (2006) RAXML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* (Oxford, England) 22(21): 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Thiers B (2013, continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. The New York Botanical Garden, New York. <http://sweetgum.nybg.org/ih/> [accessed 13 October 2021]
- Wu CY, Huang YC (1977) *Pogostemon* and *Dysophylla*. In: Wu CY, Li HW (Eds) *Flora Reipublicae Popularis Sinicae* 66. Science Press, Beijing, 366–387.
- Yao G, Deng YF, Ge XJ (2015) A taxonomic revision of *Pogostemon* (Lamiaceae) from China. *Phytotaxa* 200(1): 1–67. <https://doi.org/10.11646/phytotaxa.200.1.1>
- Yao G, Drew BT, Yi TS, Yan HF, Yuan YM, Ge XJ (2016) Phylogenetic relationships, character evolution and biogeographic diversification of *Pogostemon* s.l. (Lamiaceae). *Molecular Phylogenetics and Evolution* 98: 184–200. <https://doi.org/10.1016/j.ympev.2016.01.020>
- Yao G, Xue B, Liu K, Li Y, Huang J, Zhai J (2021) Phylogenetic estimation and morphological evolution of Alsineae (Caryophyllaceae) shed new insight into the taxonomic status of the genus *Pseudocerasium*. *Plant Diversity* 43(4): 299–307. <https://doi.org/10.1016/j.pld.2020.11.001>
- Zhao F, Chen YP, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksyby M, Wang Q, Min D-Z, Peng H, Olmstead RG, Li B, Xiang C-L (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. *BMC Biology* 19(1): e2. <https://doi.org/10.1186/s12915-020-00931-z>

Appendix I

Sequences and taxa information for all samples used in the present study (–, missing data; *, newly-generated sequences). Accession numbers are given for nrITS, *matK*, *rbcL*, *rps16*, *trnH-psbA*, *trnL-F* (c-f or c-d/e-f; the characters “c”, “d”, “e” and “f” indicate different primers used for PCR and sequencing of the marker *trnL-F*, referred from Yao et al. 2016).

Pogostemon amaranthoides Benth._28: –, KR608424, KR608489, KR608613, KR608548, KR608676; *P. amaranthoides*_42: KR608745, KR608425, KR608490, KR608614, KR608549, KR608677; *P. aquaticus* (C. H. Wright) Press_839: KR608767, KR608468, KR608527, KR608655, KR608592, KR608717; *P. auricularius* (L.) Hassk._8: KR608761, KR608451, KR608513, KR608638, KR608575, KR608700; *P. barbatus* Bhatti & Ingr._33: KR608762, KR608452, KR608514, KR608639, KR608576, KR608701; *P. barbatus*_118: KR608763, KR608453, KR608515, KR608640, KR608577, KR608702; *P. benghalensis* (Burm. f.) Kuntze: –, KR608444, –, HQ911592, KR608568, HQ911663/HQ911731; *P. brachystachyus* Benth._2: KR608775, KR608455, KR608517, KR608642, KR608579, KR608704; *P. brachystachyus*_12: KR608774, KR608454, KR608516, KR608641, KR608578, KR608703; *P. cablin* (Blanco) Benth._37: KR608757, KR608438, KR608503, KR608627, KR608562, KR608690; *P. cablin*_38: KR608752, KR608439, KR608504, KR608628, KR608563, KR608691; *P. cablin*_44: KR608753, KR608440, KR608505, KR608629, KR608564, KR608692; *P. cablin*_45: KR608754, KR608441, KR608506, KR608630, KR608565, KR608693; *P. cablin*_46: KR608755, KR608442, KR608507, KR608631, KR608566, KR608694; *P. cablin*_47: KR608756, KR608443, KR608508, KR608632, KR608567, KR608695; *P. chinensis* C. Y. Wu & Y. C. Huang_31: KR608743, KR608426, KR608491, KR608615, KR608550, KR608678; *P. chinensis*_117: KR608742, KR608449, KR608512, KR608637, KR608573, KR608699; *P. crassicaulis* (Benth.) Press_15: KR608770, KR608469, KR608528, KR608656, KR608593, KR608718; *P. cruciatus* (Benth.) Kuntze_25: KR608771, KR608466, KR608525, KR608653, KR608590, KR608715; *P. dielsianus* Dunn: MW194872, –, MW194874, MW194875, MW194873, MW194876; *P. elsholtzioides* Benth._63: –, KR608445, –, KR608633, KR608569, KR608720; *P. formosanus* Oliver_62: KR608744, KR608434, KR608499, KR608623, KR608558, KR608686; *P. formosanus*_90: KR608779, KR608435, KR608500, KR608624, KR608559, KR608687; *P. fraternus* Miq.: KR608781, KR608461, –, KR608648, KR608585, KR608710; *P. gardner* Hook. f._1: MF303612, MF303632, –, –, –, –, *P. gardner*_2: MF303603, MF303622, –, –, –, –, *P. glaber* Benth._3: KR608740, KR608431, KR608496, KR608620, KR608555, KR608683; *P. glaber*_13: KR608739, KR608429, KR608494, KR608618, KR608553, KR608681; *P. glaber*_214: KR608741, KR608430, KR608495, KR608619, KR608554, KR608682; *P. hainanensis* L.X. Yuan & Gang Yao_YG268: OL625022, OL616075, OL616077, OL616079, OL616081, OL616083; *P. hainanensis*_YG282: OL625023, OL616076, OL616078, OL616080, OL616082, OL616084; *P. heyneanus* Benth._1: KR608751, KR608427, KR608492, KR608616, KR608551, KR608679; *P. heyneanus*_2: –, HQ911401, –, FJ854069, –, FJ854297/FJ854184; *P. hirsutus* Benth.: –, HQ911397, –, FJ854070, –, FJ854298/FJ854185; *P. hispidocalyx* C. Y. Wu & Y. C. Huang_26: KR608780, KR608457, –, KR608644, KR608581, KR608706; *P. linearis* (Benth.) Kuntze_5: KR608764, KR608462, KR608521, KR608649, KR608586, KR608711; *P. linearis*_108: KR608765, KR608463, KR608522, KR608650, KR608587, KR608712; *P. litigiosus* Doan ex Suddee & A. J. Paton_53: KR608776, KR608458, KR608519, KR608645, KR608582, KR608707; *P. litigiosus*_54:

KR608777, KR608459, KR608520, KR608646, KR608583, KR608708; *P. macgregorii* W. W. Sm._99: KR608778, –, –, –, –, –; *P. paniculatus* (Willd.) Benth._1: –, KR608450, –, –, KR608574, KR608721; *P. paniculatus*_2: –, HQ911399, –, FJ854071, –, FJ854299/FJ854186; *P. parviflorus* Benth._18: KR608749, KR608436, KR608501, KR608625, KR608560, KR608688; *P. parviflorus*_19: KR608750, KR608437, KR608502, KR608626, KR608561, KR608689; *P. petelotii* Doan ex G. Yao, Y. F. Deng & X. J. Ge_41: KR608772, KR608470, KR608529, KR608657, KR608594, KR608719; *P. plectranthoides* Desf._74: KR608760, KR608446, KR608509, KR608634, KR608570, KR608696; *P. plectranthoides*_109: KR608758, KR608447, KR608510, KR608635, KR608571, KR608697; *P. plectranthoides*_110: KR608759, KR608448, KR608511, KR608636, KR608572, KR608698; *P. quadrifolius* (Benth.) F. Muell._100: KR608773, KR608456, KR608518, KR608643, KR608580, KR608705; *P. rogersii* N E. Br.: KR608782, KR608460, –, KR608647, KR608584, KR608709; *P. sampsonii* (Hance) Press_11: KR608769, KR608465, KR608524, KR608652, KR608589, KR608714; *P. septentrionalis* C. Y. Wu & Y. C. Huang_39: KR608747, KR608432, KR608497, KR608621, KR608556, KR608684; *P. septentrionalis*_40: KR608748, KR608433, KR608498, KR608622, KR608557, KR608685; *P. stellatus* (Lour.) Kuntze_851: KR608768, KR608464, KR608523, KR608651, KR608588, KR608713; *P. wightii* Benth.: MF303601, MF303620, –, –, –, –; *P. xanthiifolius* C. Y. Wu & Y. C. Huang_27: KR608746, KR608428, KR608493, KR608617, KR608552, KR608680; *P. yatabeanus* (Makino) Press_48: KR608766, KR608467, KR608526, KR608654, KR608591, KR608716;

Outgroups: *Achyrosperrum africanum* Hook. f. ex Baker: –, HQ911418, –, FJ853999, –, FJ854246/FJ854133; *A. carvalhoi* Gürke: –, HQ911412, –, FJ854001, –, FJ854248/FJ854135; *A. cryptanthum* Baker: –, HQ911415, –, FJ854002, –, FJ854249/FJ854136; *A. wallichianum* (Benth.) Benth. ex Hook. f.: –, –, –, HQ911594, –, HQ911666/HQ911734; *Anisomeles heyneana* Benth.: –, HQ911394, –, HQ911589, –, HQ911659/HQ911727; *A. indica* (L.) Kuntze_55: KR608726, KR608406, KR608471, KR608595, KR608530, KR608658; *A. indica*_115: –, KR608407, KR608472, KR608596, KR608531, KR608659; *A. indica*_116: KR608727, KR608408, KR608473, KR608597, KR608532, KR608660; *A. indica*_119: KR608728, KR608409, KR608474, KR608598, KR608533, KR608661; *A. malabarica* (L.) R. Br. ex Sims: –, HQ911396, –, FJ854013, –, FJ854260/FJ854147; *Colebrookea oppositifolia* Sm._21: KR608732, KR608414, KR608479, KR608603, KR608538, KR608666; *C. oppositifolia*_22: KR608733, KR608415, KR608480, KR608604, KR608539, KR608667; *C. oppositifolia*_23: KR608734, KR608416, KR608481, KR608605, KR608540, KR608668; *Comanthosphace japonica* (Miq.) S. Moore: –, HQ911407, –, FJ854029, –, FJ854272/FJ854159; *C. stellipila* S. Moore: –, HQ911408, –, FJ854030, –, FJ854273/FJ854160; *Craniotome furcate* (Link) Kuntze_1: KR608730, KR608412, KR608477, KR608601, KR608536, KR608664; *C. furcate*_6: KR608731, KR608413, KR608478, KR608602, KR608537, KR608665; *Eurysolen gracilis* Prain_16: KR608735, KR608417, KR608482, KR608606, KR608541, KR608669; *E. gracilis*_17: KR608736, KR608418, KR608483, KR608607, KR608542, KR608670; *Leucosceptum canum*

Sm._4: KR608738, KR608419, KR608484, KR608608, KR608543, KR608671; *L. canum*_20: KR608737, KR608420, KR608485, KR608609, KR608544, KR608672; *Microtoena* sp._9: KR608729, KR608410, KR608475, KR608599, KR608534, KR608662; *M. sp.*_10: –, KR608411, KR608476, KR608600, KR608535, KR608663; *Rostrinucula dependens* (Rehder) Kudô: –, HQ911405, –, FJ854074, –, FJ854302/FJ854189; *R. sinensis* (Hemsl.) C. Y. Wu: –, HQ911406, –, FJ854075, –, FJ854303/FJ854190; *Colquhounia coccinea* Wall._14: KR608722, KR608421, KR608486, KR608610, KR608545, KR608673; *Gomphostemma* sp._113: KR608723, KR608422, KR608487, KR608611, KR608546, KR608674; *G. sp.*_114: KR608724, KR608423, KR608488, KR608612, KR608547, KR608675.

Supplementary material I

Figure S1

Authors: Langxing Yuan, Gan Tan, Wenhua Zhang, Bine Xue, Jiwen Deng, Lei Liu, Gang Yao

Data type: Phylogenetic tree

Explanation note: Supplementary Figure 1 Maximum likelihood (ML) tree of *Pogostemon* and its relatives inferred from the cpDNA dataset (including matK, psbA-trnH, rbcL, rps16, trnL-F).

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Link: <https://doi.org/10.3897/phytokeys.188.76611.suppl1>

Supplementary material 2

Figure S2

Authors: Langxing Yuan, Gan Tan, Wenhua Zhang, Bine Xue, Jiwen Deng, Lei Liu, Gang Yao

Data type: Phylogenetic tree

Explanation note: Supplementary Figure 2 Maximum likelihood (ML) tree of *Pogostemon* and its relatives inferred from the nrITS dataset.

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