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Carpinus tibetana (Betulaceae), a new species from southeast Tibet, China

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Abstract

A new species *Carpinus tibetana* Z. Qiang Lu & J. Quan Liu from southeast Tibet is described and illustrated. The specimens of this new species were previously identified and placed under *C. monbeigiana* Hand.-Mazz. or *C. mollicoma* Hu. However, the specimens from southeast Tibet differ from those of *C. monbeigiana* from other regions with more lateral veins (19–24 vs 14–18) on each side of the midvein and dense pubescence on the abaxial leaf surface, while from those of *C. mollicoma* from other regions differ by nutlet with dense resinous glands and glabrous or sparsely villous at apex. Principal Component Analyses based on morphometric characters recognise the Tibetan populations as a separate group. Nuclear ribosomal ITS sequence variations show stable and distinct genetic divergences between the Tibetan populations and *C. monbeigiana* or *C. mollicoma* by two or three fixed nucleotide mutations. Phylogenetic analysis also identified three respective genetic clusters and the *C. mollicoma* cluster diverged early. In addition, the Tibetan populations show a disjunct geographic isolation from the other two species. Therefore, *C. tibetana*, based on the Tibetan populations, is here erected as a new species, distinctly different from *C. monbeigiana* and *C. mollicoma*.

Keywords

Carpinus tibetana, new species, Tibet

Introduction

The birch family (Betulaceae) comprises six genera and approximately 167 species (Christenhusz and Byng 2016). In this family, the hornbeams in the genus *Carpinus* (Linnaeus, 1753) are small to medium-size trees (Li and Skvortsov 1999; Holstein and Weigend 2017). In *Flora of China*, 33 hornbeam species are described and 28 of which are endemic (Li and Skvortsov 1999). The endemic species *C. monbeigiana* Hand.-Mazz. is mainly distributed in southeast (SE) Tibet and northwest (NW) Yunnan. This species is recognised due to the leaves doubly or simply setiform serrate along the margin, nutlets with dense resinous glands, peduncles and rachises with densely yellow hirsute and densely hispidulous bracts with an inflexed auricle at the base of the inner margin. However, a small number of specimens from SE Tibet were also identified as *C. mollicoma* Hu because of the numerous lateral veins and dense pubescence on the abaxial leaf surface (Li and Skvortsov 1999). Another species, *C. viminea* Wall. ex Lindl. is also distributed to SE Tibet and NW Yunnan (Wu 1991, Li and Skvortsov 1999). However, *C. viminea* is distinctly different from both *C. monbeigiana* and *C. mollicoma* with the long leaf petiole and a lobe at the base of the inner margin of bract. After examining all specimens of *C. monbeigiana* and *C. mollicoma* preserved in the Chinese Virtual Herbarium (http://www.cvh.org.cn) and Lanzhou University (LZU) in 2015, we found that the specimens from Tibet under *C. monbeigiana* or *C. mollicoma* might stand as a new species because they are clearly different from specimens of the two species collected from Yunnan (Figure 1). In order to further test this hypothesis, we conducted field surveys and an examination of morphological variation and genetic divergence. All lines of evidence support the establishment of a new species to accommodate the Tibetan populations as distinct from both *C. monbeigiana* and *C. mollicoma*.

Material and methods

Field surveys

After examining *Carpinus* specimens preserved in KUN and PE (Table 1), we found that the nutlet sizes of *C. monbeigiana* become stable after July. This was further confirmed by the measurements of the nutlet sizes of *C. monbeigiana* collected between July and September in 2015 from the same locality (Xishan, Kunming, Yunnan Province). Hence, collections from before July were excluded in our measurements of the morphological variation of specimens. We conducted the field surveys in Tibet and Yunnan from July to September in 2015 and 2016 in order to collect enough samples from different individual trees for morphological analyses and later genetic analyses. For the latter purpose, fresh leaves of each tree were immediately dried by silica gel in a plastic bag. All sampled populations of *C. monbeigiana* and *C. mollicoma* in the field are listed in Table 2. Voucher specimens were deposited in Lanzhou University Herbarium (**LZU**).

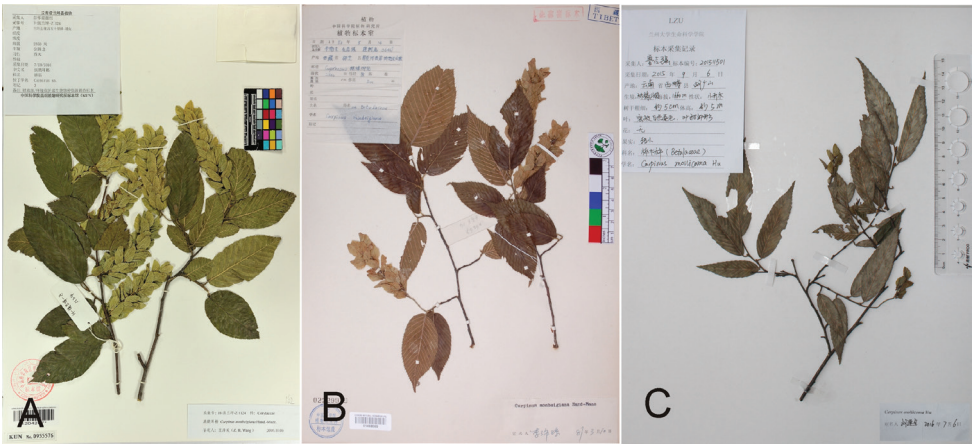


Figure 1. The gross morphology of two specimens had been identified as *Carpinus monbeigiana* and one as *C. mollicoma*. **A** *C. monbeigiana* from Yunnan (*H. Peng et al. H-Lanping-Z1124*, KUN) **B** *C. monbeigiana* from Tibet (*B.S. Li et al. 6467*, PE) **C** *C. mollicoma* from Yunnan (*Z.Q. Lu 201511501*, LZU). The number of the lateral leaf veins is totally different between two specimens from Yunnan and Tibet (**A** and **B**).

Morphological analysis

A total of 90 specimens (19 from southeast Tibet, 17 for *C. mollicoma* and 54 for *C. monbeigiana*) from individual trees were used for morphological comparisons. We examined morphological variations within and between the Tibetan populations and *C. monbeigiana* and *C. mollicoma* from other regions (Table 1) and measured 22 characters for morphological Principal Component Analyses (PCA) (Table 3).

Genetic analysis

For genetic analyses of the nuclear ITS region, 33 individuals from 7 populations of three groups were used. Amongst them, 8 individuals from two populations were collected from southeast Tibet while 9 individuals for *C. mollicoma* and 16 individuals for *C. monbeigiana*. *Carpinus viminea* was also included because this species also occurs in SE Tibet and NW Yunnan (Wu 1991, Li and Skvortsov 1999). Total DNA was extracted from 15–25 mg silica gel dried leaves using the modified CTAB method (Doyle and Doyle 1990). Nuclear ribosomal ITS sequence was used to confirm the species status of the Tibetan populations because the sequence variation of this fragment is stable within and between species with high species discrimination power (Lu et al. 2016). PCR amplifying and sequencing of the ITS fragment followed Lu et al. (2016). All newly available ITS sequences were uploaded to GenBank under the accession numbers KY436145–KY436155 and KY683787–KY683789. We used RAxML-8.1.17 (Stamatakis 2014) to conduct the Maximum likelihood (ML) analyses under the GTR + G model. Bootstrap replicates (1000) were set to calculate the support values.

Table 1. Specimens used for Principal Component Analyses (PCA) of morphological variations.

Species	Collector	Collection number	Collection site	Herbarium	No. of specimen
C. tibetana	*B.S. Li*	*06467/6467*	Yigong, Linzhi, Xizang	PE	2
C. tibetana	*W.L. Chen*	*10780*	Motuo, Xizang	PE	1
C. tibetana	*Anonymous*	*15079*	Ani to Hanmi, Motuo, Xizang	PE	1
C. tibetana	*Wu*	*5649*	Yigong, Bomi, Xizang	KUN	1
C. tibetana	*Anonymous*	*2505*	Tongmai, Bomi, Xizang	PE	1
C. tibetana	*H. Sun et al.*	*SunH-07ZX-2725*	Yigong, Bomi, Xizang	KUN	1
C. tibetana	*H. Sun et al.*	*6008*	Dexing, Motuo, Xizang	PE	1
C. tibetana	*Z.Q. Lu*	*2016QTP001-2016QTP011*	Tongmai, Bomi, Xizang	LZU	11
C. mollicoma	*Z.Q. Lu*	*201511501-201511517*	Xisha, Xichou, Yunnan	LZU	17
C. monbeigiana	*G.M. Feng*	*23645*	Huanfuping, Deqin, Yunnan	KUN	1
C. monbeigiana	*G.M. Feng*	*21595*	Jiazi, Lijiang, Yunnan	PE	1
C. monbeigiana	*G.M. Feng*	*50081/10121*	Xishan, Kunming, Yunnan	KUN	2
C. monbeigiana	*X.H. Yang*	*101202*	Xishan, Kunming,Yunnan	KUN	1
C. monbeigiana	*Z.Q. Lu*	*2015KM001-2015KM005*	Xishan, Kunming,Yunnan	LZU	5
C. monbeigiana	*W.Z. Li*	*147/149*	Xishan, Kunming,Yunnan	CSFI	2
C. monbeigiana	*Anonymous*	*30081*	Xishan, Kunming, Yunnan	KUN	1
C. monbeigiana	*Q.W. Wang*	*66847/67245*	Dela, Gongshan,Yunnan	PE	2
C. monbeigiana	*Anonymous*	*7340/7935/7940/7950/7954/8024*	Bingzhongluo, Gongshan,Yunnan	PE	6
C. monbeigiana	*Anonymous*	*22012*	Pengdang, Gongshan,Yunnan	KUN	1
C. monbeigiana	*T.T. Yu*	*19184*	Gongshan, Yunnan	PE	1
C. monbeigiana	*T.T. Yu*	*19103*	Mekong-Salwin divide, Gongshan, Yunnan	PE	1
C. monbeigiana	*Anonymous*	*22904*	Mekong-Salwin divide, Gongshan, Yunnan	KUN	1
C. monbeigiana	*S.D. Liu et al.*	*03-103*	Wumulong, Yongde, Yunnan	KUN	1
C. monbeigiana	*H. Peng et al.*	*H-LP-Z1124*	Tongdian, Lanping, Kunming	KUN	1
C. monbeigiana	*Z.Q. Lu*	*2016WXYZ001-2016WXYZ019*	Yezhi, Weixi, Yunnan	LZU	19
C. monbeigiana	*Z.Q. Lu*	*2016WXKP001-2016WXKP005*	Kangpu, Weixi, Yunnan	LZU	5
C. monbeigiana	*P.Y. Mao*	*00356/00370/00836*	Kangpu, Weixi, Yunnan	PE	3

Results

Morphologically, the Tibetan populations (Table 1; Figures 2–3) differ distinctly from those of *C. monbeigiana* from Yunnan with more lateral veins (19–24 vs 14–18) on each side of the midvein and more densely pubescent on the abaxial leaf surface and the difference was also found in the narrower distance between lateral veins (4–5 mm vs 5–8 mm) and smaller nutlet (Table 3). Meanwhile, plants of the Tibetan populations also differ from *C. mollicoma* by the nutlet having dense resinous glands and being glabrous or sparsely villous at apex. The difference was also found in the size of infructescence (2.5–4.5 cm × 1–1.5 cm vs 4–7 cm × 1.5–2.5) and bract (0.9–1.9 cm × 0.4–0.6 cm vs 1.5–1.9 cm × 0.6–0.9 cm). A Principal Component Analyses (PCA) distinguished samples from the two species and the Tibetan populations into three dif-

Table 2. Locations of the sampled populations from which individuals were used for genetic analyses of the nuclear ribosomal ITS sequence variations.

Species (individual number)	Location	Latitude (N)	Longitude (E)	Altitude (m)
C. tibetana (6)	Tongmai, Bomi, Tibet	30°06’	95°05’	2060
C. tibetana (2)	Tongmai, Bomi, Tibet	30°01’	95°03’	2080
C. monbeigiana (5)	Xishan, Kunming, Yunnan	24°58’	102°38’	2355
C. monbeigiana (8)	Yezhi, Weixi, Yunnan	27°48’	99°02’	1790
C. monbeigiana (2)	Kangpu, Weixi, Yunnan	27°38’	99°01’	1660
C. monbeigiana (1)	Weideng, Weixi, Yunnan	27°06	99°07’	1685
C. mollicoma (9)	Xisha, Xichou, Yunnan	23°26’	104°40’	1660

Table 3. Morphological characters of *C. tibetana*, *C. monbeigiana* and *C. mollicoma* at the population level.

Characters	*C. mollicoma*	*C. tibetana*	*C. monbeigiana*
LEAF			
Shape and size	Leaf blade oblong-lanceolate, or elliptic-lanceolate, rarely ovate-lanceolate, 4.5–8 cm × 1.5–3 cm; apex acute, acuminate or caudate-acuminate	Leaf blade ovate-elliptic or elliptic, 6–9 cm × 3–4 cm; apex attenuate-acuminate or caudate-acuminate	Leaf blade oblong-lanceolate, ovate-lanceolate, or elliptic-lanceolate, 6–13 cm × 3–4.5 cm; apex acute, acuminate, rarely caudate-acuminate
Length of petiole	3–8 mm	5–8 mm	6–12 mm
Number of lateral veins on each side of midvein	15–21	19–24	14–18
Average distance between lateral veins located in the middle of leaf	4–5 mm	4–5 mm	5–8 mm
Abaxially densely pubescent or glabrescent	Densely pubescent	Densely pubescent	Usually glabrescent
INFRUCTESCENCE			
Size of infructescence	2.5–4.5 cm × 1–1.5 cm; peduncle 1–1.5 cm	4–7 cm × 1.5–2.5 cm; peduncle 1–2.5 cm	4–13 cm × 1.5–3 cm; peduncle 1–3 cm
BRACT			
Size of bract	0.9–1.9 cm × 0.4–0.6 cm	1.5–1.9 cm × 0.6–0.9 cm	1.2–2.3 cm × 0.5–1.2 cm
NUTLET			
The number of ribs	6–9	7–11	6–10
Densely villous or glabrous	Densely villous	glabrous or sparsely villous at apex	glabrous or sparsely villous at apex
Densely resinous glandular or not	Not	Densely resinous glandular	Densely resinous glandular
Shape and size of nutlet	Broadly ovoid or ovoid-ellipsoid, 3.1–3.7 mm × 2–2.6 mm	Ovoid-ellipsoid, 3.0–3.9 mm × 2.2–2.8 mm	Broadly ovoid, 3.2–4.6 mm × 2.9–4.1 mm

ferent groups (Table 4; Figure 4). The first principal component axis (PC1; accounting for 43.16% of the variation) significantly separated *C. mollicoma* from *C. monbeigiana* and Tibetan populations, where there was a slight overlap between them. However, the

Figure 2. *Carpinus tibetana* Z. Qiang Lu & J. Quan Liu was drawn from *Z.Q. Lu 2016QTP001* (LZU).

second principal component axis (PC2; 14.51%) significantly separated the Tibetan populations from the other two species.

Genetically, the aligned 33 ITS sequences were 611 base pairs in length. In addition, three ITS sequences from *C. monbeigiana* were also downloaded from NCBI (AF432043, AF432044 and AF432048). In total, 16 types were identified from these

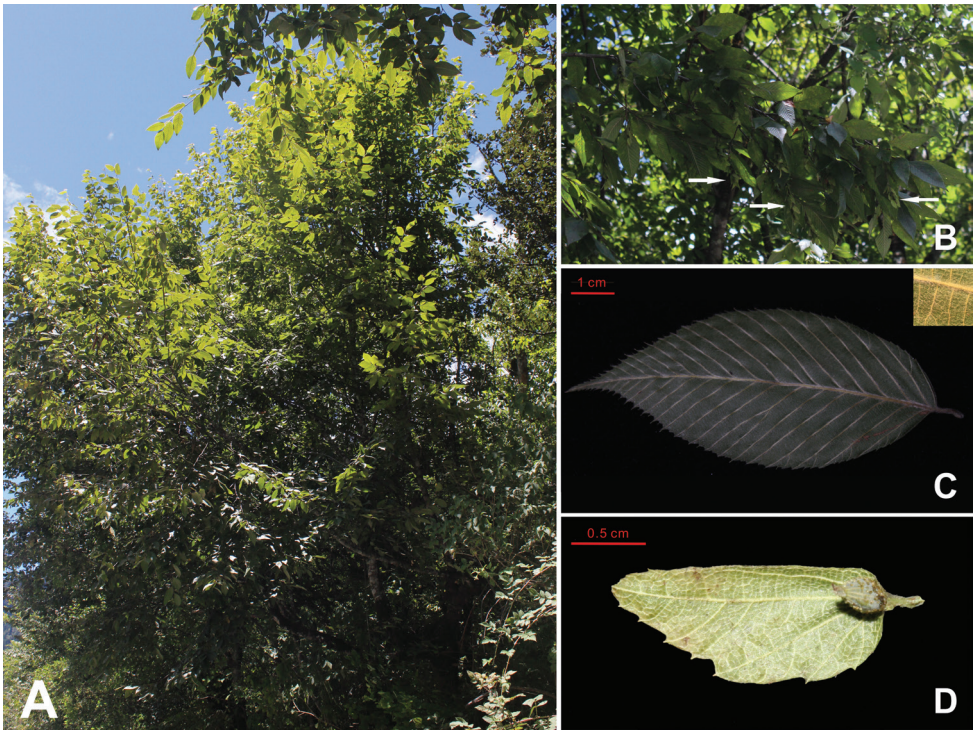


Figure 3. *Carpinus tibetana* Z. Qiang Lu & J. Quan Liu. **A** The whole plant **B** Branches with infructescences, the small white arrows pointing to the infructescences **C** Leaf **D** Bract and fruit.

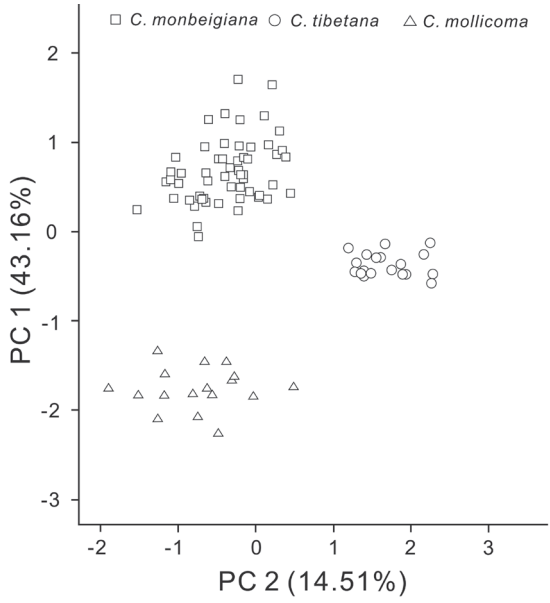
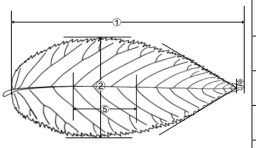


Figure 4. Morphological clustering based on Principal Component Analyses separated three different groups responding to the respective species.

Table 4. Morphological characters measured for Principal Component Analysis (PCA).

Character number	State	Unit	Coding (if qualitative)	PC1 (43.16%)	PC2 (15.51%)
	LEAF				
1	Length	cm		0.672	0.316
2	Width	cm		0.783	0.432
3	Length to width ratio	Ratio		-0.584	-0.366
4	Length of petiole	cm		0.224	-0.246
5	Distance between 5-8 lateral veins located in the middle of leaf	cm		0.853	-0.196
6	Length of apex	mm		-0.357	0.633
7	Average petiole diameter in the middle	mm		0.592	-0.094
8	Character1/ Character5	Ratio		0.544	0.703
9	Number of lateral veins on each side of midvein	Count		-0.488	0.754
10	Abaxial leaf pubescence	Qualitative	2 = Dense; 1 = Glabrescent	-0.694	0.308
	INFRUCTESCENCE				
11	Length of peduncle	cm		0.690	0.250
12	Length of infructescence	cm		0.754	-0.430
13	Width of infructescence.	cm		0.736	0.174
	BRACT				
14	Length	cm		0.697	0.283
15	Width	cm		0.663	0.377
16	Length to width ratio	Ratio		-0.410	-0.195
	NUTLET				
17	Densely villous or not	Qualitative	2 = Dense; 1 = None or sparsely villous at apex	0.856	0.360
18	Densely resinous glandular or not	Qualitative	2 = Dense; 1 = None	-0.856	-0.360
19	Number of ribs	Count		0.189	0.434
20	Length of nutlet	cm		0.586	-0.511
21	Width of nutlet	cm		0.861	-0.344
22	Length to width ratio	Ratio		-0.884	-0.160

sequences and the individual number of shared types is presented in Table 5. Phylogenetic analysis of these sequences suggested that the sampled individuals of *C. monbeigiana*, *C. mollicoma* and the Tibetan populations separated into three genetic clades with *C. mollicoma* diverging first and *C. monbeigiana* and plants from the Tibetan populations forming a sister relationship (Figure 5). The sequence variations of the Tibetan individuals showed a combination of the mutations found for *C. mollicoma* or *C. monbeigiana* (Table 2).

Geographically, all specimen records in the present study and those from Chinese Virtual Herbarium (http://www.cvh.org.cn/) suggested that the Tibetan populations are disjunct in geographical distributions from both *C. monbeigiana* and *C. mollicoma* (Figure 6).

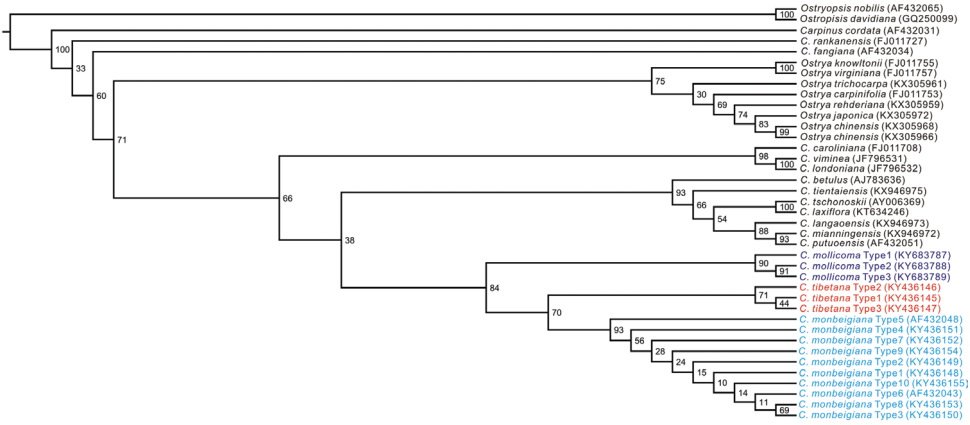


Figure 5. The ML tree based on nuclear ribosomal ITS sequence data from related species. GenBank accession numbers are shown after each species name.

Table 5. Nuclear ribosomal ITS sequence variations between three closely related species. The fixed nucleotide mutations were presented in bold type. Three ITS sequences (Type 5 and Type 6) of *Carpinus monbeigiana* (from Yunnan) were downloaded from NCBI (AF432043, AF432044 and AF432048).

	19 variable positions																		
Types of ITS sequences	1	1	1	1	1	1	1	3	3	3	4	4	4	4	5	5	5	5	5
(Individual number of the shared types)	9	4	5	8	8	8	9	0	9	9	2	2	4	5	4	5	5	5	8
		6	1	0	4	9	9	3	1	6	7	8	0	5	5	4	5	6	0
C. tibetana Type1 (5)	**A**	A	G	G	A	C	T	G	A	T	G	T	C	A	G	C	T	G	**G**
C. tibetana Type2 (2)	**A**	A	G	G	A	C	T	G	A	T	G	T	C	A	G	C	W	G	**G**
C. tibetana Type3 (1)	**A**	A	G	G	A	C	T	G	A	Y	G	T	C	A	G	C	T	G	**G**
C. monbeigiana Type1 (4)	G	A	G	R	A	Y	Y	G	R	T	S	K	Y	A	S	Y	T	G	A
C. monbeigiana Type2 (4)	G	A	G	R	A	Y	Y	G	R	T	S	K	Y	A	S	C	T	G	A
C. monbeigiana Type3 (2)	G	A	G	G	A	Y	C	R	A	T	G	T	Y	A	G	C	T	G	A
C. monbeigiana Type4 (2)	G	A	G	G	A	Y	Y	G	A	T	G	T	T	A	G	C	T	G	A
C. monbeigiana Type5 (2)	G	A	G	G	A	C	T	G	A	T	G	T	T	A	G	C	T	G	A
C. monbeigiana Type6 (1)	G	A	G	G	A	T	T	G	A	T	G	T	T	A	G	C	T	G	A
C. monbeigiana Type7 (1)	G	A	G	G	A	Y	Y	G	R	T	G	T	T	A	G	C	T	G	A
C. monbeigiana Type8 (1)	G	A	G	G	A	Y	C	R	A	T	G	T	Y	A	G	C	T	G	A
C. monbeigiana Type9 (1)	G	A	G	G	A	Y	Y	G	R	T	G	T	Y	A	S	C	T	G	A
C. monbeigiana Type10 (1)	G	A	G	R	A	Y	Y	R	R	T	S	K	Y	A	S	Y	T	G	A
C. mollicoma Type1 (5)	**A**	A	**C**	A	**G**	C	T	G	A	T	G	T	C	**G**	G	C	T	G	**G**
C. mollicoma Type2 (2)	**A**	R	**C**	A	**G**	C	T	G	A	T	G	T	C	**G**	G	C	T	G	**G**
C. mollicoma Type3 (2)	**A**	G	**C**	A	**G**	C	T	G	A	T	G	T	C	**G**	G	C	T	R	**G**

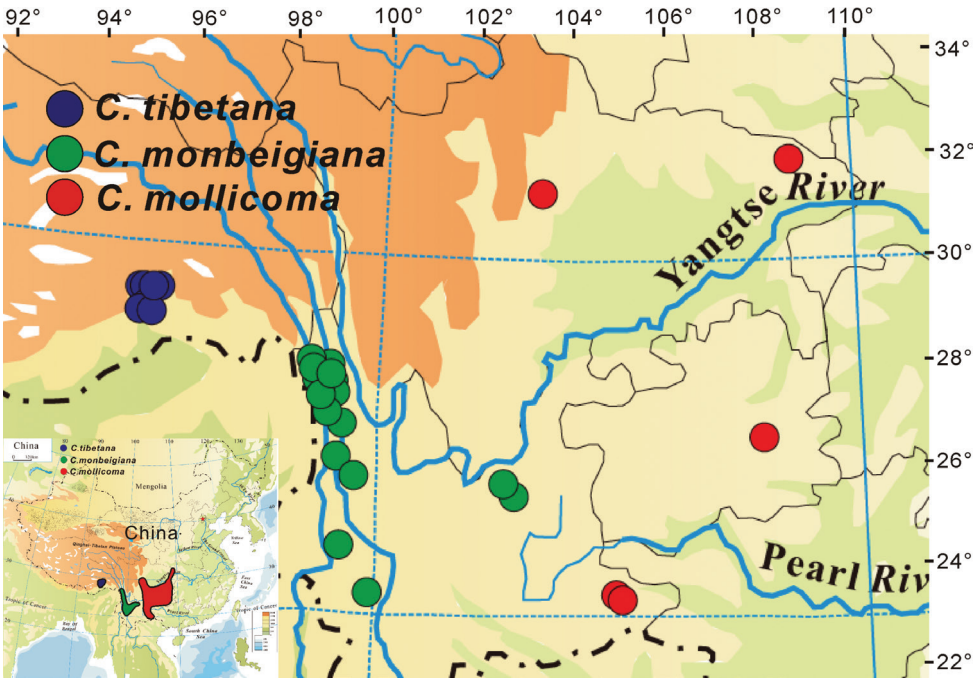


Figure 6. The distributions and locations of *C. monbeigiana*, *C. mollicoma* and *C. tibetana* based on the field investigation and Chinese Virtual Herbarium (http://www.cvh.org.cn/).

Discussion

Different species concepts emphasise the different criteria used to define and publish a new species (Wheeler and Meier 2000). An integrative practice using multiple criteria to circumscribe species boundaries will produce relatively objective and operational taxonomy (Su et al. 2015, Hu et al. 2015, Liu 2016; Lu et al. 2017). In this study, we demonstrated that the Tibetan populations previously placed under *C. monbeigiana* or *C. mollicoma* should be erected as a distinct new species based on the following lines of evidence. Firstly, these populations were obviously distinct from those of *C. monbeigiana* by the more lateral veins and dense pubescence on the abaxial leaf and from *C. mollicoma* by the nutlet with dense resinous glands and glabrous or sparsely villous at apex. All statistical analyses of the morphological traits clustered them into three separated groups. These populations seem to be characterised by a morphological combination of the other two species. Secondly, genetic divergences amongst these three groups are distinct; all of the sampled individuals from the Tibetan populations have a combination of unique genetic mutations that are found in the other two species but in a combination that is distinct from them. Phylogenetic analysis of nuclear ribosomal ITS sequence variations suggested that all sampled individuals from the Tibetan populations comprised a genetic cluster which seems to be more closely related to *C. monbeigiana* than to *C. mollicoma*. Finally, the Tibetan populations occupy

a distinct distribution disjunct from others of *C. monbeigiana* and *C. mollicoma.* All lines of evidence suggest that the divergence amongst these populations is consistent with warranting three distinct species. Given this, we here recognise the Tibetan populations as a new species. In addition, this new species probably originated through the geographic isolation from hybrid (homoploid or allopolyploid) speciation between *C. monbeigiana* and *C. mollicoma* because of its morphological and genetic combination of the other two species. However, this hypothesis needs further tests from multiple lines of evidence, including both chromosomal and population genetic observations.

Taxonomic treatment

***Carpinus tibetana* Z. Qiang Lu & J. Quan Liu, sp. nov.**
urn:lsid:ipni.org:names:60476297-2
Figures 2, 3

Diagnosis. *Carpinus tibetana* differs from *C. monbeigiana* by 19–24 lateral veins on each side of the midvein and dense pubescence on the abaxial leaf and from *C. mollicoma* by the nutlet with dense resinous glands and glabrous or sparsely villous at apex.

Type. CHINA. Tibet: Bomi County, Yigong, Tongmai, 95°04'31"E, 30°06'05"N, 2060 m, forest edge, 28 Aug 2016, *Z.Q. Lu 2016QTP001* (holotype, LZU; isotypes, LZU, PE, KUN).

Description. Trees to 10 m tall, deciduous; bark grey, smooth. Branchlets dark grey, densely yellow or white pubescent when young, glabrescent the next year. Stipules deciduous. Petiole 5–8 mm, densely white or yellow puescent; leaves alternate, leaf blade ovate-elliptic or elliptic, usually 5–8 cm × 2–3 cm, abaxially sericeous-villous along veins, pubescent elsewhere, base rounded or rounded-cuneate, margin irregularly and doubly setiform mucronate serrate, apex attenuate-acuminate or caudate-acuminate; lateral veins (17) 19–23 on each side of midvein. Male inflorescence pendulous, spicate-cymose, cylindric, enclosed by buds during winter, with many overlapping bracts, 1.5–5.0 cm × 5.0–8.0 mm when mature; flowers without bracteoles, inserted at base of bracts. Female inflorescence terminal or axillary on dwarf shoots, racemose; flowers paired; bracts leaflike, complanate, overlapping. Mature infructescence 5–10 cm × 2.0–3.5 cm; peduncle ca. 1.2 cm, densely yellow hirsute; bracts of female flowers loosely overlapping, 1.5–1.9 cm × 6–8 mm, abaxially densely yellow hirsute along reticulate veins, outer margin coarsely dentate, without basal lobe, inner margin entire, with inflexed basal auricle, apex acuminate or caudate-acuminate; veins 5–6. Nutlet ovoid-ellipsoid, 3.2–3.6 mm × 2.2–2.5 mm, glabrous or sparsely villous at apex, densely brown or orange resinous glandular, prominently 8- or 9-ribbed. Fl. Apr–May, fr. Jul–Sep.

Etymology. Due to its narrow distribution in Tibet, we give the specific epithet (*Carpinus tibetana*) referring to the name of the Xizang Autonomous Region (Tibet) of China where it is distributed.

Phenology. Flowering from April to May and fruiting from May to September.

Habitat and distribution. Up to now, according to our field surveys and sampling records in Chinese Virtual Herbarium (CVH), *Carpinus tibetana* has only been collected in Bomi and Motuo Counties (Figure 4). The new species usually grows at the forest edge and miscellaneous wood forest at elevations from 1550–2300 m a.s.l. This species probably extends its distribution to other Himalayan and adjacent regions in India, Nepal and Bhutan. Therefore, the *Carpinus* specimens collected from these regions need to be examined and confirmed and further field investigations to these regions should be conducted.

Additional specimens examined. CHINA. Tibet: Linzhi City, Yigong River, forest edge, 2300 m, 8 Aug 1983, *B.S. Li et al. 06467 & 6467* (PE); Bomi County, near to Yigong Town, secondary forest, 2100 m, 8 Sep 1976, *Wu 5649* (PE); Bomi County, Tongmai, mixed forest, 2080 m, 24 Jun 1976, *Anonymous 2505* (PE); Bomi County, Tongmai to Lulang along the G318 National Road, forest edge, 95°00'48" E, 30°02'35"N, 2060 m, 26 Sep 2009, *H. Sun et al. SunH-07ZX-2725* (KUN); Motuo County, Dexing, 26 Apr 1993, *H. Sun et al. 6008* (PE); Motuo County, from Ani to Hanmi, forest edge, 1550 m, 19 Sep 1980, *Anonymous 15079* (PE); Motuo County, forest edge, 1500 m, 29 Jun 1980, *W.L. Chen 10780* (PE); Bomi County, Yigong, Tongmai, 95°04'31"E, 30°06'05"N, 2060 m, forest edge, 28 Aug 2016, *Z.Q. Lu 2016QTP002–Z.Q. Lu 2016QTP011* (LZU).

Key for identification of these four related species in Yunnan and Tibet, China

1 Bracts with lobes at bases of inner and outer margins; petioles slender, (1.0–)1.5–3.0 cm ..... ***C. viminea***

– Bracts with an inflexed auricle at base of inner margin; petioles robust, 0.3–1.2 cm **2**

2 Infructescences 4–13 cm × 1.5–3 cm; bracts 1.2–2.3 cm × 0.5–1.2 cm; nutlets ovoid-ellipsoid or broadly ovoid, with dense resinous glands, glabrous or sparsely villous at apex **3**

– Infructescence 2.5–4.5 cm × 1–1.5 cm; bracts 0.9–1.9 cm × 0.4–0.6 cm; nutlets broadly ovoid or ovoid-ellipsoid, without resinous glands, densely villous ..... ***C. mollicoma***

3 Leaf blade oblong-lanceolate, ovate-lanceolate, or elliptic-lanceolate, abaxial leaf surface glabrescent, with 14–18 lateral veins on each side of midvein, average distance between lateral veins 5–8 mm; nutlets broadly ovoid, 3.2–4.6 mm × 2.9–4.1 mm ..... ***C. monbeigiana***

– Leaf blade ovate-elliptic or elliptic, abaxial leaf surface densely pubescent, with 19–24 lateral veins on each side of midvein, average distance between lateral veins 4–5 mm; nutlets ovoid-ellipsoid, 3.0–3.9 mm × 2.2–2.8 mm.... ..... ***C. tibetana***

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RESEARCH ARTICLE

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Deciphering the sexual diploid members of the *Boechera suffrutescens* complex (Brassicaceae, Boechereae)

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Abstract

Boechera is a model genus that is of particular interest for understanding apomixis due to the presence of numerous apomictic diploid lineages that are tightly correlated with hybridisation events. *Boechera* includes many narrowly distributed endemics and apomictic hybrid lineages that obscure morphological boundaries amongst taxa. In this study, we focus on the *Boechera suffrutescens* complex, a phylogenetically well-supported but taxonomically complex north-western United States clade whose diploid species currently include the widespread *B. suffrutescens* and two narrowly distributed serpentine endemics, *B. constancei* and *B. rollei*. Using a 15-locus microsatellite dataset, we infer ploidy and sexual vs. apomictic reproduction for all individuals and then assess species limits for all sexual diploid samples. Our results support the recognition of *B. rollei* and *B. constancei* as distinct species and reveal three divergent sexual diploid lineages within *B. suffrutescens* sensu lato. The latter three lineages exhibit geographic, genetic and morphological coherence and consequently warrant recognition at the species rank. These include *Boechera suffrutescens* s.s., which is restricted to Idaho and eastern Oregon, *Boechera botulifructa*, a newly described species distributed along the Cascade Mountain Province from Lassen County, California north to Deschutes County, Oregon and the heretofore dismissed species *Boechera duriuscula* (basionym ≡ *Arabis duriuscula*), which occurs along the Sierra Nevada Province from Plumas County southwards to Fresno County, California. Our data also reveal

substructure in *B. constancei* that is likely attributable to the highly fragmented distribution of its serpentine habitat. This refined taxonomic framework for the *B. suffrutescens* complex enhances *Boechera* as a model system, adds to our knowledge of speciation in edaphically extreme environments and provides information on ongoing conservation efforts for these taxa.

Keywords
Boechera duriuscula, *Boechera botulifructa*, Apomixis, Taxonomy, Sexual Diploid

Introduction

The genus *Boechera* Á.Löve & D.Löve was first recognised in 1976, but it was not widely accepted as distinct from *Arabis* L. prior to 2003 (Al-Shehbaz 2003). This largely North American lineage represents a complex assemblage of ±83 sexual diploid (S2X) taxa that have given rise to hundreds of apomictic hybrids, a situation that has confounded morphological classification since the first species were described in the 1820s (Li et al. 2017). The causes of this complexity include recent divergence and ongoing hybridisation, limited morphological disparity, edaphic shifts and the presence of both apomictic diploid (A2X) and triploid (A3X) hybrid lineages that are common and persistent across the distribution (Beck et al. 2011; Windham and Al-Shehbaz 2006; Windham and Al-Shehbaz 2007a; 2007b). *Boechera* is nearly unique amongst flowering plants in possessing numerous diploid apomictic lineages (Bicknell and Koltunow 2004; Koltunow and Grossniklaus 2003). Understandably, the confluence of these characteristics has attracted considerable attention and *Boechera* has become a focal point for studies of biogeography, speciation, adaptation, apomixis and ecological genomics.

Despite widespread interest in *Boechera* as a model system (e.g. Rushworth et al. 2011) ongoing research has been hindered by limited understanding of species-level diversity, biogeography and phylogeny. Only recently, through a combination of molecular phylogenetic and population genetic studies, has genuine progress been made towards a coherent *Boechera* classification. This has involved a modified "diploids first" approach (Brown et al. 2002), acknowledging that it is nearly impossible to identify and study apomictic hybrids without an in-depth understanding of the sexual diploid species that gave rise to them. This approach has proven highly effective in *Boechera*, documenting cryptic biodiversity and bringing new clarity to both the *B. fendleri* and *B. lignifera* species complexes (Alexander et al. 2015). Here, we apply this method to another poorly known group, the *B. suffrutescens* complex.

The ability to distinguish amongst different ploidy levels and reproductive modes in *Boechera* rests on several well-documented correlations derived from chromosomal, microsatellite heterozygosity and pollen data (Alexander et al. 2015; Beck et al. 2011; Li et al. 2017). Initially, pollen morphology was used as the primary indicator (Al-Shehbaz and Windham 2010; Windham and Al-Shehbaz 2006; Windham and Al-Shehbaz 2007a; 2007b). S2X lineages produce pollen in tetrads through normal meiosis; the individual grains are mostly uniform, narrowly ellipsoid, 13–16 μm wide, with three symmetrical colpi (Suppl. material 1: fig. 1A). A3X lineages produce any

functional pollen in dyads by means of apomeiosis; these grains are more irregular, ovoid-spheroid, 22–30 μm wide, with more than three asymmetric colpi (Suppl. material 1: fig. 1B) (Windham and Al-Shehbaz 2006) . A2X lineages usually produce predominantly malformed pollen (resulting from irregular meiotic events) mixed with functional meiotic and/or apomeiotic pollen (Suppl. material 1: fig. 1C) (Beck et al. 2011). More recently, an extensive 15-locus microsatellite database, encompassing nearly all known sexual diploid taxa and over 4400 accessions (Li et al. 2017), has made it possible to determine both ploidy level and reproductive mode through microsatellite analysis on a simple DNA sample. This dataset has confirmed previous reports of a bimodal distribution of heterozygosity across the genus (Alexander et al. 2015; Beck et al. 2011). Comparative meiotic studies of over 134 individuals representing 84 lineages of *Boechera* reveal that the left peak of this bimodal distribution (heterozygosity <0.5) consists almost entirely of S2X individuals while the right peak includes mostly apomicts. Amongst the apomicts, A3X lineages can then be distinguished from A2X lineages by the presence of three alleles at one or more of the 15 microsatellite loci (Alexander et al. 2015; Beck et al. 2011).

Our improved ability to sort *Boechera* specimens into natural groups, combined with cluster analysis of microsatellite data and phylogenetic analysis of DNA sequence data, have greatly improved our understanding of several S2X species complexes (Alexander et al. 2015; Windham et al. 2015). Nevertheless, there are many groups that require additional study to characterise extant sexual diploid diversity. One such group is the *B. suffrutescens* complex. This complex currently includes three S2X species (*B. constancei* (Rollins) Al-Shehbaz, *B. rollei* (Rollins) Al-Shehbaz and *B. suffrutescens* (S. Wats.) Dorn) that formed a maximally supported clade in genus-wide molecular phylogenetic analyses (Alexander et al. 2013). Two A3X species (*B. horizontalis* (Greene) Windham & Al-Shehbaz and *B. rigidissima* (Rollins) Al-Shehbaz) are believed to be hybrids between members of the *B. suffrutescens* complex and more distantly related species of *Boechera* (Al-Shehbaz and Windham 2010).

The group takes its name from *Arabis suffrutescens* S. Wats., which has been broadly defined to include populations from the Sierra Nevada, Trinity Alps, Cascades and isolated mountain peaks across the northern Great Basin, southern Columbia Plateau and Rocky Mountains of central Idaho. This highly variable taxon includes both S2X and A3X populations (Al-Shehbaz and Windham 2010), which occur in close proximity near the type locality along the Snake River Gorge in eastern Oregon. Eighteen years after Watson named *A. suffrutescens*, Greene described a segregate species, *Arabis duriuscula* Greene. The taxon was typified based on collections from Donner Lake, California, which Rollins (1941) subsequently treated as a taller and less suffrutescent phenotype of *A. suffrutescens*. *Arabis dianthifolia* Greene, described from the vicinity of Crater Lake (Greene 1910), has also been viewed as synonymous with *A. suffrutescens* (Al-Shehbaz and Windham 2010).

Two other taxa were segregated from *Arabis suffrutescens* by Rollins (1993b) and subsequently transferred to *Boechera* by Windham and Al-Shehbaz (2006). *Boechera rollei* is the most narrowly distributed taxon in the group, known only from the Trinity Mountains in Siskiyou County California (Fig. 1) and an isolated population along upper Beaver Creek, Jackson County, Oregon. The relative showiness of its flowers

indicates that it is likely an outcrossing S2X lineage (Schmidt and Bancroft 2011). The other commonly accepted segregate is *B. constancei*, a narrow endemic apparently confined to Plumas and Sierra Counties, California. This taxon is known to be diploid based on a published chromosome count from the type locality (Rollins and Rüdenberg 1971) and it exhibits protogyny with distinctly elongated styles, which is suggestive of outcrossing (Schmidt and Bancroft 2011). In addition to being of conservation concern, both *B. constancei* and *B. rollei* appear to be restricted to serpentine soils, a model substrate for studying the links between edaphically extreme environments and divergent plant speciation (Kruckeberg 1951; 1984; 2002).

Although *B. constancei* and *B. rollei* are generally separable from the wide-ranging *B. suffrutescens*, there are some collections that appear to be morphologically intermediate (Rolle, pers. comm.). There also are unresolved questions regarding the placement of *Arabis duriuscula* and *A. dianthifolia* in synonymy under *Boechera suffrutescens*, as well as the relationship between S2X and A3X populations of the latter (Al-Shehbaz and Windham 2010). The purpose of this study is to identify and characterise the S2X lineages (taxa) within the *B. suffrutescens* complex to provide a framework for future investigations into the origins of related A2X and A3X lineages. Along with traditional macro-morphological and pollen analyses, we apply the set of 15 microsatellite loci, previously employed by Beck et al. (2011) and Alexander et al. (2015), to both herbarium specimens and extensive recent field collections. Pollen and microsatellite data are used to infer the ploidy and reproductive mode of each accession. The S2X individuals singled out by this process are used in a series of population genetic analyses to identify genetically coherent lineages worthy of species-level recognition.

Materials and methods

Sampling

Samples for the project were obtained from individuals representing the morphology and known geographic range of the complex (Fig. 1), including 150 newly added collections and 348 previously collected herbarium samples. Holotype specimens of *Arabis suffrutescens*, *A. suffrutescens* var. *perstylosa*, *A. rollei*, *A. constancei* and an isotype of *A. duriuscula* were included. The holotype of *A. dianthifolia* was observed online through the Smithsonian plant database (https://collections.nmnh.si.edu).

Correlation between pollen morphology and reproductive mode

In concert with other data, pollen morphology was used to assign or confirm the assignment of individual plants to S2X, A2X or A3X categories. Pollen samples of adequate quality were obtained from 45 individuals and were analysed and categorised following Beck

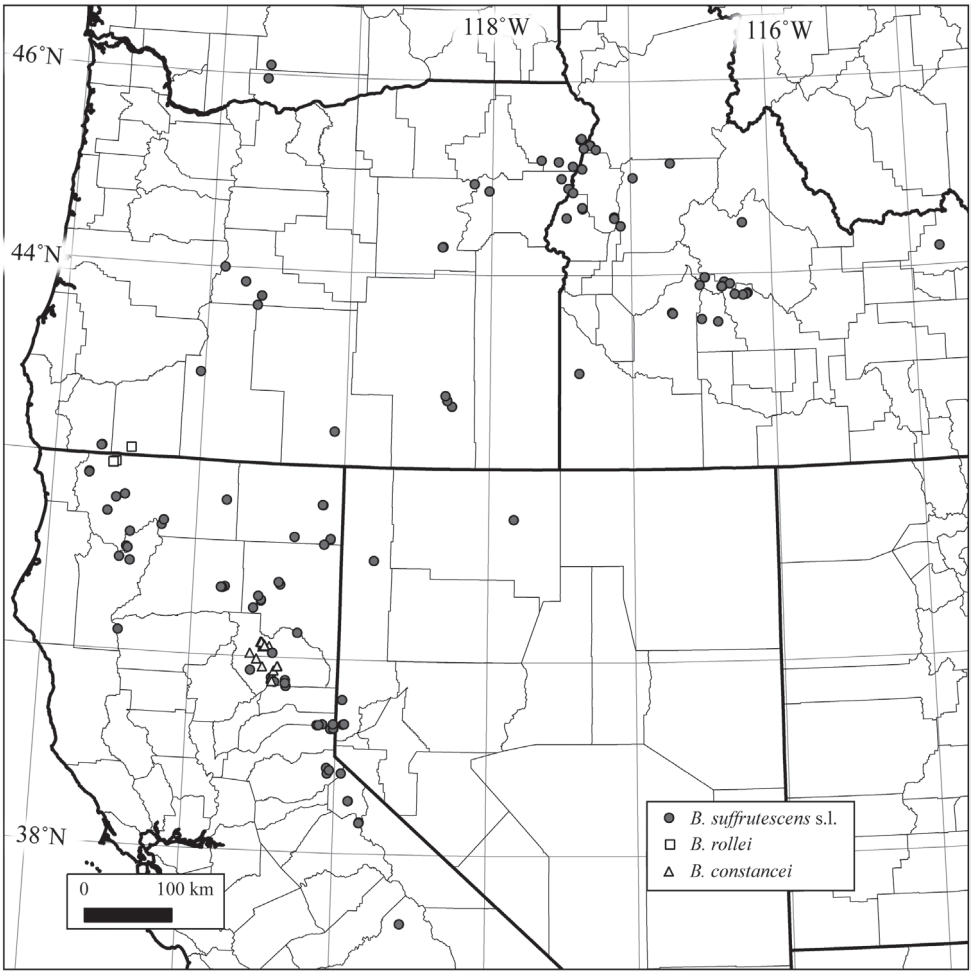

Figure 1. Geographic distribution of 498 initial individuals. Only 307 diploids were retained for diploid-level molecular analyses. All maps were created in QGIS (Quantum GIS 1137 Development Team 2013).

et al. (2011). Pollen was mounted in glycerol and immediately observed, characterised and photographed on an Olympus CH-2 objective microscope at various magnifications.

DNA extraction

Genomic DNA was extracted using a modified version of the protocol outlined in Alexander et al. (2015). The deviation included: dried leaf samples being homogenised without buffer, eluting the pulverised material in grinding buffer plus 12 µl (>600 mAU/ml) of Proteinase K and an incubation with agitation at 65 °C for 12–15 hours prior to moving on to the next step.

Amplification of microsatellite loci

Fifteen previously published microsatellite loci (ICE3, ICE14 (Clauss et al. 2002), BF3, BF9, BF11, BF15, BF18, BF19, BF20, Bdru266 (Song et al. 2006), a1, a3, b6, c8 and e9 (Dobes et al. 2004) were amplified via five multiplex polymerase chain reactions (PCR) following Beck et al. (2011). Forward primers were 6-FAM or HEX labelled. Amplicons were sized with the 500 LIZ standard (Applied Biosystems Corp., Carlsbad, CA) on an Applied Biosystems 3730 xl at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility. Allele sizes were determined using GeneMarker 1.91 (SoftGenetics, State College, PA). Locus a3 was excluded from downstream analyses due to potential unresolved paralogy consistent with the findings of Beck et al. (2011) and Alexander et al. (2015).

Identification of S2X individuals and populations

All samples with data for at least 8 of the 15 microsatellite loci were retained for analysis. The ploidy level of each sample was then estimated using the criteria outlined by Beck et al. (2011). In short, if an individual exhibited no more than two alleles per locus, it was inferred to be diploid; if three alleles were present at one or more loci, it was inferred to be triploid. Following Alexander et al. (2015), the mode of reproduction amongst diploids (e.g. S2X vs. A2X) was then inferred via an average number of alleles per non-null locus (ANA/NNL) approach. The S2X category was initially set to a mean ANA/NNL ≤1.5 (Alexander et al. 2015) and later reduced to ≤1.35 or less following downstream population genetic analyses that identified hybrid "*B. constancei*" with values above 1.35 (discussed below).

Analyses of population structure within and amongst S2X taxa

Following Alexander et al. (2015), we employed a hierarchical approach to investigate patterns of microsatellite variation and population differentiation amongst putative species-level lineages. STRUCTURE employs a parametric Bayesian approach to investigate the most likely number of differentiated (*K*) population systems (Falush et al. 2003; Pritchard et al. 2000). Exploratory STRUCTURE analyses were performed with the admixture model using default settings with 50,000 burn-in and 500,000 post-burn in generations with five iterations at each value of *K* from 1–11. Final STRUCTURE analyses included 100,000 burn-in and 1,000,000 post-burn in generations with 10 iterations for each value of *K* from 1–11. The most likely value of *K* for each analysis was identified using the Δ*K* method of Evanno et al. (2005) as implemented in STRUCTURE *Harvester* (Earl and vonHoldt 2012). Null alleles at seven loci (B11, C8, BF15, Brdu266, e9, BF3 and BF19), clearly corresponding to either prior taxonomic assignment and/or geographic structure, were coded in STRUCTURE using RECESSIVE ALLELES = 1 (Falush et al. 2007).

AWclust utilises a nonparametric approach to infer population structure based on allele sharing distance (Gao and Starmer 2008). Critically, this approach does not incorporate a model of within-group Hardy-Weinberg equilibrium, an assumption that is likely unrealistic considering the interspecific, biogeographic and temporal (inclusion of historical specimens) scope of our sample set. Multidimensional scaling plots (MDS) were generated in AWclust to visualise relative coherence and distinctness of clusters based on allele sharing distance. AWclust estimates the optimal number of clusters (*K*) via the gap statistic (Tibshirani et al. 2001), whereby individuals are assigned to clusters at the optimal *K* through the implementation of Ward's minimum variance hierarchical clustering. Gap statistics were calculated for a given data set with 100 null simulations for *K* values 1–11. The aforementioned null alleles were also treated as characters in AWclust.

Morphological assessment a posteriori

Individuals inferred as representing S2X species-level lineages through the aforementioned analyses were subsequently studied in detail to identify diagnostic morphological characteristics for the taxonomic treatment.

Results

Correlation between pollen morphology and reproductive mode

Forty-five accessions harboured pollen of sufficient quality and quantity for morphotyping. Of these, 26 individuals exhibited ovoid-spheroid, multicolpate pollen consistent with apomictic reproduction, 14 exhibited narrowly elliptic, tricolpate pollen consistent with sexual reproduction and five exhibited presumably non-viable pollen with a spheroid, ecolpate morphology. There was 92% agreement between mode of reproduction inferred via pollen morphology and that inferred by the maximum number of alleles per locus (see below). This high correlation is consistent with prior studies by Beck et al. (2011), who reported a 96% correlation in a sample of 330 specimens.

Identification of S2X individuals and populations

The maximum number of alleles per locus criterion (Alexander et al. 2015; Beck et al. 2011) identified 191 triploid (which were excluded from further analysis (see Appendix 1)) and 307 diploid (S2X and A2X) individuals (see Table 1, "Additional specimens examined" and Appendix 1). For ease of data presentation in Table 1 and this text, populations are represented by abbreviations that include a locality prefix (CD = Canyon Dam, CP = Cascade Province, GB = Great Basin, OVR = Onion Valley Reservoir,

Table 1. Summary of sexual diploid (S2X), apomictic diploid (A2X), and apomictic polyploid (A3X and A4X) assignments and clusters inferred from preliminary analyses. Polyploids, A2X clusters, and singletons were excluded from the final S2X analyses (see text).

	Clusters										
	CD-co	**CP-su**	**TL-co**	**PLSI-co**	**OVR-xco**	**SNP-su**	**TL-su**	**TL-ro**	**GB-xsu**	**Singletons**	**Polyploids**
# individuals	18	38	18	35	16	59	31	36	48	8	191
Inferred Reproductive mode	S2X	S2X	S2X	S2X	A2X	S2X	S2X	S2X	A2X	A2X, S2X	A3X, A4X
Mean ANA/NNL with range	1.295 (1.0–1.5)	1.004 (1.000–1.083)	1.044 (1.000–1.091)	1.150 (1.000–1.300)	1.362 (1.077–1.500)	1.087 (1.000–1.385)	1.142 (1.000–1.462)	1.180 (1.000–1.462)	1.547 (1.385–1.692)	1.471 (1.182–1.667)	2.006 (1.167–2.750)
# Individuals with analyzed pollen or meiotic counts	7	1	–	4	–	2	3	8	2	–	30

PLSI = Plumas and Sierra Counties, SNP = Sierra Nevada Province, TL = type locality region,) and a species suffix (co = *constancei*, ro = *rollei* and su = *suffrutescens* s.l.). If the specific identifier is preceded by an 'x' (e.g. OVR-xco), the group is a putative hybrid lineage assigned to the A2X category. Ploidy assignment for a small number of individuals was inconsistent with prior inferences for their taxon. In particular, 9 of 96 *B. constancei* individuals were inferred to be triploids despite prior diploid inference from a smaller sample of individuals (Rollins and Rüdenberg 1971).

Subsequent analyses focused on differentiating A2X and S2X individuals

The 1.5 ANA/NNL criterion (Alexander et al. 2015; Beck et al. 2011) identified 238 putative S2X individuals. Preliminary STRUCTURE runs were then employed to fine-tune the ANA/NNL cutoff. These preliminary studies identified nine putative population systems (excluding "singletons") with 16–59 individuals per group (S2X and A2X in Table 1). A small subset of three individuals from Falcon Valley, Washington did not cluster with other population systems. "Falcon Valley" is an anomalous place name used by W.N. Suksdorf and we are unable to determine from where these specimens were collected. Given the geographic uncertainty and poor sampling of this lineage, plants from "Falcon Valley" were excluded from further analysis. A group of individuals (OVR-xco) from Onion Valley Reservoir that is morphologically assignable to *B. constancei* showed genetic admixture. These individuals, inferred to represent a previously undetected A2X hybrid lineage, exhibited a mean ANA/NNL of 1.36. In light of this, the S2X mean cutoff was reset to <1.35 to provide a more conservative circumscription of the S2X category. After applying these filters, we were left with 235 inferred S2X individuals to be included in the final analyses.

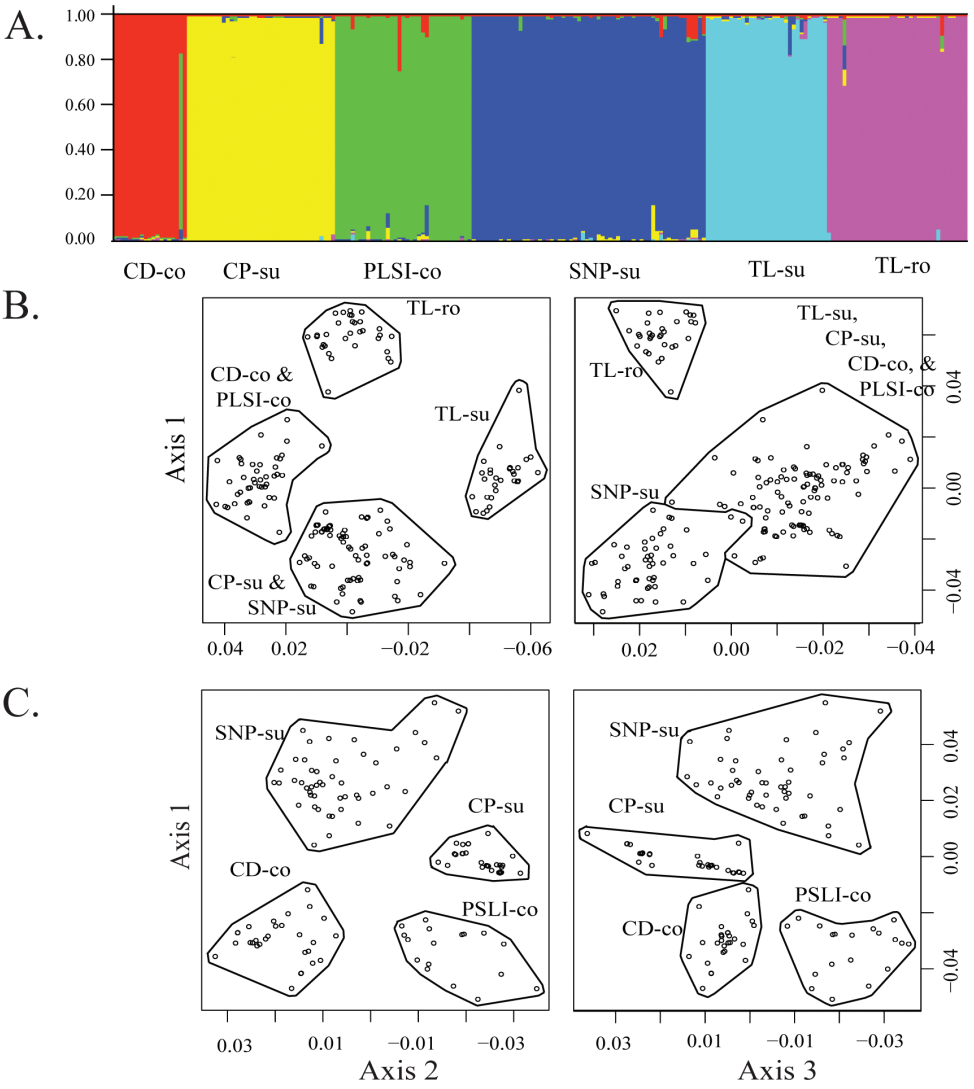

Figure 2. Results from Analysis 2 (which excluded TL-co). **A** STRUCTURE bar plot with K = 6 **B** MDS microsatellite plot of first three axes at K = 6 as found with the gap statistic **C** MDS plot with TL-su and TL-ro, the most divergent clusters, excluded to demonstrate coherence of the remaining four clusters (gap statistic K = 4).

Analyses of population structure within and amongst S2X taxa

Our diploid only (see above) and preliminary S2X only analyses revealed conflict and instability in the optimal *K* inferred by STRUCTURE as well as between AWclust and STRUCTURE. Analysis 1, including all 235 S2X individuals, yielded two equally optimal *K* (3 and 8) in STRUCTURE and two equally optimal *K* values (4 and 8) in

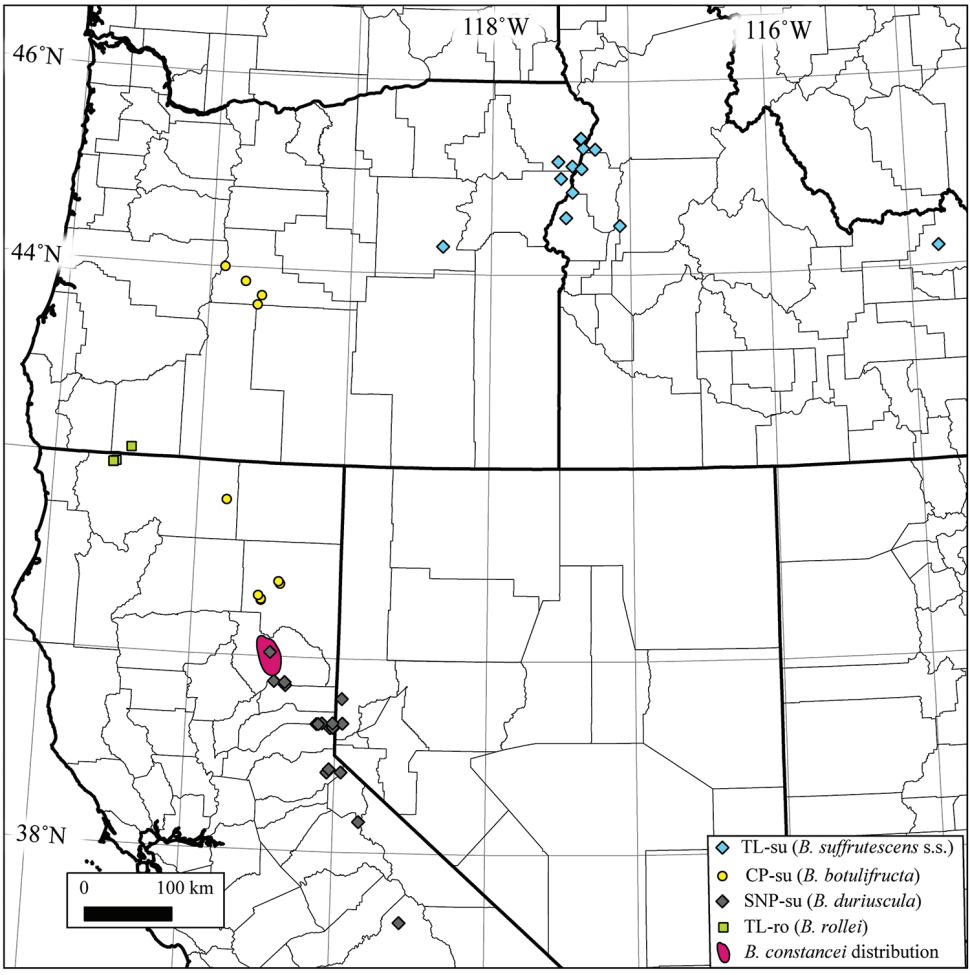

Figure 3. Geographic distribution of *B. suffrutescens* (CP-su, SNP-su, and TL-su) and *B. rollei* (TL-ro) lineages inferred from Analysis 2. A map of Plumas Co., California with distributions of the B. constancei clusters is presented in Fig. 4.

AWclust. The instability observed within and between these analyses was the result of conflicting assignments for individuals of *B. constancei* from the type locality (TL-co). TL-co individuals either formed a unique cluster (Suppl. material 2: fig. 2A, $K = 6$ and 8) or occasionally grouped with CP-su individuals (Suppl. material 2: fig. 2A, $K = 5$). These findings were consistent with potential introgression involving TL-co and CP-su. Given the instability associated with TL-co, we performed a second round of analyses (Analysis 2) without TL-co, which yielded an unambiguous $K = 6$ from both STRUCTURE and AWclust. This array specifies *B. rollei* as a single cluster, but supports two distinct clusters (CD-co and PLSI-co) within *B. constancei* and three distinct clusters (CP-su, SNP-su and TL-su) within *B. suffrutescens* s.l. (Fig. 2). Each of these clusters also occupies a discrete geographic range (Figs 3, 4) with possible introgressant

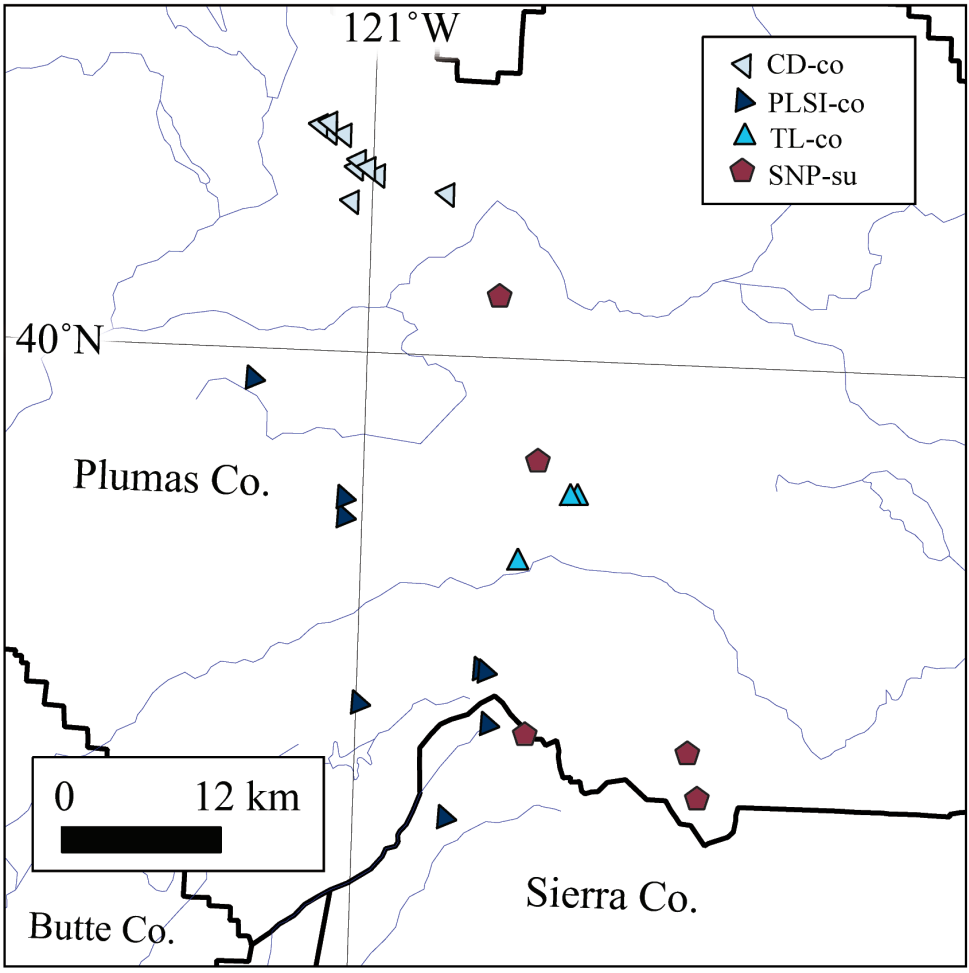

Figure 4. Distribution of diploid *B. constancei* s.l. and SNP-su clusters in Plumas County, California. CD-co lies to the north of the east branch of the north fork of the Feather River. The type locality cluster (TL-co) exhibited unstable placement in both genetic clustering analyses.

populations (TL-co) located in close proximity to the most similar putative parent (*B. constancei*) but nearly 100 km south of the documented range of the other (CP-su).

Morphological Assessment a Posteriori

Morphological comparisons of the clusters identified by STRUCTURE and AWclust revealed a variety of features useful for distinguishing these putative taxa. Character state differences in petal length, style length and the presence or absence of auricles on the cauline leaves have been used to separate *B. rollei* and *B. constancei* from *B. suffrutescens* s.l. Each of these features is consistent with differentiation amongst these lineages.

Additionally, we have identified a series of morphological features that support the recognition of the three clusters belonging to *B. suffrutescens* s.l. (CP-su, SNP-su, and TL-su). These include basal leaf pubescence, shape of the fruit apex and the length-to-width ratio of mature basal leaves (see "Taxonomic Account" section below).

Discussion

Assignment of ploidy and characterisation of S2X

The stepwise process employed to first parse diploids from polyploids and then S2X from A2X, identified 238 S2X individuals out of a total sample of 498 *B. suffrutescens* complex samples. The fact that more than half of the individuals were A3X or A2X clearly illustrates that the *B. suffrutescens* complex harbours the hybridisation, apomixis and polyploidy trifecta that has severely hindered species-level taxonomy in the genus as a whole (Alexander et al. 2015; Windham and Al-Shehbaz 2007a; 2007b). The analytical approach taken here supports the recognition of five sexual diploid taxa within the *B. suffrutescens* complex. These lineages include the current circumscriptions of *B. constancei* and *B. rollei* and a recircumscription of *B. suffrutescens* that recognises three distinct taxa. Each of these five taxa is discussed below.

Boechera rollei

All 36 individuals of *Boechera rollei* were clearly defined as S2X by allele numbers (Table 1) and as a distinct group by cluster analyses (Fig. 2). The taxon is very rare, with just three known populations restricted to serpentine soils in Siskiyou County, California and Jackson County, Oregon. Its distribution overlaps with the broadly distributed *B. suffrutescens* s.l. (Fig. 1), but the two have not been observed growing together. *Boechera rollei* is separable morphologically from all other S2X members of the complex by its unusually large (8–11 mm long) cream-coloured (vs. lavender) petals and non-geniculate fruiting pedicels. It is further separable from *B. constancei* by its auriculate cauline leaves and shorter styles (≤1.5 mm). Genetics, ecology, geography and morphology all support recognition at the species level, a conclusion that is consistent with prior taxonomic treatments (Al-Shehbaz and Windham 2010; Rollins 1993a) and meets the criteria proposed by the genetic species concept (Baker and Bradley 2006; Bateson 1909), the phylogenetic species concept (Nixon and Wheeler 1990) and the genotypic cluster concept (Mallet 1995) for species level recognition.

Boechera suffrutescens sensu lato

Boechera suffrutescens s.l. is by far the most widespread and morphologically heterogeneous taxon in the complex. With regard to the S2X lineages, STRUCTURE and

AWclust analyses subdivided individuals identified as S2X *B. suffrutescens* s.l. into three geographically distinct clusters (CP-su, SNP-su and TL-su) with little or no evidence of admixture (Fig. 2 and Table 2). The population system (TL-su) that includes the type locality for *B. suffrutescens* is S2X based on ANA/NNL results (mean 1.142) and pollen morphology (Table 1). It is separable form other S2X lineages based on several lines of evidence. It is genetically distinctive, forming a cohesive cluster in both *STRUCURE* and AWclust analyses (Fig. 2) and it exhibits unique fixed alleles at both the e9 and BF9 microsatellite loci. Its geographic range, extending ca. 100 km north-south along the Idaho-Oregon border with outlying populations in Fremont County, Idaho and Grant Counties, Oregon (Fig. 3), is separate from those of the other S2X taxa. This lineage is morphologically distinctive as well, characterised by having narrower basal leaves (length/width ratio usually ≥8:1) and sparser pubescence relative to the CP-su and SNP-su lineages (see "Taxonomic Treatment"). The combination of these features support recognition of this cluster as *B. suffrutescens* s.s.

The two remaining S2X lineages currently included within *B. suffrutescens* s.l. are distributed from the southern Sierra Nevada north into the central Cascades (Fig. 3). Both exhibit mean ANA/NNL values and pollen morphologies consistent with S2X assignment (Table 1). Like the TL-su cluster, members of the SNP-su group formed a cohesive cluster in both STRUCTURE and AWclust analyses (Fig. 2). Our sampling, comprising 59 individuals representing 23 populations of SNP-su, is distributed along the Sierra Nevada from Fresno to Plumas Counties, California (Fig. 3). At the northern end of its range, SNP-su overlaps with the distribution of *B. constancei* and hybridisation between these taxa may have given rise to the presumed A2X lineage OVR-xco (see discussion below). The SNP-su cluster is separable from sympatric populations of *B. constancei* by having shorter styles (≤1.5 mm) and auriculate cauline leaves and is distinguished morphologically from the other *suffrutescens* s.l. S2X taxa by fruit and pubescence characters. This cluster included an isotype of *Arabis duriuscula* Greene, a taxon that has been treated as a synonym of *B. suffrutescens* (e.g. Al-Shehbaz 2003; Al-Shehbaz and Windham 2010; Rollins 1993a; Windham and Al-Shehbaz). The findings presented here support the recognition of a distinct taxon requiring the recognition of *Arabis duriuscula* at the species level in *Boechera* (see "Taxonomic Treatment").

The second group previously assigned to *B. suffrutescens* s.l., the CP-su cluster, is represented by 38 individuals. Like the other two *suffrutescens* s.l. clusters, CP-su is genetically distinct in both STRUCTURE and AWclust analyses (Fig. 2). Current sampling suggests that it occupies a discrete geographic range in the Cascade Moun-

Table 2. Summary S2X only analyses one and two. 'X' indicates inclusion in the analysis using both AWClust and STRUCTURE.

	Clusters										
Formal Analysis	**CD-co**	**CP-su**	**TL-co**	**PLSI-co**	**SNP-su**	**TL-su**	**TL-ro**	**# of Individuals**	**ΔK**	**Gap Statistic K**	**Result**
1	X	X	X	X	X	X	X	235	3.8	5.7	Unstable
2	X	X	–	X	X	X	X	219	6	6	K = 6

tain Province extending from Lassen and Siskiyou Counties, California to Deschutes County, Oregon (Fig. 3). In addition to being genetically and geographically distinct, members of the CP-su cluster are separable from the other S2x taxa previously assigned to *B. suffrutescens* s.l. based on the distinctive, ovoid-shaped fruit apices. None of the species-level names, previously formalised in *Boechera* or *Arabis*, appear to be applicable to this taxon and we therefore propose a new name: *B. botulifructa* (see "Taxonomic Treatment").

Boechera constancei

Previous studies of the obligate serpentine endemic *B. constancei* considered it S2X based on chromosome counts (Rollins and Rüdenberg 1971) and pollen morphology (Al-Shehbaz and Windham 2010). Amongst our sampling of 87 individuals representing 28 unique geographic sites, 71 samples from 26 localities were indeed assigned to the S2X category based on ANA/NNL ratios (Table 1). The 16 samples from Onion Valley Reservoir (OVR-xco) were inferred to be A2X. Even with OVR-xco removed, population genetic analyses including all S2X *B. constancei* revealed considerable instability. STRUCTURE and AWclust analyses recovered as many as three clusters, CD-co, PLSI-co and TL-co (Fig. 4). The apparent instability was associated with an affinity between TL-co and CP-su, potentially indicative of hybridisation or introgression between these S2X groups. The species is well separated morphologically from the other S2X members of the *B. suffrutescens* complex by its unusually long styles (≥1.5 mm) and consistently non-auriculate cauline leaves.

The isolated island-like biogeography (Ellstrand and Elam 1993; Young et al. 1996) of *B. constancei* on serpentine soils could explain the patterns of geographically defined genetic sub-structure observed in this taxon (Suppl. material 2: fig. 2B). Future work is especially needed to investigate the complex substructure observed in *B. constancei* and to determine whether segregate taxa worthy of recognition are contained within the species.

Evaluation of A2X lineages in the *Boechera suffrutescens* complex

Two major A2X clusters were evident in the complex. The aforementioned OVR-xco cluster consisted of 16 individuals collected from serpentine in the vicinity of Pilot Peak and Onion Valley Reservoir in Plumas County, California. The STRUCTURE allele assignment profiles for these individuals exhibited apparent admixture between SNP-su and *B. constancei* and they resolved in intermediate positions between these putative parents in AWclust plots (data not shown). No pollen data were available from which to infer mode of reproduction. Further research will be required to confirm this assertion and investigate the origin of this cluster.

The second major A2X cluster (GB-xsu) is broadly distributed across the mountain ranges of the Great Basin in California, Oregon and Idaho. It is morphologically

assignable to *B. suffrutescens* s.l. Both the mean ANA/NNL of GB-xsu and pollen morphology from two individuals indicate that this is an A2X lineage (Table 1). Some additional preliminary analyses suggest it may have arisen through hybridisation between SNP-su and CP-su (Windham, unpubl. data).

The focus of this study was on circumscribing the sexual diploid taxa of the *B. suffrutescens* complex and our combined data support the recognition of at least five S2X species. Although not discussed in detail here, at least two A2X lineages and an even greater diversity of A3X hybrid lineages were also evident. More than half of the individuals included in this study ultimately were assigned to asexual groups and preliminary analyses imply that some of these lineages incorporate one or more genomes from outside of the *B. suffrutescens* complex (Morin, unpubl. data). This is consistent with prior observations that hybridisation and a transition to apomixis may be linked (e.g. Beck et al. 2011) and that recognising the impact of these phenomena is a critical part of deciphering *Boechera* diversity and evolution.

Taxonomic treatment

Members of the *B. suffrutescens* complex are distinguished from congeneric taxa by having relatively wide (2.5–6 mm) pendent fruits containing a single row of broadly winged (0.3–1.5 mm) seeds per locule. Previous molecular analyses (Alexander et al. 2013) have established that sexual diploid accessions of *B. constancei*, *B. rollei* and *B. suffrutescens* s.l. (represented by the SNP-su lineage) form a well-supported clade. In addition to the five S2X taxa characterised below, we encountered many A2X and A3X individuals. Some of these exhibit morphological characters that clearly set them apart from S2X taxa, suggesting that they are products of hybridisation with other species groups. More problematic were the apomictic hybrids that have arisen within the *B. suffrutescens* group, blurring the already subtle distinctions amongst the S2X members of the complex. It should be noted that the taxonomic treatment provided below applies only to S2X individuals and that pollen morphology and/or allelic diversity are the only reliable means for distinguishing closely related sexual and apomictic lineages in *Boechera* as a whole (Beck et al. 2011; Windham and Al-Shehbaz 2006).

Key to sexual diploid taxa of the *B. suffrutescens* complex

Given the frequency of hybridisation in *Boechera*, pollen morphology should be characterised prior to proceeding with this key. An inference of sexual diploidy can be made for individuals that produce mostly well-formed, narrowly ellipsoid symmetrically tricolpate pollen (Suppl. material 1: fig. 1). In terms of the macromorphological characters used in the key, there is considerable variation within species and some inevitable morphological overlap between species. Multiple plants should be examined if possible. *Caute procedere*.

1 Petals mostly more than 6 mm long, cream white, but occasionally with rose-coloured apices (*B. rollei*); mature fruiting pedicels curved-descending or reflexed but never distinctly geniculate proximally; plants only known from serpentine (ultramafic) substrates in the Klamath Mountains or on the west slopes of the Sierra Nevada near Lake Delahunty **2**

– Petals mostly less than 6 mm long, usually lavender-purple but occasionally cream with rose-coloured apices; many mature fruiting pedicels distinctly geniculate proximally, more or less straight distally; plants found mostly on non-serpentinic (felsic) substrates across a wide range from the southern Sierra Nevada north through the central Cascade Province and east to central Idaho **3**

2 Upper cauline leaves with distinct auricles 0.5–2.5 mm long; styles 0.5–1.5 mm long; petals 8–11 mm long; fresh herbage without a distinct bluish cast. Klamath Mountains..... ***Boechera rollei***

– Upper cauline leaves without auricles; style 1.5–5.5 mm long; petals 6–8 mm long; fresh herbage usually with a distinct bluish cast. Plumas and Sierra Counties in the vicinity of Lake Delahunty..... ***Boechera constancei***

3 Basal leaves on most plants glabrous or glabrate with few 1–2(3) rayed trichomes on the leaf margins and apices; length-to-width ratio of mature basal leaves usually ≥8:1; plants distributed from Grant County, Oregon east to central Idaho..... ***Boechera suffrutescens***

– Basal leaf surfaces pubescent and the leaves ciliate, with 2–4(–5) rayed trichomes; length-to-width ratio of mature basal leaves 4:1–9:1; plants of the Sierra Nevada and Cascade Provinces..... **4**

4 Mature fruit apex abruptly tapered into an ovoid tip with an apical angle (measured from the style base to a point 5 mm proximal to it) mostly greater than or equal to 30°; plants distributed in the Cascade Province from Lassen County, California to Deschutes County, Oregon..... ***Boechera botulifructa***

– Mature fruit apex more gradually tapered, with an apical angle (measured from the style base to a point 5 mm proximal to it) less than 25°; plants found in the Sierra Nevada from Fresno County, California north to Plumas County, California and near Lake Tahoe in Washoe County, Nevada..... ***Boechera duriuscula***

Boechera botulifructa **D.P. Morin, sp. nov.**
urn:lsid:ipni.org:names:60476298-2
Figures 3, 5

Type. U.S.A. California. Lassen County: 1.75 mi SSE of Coulthurst Flat on E road cut berm of Champs Flat Road. 1.35 air mi SSW of Cleghorn Reservoir, 26 Jun 2012, *C.D. Bailey & D.P. Morin 24* (holotype: NMC!; isotypes: DUKE!, MO!).

Diagnosis. As a member of the *B. suffrutescens* complex, *B. botulifructa* can be distinguished from most other species of *Boechera* by pendent relatively wide siliques (2–6 mm). Within the complex, the species is one of just five that produces narrowly ellipsoid symmetrically tricolpate pollen (Suppl. material 1: fig. 1A) indicative of dip-

Figure 5. *Boechera botulifructa*, Morin 24 (MO).

loid sexual reproduction. *Boechera botulifructa* is distinguishable from four other sexual diploid *B. suffrutescens* complex species by the presence of small petals (4–6 mm long), abruptly tapered silique distal apices and a geographic distribution along the Cascade Province in California and Oregon.

Description. Plants long-lived perennials, with woody caudices raised above ground level 1–5 cm, lacking crowded, persistent leaf bases; herbage without an obvious bluish cast. Fertile stems 1(–3) per caudex branch, each arising from a basal

rosette, lower parts pubescent to densely pubescent with short-stalked, 2–3(4) rayed trichomes 0.1–0.3 mm. Leaves: at stem bases oblanceolate, 1.7–5.8 mm wide, entire, ciliate with 2–3(–4) rayed trichomes to 0.07–0.40 mm; cauline leaves (4–)6–12, occasionally concealing stem proximally, the uppermost glabrous, with auricles (0)0.3–1.4 mm. Inflorescences mostly unbranched, 6–12 flowered; mature fruiting pedicels 9–17 mm, reflexed, distinctly geniculate proximally, but straight distally, glabrous. Flowers pendent at anthesis; sepals glabrous; petals 4.5–6.0 long × 2.0–2.5 mm wide, pale lavender or whitish with rose apices; anthers with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen; ovules 20–30 per fruit. Fruits 3–7(–10) cm long × 2.0–2.5 mm wide, pendent, straight to somewhat curved, with undulate edges; apical angle of fruit valve 30–38° (measured from base of style to 5 mm proximate); style persistent 0.2–1.2 mm long. Silique apex mostly rounded apically. Seeds uniseriate, 2.5–5.5 × 1.8–3.5 mm; wing continuous, 0.8–1.5 mm wide.

Distribution, habitat and phenology. As currently known, the species occupies three distinct regions in the Cascade Province: western Deschutes County, Oregon, near Medicine Lake, Siskiyou County, California and the area west of Eagle Lake in Lassen County, California. It favours rocky slopes and gravelly felsic soils in association with *Artemisia tridentata*, *Purshia tridentata*, *Pinus jeffreyi*, *Pinus contorta* and *Juniperus* at elevations of 1300–2100 m; flowering from May to July.

Comments. Morphologically, *B. botulifructa* is most similar to *B. duriuscula* and these two taxa are parapatric along the southern distribution of *B. botulifructa*. The species is distinguished from close relatives primarily by the abrupt tapering of the apex of the fruit, resulting in a sausage-like profile to which the specific epithet refers. Like most other members of the *B. suffrutescens* complex, it has a suffrutescent habit and wide (>3 mm), reflexed, often secund, fruits. Molecular data suggest that the specimens from the southernmost population in Lassen County, California, have diverged from the northern populations and may have a history of gene flow with *B. constancei* from the vicinity of its type locality. The latter *B. botulifructa* individuals, from Lassen County, also have reduced cauline leaf auricles relative to other non-serpentinicolous members of the complex.

Though the species spans a large geographic range, we have only identified nine populations systems thus far, suggesting a need for future investigation of conservation status. Within the populations we visited, individuals were sparsely dispersed across broad areas. The species occurs on public lands with noted impacts from grazing activity and potential impacts from logging of local native forests.

The holotype for *Arabis dianthifolia* Greene was collected at Crater Lake, Oregon, but our access to this type was limited to high resolution images. Although Crater Lake lies within the overall range of *B. botulifructa*, the specimen observed lacked the diagnostic fruit apex character. Furthermore, preliminary microsatellite analyses of specimens collected near the type locality indicate that *A. dianthifolia* is probably A2X (Windham, unpubl. data).

Specimens examined. California. Lassen County: Pine Creek near Bogard Ranger Station, 23 Jun 1960, *S.K. Harris 21448* A, B (GH); USFS 22N02. 1.25 road mi N of highway 44, 26 Jun 2012, *D.P. Morin 22* A - I (NMC); 1.75 mi SSE of Coulthurst

Flat on E road cut of Champs Flat Road. 1.35 air mi SSW of Cleghorn Reservoir, 26 Jun 2012, *D.P. Morin 24* A, B, C (NMC), D, E, F, (DUKE), G, H, I (MO); Coulthurst Flat area (T34N, R10E, S27, SW), 29 Jun 1983, *G.D. Schoolcraft 1038* (NY). Siskiyou County: Medicine Lake, 28 Jul 1921, *A. Eastwood 10885* A, B, C (GH). **Oregon.** Deschutes County: Along Elk Lake, 13 Jun 1925, *C.H. Peck 14337* (WILLU); Take unnamed dirt road E 0.4 mi from Jones Well Rd. 7 air mi SSW of Paulina Lake, 26 Jun 2012, *D.P. Morin 17* A, B, C, D - J (NMC); Deschutes NF, Ann's Butte, ca. 3.5 mi W of Sunriver on Rd. 40, 26 Jun 1992, *D.W. Taylor 12889* A, B (NMC); Paulina Lake, 29 Jul 1894, *J.B. Leiberg 584* A, B, (OSC).

Boechera constancei **(Rollins) Al-Shehbaz, Novon 13: 384. 2003.**
Figures 3, 4, 6

≡ *Arabis constancei* Rollins, Contr. Gray Herb. 201:5. 1971. **Type: U.S.A**. **California**. Plumas County: 7.6 mi SE of Quincy (at Spring Garden Overpass), on road to Blairsden, 11 Jul 1969, *L. Constance* and *T. Chuang 3875* (holotype: GH!; isotype: UC!).
GH holotype image – http://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id=49339

=*A. suffrutescens* S. Watson var. *perstylosa* Rollins, Rhodora 43: 471. 1941. **Type: U.S.A**. **California**. Plumas County: Above the Middle Fork of the Feather River 7.3 mi SE of Quincy, 9 Jun 1938, *L. Constance 2309* (holotype: GH!; isotypes: DS, NY, UC!, WS, WTU).
GH holotype image – http://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id=122692

Description. Plants long-lived perennials, with woody caudices raised above ground level 1–5 cm, lacking crowded, persistent leaf bases; herbage often with a distinct bluish cast. Fertile stems 1(–3) per caudex branch, each arising from a basal rosette, 1.2–3.0 dm, glabrous or glabrate proximally with few 1–2 rayed trichomes. Leaves at stem bases narrowly oblanceolate, 1.5–4.0 mm wide, entire, ciliate with simple and stalked 2–3(4) rayed trichomes 0.3–0.8 mm, basal leaf surfaces glabrous with ciliate margins to pubescent; cauline leaves 6–12, glabrous, lacking auricles, usually not concealing the stem proximally. Inflorescences unbranched, 5–15 flowered; mature fruiting pedicels 4–12 mm, strongly recurved or reflexed proximally but not distinctly geniculate proximally, glabrous. Flowers divaricate-ascending at anthesis; sepals glabrous; petals 6–8 mm long × 1.5–2 mm wide, creamy white, glabrous; anthers with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen; ovules 18–28 per fruit. Fruiting pedicels glabrous, 5–15 mm, recurved but not distinctly geniculate proximally. Fruits 3.6–7.5 cm long×3.0–3.5 mm wide, pendent or reflexed, usually secund, straight or slightly curved, with undulate margins, glabrous; apical angle of fruit valve 16–25° (measured from base of style to 5 mm proximate); style persistent 1.5–5.5 mm. Seeds uniseriate, 3–4 × 2.5–3 mm; wing continuous, 0.5–1.0 mm wide.

Distribution, habitat and phenology. *Boechera constancei* is only known from the western slope of the Sierra Nevada in the vicinity of Lake Delahunty in Sierra County and adjacent southern Plumas County, California. It appears to be confined to a variety of serpentinic substrates in association with *Pinus jeffreyi* and other "serpentine barren" vegetation types at elevations from 1200–1900 m; flowering from Apr–Jun.

Comments. *Boechera constancei* was originally treated as a variety of *Arabis suffrutescens* s.l., but it is distinguished from members of that group by its non-auriculate cauline leaves, longer (6.0–8.0 vs. 4.5–6.0 mm) petals that are creamy white and longer (1.5–5.0 vs. 0.4–1.2 mm) style. Although restricted to serpentine substrates, it generally shows greater local abundance and higher population densities within its narrow geographic range than *B. duriuscula*, *B. botulifructa* and *B. suffrutescens* s.s.

Specimens examined. California. Plumas County: Central Sierra Nevada, Plumas National Forest, N side of F.R. 24N20 above East Branch Rock Creek, 1.0 road miles from the junction with 24N28, 1.1 miles NE of Deanes Valley Campground, 4.3 miles WSW of central Quincy, 4 Aug 2009, *P.J. Alexander 997* A (DUKE), B, E, F (NMC); Above the Middle Fork of the Feather River, 7.3 mi SE Quincy, 9 Jun 1938, *L. Constance 2309* A (GH), A, B (UC); 7.6 mi SE of Quincy (at Spring Garden Overpass) on road to Blairsden, 11 Jul 1969, *L. Constance 3875* (UC), (GH); Sierra Nevada, about 2 miles from Spring Garden on road to Quincy, 20 Sep 1974, *J.T. Howell 50896* A - D (CAS); Sierra Nevada. 2 mi. northwest of Spring Garden, 16 Jun 1975, *J.T. Howell 51131* A, B (CAS); 2.65 mi W of Round Valley Reservoir dam, 23 Jun 1981, *J.T. Howell 54150* (NY); Plumas Nat'l Forest 7.6 mi. SE of Quincy (at Spring Creek Overpass) on road to Blairsden, 200 yards up steep serpentine slope from train track, 2 Jul 2012, *D.P. Morin 36* A - F (NMC); Plumas Nat'l Forest. 16.6 road miles S on La Porte Rd to E turnoff toward Onion Valley Reservoir 30 yards from La Porte Rd as slope steepens, 2 Jul 2012, *D.P. Morin 39* A (NMC); 1/2 mi. S of La Porte Rd, 1/2 mi SE (and above) serpentine cliffs. 1 mi NW of Pilot Peak, 3 Jul 2012, *D.P. Morin 44* A, B, C, D (NMC), E, F (DUKE); Plumas Nat'l Forest. 0.5 air mi WSW of Onion Valley Reservior. +/- 16.75 road mi from La Porte turnoff N in East Quincy. Above cliffs ESE of La Porte Rd., 3 Jul 2012, *D.P. Morin 45* F, H, L (NMC); Plumas NF; along all roads near Clear Creek NE of Mine Pit, E of Clear Creek, 8 Jun 1983, *J.H. Robertson 17217* (UNR); At junctions of roads 26N18, 26N92 and 27N92, ca. 0.5 mi east of Long Valley Mine, ca. 4 air miles west of Greenville, 5 Jul 1981, *M.S. Taylor 3649* A, B (CAS); S side of Hwy 70 ca. 200 yards S of Spring Garden Overpass ca. 7.5 mi SE of Quincy (T24N, R10E, S25; type locality), 2 Jun 1981, *M.S. Taylor 3827* (MO); East side of 25N17, ca. 0.125 mi south of Bean Creek, ca. 0.25 mi north of jct. 25N17 with 25N81 (Old Mt House Rd.). Ca. 3.5 air mi NW of Meadow Valley, 22 Jun 1981, *M.S. Taylor 3944* (CAS); Both sides of spur road off 26N18, ca. 0.5 mi southeast of Long Valley Mine, ca. 4 air mis west of Greenville, 18 May 1982, *M.S. Taylor 4471* A, B (CAS); South side of 27N92, ca. 1.5 air mi southeast of Canyon Dam, 18 May 1982, *M.S. Taylor 4474* A, B (CAS); South side of 27N92, ca. 2 air mi southeast of Canyon Dam, 18 May 1982, *M.S. Taylor 4478* A, B (CAS); Both sides of

Figure 6. *Boechera constancei*, Ahart 12,874 (JEPS).

road 27N92, near Goldstripe Mine, ca. 2.25 mi SE of Canyon Dam., 18 May 1982, *M.S. Taylor 4479* A, B, C (CAS); North side of 204, ca. 1 air mi southeast of Long Valley Mine, ca. 4 air mis west of Greenville, 1 Jun 1982, *M.S. Taylor 4582* C (CAS); County Rd 204 ca. 2 mi W from Round Valley (site); Crescent Mills 7.5 USGS quadrangle; NE .25 Section 8, 26 May 2004, *D.W. Taylor 19075* A (ORE), A, B (JEPS). Sierra County: About 3/4 mile (air) east of Lake Delahunty, about 2 miles (air) northeast of Gibsonville (39°45'01"N by 120°52'39.6"W), 28 Jun 2006, *L. Ahart 12874* (JEPS); West slope of the northern Sierra Nevada, 1.8 miles (linear) NE of Gibsonville on the road to Johnsville, 2.3 miles WNW of Mount Etna, 13.2 miles SSE of Quincy, Plumas National Forest, 26 Jun 2009, *P.J. Alexander 1046* A (BRY), B, D - F (NMC); Plumas Nat'l Forest. Open serpentine knoll 300 m WSW of Lake Delahunty. 50 m S of sign "Entering and Welcome to Delahunty Lake." 1 mi E from La Porte Rd on McCrea/Johnsonville Rd. Large, healthy population, 3 Jul 2012, *D.P. Morin 40* A - D, H (NMC), E, F, G (DUKE), I, J (MO); Plumas Nat'l Forest. From La Porte Rd, 1.8 mi road mi E on McRea/Johnsonville Rd 2ft from N side of road in washed out open serpentine slope, 3 Jul 2012, *D.P. Morin 41* A, B, C, D (NMC); Plumas Nat'l Forest. From La Porte Rd, 1.8 road mi E on McRea/Johnsonville Rd 70 ft from S side of Rd., 3 Jul 2012, *D.P. Morin 42* A - D (NMC).

***Boechera duriuscula* (Greene) D.P. Morin, comb. nov.**
urn:lsid:ipni.org:names:77178481-1
Figures 3, 7

≡ *Arabis duriuscula* Greene, Pittonia 4: 191. 1900. **Type: U.S.A. California.** Nevada County: Donner Lake, *Michener s.n.* (1893) and *Sonne s.n.* (1882). The repository of the Sonne syntype(s) is unknown at this time; the Michener syntype deposited at NDG has been included in our morphological and molecular analyses and is here designated as lectotype.

Description. Plants long-lived perennials, with ±woody caudices occasionally raised above ground level, lacking crowded, persistent leaf bases; herbage without an obvious bluish cast. Fertile stems 1–3(–4) per caudex branch, each arising from a basal rosette; lower parts pubescent to densely pubescent with short-stalked, 2–3(4) rayed trichomes 0.1–0.3 mm. Leaves at stem bases oblanceolate, 1.7–5.8 mm wide, entire, ciliate with 2–3(–4) rayed trichomes to 0.07–0.40 mm; cauline leaves (4–)6–12, occasionally concealing stem proximally, the uppermost glabrous, with auricles (0)0.3–1.4 mm. Inflorescences mostly unbranched, 6–12 flowered; mature fruiting pedicels 9–17mm, reflexed, distinctly geniculate proximally, but otherwise straight, glabrous. Flowers pendent at anthesis; sepals glabrous; petals pale lavender or whitish with rose tips apically 4.5–6.0 mm long × 2.0–2.5mm wide; anthers with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen; ovules 20–30 per fruit. Fruits 3–7(–10) cm

Figure 7. *Boechera duriuscula*, Tiehm and Nachlinger 8279 (CAS).

long × 2.0–2.5 mm wide, pendent, straight to somewhat curved, with minutely undulate edges; apical angle of fruit valve <25° (measured from base of style to 5 mm proximate); style glabrous, 0.2–1.2 mm. Seeds uniseriate, 2.5–5.5 × 1.8–3.5 mm; wing continuous, 0.8–1.5 mm wide.

Distribution, habitat and phenology. *Boechera duriuscula* is distributed in the Sierra Nevada from Kaiser Crest in Fresno County north to Mt. Hough in central Plumas County California. A few populations have been documented in Washoe, County Nevada, in the vicinity of Lake Tahoe. It is found on rocky or gravelly felsic substrates, often in association with *Abies magnifica*, *Pinus jeffreyi* and open *Wyethia* meadows at elevations from 2200–2750 m; flowering May–July.

Comments. *Boechera duriuscula* is distinguished from *B. suffrutescens* s.s. by being persistently pubescent basally. It differs from *B. botulifructa* by having fruits that taper more gradually apically (< 25° versus ≥30° as measured from the apex to a point 5 mm proximal to it).

Specimens examined. California. Alpine County: Armstrong Pass, 9 miles south of South Tahoe, 12 Aug 1978, *G.L. Stebbins 78149* A, C, D (CAS). El Dorado County: Summit area Echo Peak, 27 Jul 2012, *G.L. Smith 2497* (JEPS); ENE of Kyburz below Cup Lake near head of Tamarack Creek ca. 1.17 km SSE of the summit (9235) of Ralston Peak. T11N, R17E, Sec. 9. Lat.: 38°49'22"N Long.: 120°05'57"W (WGS84 Datum), 18 Jun 2002, *M.D. Windham 2579* A (MO), A (NMC). Fresno County: Kaiser Crest, 27 Jul 1914, *F.J. Smiy 621* (GH). Nevada County: Donner Lake, 1893, *E. Michener s.n.* (NDG); Truckee, Sierra Nevada Mountains, Jun 1892, *C.F. Sonne 9* A, B, C (UC); Sierra Nevada. Just W of Truckee near Donner State Park., 30 Jun 1965, *G.H. True 2142* A, B (CAS). Placer County: NE from Highway 267, travel 3.4 mi on Martis Peak Road toward Martis Peak Lookout, 1 Jul 2012, *D.P. Morin 32* A - E (NMC), F, G (DUKE), H, I, J (MO); Tahoe Nat'l Forest 3.5 air mi N of Lake Tahoe. 2.7 air mi NE of Brockway Summit. 3.7 road mi on Martis Peak Road from northern turnoff from Highway 267, 1 Jul 2012, *D.P. Morin 34* A, B, C, (NMC); Tahoe Nat'l Forest. Turnout on N side of Road to Martis Peak Lookout. +/- 2.6 air mi from Brockway Summit., 1 Jul 2012, *D.P. Morin 35* A, B, C, (NMC), D, E, F (DUKE), G, H, I (MO); Martis Peak, western flank near headwaters Monte Carlo Creek (T17N, R17E, S34, NE), 15 Jul 2005, *D.W. Taylor 19408* (JEPS). Plumas County: North side of the summit of Mount Elwell, ±0.1 miles N of the highest point, ±1.8 miles SE of Mt. Washington, ±14 miles NE of Downieville, northern Sierra Nevada, Plumas National Forest, 19 Apr 2009, *P.J. Alexander 864* A, B (NMC); Sierra Nevada. Jamison Creek., 27 Jun 1951, *J.T. Howell 27628* A, B, C (CAS); Summit ridge of Mt. Hough, 11 Jul 1967, *J.T. Howell 43348* A (GH), (CAS); Mount Hough Summit, ca. 7 mi NNE of Quincy., 5 Aug 1982, *M.S. Taylor 4927* A, B, C (CAS). Sierra County: At Verdi Peak Lookout, Verdi Range., 14 Jul 1970, *J.T. Howell 5522* A, C, D (CAS); Sierra Nevada. Near Mount Etna, 4 mi E of Gibsonville. Sierra-Plumas county line., 20 Jul 1975, *A. Tiehm* B (CAS). Tuolumne County: 2 mi w Sonora Pass, 27 Jul 2005, *R.C. Rollins 2993* (UC), (RSA). **Nevada.** Washoe County: Peavine Mountain, S of Murpheys meadow, T18N R20E sec. 17, 8 Aug 1974, *A. Tiehm 505* (UNR); Sierra Nevadas, Carson Range, N side of Galena Creek on the south side of Mt. Rose, 5 Aug 1983, *A. Tiehm 8279* B, C (CAS).

Boechera rollei **(Rollins) Al-Shehbaz, Novon 13: 389. 2003**
Figures 3, 8

≡ *Arabis rollei* Rollins, Harvard Pap. Bot. 4: 43. 1993. **Type: U.S.A. California.** Siskiyou County: Divide between the Applegate and Klamath rivers, Red Butte-Kangaroo Mt, Lilypad Lake –Towhead region, 4 Aug 1983, *W.E. Rolle 831* (holotype: GH!; isotypes: JEPS!, MO).

GH holotype image – http://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id=67660

Description. Plants long-lived perennials, with woody caudices raised above ground level 1–5 cm, lacking crowded, persistent leaf bases; herbage without an obvious bluish cast. Fertile stems usually 1 per caudex branch, arising from centre of basal rosettes, glabrous throughout. Leaves at stem bases oblanceolate, 3–8 mm wide, entire, ciliate proximally with 1–3 rayed trichomes 0.2–0.7 mm, blade surfaces glabrous or sparsely pubescent with short-stalked, 2–4 rayed trichomes 0.2–0.4 mm; cauline leaves 6–12, occasionally concealing stem proximally, the uppermost glabrous, with auricles 0.5–2.5 mm. Inflorescences unbranched, 3–7 flowered; fruiting pedicels 4–8 mm, arched, curved proximally (not geniculate), glabrous. Flowers divaricate-ascending at anthesis; sepals glabrous; petals creamy-white and occasionally blushed lavender, 8–11 mm long × 2.0–2.5 mm wide, glabrous; anthers with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen; ovules 14–22 per fruit. Fruits 35–65 mm × 2.0–3.5 mm, pendent to reflexed, not appressed to rachises, often secund, straight to somewhat curved, with undulate edges, glabrous; apical angle of fruit valve <25° (measured from base of style to 5 mm proximate); style persistent 0.6–1.2 mm. Seeds uniseriate, 3–4 × 1.5–2.0 mm; wing distal and proximal, 0.3–0.6 mm wide.

Distribution, habit and phenology. Populations of *B. rollei* are known from the Klamath Mountains Province primarily in the vicinity of Lilypad Lake in Siskiyou County, California. However, a single individual from Beaver Creek in Jackson County, Oregon has been confirmed morphologically and genetically as *B. rollei*. It is noteworthy that the collector of this specimen (F.W. Hoffman 2551) mentioned that it came from serpentine soil, to which *B. rollei* appears to be restricted. The species is often associated with *Pinus jeffreyi* and *Calocedrus decurrens* on sparsely forested slopes at elevations of 1600–1800 m; flowering June–Aug.

Comments. *Boechera rollei* is distinguished from the three S2X clusters of *B. suffrutescens* s.l. by its showier flowers with longer (8.0–11.0 vs. 4.5–6.0 mm) petals and narrower (0.3–0.6 vs. 0.8–1.5 mm) seed wings. It is easily distinguished from *B. constancei* by its markedly auriculate cauline leaves, shorter (0.6–1.2 vs. 1.5–5.5 mm) styles and lack of herbage with bluish cast.

Specimens examined. California. Siskiyou County: Below the Pacific Crest Trail on the W side of Lilypad Lake, ±0.5 mile S of Red Butte, ±5.5 miles N of Seiad Valley, Siskiyou Mountains, Rogue River National Forest, 24 Jul 2008, *P.J. Alexander 869* A, D, E (DUKE), C, D, E (NMC); On the Pacific Crest Trail ±0.7 miles SSW of Cook and Green Pass, ±1.9 miles E of Red Butte, ±7 miles NNE of Seiad Valley, Siskiyou

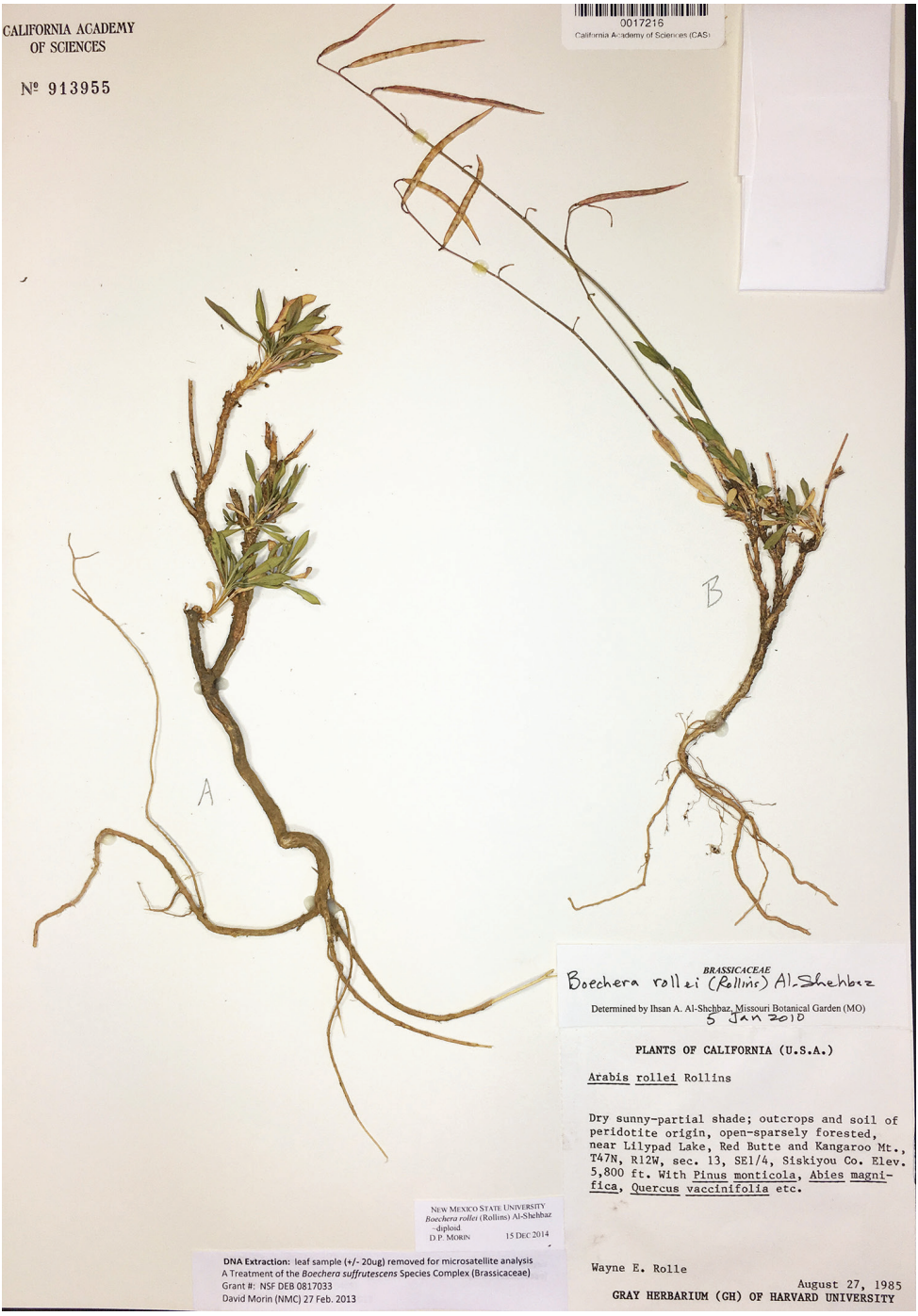

Figure 8. *Boechera rollei*, Rolle s.n. (CAS).

Mountains, Rogue River National Forest, 17 Apr 2009, *P.J. Alexander 873* (MO), A, B, C (NMC); 0.1 mi SW of border between Siskiyou National Forest and Klamath National Forest. NE facing slope above Lilypad Lake on Pacific Crest Trail. 0.7 air miles SSW of Red Butte summit. 1/4 mi SW of Lilypad Lake, 29 Jun 2012, *D.P. Morin 13* A, B (NMC) (these represent the DNA vouchers for samples A – O); Divide between the Applegate and Klamath Rivers, Red Butte-Kangaroo Mt.-Lilypad Lake-Towhead Lake region (T47N, R12W, S13), 8 Apr 1983, *W.E. Rolle 831* A - D (GH), A (JEPS); Red Buttes Wilderness Area about 3/5 mi S of Towhead Lake, or ½ mi W of Lily Pad Lake. T47N R12W Sec. 13 NW1/4 of SW1/4., 8 Apr 2009, *W.E. Rolle 1538* (DUKE); Near Lilypad Lake, Red Butte and Kangaroo Mt., 27 Aug 1985, *W.E. Rolle s.n.* (TEX), (GH), A, B (JEPS), A, B (CAS). **Oregon.** Jackson County: Upper Beaver Creek, 10 Jul 1948, *Hoffman 2551* (UC).

***Boechera suffrutescens* (S. Watson) Dorn, Brittonia 55: 3. 2003**
Figures 3, 9

Type. U.S.A. Oregon. Wallowa County/Baker County: Bluffs of Snake River and vicinity, 1881, *W.C. Cusick 919* (holotype: GH!; isotype: ORE).

GH holotype image – http://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id=27057

Description. Plants long-lived perennials, with woody caudices raised above ground level 1–5 cm, lacking crowded, persistent leaf bases; herbage without an obvious bluish cast. Fertile stems usually 1 per caudex branch, arising centrally from basal rosettes, lower parts glabrous or glabrate with 1–2(3) rayed trichomes (0.1–0.3 mm). Leaves at stem bases narrowly oblanceolate to obsubulate, 1.5–6.0 mm wide, entire, not ciliate or rarely with simple trichomes to 0.7 mm, blade surfaces usually glabrous or sparsely pubescent with 1–3(4) rayed trichomes, but occasionally plant herbage basally pubescent with short-stalked 1–4(5)-rayed trichomes (0.07–0.40 mm) if stressed or sterile; cauline leaves (4–)6–12, sometimes concealing stem proximally, the uppermost glabrous, with auricles 0.8–4.5 mm long. Inflorescences mostly unbranched, 6–12-flowered; fruiting pedicels 4–18 mm, reflexed, usually distinctly geniculate proximally but otherwise straight, glabrous. Flowers pendent at anthesis; sepals glabrous; petals purple or whitish with rose tips, 4.5–6.0 mm long × 2.0–2.5 mm wide, glabrous; anthers with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen; ovules 20–30 per fruit. Fruits 1.7–5.5 cm long × 3.3–4.0 mm wide, reflexed, pendent, occasionally appressed to rachises, often secund, straight to somewhat curved, with undulate edges, glabrous; apical angle of fruit valve 15°–23° (measured from base of style to 5 mm proximate); style persistent, 0.4–1.2 mm in length. Seeds uniseriate, 2.5–5.5 × 1.8–3.5 mm; wing continuous, 0.8–1.5 mm wide.

Distribution, habit and phenology. *Boechera suffrutescens* is distributed north and east of the Great Basin; concentrated in the vicinity of the Snake River Gorge (Hells

Figure 9. *Boechera suffrutescens*, Morin, Windham, Allphin 14 (NMC).

Canyon), but extending from Grant County, Oregon to central Idaho on steep, rocky, basaltic substrates in alpine and subalpine ecozones at elevations from 1800–2500 m; flowering from May–July.

Comments. Although geographically isolated from the other S2X species of the complex, *B.suffrutescens* s.s. is the least distinct morphologically. The most useful character for distinguishing this species is that individuals usually have basal leaves that are glabrate, with a few 1(–2) rayed trichomes scantily dispersed along the margins and apices. However, plants are occasionally encountered that are pubescent basally with 1–3(–4) rayed trichomes. These individuals often appear stressed or lack flowering stems, suggesting that pubescence may be more prevalent amongst plants growing in unfavourable environments. On the holotype specimen, one of each morphotype is present and the plant lacking a flowering stem is pubescent. All other taxa in the complex are consistently pubescent basally. On robust individuals of *B. suffrutescens* s.s., the basal leaves are generally narrower and the fruits are generally wider than those of the other S2X taxa.

Specimens examined. Idaho. Adams County: Confluence of Wildhorse River and No Business Cr. On N & W exposures, 13 May 1987, *D. Atwood 12561* (GH). Valley County: In basaltic outcrop on E side of high ridge W of Cascade. Payette NF, 15 Jul 1937, *R.C. Rollins 13852* (UC). Wash County: Seven Devils Mts., 10 Jul 1899, *M.E. Jones 6164* A, B (RSA). Washington County: Middle slopes of Hitt Mountain, 15 Jun 1943, *C.H. Christ 14044* (OSC); Dry hillside above Spring Creek. Ida Range 5 W Twsp. 14 North, 22 Jun 1940, *R.J Davis 2184* A, B, C (GH). **Oregon.** Grant County: West rim of High Lake Basin, Blue Mts., 4 Aug 1946, *B. Maguire 26497* (UC). Union (Baker/Wallowa) County: Bluffs of Snake River and vicinity, 1881, *W.C. Cusick 919* (GH); Stony hills near Snake River, 26 May 1898, *W.C. Cusick 1808* (UC); Overlooking Hells Canyon from west. Northeast of Hells Canyon Overlook. E +/- 100 yards from NFD 490. Take unpaved NFD 490 NE +/-3mi from Wollawa Mountain Loop, 22 Jun 2012, *D.P. Morin 10* A, B, C, D (NMC), E, F (DUKE); ESE facing slope 1/4 mi NE from dirt Hat Point Road on E side of Saddle Creek Campsite. Overlooks the Seven Devils Mtns and Saddle Creek to the east, a tributary to Snake River, 25 Jun 2012, *D.P. Morin 11* A (NMC); Hat Point Road and Saddle Creek Campground. Overlooks the Seven Devils Mtns and Saddle Creek Canyon to the E., 26 Jun 2012, *D.P. Morin 12* A, B, C (NMC); ENE facing rocky basalt on W side of Wallowa Mountain Loop. +/- 1.5 mi N of turnoff to Lick Creek Campground. 16.5 air miles SE of Joseph., 25 Jun 2012, *D.P. Morin 14* A, B, C, D (NMC), E, F, G (DUKE); Snake River Canyon near the mouth of Battle Cr., 12 Jul 1933, *M.E. Peck 17616* (OSC); SE of Enterprise near crest overlooking McGraw Creek E of Forest Route 490 ca. 0.25 road mi NE of the parking area at Hells Canyon Overlook, 22 Jun 2012, *M.D. Windham 4110* (DUKE); SE of Enterprise on slope above Lick Creek along Wallowa Mountain Loop Rd. ca. 14.1 road mi W of its junction with Upper Imnaha Rd., 22 Jun 2012, *M.D. Windham 4132* (DUKE).

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Appendix 1

Additional specimens examined for individual samples that were deemed singeltons (2X), apomictic diploid (A2X), triploid (A3X) or polyploid (≥ 4x). All individuals were collected in the U.S.A. If present, suffixes (A, B, C etc.) indicate specific individuals from a collection event in which vouchers of leaf samples from multiple individuals were collected. All populations are represented by voucher specimens deposited in the herbaria indicated (acronyms from *Index Herbariorum*).

***Boechera* cf. *constancei* (A2X): California.** Plumas County: Plumas Nat'l Forest. SSE aspect, 1 mi NW of Pilot Peak. 0.5 mi WNW of Onion Valley Resevoir. 0.4 mi WSW from W turnoff 23N24 from La Porte Rd., 2 Jul 2012, *D.P. Morin 38* A - D (NMC),

E, F (DUKE); La Porte Highway. +/-17 mi road mi S of East Quincy, 3 Jul 2012, *D.P. Morin 43* A, B (NMC); Plumas Nat'l Forest. 0.5 air mi WSW of Onion Valley Reservior. +/- 16.75 road mi from La Porte turnoff N in East Quincy, 3 Jul 2012, *D.P. Morin 45* A - E, G, I (NMC), J, K (DUKE). Sierra County: West slope of the northern Sierra Nevada, 1.8 miles (linear) NE of Gibsonville on the road to Johnsville, 2.3 miles WNW of Mount Etna, 13.2 miles SSE of Quincy, Plumas National Forest, 9 Apr 2009, *P.J. Alexander 1046* (NMC). ***Boechera* cf. *suffrutescens* (A2X)** California. Lassen County: 3.9 car mi NE of Blue Lake. 6.5 air mi WNW of Hat Mtn., 30 Jun 2012, *D.P. Morin 26* A, B (NMC). Modoc County: NE slope Emerson Peak; Warner Mountains, 23 Jul 1946, *A.M. Alexander 5054* A, B (UC), B (GH); Cedar Peak, Warner Mts., 1 Jul 1940, *M. Ownbey 2147* (CAS), (WS); Warner Mountains, E face of Emerson Peak, Emerson Creek drainage Emerson Peak; Warner Mountains, Emerson Peak, 5 Aug 1989, *D.W. Taylor 10436* A (JEPS). Plumas County: Summit ridge of Thompson Peak, Diamond Range; Canadian Zone forest, areas of volcanic rock, 31 Jul 1973, *J.T. Howell 50117* B (CAS). Shasta County: Vicinity of Magee Peak (Summit of Crater Peak, Lassen National Forest, Thousand Lakes Wilderness); Lassen National Forest, Thousand Lakes Wilderness, 24 Aug 1975, *D.W. Taylor 5430* A, B (JEPS). Siskiyou County: Ridge W of Baldy Mt. L. O., 6 mi by air W of Happy Camp, Siskiyou Mts., 2 Jul 1952, *P.A. Munz 17911* A, B (RSA). Trinity County: North slope of North Yolla Bolly Peak, Yolla Bolly Mtns. Line of Tehama and Trinity Counties., 18 Jul 1951, *P.A. Munz 16636* A, B, C (RSA). **Nevada.** Humboldt County: Humbolt NF, Santa Rosa Range, ca. 50 air mi. NNW of Winnemucca, a. 5 mi W of Paradise Valley. Mostly on the ridge between Singas Creek and Morey Creek drinages. Access was gained by driving up the dirt road to Singa. A few plants found on the granitic, 26 Jul 2009, *C.D. Bailey 306* (DUKE). Washoe County: Fox Mountain approx. 26.5 air mi NNW of Gerlach, NV. Approximately 0.33 mi N (downslope) from Fox Mountain Summit (radio tower access route Old Camp Canyon Rd.) descending into NE facing gully, E facing aspect., 30 Jun 2012, *D.P. Morin 28* A, - E, G (NMC); Sierra Nevadas, Carson Range, N side of Galena Creek on the south side of Mt. Rose, 5 Aug 1983, *A. Tiehm 8279* A (CAS), A, B, C (GH); Granite Range, Fox Mt. on the NW end of the range, just N of the peak, 30 Jul 1986, *A. Tiehm 10818* A, C (OSC), A, B, C (RSA), A, B (GH). **Oregon.** Deschutes County: Paulina Lake., 30 Jun 1931, *J.T. Howell 7097* A, B (GH). Grant County: High Lake Basin, Malheur NF, Blue Mts, 4 Aug 1946, *B. Maguire 26497* (TEX); Malheur NF, along trail near road 1640, near bend in road on S side of Strawberry Mt., 19 Aug 2010, *N. Otting* 16442 A (OSC); About 5 mi SW of Anthony Lake, on granitic soil, 17 Jul 1952, *C.L. Hitchcock 19715* A, B (RSA). Harney County: SE of Frenchglen near crest of Steens Mountain along North Loop Road ca. 1.3 road mi WSW of the turnoff to Kiger Gorge Overlook. Lat.: 42.69940N; Long.: 118.59760W (WGS84 Datum), 4 Aug 2009, *M.D. Windham 3829* (DUKE); Steens Mtn. along road on W side of McCoy Creek, 12 Jul 1979, *Wright 1127* (OSC). Klamath County: SE side of Crater Lake on slope overlooking Kerr Valley along Rim Drive ca. 0.9 road mis ESE of its junction with Pinnacles Road. Lat.: 42.90910N; Long.: 122.05950W (WGS84 Datum), 8 May 2009, *M.D. Windham 3843* (DUKE). Lake County: Fremont N.F.; E of McDowell Peak (T37S, R22E, S20, NE, NW); in

rocky areas, 24 Jul 1991, *B. Rittenhouse 733* (OSC). **Washington.** Yakima County: Mt. Adams, 15 Aug 1882, *J.T. Howell, s.n.* (OSC). Unassigned ***Singletons* (2X): California.** Plumas County: 1 mi NW of Pilot Peak, 14 mi NE of La Porte, 8 July 1986, *Ahart 5356* (CAS); Jameson Creek W of Johnsville; rocky outcrop in yellow pine forest, 28 May 1985, *D. Anderson 2805* (GH). Shasta County: Trinity Mountains; Grey Rocks; ca. 10 air mis WSW of Dunsmuir (T38N, R5W, S21, SW), 9 Jul 1993, *D.W. Taylor 13824* (JEPS). Siskiyou County: Along Forest Service road 17, 0.5 mi north of summit at county line. China Mountain Quad., 17 Jul 1980, *T.W. Nelson 6094* (CAS). **California.** Siskiyou County: Red Butte-Kangaroo Mt.-Lilypad Lake- Towhead Lake region, T47N R12W, Sec. 13, 8 Apr 2004, *W.E. Rolle 831* (MO). **Oregon.** Baker County: Largest rocks of Pine Creek near Snake River (possible topotype), May n/a, *W.C. Cusick* (ORE). **Washington.** Unknown County: Falcon Valley, 18 May 1884, *W.N. Suksdorf s.n.* A, B, C (UC). ***Boechera* cf. *suffrutescens* Complex (A3X): California.** El Dorado County: Tallac Trail between Lake Gilmore and Camp Lake Tahoe Region, 24 Jul 1907, *R.L. Pendleton 1126* (UC). Elmore County: 10 mi W of Featherville Divide above Trinity Lakes, 25 Jul 1944, *C.L. Hitchcock 10347* A, B (UC). Plumas County: About 1/2 mi S of Bucks Lake (N of a poor logging road), 29 Jun 1994, *L. Ahart 7409* (JEPS). **California.** Plumas County: Sierra Nevada. On road from Round Valley to Long Valley, 31 May 1974, *W. Dakan* A, B (CAS); Summit ridge of Thompson Peak, Diamond Range, 31 Jul 1973, *J.T. Howell 50117* (CAS); East side of 25N17, ca. 0.125 mi south of Bean Creek, ca. 0.25 mi north of jct. 25N17 with 25N81 (Old Mt House Rd.). Ca. 3.5 air mi NW of Meadow Valley., 22 Jun 1981, *M.S. Taylor 3944* A (CAS); Red Hill Lookout, ca. 3 air mi NE of Belden., 25 Aug 1981, *M.S. Taylor 4237* (CAS); North side of 27N92, ca. 2 air mi SE of Canyon Dam, 18 May 1982, *M.S. Taylor 4476* (CAS); Between spur road and 27N92 NW of Goldstripe Mine ca. 4 air mi W of Greenville (T27N, R8E, S36, SW), 1 Jun 1982, *M.S. Taylor 4579* (MO). Shasta County: Thousand Lake Basin; forest floor of the interlake region, 11 Jul 1932, *F.W. Peirson 10151* (RSA); s of Burney; Thousand-Lake Basin, 11 Jul 1932, *F.W. Peirson 10151* A, B, C (UC). Sierra County: West slope of the northern Sierra Nevada, 1.8 miles (linear) NE of Gibsonville on the road to Johnsville, 2.3 miles WNW of Mount Etna, 13.2 miles SSE of Quincy, Plumas National Forest., 9 Apr 2009, *P.J. Alexander 1046* (NMC). Siskiyou County: South Fork of Salmon River near Big Flat., 21 Jul 1937, *J.T. Howell 13204* A, B (GH); Caribou Basin, Salmon-Trinity Alps, 24 Jul 1937, *J.T. Howell 13379* (GH); East side of Hiway 93 between Calahan and Cecilleville at Carter Summit Trailhead. +/- 11 mi SW of Calahan, 28 Jun 2012, *D.P. Morin 20* A, B, D - J (NMC), C (DUKE); N of Hancock Lake (W side of Red Hill, vicinity of English Peak); Salmon Mountains, Marble Mountain Wilderness Area, English P, 16 Aug 1968, *F.W. Oettinger 540* (UC); Small ridge near Wolverine Lake, Marble Mts., 25 Sep 1985, *W.E. Rolle s.n.* (GH); Trinity Alps, above S Fork of Salmon R, along Yellow Rose Mine trail, top of Pass between Yellow Rose and Dorleska mines, 15 Jul 2005, *R.C. Wenk 206* A, B (CAS); Mt. Eddy, 12 Jun 1976, *J. Whipple 981* A (GH); Caribou Lake Salmon/Trinity Alps Primitive Area, 27 Jul 1955, *I.L. Wiggins 13534* A, B (CAS). **Idaho.** Adams County: Micah Summit, 9 mi S of Cabin Creek Campground, W side of dirt road on embankment. 4.75 mi due W of Cascade Re-

sevoir. Near intersection of NFD 206 and NFD 207. 3/4 mi ENE of Indian Point Lookout, 21 Jun 2012, *D.P. Morin 8* B, C, D (NMC), E, F (DUKE); South of Micah Summit, on W side of Anderson Creek Rd. (NFD 206); 0.2 mi W of Weiser River, 21 Jun 2012, *D.P. Morin 9* A, B, C (NMC), D, E, F (DUKE); SE of Council near Mica Saddle along FR 206 ca. 100 meters NNE of its junction with FR 207, 21 Jun 2012, *M.D. Windham 4104* (DUKE); SE of Council below Mica Saddle along Forest Route 206 ca. 1.2 road miles S of its junction with Forest Route 207, 21 Jun 2012, *M.D. Windham* (DUKE). Blaine County: West side of Galena Pass on the N side of ID Hwy 75, N end of the Smoky Mountains, ±23 miles NW of Ketchum, Sawtooth National Forest, 20 Apr 2009, *P.J. Alexander 884* A - E (NMC); Dry gravelly hillside of Galena Summit, 29 Jul 1941, *A. Cronquist 3521* A (GH); 7.3 mi N of Ketchum, 19 Jun 1979, *R.J. Davis 79276* B (GH); Above Galena, Central Idaho, Jul 1895, *L.T. Henderson 3537* A, B, C (ORE); Iron Mt., 5 mi W of Martin, 26 Jun 1938, *C.L. Hitchcock 3826* A, B (GH); 7.3 mi N of Ketchum, 19 Jun 1979, *R.C. Rollins 79276* A, B (UNR), A (UC); Rocky ridge ca. 1.5 mi. N of Galena Summit, between Stanley and Kethcum, 27 Jun 1986, *R.C. Rollins 86133* (TEX); N side of Galena Summit, off State Hwy. 75, between Stanley and Ketchum, 27 Jun 1986, *R.C. Rollins 86117* (TEX), C (GH); Ca. 1.5 mi N of Galena Summit, off State Hwy. 75, between Stanley and Ketchum, 27 Jun 1986, *R.C. Rollins 86133* A - E (GH). Camas County: Crouch Summit, 7 mi N of Soldier, 5 Jul 1965, *C.L. Hitchcock 23813* A, B (CAS). Custer County: Ridge above Mill Creek, 12 mi W of Challis, 8 Jul 1941, *A. Cronquist 2966* A, B (GH); Ca. 0.5 mi NE of Toxaway Lake. 10 mi WSW of Obsidian; Sawtooth Mts., 11 Aug 1944, *C.L. Hitchcock 5739* A (UC), B (GH). Elmore County: On highest slope of Trinity Peak; 17 mi. N of Fall Creek, 12 Jul 1950, *J.H. Christ 20051* A - F (OSC); Sawtooth Prmitive Area; headwaters of Middle Fork Boise River above Atlanta. Rocky slope ca. 4 mi S of Spangle Lakes, 19 Jul 1944, *C.L. Hitchcock 10167* A, B (UC). Idaho County: Hibbs Cow Camp, Seven Devils Mts., 26 Jun 1940, *M. Ownbey 2083* A (UC), A, B (OSC), A, B (GH). Ketchum County: Road towards Galena Summit, 29 mi N of Ketchum, 19 Jun 1979, *R.C. Rollins 79279* A, B, E (GH). Owyhee County: Above Sawpit Creek. Ca. 3 mi SW of Silver City, 12 Jul 1951, *W.H. Baker 7891* (GH). Valley County: Box Lake ca. 9 air mi NE of McCall, E-facing bare granite slope on opposite side of ridge SE of lake; overlooking Lick Creek Road, 27 Jul 1989, *B. Ertter 8793* A, B (UC); Elk Summit, Range 8E, Township 21N. Moist sliding soil, 7 Jul 1940, *R.J. Davis 2652* A, B, C (UC); In basaltic outcrop on E side of high ridge W of Cascade, Payette NF, 15 Jul 1937, *J.W. Thompson 13852* A, B, C (GH). Washington County: Rush Creek, 7 Jul 1899, *M. Jones 6164* (UC); Rush Creek, 5 Aug 1899, *M.E. Jones s.n.* A, B (RSA). **Nevada.** Washoe County: Granite Range, Fox Mt. on the NW end of the range, just N of the peak, 30 Jul 1986, *A. Tiehm 10818* B (OSC). **Oregon.** Baker County: Ekhorn Range, Hunt Mtn.; Pine Creek drainage, 20 Jul 1986, *E. Joyal 1235* (OSC); Rocky granitic hillside along Wood River. 10 mi N of Ketchum, 29 Jul 1941, *A. Cronquist 3481* A, B (GH). Grant County: About 5 mi SW of Anthony Lake, 17 Jul 1952, *C.L. Hitchcock 19715* A, B (RSA). Harney County: Steens Mts. Opposite Devine Ranch, 5 Jul 1896, *J.B. Leiberg 2514* A (GH). Wallowa County: Between Douglas Lake and Moccasin Lake, 23 Aug 1946, *B. Maguire 27157* (UC). **Nevada.**

Washoe County: Granite Range, Fox Mountain on the NW end of the range, just N of the peak, 30 Jul 1986, *A. Tiehm 10818* A (CAS). **Oregon.** Josephine County: Near the summit of Lake Mountain, Oregon Caves National Monument & vic., 3 Jul 1949, *Baker 265* (WTU); Sand Ridge, Lake Mountain Trail; Oregon Caves National Monument & vicinity, 16 Aug 1949, *Baker 646* (UC).

Supplementary material 1

Representative pollen morphologies

Authors: Morin DP, Alexander PJ, Beck JB, Windham MD, Bailey CD

Data type: measurement

Explanation note: **A**. S2X pollen of *B. rollei* (Morin 13, NMC). **B**. Potentially functional pollen from an A3X individual (Morin 8, NMC). **C**. Representative malformed non-functional pollen from A2X and some A3X individuals (Christ 20051, OSC).

Link: https://doi.org/10.3897/phytokeys.98.24296.suppl1

Supplementary material 2

Analysis 1

Authors: Morin DP, Alexander PJ, Beck JB, Windham MD, Bailey CD

Data type: statistical data

Explanation note: **A**. STRUCTURE bar plots with the highest likelihood for K = 5, 6, and 8 respectively. Instability was consistently noted across iterations at all values of K. B. An MDS plot demonstrates distinctness in TL-ro in three dimensions and TL-su in two dimensions. Calculations for optimal K were ambiguous and inconsistent, as were clustering patterns with regard to the **B**. *constancei* clusters (TL-co, PLSI-co and CD-co), particularly with regard to TL-co. MDS plots do not represent analyses, but are a visual interpretation of the dataset. TL-su and TL-ro are the most distinct and coherent clusters based on allele sharing distance.

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Recognition of the genus *Thaumatophyllum* Schott – formerly *Philodendron* subg. *Meconostigma* (Araceae) – based on molecular and morphological evidence

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Abstract

Philodendron subgenus *Meconostigma* has been a well-circumscribed group since 1829. Members of this group are easily distinguished by diagnostic morphological characters as well as by a distinct ecology and geographical distribution. Based on molecular, morphological and cytological evidence, we propose the recognition of *P.* subg. *Meconostigma* as a distinct genus, *Thaumatophyllum* Schott. We also present the necessary new combinations, an emended key and some nomenclatural and taxonomic corrections regarding 21 names of *Thaumatophyllum*.

Keywords

chromosomes, molecular phylogeny, morphology, nomenclature, *Philodendron*, *Thaumatophyllum*

Introduction

Philodendron Schott is the second most species-rich and diverse genus in the family Araceae and also in the "*Homalomena* clade" (sensu Cusimano et al. 2011), comprising 487 formally recognised species (Boyce and Croat 2018). The genus ranges from northern Mexico to southern Uruguay (Mayo et al. 1997), most commonly in tropical humid forests as epiphytes and hemi-epiphytes. Most rarely, it also occurs as terrestrial plants in open habitats (e.g. seasonal dry forests of South America).

Cabrera et al. (2008) published a family-wide molecular phylogeny that included species from 102 genera. Cusimano et al. (2011) re-analysed and augmented a molecular data set with a more complete genus sampling and compared the resulting phylogeny with morphological and anatomical data, proposing informal names for the suprageneric clades. The "*Homalomena* clade" (composed of the genera *Adelonema* Schott, *Cercestis* Schott, *Culcasia* P.Beauv., *Furtadoa* M.Hotta, *Homalomena* Schott and *Philodendron* Schott) was recovered in both molecular and morphological analyses and was supported by the occurrence of sclerotic hypodermis and resin canals in the roots and absence of endothecial thickenings in the anthers (present in *Homalomena*). The clade is composed of two sister groups: "Culcasieae clade" (*Cercestis, Culcasia*) and "*Philodendron* clade" (*Furtadoa*, *Homalomena*, *Philodendron*). Mayo et al. (2013) gave an alphabetical table of the clades that is a useful complement to the listing in Cusimano et al. (2011).

The evolutionary history of the "*Philodendron* clade" has been discussed in several recent papers (Tam et al. 2004, Gauthier et al. 2008, Mayo et al. 2013, Wong et al. 2013, Loss-Oliveira et al. 2014, Wong et al. 2016, Loss-Oliveira et al. 2016), as well as the relationship amongst the three subgenera of *Philodendron* as independent lineages (Gauthier et al. 2008, Loss-Oliveira et al. 2014, Loss-Oliveira et al. 2016). A question recently answered was how the Asian-Neotropical distribution of the genus *Homalomena* originates (*sensu* Mayo et al. 1997). Based on molecular evidence (Gauthier et al. 2008, Wong et al. 2013, Wong et al. 2016), the American species of *Homalomena* were recognised as a separate lineage and consequently Schott's old genus *Adelonema* was recognised once more (Wong et al. 2016). The "*Philodendron* clade", still needs better phylogenetic resolution for two other lineages: *Homalomena* + *Furtadoa* and *Philodendron* subgenera *Philodendron* and *Pteromischum*. Several research articles (Wong et al. 2016, Loss-Oliveira et al. 2016) have proposed different hypotheses for the relationship amongst these lineages as summarised in Fig. 1.

The recent recognition of the genus *Adelonema* for the American species of *Homalomena* (Wong et al. 2016) makes the genus *Philodendron* paraphyletic in some of the current proposed phylogenetic hypothesis (Figs 1B, 3A). The most recent studies (Loss-Oliveira et al. 2016, Vasconcelos 2015) recovered two major lineages: *P.* subg. *Meconostigma* (= *Thaumatophyllum*) and *Philodendron* subg. *Philodendron* plus subg. *Pteromischum*. Vasconcelos (2015) recovered *P.* subgenus *Pteromischum* as monophyletic and sister clade to *P.* subg. *Philodendron*.

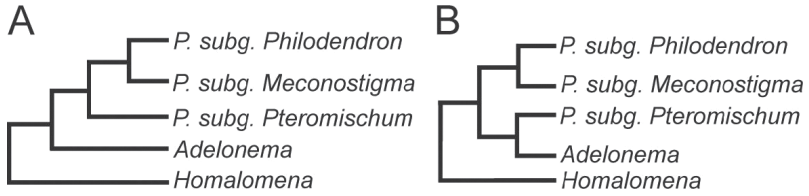

Figure 1. *Philodendron*, *Homalomena* and *Adelonema* phylogenetic relationships markers by previous publications. **A** Gauthier et al. (2008), maximum parsimony. **B** Gauthier et al. (2008), Bayesian analysis; Wong et al. (2013), Wong et al. (2016).

Philodendron subgenus *Meconostigma* (= *Thaumatophyllum*) has been a well-circumscribed group since the genus *Philodendron* was first recognised taxonomically by Schott (1829). It is now comparatively well-studied taxonomically; the last taxonomic revision included 15 species (Mayo 1991, with updates by Gonçalves and Salviani 2002, who recognised 19 species). Members of this subgenus are easily distinguished by diagnostic morphological characters as well as by a distinct ecology and geographical distribution that ranges from the Amazonian and Atlantic forests to the savannah-like landscapes of the Cerrado biome (Mayo 1991).

Based on the evidence now accumulated (most recently, by Calazans et al. 2014, Loss-Oliveira et al. 2014, 2016), we propose the recognition of *P.* sugb. *Meconostigma* as a distinct genus, *Thaumatophyllum* Schott (1859), a taxon that was accepted by experts as recently as Bunting (1980). Barroso (1962) was the first botanist to formally assign the name *Thaumatophyllum* to the synonymy of *Philodendron* and Mayo and Barroso (1979) gave a detailed account of the confusion that had misled previous authors regarding the floral morphology of *T. spruceanum*. The aim of this paper is, therefore, to formally propose this change of status and validly publish the necessary new combinations. We also provide an emended key and some nomenclatural and taxonomic corrections concerning six names in this genus.

Methods

Taxon and gene sampling

We have sampled data for 110 extant species of *Philodendron*, 21 species of *Thaumatophyllum* and six species of *Homalomena* and five of *Adelonema* of the nuclear 18S and external transcribed spacer (ETS) and the chloroplast *trn*K intron, maturase K (*matK)* genes, *trn*L intron, *trn*L-*trn*F intergenic spacer. Species from the genera *Cercestis*, *Culcasia*, *Colocasia*, *Dieffenbachia*, *Heteropsis*, *Montrichardia*, *Nephthytis*, *Furtadoa* and *Urospatha* were included as the outgroup. The species list, the voucher and GenBank accession numbers are listed in Suppl. material 1: table 1. The majority of the used sequences were generated by a previous study of our group (Loss-Oliveira et al. 2016).

Additionally, we generated a subsampled dataset comprised of species from our original data with available ETS and 18S sequences and at least two available chloroplast sequences. This strategy aimed to reduce the impact of missing data in the concatenated analysis. This taxon sampling is described in Suppl. material 1: table 1.

Alignment and phylogenetic analysis

The methodological approach of Loss-Oliveira et al. (2016) was followed in order to estimate individual gene trees and a supertree. We have used MAFFT 7 (Katoh and Standley 2013) to individually align the molecular markers and SeaView 4 (Gouy et al. 2010) to manually adjust them. Bayesian analysis was conducted in MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) for individual gene trees (Fig. 1, Suppl. material 1) using the GTR + G substitution model. The Markov chain Monte Carlo (MCMC) algorithm was run twice for 10,000,000 generations with four chains, which were sampled every 100th cycle. We have applied a burn-in of 20%. Individual gene trees were used to estimate a supertree with PhySIC_IST algorithm (http://www.atgc-montpellier.fr/physic_ist/) in order to avoid the impact of missing data in the estimation (Scornavacca et al. 2008).

We have also performed phylogenetic analysis for concatenated chloroplast markers separated from nuclear markers from the subsample consisting of species with available ETS and 18S sequences and at least two chloroplast markers in order to compare the estimated trees. Both chloroplast and nuclear datasets were used to estimate trees from Maximum Likelihood and Bayesian analysis approaches.

A maximum likelihood approach was performed in PhyML, implemented in Seaview (Gouy et al. 2010). The GTR+G model of sequence evolution was used for both chloroplast and nuclear concatenated sequences.

The Bayesian analysis were performed in MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) using the GTR + G substitution model for both chloroplast and nuclear concatenated sequences. The MCMC algorithm was run twice for 10,000,000 generations, using four chains. Chains were sampled every 100th cycle and a burn-in of 20% was applied.

Results

Phylogenetic analysis

As observed in Figure 2, *Philodendron* subg. *Meconostigma* was recovered as monophyletic and as a sister group of *P.* subg. *Philodendron* and *P.* subg. *Pteromischum.*

The subsampled chloroplast analyses (Figure 2, Suppl. material 1) were inconclusive. They presented very low posterior probabilities for Bayesian analysis (Figure 2A, Suppl. material 1), as well as very low aLRT values for Maximum Likelihood estimates

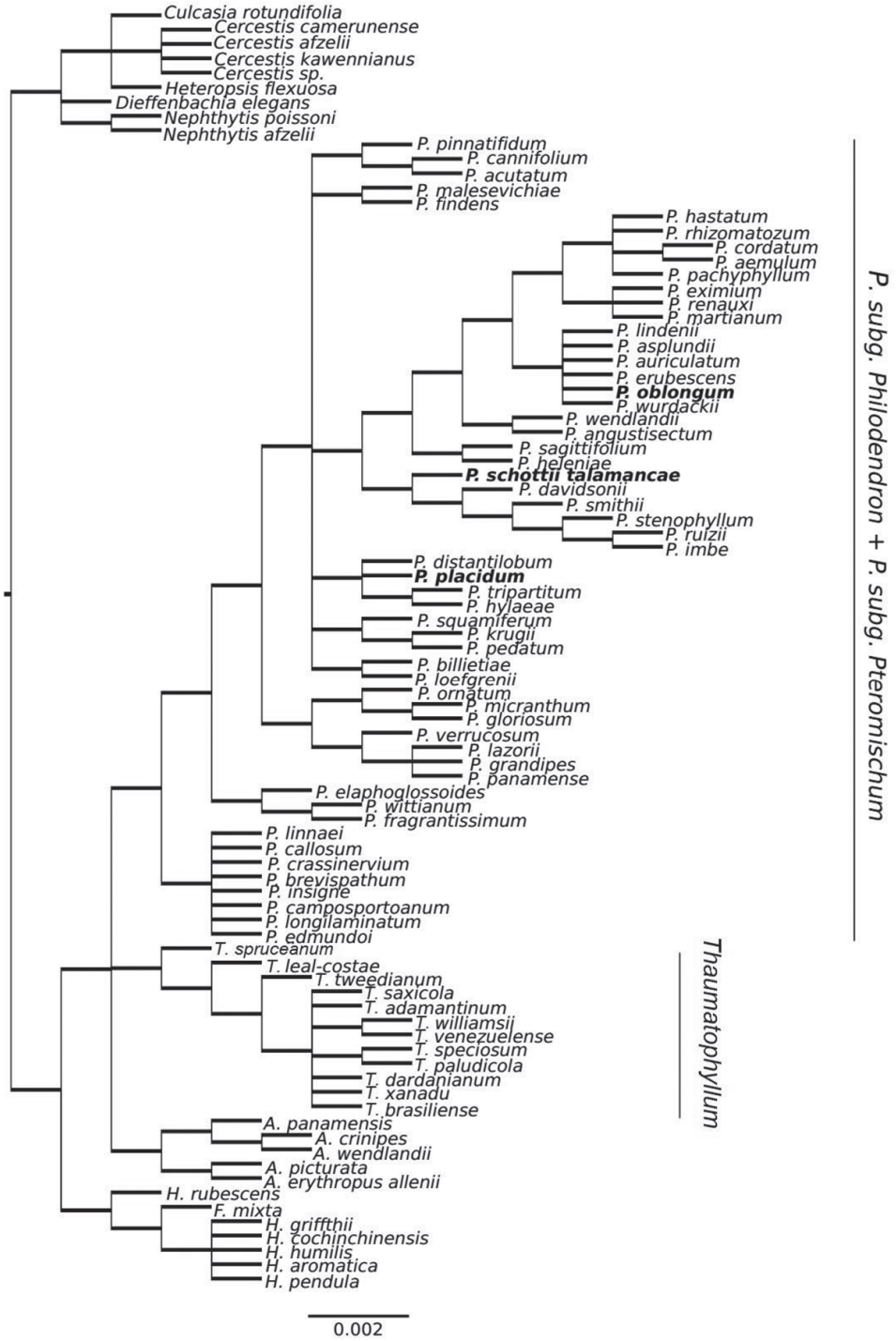

Figure 2. Supertree of *Philodendron*, *Thaumatophyllum*, *Adelonema* and *Homalomena* species. Names in bold are species of *P.* subg. *Pteromischum*.

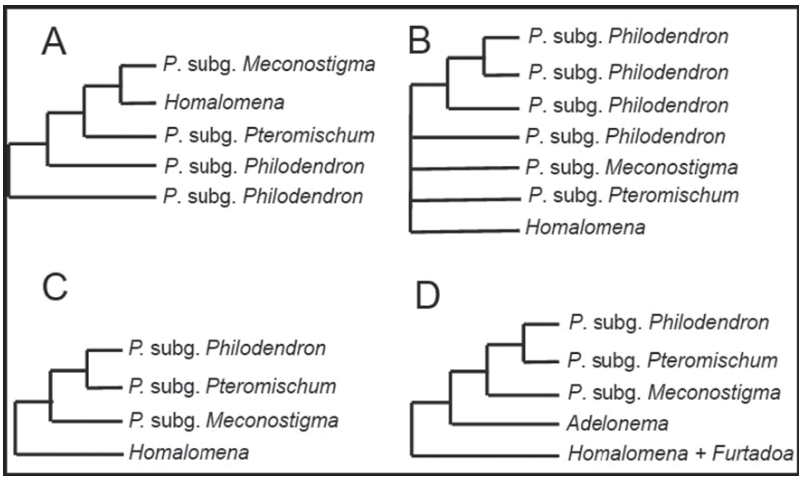

Figure 3. Phylogenetic relationships amongst *Philodendron*, *Thaumatophyllum*, *Homalomena* and *Adelonema* recovered by previous authors. **A** Barabé et al. (2002) **B** Tam et al. (2004) **C** Loss-Oliveira et al. (2014) **D** Vasconcelos (2015).

(Figure 2B, Suppl. material 1). On the other hand, the results from ETS and 18S analysis (Figure 3, Suppl. material) presented very similar results for both Bayesian analysis (Figure 3A, Suppl. material 1) and Maximum Likelihood estimates (Figure 3B, Suppl. material 1), with high posterior probabilities and aLRT support, respectively. The estimated phylogenetic relationships are also very similar to those found in the estimated supertree.

Discussion

The genus *Thaumatophyllum* Schott

Molecular evidence. Barabé et al. (2002) used the *trn*L intron and *trn*L-*trn*F intergenic region to estimate the phylogenetic relationships of 33 genera of Araceae; they included six species of *Philodendron* and found that three species of subg. *Philodendron* formed a sister group to a clade composed of species of *Thaumatophyllum*, subg. *Pteromischum* and *Homalomena*; *P. insigne* (subg. *Philodendron* sect. *Baursia*) was sister group to all these. Tam et al. (2004) analysed the *trn*L-*trn*F with the same six species of *Philodendron* within a larger analysis focused on subfam. *Monsteroideae*, but this part of their tree was largely unresolved. Gauthier et al. (2008) carried out a more complete analysis of *Philodendron* based on over 80 species using ETS and ITS markers. Their ETS tree recovered *P.* subg. *Meconostigma* as monophyletic and sister group to *P.* subg. *Philodendron*, with *P.* subg. *Pteromischum* as the basal component of *Philodendron* (Fig. 3A). In their ITS tree, the three subgenera formed a trichotomy.

Similarly, Loss-Oliveira et al. (2014) recovered *P*. subg. *Meconostigma* (= *Thaumatophyllum*) as monophyletic with 100% aLRT support and 100% posterior probability through the analysis of nuclear ETS and 18S markers and chloroplast *mat*K, *trn*K, *trn*L intron and *trn*L-*trn*F intergenic region (Fig. 3C). *Thaumatophyllum* was recovered as sister group of *P*. subg. *Philodendron* and *P*. subg. *Pteromischum*.

The analysis conducted by Vasconcelos (2015), using the chloroplast markers *rpl*32-*trn*L, *trn*V-*ndh*C and *trn*Q-5'-*rps*16 and the nuclear ITS, corroborate the monophyly of *Thaumatophyllum* and its position as sister group of *P*. subg. *Philodendron* and *P*. subg. *Pteromischum* (Fig. 3D).

Wong et al. (2016) used nuclear ITS and plastid *mat*K markers in an analysis which included Asian *Homalomena*, *Adelonema* (previously American *Homalomena*) and *Philodendron* (*sensu lato*) and also found that *Thaumatophyllum* and *P.* subg. *Philodendron* were sister groups. In this study, *P.* subg. *Pteromischum* emerged as sister to *Adelonema*.

These results are consistent with our findings that *Thaumatophyllum* is a monophyletic and isolated lineage in *Philodendron*, the sister group of *P*. subg. *Pteromischum* and *P*. subg. *Philodendron*.

Morphological evidence. As here defined, *Thaumatophyllum* is a Neotropical genus composed of 21 species. It is defined by an arborescent habit, very much thickened spathe, well developed sterile intermediate zone in the inflorescence equal or longer than the staminate zone, the gynoecium always having stylar lobes and an axial vascular system independent of the funicle supply (Mayo 1991, Calazans et al. 2014). Other distinctive features of *Thaumatophyllum* are (Mayo 1991, Calazans et al. 2014): 1. sympodial articles diphyllous, internode between prophyll and preceding foliage leaf never developed, internode between prophyll and succeeding foliage leaf sometimes developed but usually very short; 2. leaf blade cordiform, sagittate or hastate, never unlobed at the base; 3. adaxial spathe resin canals J-shaped in longitudinal section, extending to the epidermal surface and secreting resin at anthesis; 4. abaxial spathe resin canals distributed throughout the abaxial parenchyma zone; 5. stamens normally long, slender, more than 3 times longer than wide (except *T. leal-costae*); 6. stamen vascular trace unbranched (French 1986); 7. style lobes always present; 8. central style dome often present; 9. separate stylar canals occasionally absent; 10. vascular plexus normally present in style; 11. basal vascular complex of gynoecium multi-stranded; 12. lobed central vascular cylinder in the roots (V.T. Rosa, personal comm.); 13. lack of cell wall thickening in the inner root endodermis and neighbouring cortical cells; and 14. collenchyma rather than sclerenchyma sheaths around root resin canals.

Shoot morphology and arborescent habit. Stem architecture in *Thaumatophyllum* is similar to *Philodendron*, since the mature stems of both genera are sympodia composed of diphyllous articles (terminology after Ray 1987). However, in those species of *Thaumatophyllum* which have appreciably elongated internodes, the pattern of elongation is different from that of *Philodendron*. The position of the 'intravaginal squamules' (Dahlgren and Clifford 1982, Mayo 1991) is also different in the two genera and is evidence of the two contrasting patterns of internode elongation. The squamules are always found immediately above the prophyll scar in mature internodes of *Philo-*

dendron. However, in *Thaumatophyllum*, the squamules occur immediately below the prophyll scar and often surround the foliage leaf scar as well. Also in *Thaumatophyllum* the squamules frequently persist on the adult stem and are normally spinose or aculeate projections; their number, size, shape and persistence are taxonomically useful.

Inflorescence. *Thaumatophyllum* is characterised by normally solitary inflorescences in each floral sympodium and very thick, weakly constricted or unconstricted spathes with a uniformly white inner surface. In the spadix, the long staminodial zone that equals or exceeds the fertile male zone is the most useful diagnostic character and distinguishes it from the genus *Philodendron*. This long staminodial zone plays an important role in the floral biology, serving as a food resource and as the main source of the very large temperature elevations observed during flowering (Gibernau et al. 1999, Gibernau and Barabé 2000, Barabé et al. 2002, Gibernau et al. 2005).

Pistillate flowers and the Gynoecium. Unlike *Philodendron*, the style lobes are conspicuous in *Thaumatophyllum* and, together, constitute the style crown (Mayo 1991); they resemble stigma lobes as they are frequently covered by stigmatic tissue but are distinct from other kinds of lobed stigma because the lobing is caused by the style apex tissues rather than differential growth of the stigma trichomes. In many species the central region of the style apex is elongated into a more-or-less cylindrical axial portion, the central dome. The central dome may be excavated itself into a pit or even a long canal and may itself have lobed margins. The gynoecial type, typical of *Thaumatophyllum,* was designated by Mayo (1986, 1989, 1991) as type A, based on a sample of only four species. Calazans et al. (2014) studied 19 out of 21 species and recognised a further three subtypes within Mayo's type A: subtype A1: stylar body absent and stylar canals short, central stylar dome absent and compitum deep (*T. adamantinum*, *T. dardanianum*, *T. speciosum* and *T. williamsii*); subtype A2: undeveloped stylar body present with long stylar canals, central stylar dome absent and compitum shallow (*T. corcovadense*, *T. lundii*, *T. paludicola*, *T. saxicola*, *T. stenolobum*, *T. tweedieanum* and *T. uliginosum*); subtype A3: well developed stylar body present with stylar canals long, central stylar dome present and compitum shallow (*T. bipinnatifidum*, *T. brasiliense*, *T. mello-barretoanum*, *T. petraeum*, *T. spruceanum*, *T. solimoesense*, *T. undulatum* and *T. venezuelense*).

Based on molecular evidence, Loss-Oliveira et al. (2014) suggested that the common ancestor of *Thaumatophyllum* probably possessed short stylar lobes, long stylar canals, a stylar body, a vascular plexus in the gynoecium and druses in the stylar parenchyma. These authors also proposed that the morphological diversity observed in the gynoecium of *Thaumatophyllum* species is the result of an ongoing process of fusion of its floral structures and that the resulting reduction of energy wastage and increase in stigmatic surface are likely to be evolving under positive selection.

Chromosome numbers. Available chromosome numbers for *Philodendron* range from 2n = 28 to 40 (Correia-da-Silva et al. 2014) with a prevalence of 2n = 32, whereas for *Thaumatophyllum* they range from 2n = 28 to 36, with a clear prevalence of 2n = 36, indicating a distinct cytological trend (Correia-da-Silva et al. 2014, Vasconcelos et al. 2017).

Evolutionary history. Mayo (1988) hypothesised that *Thaumatophyllum* was the first lineage to emerge as a distinct clade from ancestral *Philodendron* and the Eastern and Southern South America species would present a higher number of plesiomorphic gynoecial characters (low number of locules and simple style) and the Amazonian species would have more apomorphic characters (high number of locules and elaborated style). Results from the morphology-based phylogenetic reconstruction of Calazans et al. (2014) partly agreed with Mayo's (1988) findings, recognising it as a natural group and suggesting its origin and diversification within open areas of the Cerrado biome. Loss-Oliveira et al. (2016) however, based on molecular evidence, proposed that the last common ancestor of *Philodendron* occurred in Amazonia about 8.6 Ma (11.1–6.8 Ma) during the Middle/Late Miocene, and that *Philodendron* lineages occurred exclusively in Amazonia for ca. 5.0–6.0 Ma. This implies that *Thaumatophyllum*, as well as the Atlantic forest lineages, must have diverged from Amazonian ancestors. The majority of *Thaumatophyllum* species from the Cerrado would then have evolved from Atlantic forest ancestors, from the Late Miocene to the Pliocene.

Ecology. *Thaumatophyllum* species have a preference for open environments with higher light intensity. The life forms vary from terrestrial to hemi-epiphytic, but can be rupicolous (*T. saxicola* and *T. adamantinum*), aquatic or subaquatic in freshwater swamps at lowland sites (*T. tweedieanum*, *T. undulatum*, *T. uliginosum*). More frequent are forest hemi-epiphytes which grow equally well in rupicolous habitats or even in open coastal sites on sand in the case of the *T. williamsii*, *T. corcovadense*, *T. bipinnatifidum* and *T. speciosum*. All the extant species have a notable preference for open habitats and the ability to tolerate a certain degree of drought.

Taxonomic treatment

Thaumatophyllum **Schott, Bonplandia 7: 31. 1859.**

Type. *Thaumatophyllum spruceanum* Schott, Bonplandia 7: 31. 1859.

Etymology. from Ancient Greek "θαυματ-" ("*thaumato-*", wonder, miracle) + "φύλλον" ("*phyllum*", leaf); wonderful leaf, referring to the beautiful and peculiar leaves of the type species.

Thaumatophyllum adamantinum **(Schott) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178483-1

Philodendron adamantinum Schott, Syn. Aroid. 114. 1856.

Type. Brazil, Minas Gerais, Tejuco, Serro Frio, *Martius 1208* (holotype: M).

Thaumatophyllum bipinnatifidum **(Schott ex Endl.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178497-1

Philodendron bipinnatifidum Schott ex Endl., Gen. Pl. 1(3): 237. 1837.
Type. Illustration in Schott Icones Aroideae Nº 2640 (lectotype, designated by Sakuragui et al. 2011); Brazil, Rio de Janeiro, Arraial do Cabo, 13 Feb. 2012, *L.S.B. Calazans et al. 170* (epitype, designated by Sakuragui et al. 2011: RB).
Philodendron selloum C.Koch, Index Seminum (B) 1853 (App.): 14. 1853.
Type. Plant cultivated at Berlin Botanic Garden, *C. Koch s.n.* (lectotype, designated by Sakuragui et al. 2011: K, tracing).
Philodendron pygmaeum Chodat & Vischer, Bull. Soc. Bot. Genève 11: 299. 1919 publ. 1920.
Type. Paraguay, Paraguari, 'Cerro Akahay', 1914, *R.H. Chodat & W. Vischer 358* (holotype: G).

Thaumatophyllum brasiliense **(Engl.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178484-1

Philodendron brasiliense Engl., Fl. Bras. 3(2): 168. 1878.
Type. Brazil, Minas Gerais, Caldas, Rio Verde, Feb-Mar. 1868, *S.E. Henschen in Herb. Regnell III. Nº 1292* (lectotype, designated by Sakuragui et al. 2011: S).
Philodendron cymbispathum Engl., Bot. Jahrb. 26: 555. 1899.
Type. Brazil, Minas Gerais, *A.F.M. Glaziou 16497* (**lectotype, designated here**: B; isolectotypes: C, LE, P).

Thaumatophyllum corcovadense **(Kunth) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178485-1

Philodendron corcovadense Kunth, Enum. Pl. 3: 49. 1841.
Type. illustration in Vellozo, Fl. Flum. 9: tab. 115. 1831 (lectotype, designated by Sakuragui et al. 2011); Brazil, Rio de Janeiro, Mangaratiba, Ilha da Marambaia, 19 Out. 2004, *M.A. Nadruz Coelho 1590* (epitype, designated by Sakuragui et al. 2011: RB).
Philodendron melanorrhizum Reitz, Sellowia 9:50, t.10. 1958.
Type. Brazil, Santa Catarina, Itajaí, Luís Alves, Braço Joaquim, 14 Oct. 1954, *R. Klein 917* (holotype: HBR; isotypes: NY, UC, US).

Thaumatophyllum dardanianum **(Mayo) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178498-1

Philodendron dardanianum Mayo, Kew Bull. 46: 648. 1991.

Type. Brazil, Bahia, Chapadão Oriental da Bahia, 37km N from Correntina from road to Inhaúmas, 29 Apr. 1980, *Harley et al. 21963* (holotype: CEPEC; isotypes: K, MO, US).

***Thaumatophyllum leal-costae* (Mayo & G.M. Barroso) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178499-1

Philodendron leal-costae Mayo & G.M. Barroso, Aroideana 2: 82. 1979.
Type. Brazil, Bahia, Serra do Jatobá, Nossa Senhora dos Milagres, Morro do Couro, 06 Mar. 1977, *Harley et al. 19428* (holotype: CEPEC; isotypes: K, M, MO, NY, P, RB, SEL, US).

***Thaumatophyllum lundii* (Warm.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178486-1

Philodendron lundii Warm., Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 1867: 128. 1867.
Type. Brazil, Minas Gerais, Lagoa Santa, *Warming s.n.* (holotype: C).

***Thaumatophyllum mello-barretoanum* (Burle-Marx ex G.M. Barroso) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178496-1

Philodendron mello-barretoanum Burle-Marx ex G.M. Barroso, Arch. Jard. Bot. Rio de Janeiro 15: 94. 1957.
Type. Brazil, Goiás, cultivated at Jardim Botânico do Rio de Janeiro, *Burle-Marx s.n.* (holotype: RB 97081).

***Thaumatophyllum paludicola* (E.G. Gonç. & Salviani) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178487-1

Philodendron paludicola E.G. Gonç. & Salviani, Aroideana 25: 2. 2002 publ. 2003.
Type. Brazil, Espírito Santo, São Mateus, access to Barra Nova, 21 Dec. 2000, *E.R. Salviani & L. Bernacci 1869* (holotype: UB; isotype: K).

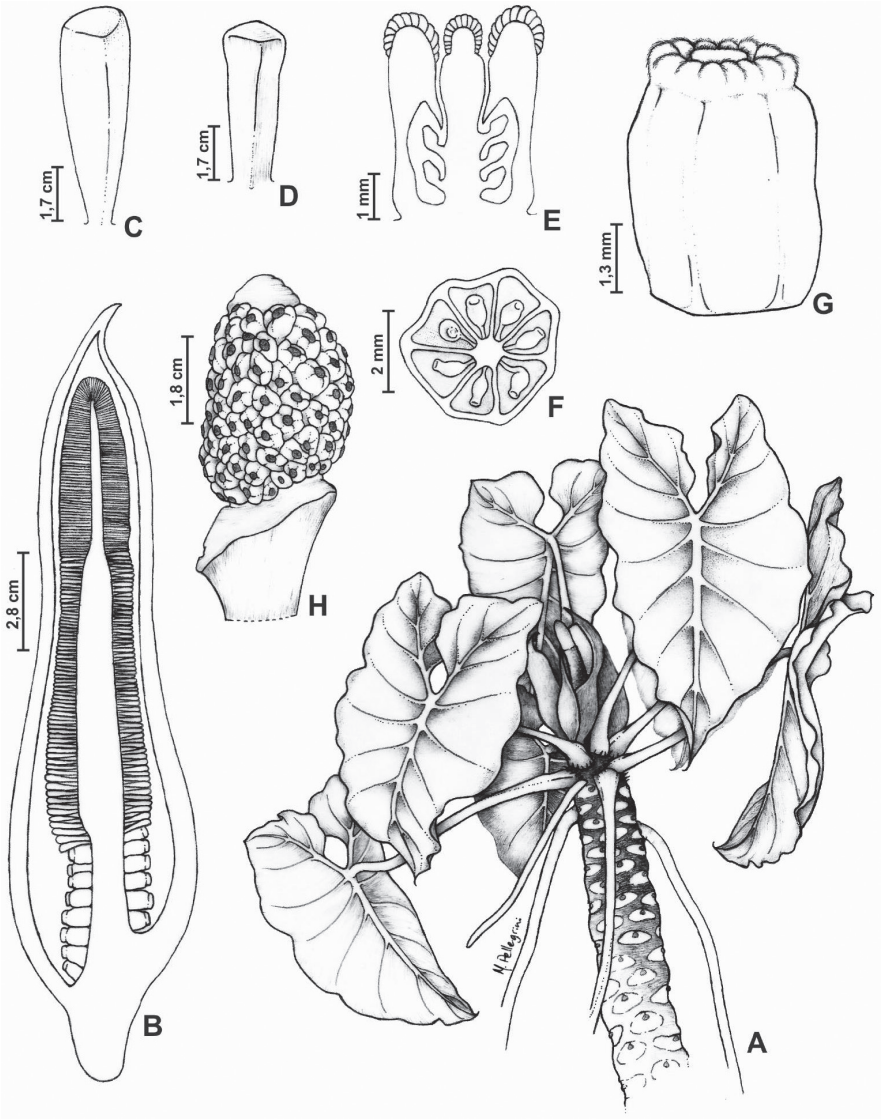

Figure 4. *Thaumathophyllum petreum*. **A** Habit **B** Longitudinal cut of the inflorescence **C** Staminode **D** Stamen **E** Longitudinal cut of a female flower **F** Transversal cut of a female flower showing the 6-locular ovary **G** Side view of a female flower **H** Infructescence. All from *Calazans & Morais 28* (RB).

Thaumatophyllum petraeum **(Chodat & Vischer) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178488-1
Fig. 4.

Philodendron petraeum Chodat & Vischer, Bull. Soc. Bot. Genève 11: 296. 1919 publ. 1920. Type. Paraguay, Tobaty between Tobaty and Barrero Grande, *R.H. Chodat & W. Vischer 349* (holotype: G).

Philodendron petraeum var. *triangulare* Chodat & Vischer, Bull. Soc. Bot. Genève 11: 299. 1919 publ. 1920.
Type. Paraguay, Tobaty between Tobaty and Barrero Grande, *R.H. Chodat & W. Vischer 347* (holotype: G).

Philodendron petraeum var. *valenzuelae* Chodat & Vischer, Bull. Soc. Bot. Genève 11: 299. 1919 publ. 1920.
Type. Paraguay, prope Valenzuela, *R. H. Chodat & W. Vischer 357* (holotype: G).

Remarks. The species was previously synonymised under *P. tweedieanum* (= *T. tweedieanum*) by Croat and Mount (1988). We propose its reinstatement as an accepted species based on the following morphological differences: herbs erect and rupiculous (x herbs decumbent or rhizomatous subterranean acaulous in *T. tweedieanum*), prophyll deciduous when still herbaceous (x marcescent and persistent-membranous in *T. tweedieanum*), denudation of posterior division absent (x present in *T. tweedieanum*), presence of stylar central dome in pistillate flowers (x absence of stylar central dome in *T. tweedieanum*). Besides having a different gynoecium type (Calazans et al. 2014), the majority-rule consensus tree based on morphological characters support these species as different lineages. Furthermore, the phylogeny based on molecular characters supports the two species as separate taxa (Loss-Oliveira et al. 2014). *Thaumatophyllum petraeum* was first described for Paraguay with four varieties and are still recorded only from this country. We have no evidence to recognise the varieties as distinct taxa, except for. *P. petraeum* var. *tobatiense* Chodat & Vischer, which is a synonymous of *P. undulatum* (= *T. undulatum*).

Thaumatophyllum saxicola **(Krause) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178489-1

Philodendron saxicola Krause, Pflanzenr. IV, 23Db: 133. 1913.
Type. Brazil, Bahia, Serra do Sincorá, Nov. 1906, *E. Ule 7568* (holotype: B; isotype L).

Thaumatophyllum solimoesense **(A.C. Smith) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178490-1

Philodendron solimoesense A.C. Smith, J. Arnold Arbor. 20: 289. 1939.
Type. Brazil, Amazonas, São Paulo de Olivença, basin of Creek Belem, Oct-Dec. 1936, *B.A. Krukoff 8861* (holotype: NY; isotype: F).

Thaumatophyllum speciosum **(Schott ex Endl.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178501-1

Philodendron speciosum Schott ex Endl., Gen. Pl. 1(3): 237. 1837.

Type. illustration in Schott Icones Aroideae N° 2522 (lectotype, designated by Sakuragui et al. 2011); Brazil, Minas Gerais, Descoberto, 10 Nov. 2001, *V.R. Almeida 18* (epitype, designated by Sakuragui et al. 2011: CESJ, RB).

***Thaumatophyllum spruceanum* Schott, Bonplandia (Hannover) 7: 31. 1859.**

Philodendron spruceanum (Schott) G.M. Barroso, Arch. Jard. Bot. Rio de Janeiro 17: 14. 1962, *nom. illeg.*
Type. based on *Thaumatophyllum spruceanum* Schott.
Philodendron goeldii G.M. Barroso, Arch. Jard. Bot. Rio de Janeiro 15: 95. 1957.
Type. Brazil, Manaus, Igarapé das Flores, 30 Sept. 1903, *A. Goeldi s.n.* (holotype: MG 3879).

Type. Brazil, inundated forest in angle between Rio Negro and Solimões, 1851, *Spruce 120* (holotype: K).

***Thaumatophyllum stenolobum* (E.G. Gonç.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178491-1

Philodendron stenolobum E.G. Gonç., Aroideana 25: 3. 2002 publ. 2003.
Type. Brazil, Espírito Santo, Colatina, road to São Domingos, 10 Oct. 2000, *E.G. Gonçalves et al. 567* (holotype: UB).

***Thaumatophyllum tweedieanum* (Schott) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178492-1

Philodendron tweedieanum Schott, Bonplandia (Hannover) 7: 29. 1859.
Type. Argentina, Entre Rios, delta region of Rio Paraná, *J. Tweedie s.n.* (**lectotype, designated here:** K; isolectotype: LE).
Philodendron dubium Chodat & Vischer, Bull. Soc. Bot. Genève 11: 295. 1919 publ. 1920.
Type. Paraguay, prope San Bernardino, *E. Hassler 1713* (**lectotype, designated here:** G); Paraguay, Lago Ypacaraí, *R.H. Chodat & W. Vischer 359* (remaining syntype: G, not found).

***Thaumatophyllum uliginosum* (Mayo) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178502-1

Philodendron uliginosum Mayo, Kew Bull. 46: 666. 1991.
Type. Brazil, Minas Gerais, Santana do Riacho, 25 Oct. 1974, *G. Hatschbach & Koszicki 35350* (holotype: MBM; isotypes K, US).

***Thaumatophyllum undulatum* (Engl.) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178493-1

Philodendron undulatum Engl., Monogr. Phan. 2: 428. 1879.
Type. Paraguay, Aregua plains, Jul. 1875, *B. Balansa 576* (**lectotype, designated here:** P; isolectotype: G, not found).
Philodendron eichleri Engl., Bot. Jahrb. Syst. 26: 556. 1899.
Type. Brazil, Minas Gerais, Carandaí, 15 Nov. 1887, *A.F.M. Glaziou 17332* (lectotype, designated by Sakuragui et al. 2011: K; remaining syntype: P).
Philodendron petraeum var. *tobatiense* Chodat & Vischer, Bull. Soc. Bot. Genève 11: 297. 1919 publ. 1920.
Type. Paraguay, Cerro Tobaty, *R.H. Chodat & W. Vischer 350* (holotype: G).

***Thaumatophyllum venezuelense* (Bunting) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178494-1

Philodendron venezuelense Bunting, Acta Bot. Venez. 10: 315. 1975.
Type. Venezuela, Territorio Federal Amazonas, Departamento Casiquiare, environs of Yavita on the Temi and near the Yavita-Pimichín road, 6-19 Jul. 1969, *Bunting et al. 3864* (holotype: MY; isotypes: NY, U).

***Thaumatophyllum williamsii* (J.D. Hooker) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178503-1

Philodendron williamsii J.D. Hooker, Bot. Mag. 97: t. 5899. 1871.
Type. Brazil, Bahia, region of Salvador, cultivated at Kew, Aug. 1870, *Williams s.n.* (holotype: K).

***Thaumatophyllum xanadu* (Croat, Mayo & J. Boos) Sakur., Calazans & Mayo, comb. nov.**
urn:lsid:ipni.org:names:77178495-1

Philodendron xanadu Croat, Mayo & J. Boos, Aroideana 25: 63. 2002 publ. 2003.
Type. origin unknown, based on plant cultivated in Wellington, West Palm Beach, Florida, *T.B. Croat 81537* (holotype: K; isotypes: B, F, COL, GH, INPA, K, MO, NY, R, RSA, SP, TRIN, UB, US).

Key to the species of *Thaumatophyllum*

(adapted from Mayo (1991) and Gonçalves and Salviani (2002))

1 Leaf blade transverse-cordiform in outline, pedately compound.....**2**
– Leaf blade cordiform-sagittate, sagittate or hastate in outline, margins entire, repand, sinuately lobed, pinnatifid or bipinnatifid **3**
2 Ovary locules 3–4; leaflets 8–11, central leaflet 10–17 cm long; occurring on rocks in semi-arid areas or terrestrial in coastal restinga scrub on sand; usually in association with populations of Bromeliaceae; northeast Brazil .... ***T. leal-costae***
– Ovary locules 10–26; leaflets 10–20, central leaflet 18–50 cm long; hemiepiphytic or terrestrial; most common along river margins; Amazon basin .... ***T. spruceanum***
3 Leaf margin sinuately lobed, pinnatifid or bipinnatifid **4**
– Leaf margin entire or repand or, if sinuately lobed, then peduncle 16 cm long or more.....**11**
4 Leaf margin bipinnatifid, rarely pinnatifid but then with primary lateral veins of anterior division (5-)6–9(-10) per side; leaf blade over 50 cm long, primary lateral lobes (12-)17–35(-55) cm long.....**5**
– Leaf margin undulate or pinnatifid; if pinnatifid, then with primary lateral veins of anterior division 3–4(-10) per side; leaf blade up to 50 cm long, usually smaller; primary lateral lobes 5–17.5 cm long.....**7**
5 Petioles flattened or slightly convex adaxially; intravaginal squamules never persistent, foliage leaf scars always concolorous with the internodes; uplands of Cerrado (Minas Gerais, Bahia and Goiás states – 700–1200 m)..... ***T. lundii***
– Petioles conspicuously sulcate adaxially; intravaginal squamules persistent, very rarely deciduous (if deciduous, then foliage leaf scars discolorous with the internodes).....**6**
6 Intravaginal squamules numerous and dense, 5–12 mm long, 2–4 mm wide at base, persistent but easily detachable, rarely deciduous; female portion of the spadix adnate to the spathe for 60–80% of its length; southern and western (coastal) Brazil, Argentina, Paraguay ..... ***T. bipinnatifidum***
– Intravaginal squamules few and scattered, robust, 8–20 x 5–10 mm, always persistent, hardly detachable; female portion of the spadix adnate to spathe for 40–50% of its length; northern Goiás and possibly Mato Grosso states... ..... ***T. mello-barretoanum***
7 Plants aquatic or rarely terrestrial; leaf margin sinuately lobed (sinuses penetrating less than halfway to midrib), primary lateral lobes of anterior division 1.5–6.5(-14) cm long, usually oblique and turned towards leaf apex; female zone of the spadix (1.5-)4–5 cm long.....**8**
– Plant rupiculous or terrestrial; leaf margin pinnatifid (sinuses penetrating at least halfway to midrib), primary lateral lobes of anterior division 5–17.5 cm long, not oblique, female zone of the spadix 1.4–3.4 cm long.....**9**

8 Stems with long and thorn-like intravaginal squamules; leaf blades never erect in living plants; Argentina, Paraguay, Bolivia and Brazil (South and Southeastern) ..... ***T. undulatum***

– Stems without persistent intravaginal squamules; leaves always erect or semi-erect in living plants; Eastern Brazil (northern Espírito Santo and southern Bahia) ..... ***T. paludicola***

9 Petiole green or glaucous green at apex; leaf blade broadly ovate in outline, dark to subglossy green, sometimes glaucous, primary lateral lobes 3–4(-5); spathe 6.4–16 cm long, green outside, opened at anthesis; ovary locules 4–8(-11) **10**

– Petiole purplish at apex; leaf blade triangular to ovate in outline, glossy dark green, primary lateral lobes 5–10; spathe (8.2-)12–18 cm long, dark purple outside, tightly clasped around spadix at anthesis; ovary locules (6-)7–8 ..... ***T. xanadu***

10 Leaf blade (32-)35–50 cm long, primary lateral lobes of anterior division 3.5–6.3(-7.5) cm wide, distance between sinuses and midrib progressively greater towards base of anterior division; fertile male zone of the spadix 1.5–2.2 cm diam. ..... ***T. saxicola***

– Leaf blade 17-33 cm long, primary lateral lobes of anterior division 1.4–3.7(-7.4) cm wide, distance between sinuses and midrib usually becoming progressively less towards base of anterior division; fertile male zone of spadix 0.85–1.3 cm diam. ..... ***T. adamantinum***

11 Overall length of adult leaf blade more than 60 cm (sometimes 50–60 cm in *T. solimoesense*); petiole apex often minutely rugose-verruculate (may be smooth in *T. stenolobum*) **12**

– Overall length of leaf blade less than 60 cm, petiole apex smooth, never occurring in Amazonia **16**

12 Species from Eastern Brazil; stamens 6 mm long or more; staminodes more than 1.6 mm wide at apex, less than 2.5× longer than wide; ovary locules 6–13 per ovary **13**

– Species from Amazonia; stamens less than 6 mm long; staminodes less than 1.6 mm wide at apex, more than 2.5× longer than wide; ovary locules 17–34(-47) per ovary **15**

13 Leaf blade narrowly sagittate, sometimes subhastate; anterior division 2.1–3.3× longer than wide; intravaginal squamules deciduous ..... ***T. stenolobum***

– Leaf blade broadly sagittate; anterior division 1–1.5× longer than wide; intravaginal squamules small but persistent **14**

14 Leaf blade less than twice as long as wide; spathe outside lacking extrafloral nectaries, inside carmine magenta at anthesis; central style dome lacking ..... ***T. speciosum***

– Leaf blade more than twice as long as wide; spathe outside with punctate, pale brown extrafloral nectaries, inside cream-white at anthesis; central style dome present ..... ***T. williamsii***

15 Cataphylls persistent; primary lateral veins of anterior division of leaf blade (5-)6–7; ovary locules 17–22; style elongated, distinctly narrower than ovary and lacking an axial canal..... ***T. venezuelense***
– Cataphylls deciduous; primary lateral veins of anterior division of leaf blade (3-)4–5(-6); ovary locules 26–34(-47); style short, as broad as ovary with an axial canal or cavity which is very conspicuous in fruit..... ***T. solimoesense***
16 Peduncle subequal to twice as long as spathe; plants aquatic or rupiculous, aerial portion of the stem unbranched; internodes shorter than prophyll scars.....**17**
– Peduncle only about one third of spathe length; plant hemi-epiphytic or terrestrial; aerial stem branching frequently; internodes usually longer than prophyll scars ..... ***T. corcovadense***
17 Leaf blade at least twice as long as broad; style longer than ovary..... ..... ***T. dardanianum***
– Leaf blade much less than twice as long as broad; style shorter than ovary **18**
18 Intravaginal squamules abundant; broadly triangular, 3–12 mm long, (1.5-)3–7(-9) mm broad at base; style body as wide as ovary ..... ***T. brasiliense***
– Intravaginal squamules few, more narrowly triangular, 1.5–5 mm long, 0.5–2.5 mm broad at base; style body slightly narrower than ovary.....**19**
19 Leaf blades subglossy to glaucous green, margins weakly repand; Argentina, Paraguay, Uruguay, South Brazil **20**
– Leaf blades dark glossy green, margins entire, rarely repand; central Brazil.... ..... ***T. uliginosum***
20 Plants rupiculous, stem erect; prophyll deciduous; stylar central dome present ..... ***T. petraeum***
– Plants aquatic, stem decumbent or rhizomatous subterranean; prophyll marcescent and persistent; stylar central dome absent..... ***T. tweedieanum***

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Supplementary material 1

Taxon sampling, voucher information and GenBank

Authors: Cassia Mônica Sakuragui, Luana Silva Braucks Calazans, Leticia Loss de Oliveira, Érica Barroso de Morais, Ana Maria Benko-Iseppon, Santelmo Vasconcelos, Carlos Eduardo Guerra Schrago, Simon Joseph Mayo

Data type: molecular data

Explanation note: Taxon sampling, voucher information and GenBank accession numbers of *Philodendron*, *Homalomena* and outgroup species.

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RESEARCH ARTICLE

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Chiococca grandiflora (Rubiaceae), a new species from Northern Mexico

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Abstract

The new species *Chiococca grandiflora* Lorence & T.Van Devender from Sinaloa and Sonora, Mexico differs from its congeners by its larger, showy white flowers in compact cymes of 3–9, and infundibuliform corollas 16–20 mm long with tubes 13–17 mm long and lobes 3–3.5 mm long. Its distribution, habitat, and relationships are outlined. The conservation status for this species is estimated to be Endangered (EN) based on IUCN Red List Criteria.

Resumen

La nueva especie, *Chiococca grandiflora* Lorence & T.Van Devender, de Sinaloa y Sonora, México, difiere de sus congenéricas por sus grandes y vistosas flores blancas, en cimas compactas de 3–9 flores, y corolas infundibuliformes de 16–20 mm de longitud, con tubos de 13–17 mm y lóbulos de 3–3.5 mm de longitud. Se presentan datos de distribución, hábitat y relaciones. De acuerdo con los criterios de la Lista Roja de la IUCN, se estima que el estado de conservación de esta especie debe ser como En Peligro (EN, por sus siglas en Inglés).

Keywords

Chiococca, conservation, Mexico, Rubiaceae, Sinaloa, Sonora

Introduction

Chiococca P. Browne (Rubiaceae, Cinchonoideae: Chiococceae) is a taxonomically complex genus of about 25 species of Neotropical shrubs and small trees. The type species is *Chiococca alba* (L.) Hitchc., a widespread and morphologically extremely variable shrub or small tree ranging from northern Mexico and the Caribbean south to Argentina. Borhidi (2006) recognized 13 species for Mexico in his *Rubiáceas de México*. The genus was revised for the 10 species occurring in the Mesoamerica region by Lorence and Taylor (2012). Several additional species occur in the Caribbean and South America.

Traditionally within Rubiaceae *Chiococca* has been placed in subfamily Cinchonoideae, tribe Chiococceae which is strongly supported by recent molecular evidence (Manns and Bremer 2010). This genus is distinctive but its species are often difficult to key out, however, especially since mature flowers and fruits rarely occur together on the same collection, but these both are often essential for identification. Certain taxonomically useful vegetative and floral characters tend to be subtle and overlap or intergrade in certain species, often making identification a challenge.

A distinctive new species of *Chiococca* with large white flowers was discovered and collected by Sally Walker in 1970 in Sinaloa, Mexico, two miles west of El Palmito near the Durango-Sinaloa border, and also at 19 km west of El Palmito (Figure 3). A third specimen was collected in 1978 near El Palmito, Sinaloa by Tim Walker. Collections of the same species were made in 1992 by Paul S. Martin of the University of Arizona while conducting field work in Sonora at Sierra Saguaribo near Tepopa for the revision of Howard Scott Gentry's *Rio Mayo Plants* (Gentry 1942; Martin et al. 1998). This plant was reported as *Chiococca* sp. nov. by Martin et al. (1998) in the book *Gentry's Rio Mayo Plants*. Comparison with all other species of the genus revealed it represents a new species described below.

Taxonomy

Chiococca grandiflora **Lorence & T.Van Devender, sp. nov.**
urn:lsid:ipni.org:names:60476299-2

Type. MEXICO. Sonora, Municipio Alamos. Near Tepopa NNW of Chiribo, 27°19'N, 108°43.5'W, 1100–1400 m, 22 August 1992 (fl), *P. S. Martin, P. Comtois, C. Lindquist, S. A. Meyer, B. Risner, & D. A. Yetman s.n. sub P. Jenkins 92-135* (Holotype: ARIZ-309922!; Isotypes ARIZ-383348!, PTBG-105887!) (Figures 1, 2). [Note: The ARIZ isotype specimen label says "Abandoned orchard of Tepopa" at 1100–1400 m elevation.]

Chiococca grandiflora differs from other members of the genus by its relatively larger, showy white flowers in compact cymes of 3–9, and its infundibuliform corollas 16–20 mm long with tubes 13–17 mm long and lobes 3–3.5 mm long.

Shrubs to 3 m tall, branches erect-ascendent, branchlets glabrous or sometimes persistently short hirtellous with white trichomes 0.05–0.1 mm long. Leaves of a pair

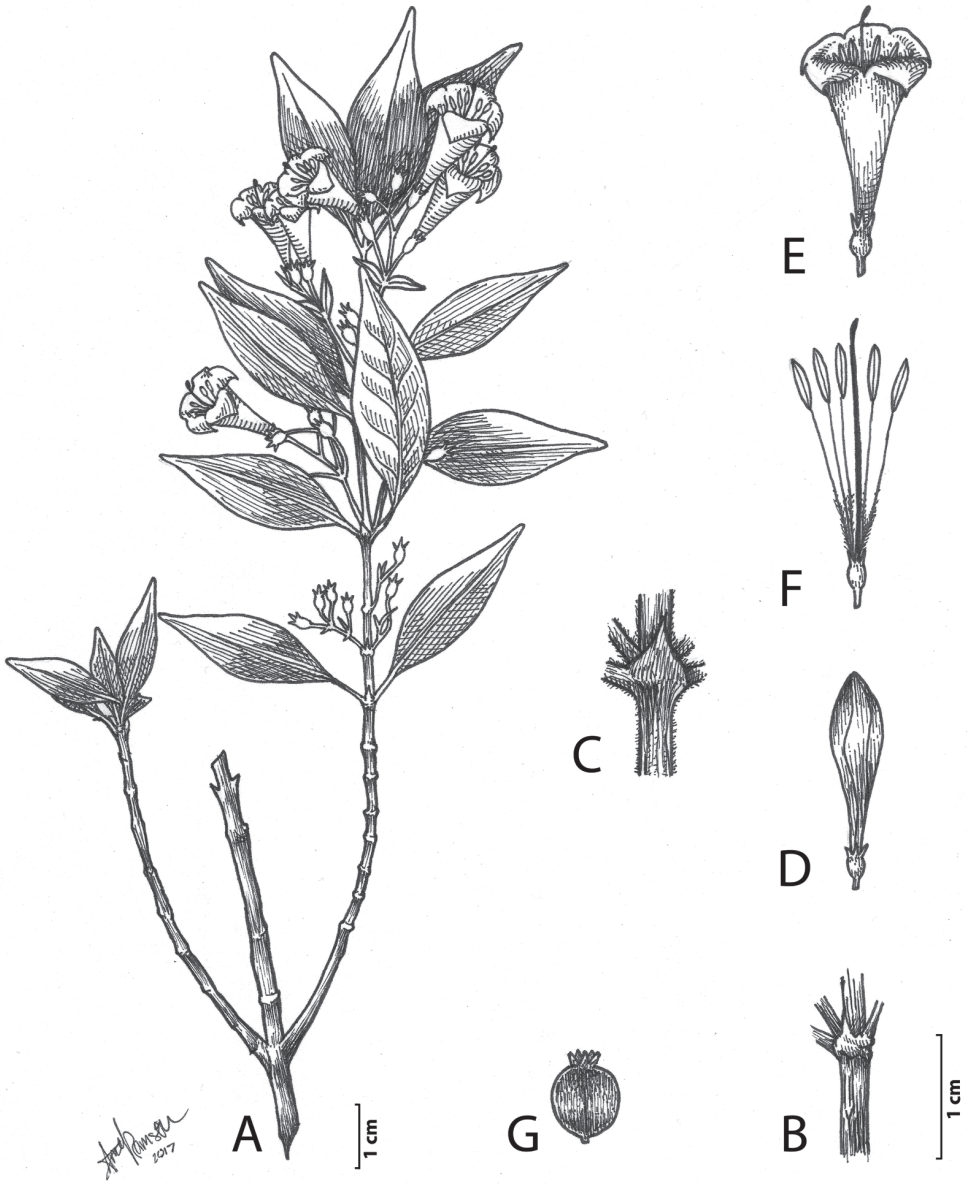

Figure 1. *Chiococca grandiflora* Lorence & T.Van Devender. **A** flowering branch **B** node showing stipule and petiole bases, glabrous form **C** node showing stipule and petiole bases, pubescent form **D** flower in bud **E** flower at anthesis **F** flower with corolla removed showing stamens and pistil **G** immature fruit. **A, B, D–F** based on P. S. Martin, P. Comtois, C. Lindquist, S. A. Meyer, B. Risner, & D. A. Yetman s.n. sub P. Jenkins 92-135 (ARIZ-383348) **C** based on S. Walker s.n. (UTC-00263027) **G** based on T. Walker s.n. (ARIZ-212520).

equal, petiolate; blades ovate, 1.8–6.5 cm long, 0.8–2 cm wide, glabrous or sometimes minutely hirtellous on both surfaces, stiffly chartaceous to subcoriaceous, apex acute or acuminate to short-acuminate, base cuneate, decurrent, margin weakly revo-

lute, secondary veins 3–5 on each side, brochidodromous, inconspicuous on both surfaces; petioles 3–4 mm long, glabrous or sometimes short-hirtellous; stipules triangular, 2–3 mm long, acute and aristate, the awn subulate, 1.5–2.5 mm long, the base 1–1.5 mm long, thick and persistent, externally glabrous or sometimes short hirtellous, internally with colleters. Inflorescences axillary, 3–4 cm long, 2–2.5 cm wide, racemose or sometimes with a pair of basal secondary branches, glabrous or sometimes short-hirtellous, rachis 5–12 mm long, floral bracts 1–3 mm long, triangular to linear-subulate, acute, glabrous or the margins short-hirtellous; flowers 3–9, pendulous, hypanthium 1.5–2.2 mm long, obovoid-ellipsoid, laterally compressed, glabrous or sometimes short-hirtellous; calyx limb below the lobes 0.5–0.7 mm long, tubular, short-hirtellous or only the margins hirtellous, calyx lobes 5, 0.4–1.0 mm long, triangular-subulate, acute, recurved, glabrous or the margins hirtellous, glabrous within; corolla infundibuliform, 16–20 mm long, white, externally sparsely hirtellous, internally glabrous, tube 13–17 mm long, 6–11 mm wide at throat, lobes 5, triangular, 3–3.5 mm long and wide, recurved at anthesis; stamens with tips exserted for 1–2 mm, filaments 8–12 mm long, minutely strigillose, attached near base of tube, anthers linear, 3–3.2 mm long; style exserted for 2–3 mm, 16–18 mm long, glabrous, tip swollen for ca. 1 mm, with stigmatic portion decurrent for ca. 5 mm laterally. Fruits drupaceous, spongy at maturity, 5–5.5 mm in diameter, broadly ellipsoid to subglobose, compressed, white, glabrous. Seeds not seen.

Additional specimens examined (paratypes). MEXICO. Sinaloa. Municipio Concordia. Rock Slide, El Palmito, Sinaloa, elev. 7000 ft, 18 March 1978 (fr), *T. Walker s.n.* (ARIZ-212520); Municipio Concordia, 2 miles W of El Palmito, Sinaloa, 7000 ft, pine oak, 1 September 1970 (fl), *S. Walker s.n.* (UTC-00263027; ARIZ-181630); Municipio Concordia, 19 km W of El Palmito, Sinaloa, in Sinaloa, 6200 ft., 1 September 1970 (fl), *S. Walker 70,043* (K, loan # H2017/00697).

Discussion. *Chiococca grandiflora* displays some variation in vegetative and floral pubescence. We were at first inclined to select as holotype the most amply floriferous specimen, from Sinaloa 2 miles W of El Palmito collected by Sally Walker *s.n.* on 1 September 1970 (UTC-00263027), since there is a putative duplicate of this collection (ARIZ-181630; gathered at the same locality on the same date). However, the UTC specimen has uniformly short-hirtellous twigs, leaves, and inflorescences with white trichomes 0.05–0.1 mm long (Figure 1C), compared with the ARIZ specimen which is essentially glabrous except for the calyx lobes having ciliate margins. Although the collections presumably were gathered from the same population, they were almost certainly taken from different individuals. In any case, it suggests that pubescence is variable in this species, and this variation is encompassed by the description above. The other Sinaloa specimens, *T. Walker s.n* (Rock Slide, El Palmito, ARIZ-212520) and *S. Walker* 70,043 (19 km W of El Palmito, K), and the type collection from Sonora are all similarly glabrous. Field studies would help elucidate this variation. However, localities of the known populations in Sinaloa are remote and those in Sonora are rugged and relatively difficult to access (Figures 4, 5). The variation in pubescence documented here is not unusual for a species of *Chiococca*.

Figure 2. *Chiococca grandiflora* Lorence & T.Van Devender. Holotype collection, P. S. Martin, P. Comtois, C. Lindquist, S. A. Meyer, B. Risner, & D. A. Yetman s.n. sub P. Jenkins 92-135 (ARIZ-309922).

Regarding the type collection, Paul Martin was most likely the actual collector, although Martin did not assign numbers to his collections, only dates. Consequently, Phil Jenkins catalogued the specimen and assigned it his number 92-135. The paratype collections by Sally Walker (and Tim Walker) are from near the Durango border and were originally designated on the specimen label as either "west of El Palmito, Durango in Sinaloa" or "near El Palmito, in Durango" as the state line was not exactly known. El Palmito and immediate surroundings are in Sinaloa, however, and the state line is farther east than they realized.

Relationships. The relationships of *Chiococca grandiflora* within its genus are unclear, along with those of all the other species, and molecular phylogenetic studies have not yet been undertaken for the genus. Based on its exceptionally large corollas, this new species does not closely resemble any of the three other species occurring in northern Mexico. *Chiococca henricksonii* M.C.Johnst. is a distinctive, narrowly endemic microphyllous species from Coahuila having tiny leaves with petioles 1–2 mm long, blades 4–9 mm long, and small solitary flowers with corollas only 7 mm long. *Chiococca petrina* Wiggins ranges from central Sonora to Chihuahua and Sinaloa. It also has relatively small puberulent leaves with petioles 1–2 mm long, blades 0.6–2 cm long, and much smaller, externally puberulent corollas 4–6 mm long with lobes about half as long as the tube. The widespread and variable *Chiococca alba* reaches its northernmost range in southern Florida, the southern tip of Texas, and also occurs in the Río Mayo region of Sonora. It differs in having inflorescences with more numerous flowers borne on a longer floral rachis and much smaller, externally glabrous corollas 6–8 mm long. Leaf size is extremely variable with petioles 3–10 mm long and blades 1.3–13 cm long, usually glabrous except the margin sometimes minutely hirtellous. Considering its morphological variability, *C. alba* may be most closely related to *C. grandiflora*.

Habitat and Ecology. [Description by George M. Ferguson based on field notes and collections made at Tepopa, Sonora on 18 March 1992 and 16–17 March 1993 with Mark Fishbein, and from El Palmito, Sinaloa 12–13 April 1999 with Andy Sanders. The El Palmito site was visited by T. Van Devender in October 2017.] *Chiococca grandiflora* occurs in strikingly similar habitats at two known localities, despite a 500 km latitudinal distance apart on the Pacific versant of the Sierra Madre Occidental (Figure 3). Both are at the lower edge of pine-oak forest within a mountainous country of thick ignimbrite deposits of rhyolite and volcanic ash. Deep barranca canyons support a diversity of vegetation here where tropical and temperate zones intermix in a region subject to substantial rainy seasons in summer and winter, interspersed by marked dry seasons especially in late spring.

Near the paratype locality at 2 miles northwest of El Palmito in Sinaloa is Rancho Liebre (23.58, -105.85), located at ca. 2100 m elevation (Figure 4), a well visited birding spot on the north side of Mexico Highway 40, where one can find the narrowly endemic tufted jay or "urraca pinta" (*Cyanocorax dickey* Moore) discovered in 1934 (Crossin 1967). The area lies within the Madrean-Tropical subregion of the Madrean Ecoregion (González-Elizondo et al. 2013) which was originally defined as a Mixed Boreal-Tropical faunal region (Webb 1984). The vegetation is pine-oak forest con-

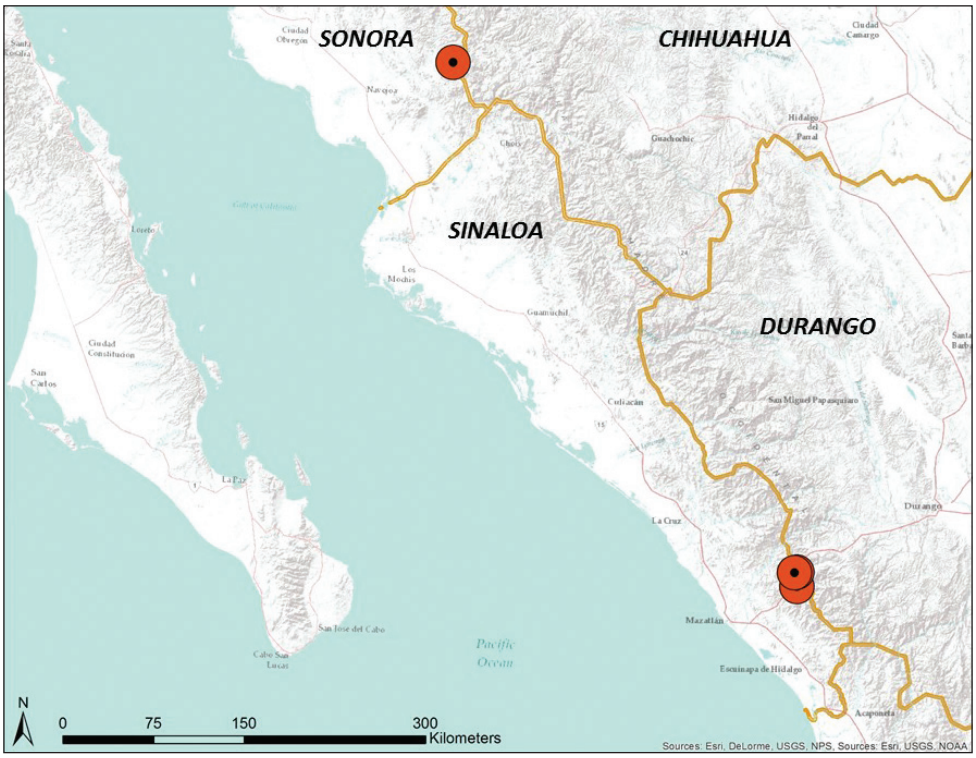

Figure 3. Distribution of *Chiococca grandiflora* in western Mexico.

taining elements of southern affinity with the dominant trees being *Pinus devoniana* Lindl., *P. herrerae* Martínez, *P. lumholtzii* B.L.Rob. & Fernald, *P. yecorensis* Debreczy & I.Rácz, *Quercus castanea* Née, *Q. jonesii* Trel., *Q. gentryi* C.H.Mull., *Q. mcvaughii* Spellenb., *Q. scytophylla* Liebm., *Q. subspathulata* Trel., and *Q. viminea* Trel. Other overstory trees are *Arbutus glandulosa* M.Martens & Galeotti, *A. xalapensis* Kunth, *Alnus* Mill., *Oreopanax* Decne. & Planch., and *Vachellia pennatula* (Schtdl. & Cham.) Seigler & Ebinger, and a diverse understory of shrubs, ferns and epiphytes includes *Bouvardia* Salisb., *Cuphea* P.Browne, *Garrya* Douglas ex Lindl., *Mitracarpus rhadinophyllus* (B.L.Rob.) L.O.Williams, *Oncidium* Sw., *Peperomia* Ruíz & Pavón, *Pteridium* Gled. ex Scop., *Rhus* Tourn. ex L., *Rubus* Tourn. ex L., and *Woodwardia* Sm.

The paratype localities near El Palmito are along a ridge with a divide on the rim of a spectacular, immense barranca system draining toward the northwest and another to the south. The north-facing slope of the barranca heading here has a resemblance to a cloud forest with dominant trees being *Abies durangensis* Martínez, *Quercus laurina* Bonpl., *Q. mcvaughii*, *Q. rugosa* Née, *Cornus disciflora* Moc. & Sessé ex DC., *Magnolia schiedeana* Schltdl., *Ostrya virginiana* K.Koch, *Prunus serotina* Ehrh., *Tilia mexicana* Schltdl., with species of *Clethra* Gronov. ex L., *Cercocarpus* Kunth, *Cinnamomum* Schaeffer, and *Ilex* L. At the old Rancho Liebre is an orchard and other ornamental plantings such as *Sambucus nigra* L., *Hesperocyparis* Bartel & Price, *Iris* Tourn. ex L.,

Figure 4. Habitat of *Chiococca grandiflora* near paratype location ca. 2 miles NW of El Palmito, Sinaloa, Mexico showing deep barrancas with pine-oak forest transitional to tropical deciduous forest.

and *Rosa* Tourn. ex L. At the most southern paratype locality 19 km west of El Palmito, which is near the Tropic of Cancer at 1890 m along Mexico Highway 40, the vegetation here (23.466, -105.831) is lower pine-oak forest with *Pinus oocarpa* Schiede ex Schltdl. and *Pinus yecorensis* as the dominant pines and near the lower limit of *Pinus herrerae* with an abundance of oaks including *Quercus macvaughii, Q. obtusata* Bonpl., and *Q. tarahumara* Spellenb., J.D.Bacon & Breedlove, with *Arbutus xalapensis*, and *Arctostaphylos pungens* Kunth.

The more northern holotype locality for *Chicocca grandiflora* is at Tepopa, Sonora at 1100–1400 m, which is ca. 500–1000 m lower in elevation than the paratype localities (Figure 5). It is also situated in a steep northwestward draining barranca, at the ecotone of upper tropical deciduous forest including riparian evergreen forest, and lower edge of pine-oak forest with *Pinus oocarpa* and *Quercus tarahumara* with *Dodonaea viscosa* Jacq. The orchard site of Tepopa (27.3261, -108.7316) is an old ranch homestead perched above an intermittent stream at 1100 m elevation. The type specimen was probably collected along a trail between this site and the forested mesa top at 1400 m (Figure 5). From San Bernardo, Sonora into the Sierra Madre was a trail route for mule cargo that passed by here until early in the last century. Howard Scott Gentry visited this site in 1935–36 and made plant collections. An orchard, planted on a steep slope with rock lined terraces around seeps, was abandoned when Gentry visited. Paul Martin and stu-

Figure 5. Habitat of *Chiococca grandiflora* in vicinity of holotype location near Tepopa, Sonora, at ecotone of upper tropical deciduous forest including riparian evergreen forest, and lower edge of pine-oak forest. **5a** showing abandoned orchard with fruit trees including bananas near center.

dents from the University of Arizona revisited this famous Gentry collection locale in 1992–93 (Martin et al. 1998) and found growing in the abandoned orchard avocado, banana, grapefruit, mango, orange, peach and pomegranate trees, and cultivars of native *Casimiroa edulis* La Llave and *Tecoma stans* (L.) Juss. ex Kunth.

The vegetation at Tepopa is transitional oak woodland above the uppermost tropical deciduous forest on dry slopes. Oak Woodland (with a few scattered *Pinus oocarpa* on north-facing slopes) occurs at ca. 1000–1200 m with the dominant trees being *Quercus oblongifolia* Torr. (often with an epiphytic orchid, *Laelia eyermaniana* Rchb.f.), *Q. tarahumara*, *Q. chihuahuensis* Trel., *Q. viminea* Trel., *Lysiloma watsonii* Rose, and *Ipomoea arborescens* (Humb. & Bonpl. ex Willd.) G.Don var. *pachylutea* Gentry. Understory shrubs include *Bouvardia, Cuphea, Dodonaea viscosa*, *Rhus terebinthifolia* Schltdl. & Cham., *Rubus, Tithonia calva* Sch. Bip., and the succulents *Agave bovicornuta* Gentry, *Dasylirion gentryi* Bogler, and *Ferocactus pottsii* (Salm-Dyck) Backeb.

Intergrading with oak woodland on the north-facing slopes just above Tepopa at 1200 m and upward to the mesa top at 1400 m is pine-oak forest of *Pinus oocarpa*, *P. yecorensis*, *Quercus epileuca* Trel. (with *Encyclia* Hook. orchids), *Berberis* Tourn. ex L., *Clethra, Heliocarpus* L., *Prunus serotina* Ehrh. with an understory of *Gaultheria odorata* Bredem. ex Willd., *Nolina microcarpa* S.Watson, and *Roldana hartwegii* (Benth.)

H.Rob. & Brettell. Along the streambed from 900 to 1250 m is a northern extent of tropical evergreen riparian forest consisting of *Aphananthe monoica* (Hemsl.) J.-F. Leroy, *Brahea* Mart. ex Endl., *Cinnamomum hartmannii* (I.M.Johnst.) Kosterm., *Cornus disciflora*, *Ficus* Tourn. ex L., *Ilex rubra* S.Watson, *Oreopanax peltatus* Linden ex Regel, *Persea podadenia* S. F. Blake, *Prunus zinggii* Standl., *Piper villiramulum* C.DC., *Quercus tuberculata* Liebm., *Sideroxylon tepicense* (Standl.) T.D.Penn., *Urera* Gaudich., epiphytic *Tillandsia cretacea* L.B.Sm. and a large orchid, *Stanhopea maculosa* Knowles & Westc., at its northern-most distribution.

Proposed Conservation Status. Endangered: EN B1ab(iii) + 2ab (iii). It is possible that *Chiococca grandiflora* is more widespread and/or abundant than the small number of collections suggests, since areas between and around the known sites in Sinaloa and Sonora have not been well explored botanically. However, based on the best available evidence this species falls into the IUCN Red List Criteria (IUCN 2001) category of Endangered (EN): B1: total Extent of Occurrence (EOO) is less than 5,000 km^2 (ca. 495 km^2); B1a, is severely fragmented and known to exist at no more than five locations; B1biii, continuing decline inferred in area, extent and/or quality of habitat; B2: total area of occupancy (AOO) less than 500 km^2 (ca. 12 km^2), B2a, severely fragmented or known to exist at no more than five locations, and B2b iii, continuing decline inferred in area, extent and/or quality of habitat. The suitable habitat for *Chiococca grandiflora* is a declining or endangered environment threatened by human activity (deforestation, fire).

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RESEARCH ARTICLE

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New taxonomic and conservation status of *Ossiculum* (Vandeae, Orchidaceae), a highly threatened and narrow-endemic angraecoid orchid from Central Africa

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Abstract

In the context of producing a revised phylogenetic Linnean taxonomy of angraecoid orchids, the monotypic and narrow-endemic genus *Ossiculum* is synonymised with *Calyptrochilum*. Accordingly, a new combination in *Calyptrochilum* is proposed for *Ossiculum aurantiacum*. The morphological and DNA-based evidence for this transfer is discussed. Moreover, *Calyptrochilum aurantiacum* is here firstly reported outside Cameroon, with a record from the Republic of the Congo. The Red List conservation status of this species is reassessed and it is to be downgraded from "Critically Endangered" (CR) to "Endangered" (EN), following the recent discovery of additional subpopulations in Cameroon.

Résumé

En vue de produire une classification taxonomique linnéenne des orchidées angraecoïdes, le genre monotypique *Ossiculum*, longtemps considéré comme endémique du sud-ouest Cameroun, est placé en synonymie de *Calyptrochilum*. En conséquence, une nouvelle combinaison dans *Calyptrochilum* est proposée pour *Ossiculum aurantiacum*. Les données morphologiques et moléculaires qui justifient cette combinaison sont

discutées. L'espèce est signalée pour la première fois hors du Cameroun, en République du Congo. Suite à la découverte récente de sous-populations supplémentaires au Cameroun, le statut de conservation de cette espèce est réévalué selon la liste rouge de l'UICN. Bien que toujours menacée, l'espèce *Calyptrochilum aurantiacum* est transférée de la catégorie "En danger critique" (CR) vers la catégorie "En danger" (EN).

Keywords
Angraecoid orchids, *Calyptrochilum*, *ex situ* conservation, IUCN Red List Categories and Criteria, Mungo River Forest Reserve, Odzala National Park, *Ossiculum aurantiacum*

Introduction

While describing the monotypic genus *Ossiculum* P.J.Cribb & Laan, van der Laan and Cribb (1986) were the first to point out its morphological resemblance to *Calyptrochilum* Kraenzl, an angraecoid genus comprising two species widespread in tropical Africa. In fact, both genera possess a sigmoid clavate spur and their column bears a somewhat elongate bifid rostellum. Moreover, *Ossiculum aurantiacum* P.J.Cribb & Laan and *Calyptrochilum christyanum* (Rchb.f.) Summerh. have a yellow lip and a spur of similar shape. Later, Gasson and Cribb (1986), when describing the leaf anatomy of *O. aurantiacum*, found that *Ossiculum* and *Calyptrochilum* share an array of unique features which sets them apart from other angraecoid orchids: the same epidermal cell arrangements, spiral thickenings on some mesophyll cells, the presence of water storage cells, along with the absence of hypodermal and mesophyll sclereids and that of a palisade layer. By pooling the anatomical evidence with morphological and cytological data, these authors concluded that *Ossiculum* is most closely related to *Calyptrochilum*. The study of Arends and van der Laan (1986) on the cytology and morphology of angraecoid orchids recognised four groups based on rostellum length and basic chromosome number. One of these groups (the third one) comprised only *Calyptrochilum* and *Ossiculum* (rostellum elongated, x = 17 or 18). Twenty years later, the study of Carlsward et al. (2006) on comparative vegetative anatomy and systematics of 142 angraecoid orchid species also showed that *Ossiculum aurantiacum* and the two *Calyptrochilum* species have a very similar leaf anatomy. However, with its brightly yellow-orange flowers, its rostellum lobes resembling pincers and with a bipartite basal callus on the lip, *Ossiculum* is readily distinguished from the two species of *Calyptrochilum,* which bear predominantly white and green flowers, straight rostellum lobes and a three-lobed lip lacking a basal callus. In addition, the latter two species have a horizontal to pendent habit (vs an erect habit in *Ossiculum*) with the leaves more or less flat and arranged in a single plane (vs leaves V-shaped in cross-section).

Ossiculum aurantiacum was first discovered in the Mungo River Forest Reserve, Cameroon in 1980. At that time, the only specimens known were the type and a young seedling, both collected by the Dutch botanist H. J. Beentje. The young seedling flowered in cultivation in November 1983 at the University of Wageningen (The Netherlands) from which a voucher was collected (*van der Laan 718*). During the next

20 years, *O. aurantiacum* was not seen again despite the several thousand botanical collections that were made within 50 miles of the type locality (Pollard 2011). Based on that unique collection, the species was assessed as "Critically Endangered" (CR) on the IUCN Red List by Pollard and Darbyshire (2004).

In 2004, a new locality for this species was discovered in the Banyang-Mbo Wildlife Sanctuary, Cameroon. This wildlife sanctuary is widely held to be one of the most biologically important forest complexes in West Central Africa, harbouring 325 documented bird species, 71 amphibians, 63 reptiles and 33 large mammals (Riley and Riley 2005) and several narrow endemic plants (e.g. Cheek 2002; Sonké et al. 2002). Several large-scale anthropogenic disturbances (e.g. oil palm plantations in the west, timber exploitation in the north) have taken place around the sanctuary, menacing its biodiversity, notably the narrow range species which occur within the protected area and its surroundings (Asaha and Deakin 2016). The discovery of this new specimen in an additional location in Cameroon cast away the fears that *O. aurantiacum* might already be extinct and has fostered new specific surveys and a conservation programme dedicated to this species.

Thanks to intensive fieldwork in Cameroon in 2011 and 2017, we discovered new localities of *O. aurantiacum.* Newly collected specimens of the species were included into a phylogenetic study of African angraecoid orchids (Simo-Droissart et al. 2018a). In the meantime, examination of dried material deposited in BRLU (herbarium acronym according to Thiers 2018) led one of us (V. Droissart) to discover that the species has been collected in the Republic of the Congo in 1996, but had been misidentified as *Cyrtorchis* sp. This finding, along with new collections made at the type location in Cameroon in 2017, prompted us to reassess the IUCN conservation status of the species.

Based on these new data, this paper aims to (i) reappraise the taxonomy of *Ossiculum aurantiacum* and its generic status, (ii) update its distribution in light of the new collections in Cameroon and the first record of this species for the Republic of the Congo and (iii) define its ecology and threats and reassess its IUCN conservation status.

Material and methods

On-site ecology and conservation status of *Ossiculum aurantiacum* were investigated during three main field campaigns in the Southwest Region of Cameroon (2004, 2011 and 2017). To look for additional collections, herbarium specimens of BR, BRLU, K, WAG and YA (herbaria acronyms according to Thiers 2018) were examined. The geographical distribution of the species was determined from data given on the herbarium sheets. New country records were identified by comparing the species distribution with the information provided by Govaerts et al. (2017). The species distribution map was prepared using ArcMap 10.4.1 (ESRI 2016).

Phylogenetic relationships between African angraecoid orchids, including *Ossiculum* and *Calyptrochilum*, were inferred on the basis of DNA sequence data from three regions (ITS, *matK,* and the *trnL-trnF* intergenic spacer) and 555 accessions represent-

ing 316 species from 43 genera (see Simo-Droissart et al. 2018a). We performed the parsimony and the Bayesian analyses on the combined matrix using, respectively, the computer programme PAUP* v.4.0 a 146 (Swofford 2002) and MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012).

Using the IUCN Red List Categories and Criteria (IUCN 2012), we made a preliminary risk of extinction assessment for the species. We imported georeferenced specimen data into the *ConR* package (Dauby et al. 2017) to calculate the area of occupancy (AOO) and extent of occurrence (EOO). The cell size for AOO was set 2 × 2 km as recommended by IUCN (2017). We calculated the number of 'locations' (as defined by IUCN 2017) with regard to the kind of threats, such that a single 'location' may encompass more than one adjacent population.

Results

Taxonomy

Simo-Droissart et al. (2018a) included *Ossiculum aurantiacum* in their molecular study of African angraecoid orchids and showed that, in both parsimony and Bayesian analyses, *O. aurantiacum* was sister to the two species of *Calyptrochilum*. This relationship is supported with strong bootstrap (BS 100) and posterior probability (PP 1), while the sister relationship of the two *Calyptrochilum* species is supported moderately and only by the Bayesian analysis (PP 0.77). Given the morphological and cytological similarities between *Ossiculum* and *Calyptrochilum* (see above) and the molecular data, we consider the monotypic genus *Ossiculum* as a junior synonym of *Calyptrochilum* and propose the following amended generic diagnosis.

Calyptrochilum Kraenzl., 1895: 30

Ossiculum van der Laan & Cribb, 1986: 823. Type species: *Ossiculum aurantiacum* van der Laan & Cribb, 1986: 824, **syn. nov.**

Type species. *Calyptrochilum preussii* Kraenzl. (1895: 30) (= *Calyptrochilum emarginatum* (Afzel. ex Sw.) Schltr. (1918: 84)).

Basionym. *Limodorum emarginatum* Afzel. ex Sw.(1805: 86).

Description. Epiphytic or lithophytic herbs. Stem cylindrical, erect, spreading or pendent, covered by sheathing leaf bases. Leaves coriaceous, distichous, imbricate, unequally bilobed at tip, articulate to sheathing at the base. Inflorescence axillary, few- to many-flowered, shorter than leaves; bract distichous, cucullate. Flowers white, sometimes with a green or yellow mark on labellum or bright orange with a yellow labellum. Sepals ovate-elliptic, acuminate. Petals oblanceolate, acute. Labellum entire or

trilobed, spurred at base; spur geniculate in middle, clavate at tip. Column with two pollinia, pollinia attached to an ovate or horseshoe-shaped viscidium.

Distribution and ecology. *Calyptrochilum* is a genus of three species distributed throughout tropical Africa, from sea level to 1200 m and found as epiphyte in humid evergreen forests, humid woodland, savannahs and as lithophyte.

***Calyptrochilum aurantiacum* (P.J.Cribb & Laan) Stévart, M.Simo & Droissart comb. nov.**
urn:lsid:ipni.org:names:60476300-2

Basionym. *Ossiculum aurantiacum* van der Laan & Cribb, 1986: 824.

Type. Cameroon. Mungo River Forest Reserve, 13 km on road from Kumba to Loum, 04.682°N, 09.533°E, 16 Dec. 1980, *Beentje 1460A* (holotype: WAG! isotypes: K!, WAG!, YA!).

Additional specimens examined. CAMEROON. Mungo River Forest Reserve, 13 km on road from Kumba to Loum, 04.682°N, 09.533°E, 16 Dec. 1980, *van der Laan 718* (WAG). Southwest Region, Nguti village, path between the WCS station and the camp "552", outside of the Banyang-Mbo Wildlife Sanctuary, 05.337°N, 09.473°E, 15 November 2004, *Yaoundé shadehouse series 196* (BRLU, YA). *Ibid.*, 10 May 2005, *Yaoundé shadehouse series 265* (BRLU). *Ibid.*, 20 September 2006, *Yaoundé shadehouse series 428* (BRLU). *Ibid.*, inside of the Banyang-Mbo Wildlife Sanctuary, 05.344°N, 09.517°E, 9 May 2011, *Yaoundé shadehouse series 2773* (BRLU). *Ibid.*, 13 June 2011, *Yaoundé shadehouse series 2865* (YA). *Ibid.*, 1 September 2011, *Yaoundé shadehouse series 3075* (BRLU). *Ibid.*, 7 May 2012, *Yaoundé shadehouse series 3550* (K, MO, WAG). Southwest Region, Nguti village, between the WCS station and the camp "552", on a forestry trail at the border of the Banyang-Mbo Wildlife Sanctuary, 05.355°N, 09.491°E, 4 June 2017, *Droissart & Kamdem N. 2382* (BRLU, YA). Southwest Region, north of Ebonji village, on the trail to Mahole village, on cocoa tree, 04.773°N, 09.597°E, 8 June 2017, *Droissart & Kamdem N. 2407* (BRLU, YA). *Ibid.*, on kola tree, 8 June 2017, *Droissart & Kamdem N. 2409* (BRLU, YA). Southwest Region, northeast of Ebonji village, approximately 500 m from west bank Mungo River, on *Desbordesia glaucescens* (Engl.) Tiegh. (Irvingiaceae), 04.771°N, 09.578°E, 9 June 2017, *Droissart & Kamdem N. 2420* (BRLU, YA). REPUBLIC OF THE CONGO. Grand escarpement d'Odzala, bai de l'ombrette, forêt à *Gilbertiodendron dewevrei* (De Wild.) J.Leonard (Fabaceae), 01.067°N, 14.467°E, 1996, *Lejoly 96/1063* (BRLU).

Distribution. Cameroon and the Republic of the Congo (Figure 1). This species is reported here for the first time in the Republic of the Congo. Its presence in a forest with *Gilbertiodendron dewevrei* suggests that *Calyptrochilum aurantiacum* might be more widely distributed, as this type of monodominant forest extends from Nigeria to the Democratic Republic of the Congo and northern Angola.

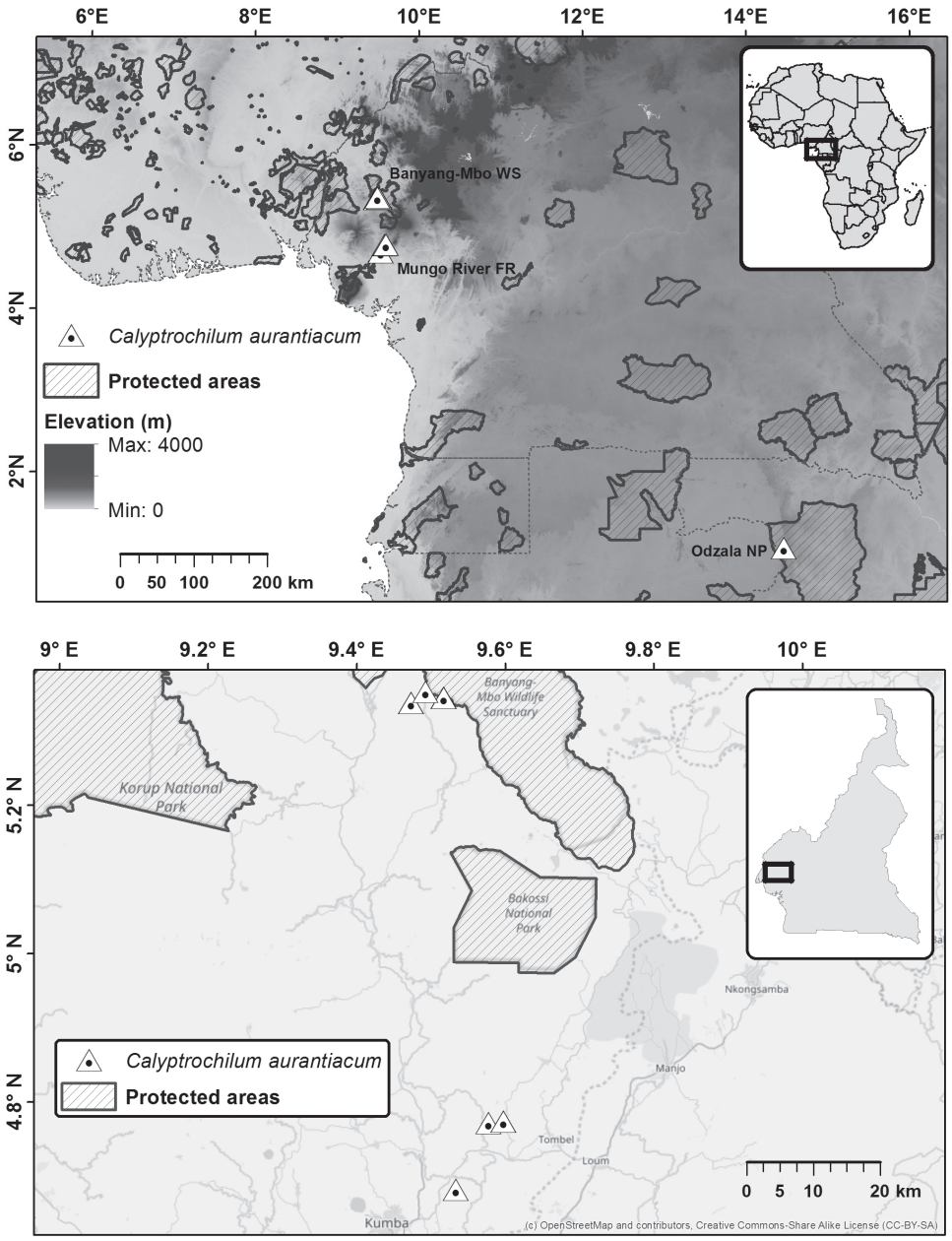

Figure 1. Current distribution of *Calyptrochilum aurantiacum* in Central Africa (upper map) and in Cameroon (lower map). WS = Wildlife Sanctuary; FR = Forest Reserve; NP = National Park.

Ecology and habitat. *Calyptrochilum aurantiacum* occurs between 240 to 600 m elevation in mature dense lowland forest (sometimes degraded), in plantations and in *Gilbertiodendron dewevrei* monodominant forest. It was initially collected growing as an epiphyte 35

Figure 2. Habitat degradation and forest clearance at the type locality of *Calyptrochilum aurantiacum* (Mungo River Forest Reserve). In less than 10 years, hundreds of hectares of mature lowland rainforest have been converted to cocoa plantations along the Mungo River (pers. comm. by local communities). **A** Freshly cleared forest where plantain and bananas will be cultivated during two years prior to being replaced by cocoa trees. **B** A ten-year old cocoa plantation on the banks of the Mungo River; only a few shade trees remain from the original forest. **C** Plantain and banana bunches that are harvested every day along the Mungo River. Photographs by V. Droissart (June 2017).

m above the ground on the lower branches of a primary forest tree (van der Laan and Cribb 1986). In 2017, we collected the species near the type locality on *Desbordesia glaucescens* (Irvingiaceae) and *Scottellia* sp. (Achariaceae) that had recently been felled to establish a new cocoa and banana plantation (Figure 2). In the same locality, the species was also collected on kola and cocoa trees in a 7-year old cocoa plantation. Within and around the Banyang-Mbo Wildlife Sanctuary, it was collected in the canopy of large trees (i.e. *Duguetia staudtii* (Engl. & Diels) Chatrou (Annonaceae) and *Klainedoxa gabonensis* (Pierre ex Engl.) (Irvingiaceae)).

Calyptrochilum aurantiacum is a heliophile epiphyte growing on branches of 5 to 20 cm in diameter under the canopy of large trees (from 50 to more than 100 cm diameter at breast high). In most observed trees (ten observations), *Calyptrochilum aurantiacum* was found growing together with the angraecoid orchid *Diaphananthe plehniana* (Schltr.) Schltr. and with the fern *Microgramma owariensis* (Desv.) Alston (Polypodiaceae) (Figure 3). On *Duguetia staudtii* and *Desbordesia glaucescens*, we observed more than 100 individuals per tree. The flowering and fruiting of *C. aurantiacum* occur during the large rainy season, from May to November.

Conservation

The extent of occurrence (EOO) of *Calyptrochilum aurantiacum* is estimated to be 19948.1 km^2, which falls within the limits for "Vulnerable" (VU) category under criterion B1, whereas its area of occupancy (AOO) is estimated to be 28 km^2, which falls within the limits for "Endangered" (EN) category under criterion B2. *Calyptrochilum aurantiacum* is an epiphyte of lowland evergreen rainforest. The species occurs within two officially protected areas: the Banyang-Mbo Wildlife Sanctuary in Cameroon and the Odzala National Park in Republic of the Congo. These two protected areas appear well managed. In other sites where *C. aurantiacum* occurs in Cameroon (within the Mungo River Forest Reserve and outside of the Banyang-Mbo Wildlife Sanctuary), its habitat is currently experiencing a great deal of human pressure (i.e. lowland forest is currently under threat from logging, farming practices, establishment of crop plantations, population growth and human migration, leading to urban expansion). From our recent observations made in June 2017 (Figure 2), the Mungo River Forest Reserve in Cameroon can no longer be considered as an effective protected area. Indeed, the recent construction of a road along the border of the Mungo River Forest Reserve provides greater accessibility, resulting in intensification of forest clearance inside and outside of the reserve's boundary. The main threats to the species are timber exploitation in the north of the Banyang-Mbo Wildlife Sanctuary, cocoa, plantain and banana plantations in the Mungo River Forest Reserve and shifting agriculture and oil palm plantation on the eastern part of the Banyang-Mbo Wildlife Sanctuary. These activities are gradually transforming the species habitat and we think that this degradation will continue in the future.

There are 20 herbarium specimens of *Calyptrochilum aurantiacum* collected in six localities that represent three subpopulations: two in Cameroon (the Mungo River Forest Reserve and the Banyang-Mbo Wildlife Sanctuary) and the third in the Republic of the Congo. These three subpopulations represent five locations (*sensu* IUCN 2017), which fall within the limits for the IUCN category "Endangered" (EN) under criterion B2. The projected ongoing loss of its habitat leads us to predict a continuous decline in mature individuals of the species. Thus, *C. aurantiacum* is assigned an IUCN conservation status of EN B2ab(iii,v).

Discussion

The integration of molecular evidence into current taxonomical practice and the adoption of a strictly phylogenetic Linnean framework in orchid classification have been altering many generic boundaries (Chase et al. 2015). For instance, two other African angraecoid genera, *Podangis* Schltr. and *Sphyrarhynchus* Mansf. considered monotypic, were recently recircumscribed and now comprise, respectively, two (Cribb and Carlsward 2012) and three species (Martos et al. 2017). Our recent molecular phylogeny on African angraecoid orchids (Simo-Droissart et al. 2018a) substantially improved the understanding of taxonomic relationships among the angraecoid orchids, leading us to describe a new genus to accomodate continental African species of *An-*

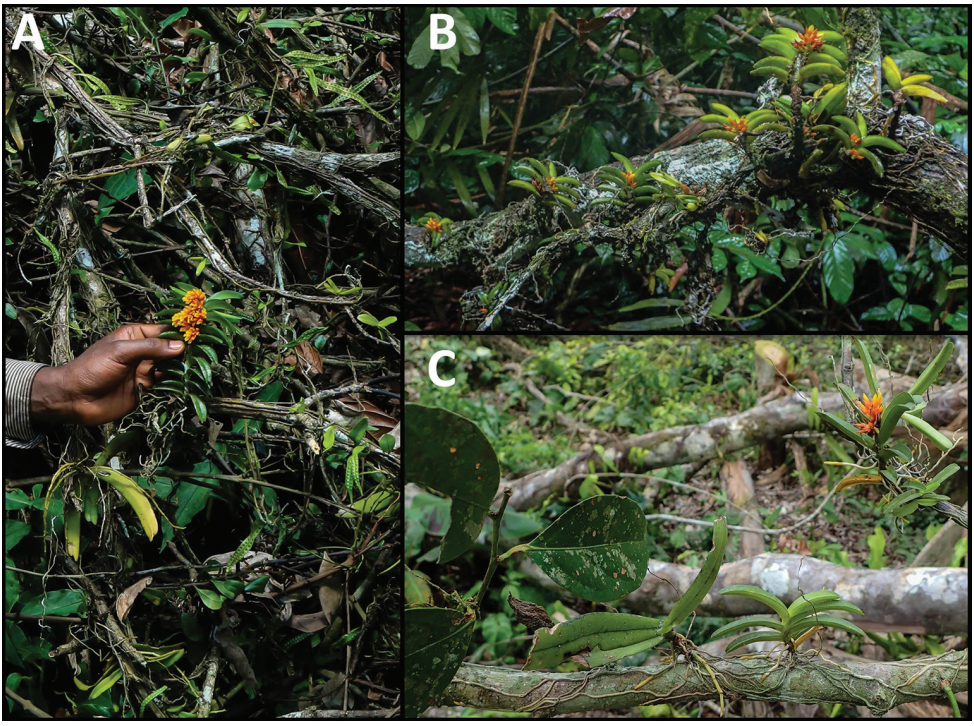

Figure 3. Ecology and habit of natural populations of *Calyptrochilum aurantiacum*. **A** Flowering individual growing on upper branches of a felled kola tree, along with the angraecoid orchid *Diaphananthe plehniana* and the fern *Microgramma owariensis.* **B** Dense population growing on 1 metre long branches of *Duguetia staudtii.* **C** *Calyptrochilum aurantiacum* and *Diaphananthe plehniana* growing side by side on *Salacia* sp. (Celastraceae). Photographs by V. Droissart (June 2017).

graecum Bory sect. *Pectinaria* (Benth.) Schltr. (Simo-Droissart et al. 2018b) and to transfer *Ossiculum aurantiacum* to *Calyptrochilum*.

Our recent observations on the rapid degradation of the natural habitat of *Calyptrochilum aurantiacum* within the Mungo River Forest Reserve and around the Banyang-Mbo Wildlife Sanctuary both in Cameroon, stress the need for urgent actions to protect the last remaining patch of intact lowland forests from human activities. The survival of several other plant species endemic to this area, such as *Afrothismia winkleri* (Engl.) Schltr. (Burmanniaceae, IUCN status = CR, IUCN SSC East African Plants Red List Authority 2013), *Cola metallica* Cheek (Malvaceae, IUCN status = CR, Cheek 2003), *Tricalysia lejolyana* Sonké & Cheek (Rubiaceae, IUCN status = EN, Sonké et al. 2004), also relies on the conservation of these forests occurring below 600 m elevation.

Due to the ongoing destruction of its natural habitat, *Calyptrochilum aurantiacum* has been the focus of an *ex situ* conservation project. The species has been cultivated since 2004 in our *ex situ* collection in Yaoundé, Cameroon. Living specimens, collected from fallen branches and trees in the field, have been successfully grown and they have flowered for several consecutive years. Presently, about 150 living individuals are being cultivated in the shadehouse. Since November 2015, we

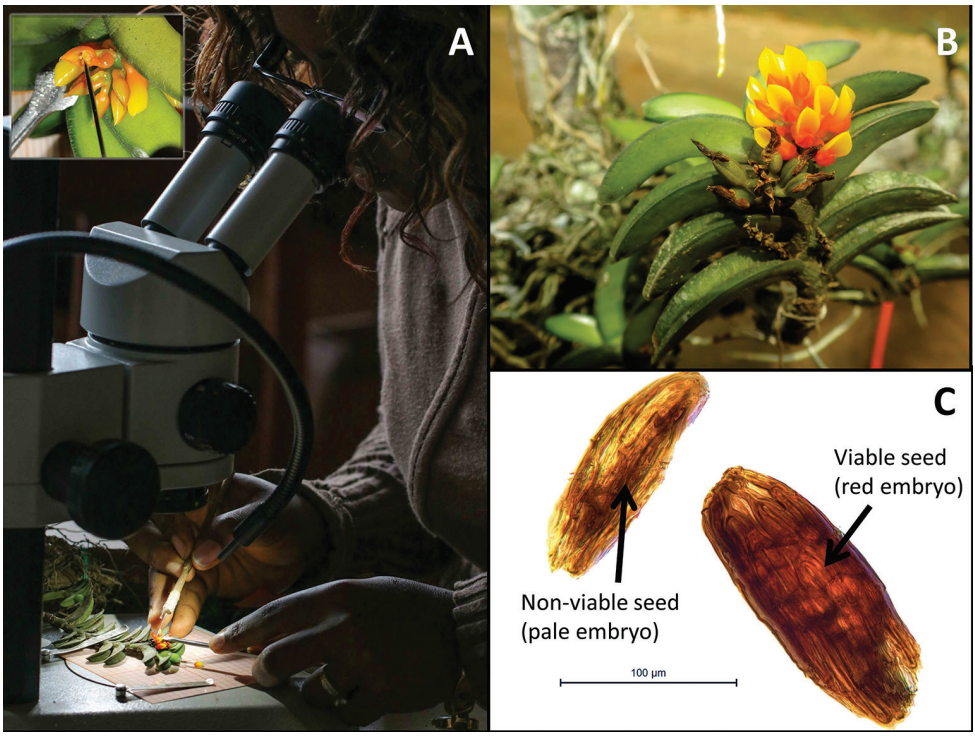

Figure 4. Seed banking of *Calyptrochilum aurantiacum* in Yaoundé (Cameroon). In 2017, an *ex situ* conservation programme was initiated to support the long term preservation of *C. aurantiacum*. **A** Due to the small size of the flowers, manual pollination has been performed under a stereomicroscope. **B** Fruit development and maturation takes place in a shadehouse established in Yaoundé. **C** Finally, viable seeds have been harvested and conserved in the freezer (-20°C). Before being preserved at low temperature, seed viability is assessed using the tetrazolium test (red colouration of living embryo). Photographs **A** and **C** by V. Droissart, B by Gyslène Kamdem.

have performed eight hand pollination experiments on *C. aurantiacum* in the shadehouse and the species has been introduced in our seed bank in Yaoundé (Figure 4). However, due to the small size of its flowers, manual pollination of *C. aurantiacum* is still challenging as the fruit set (resulting from hand self-pollination) remains low (~25%). On-site studies on reproductive biology of the species are thus needed to identify pollination mechanisms involved and to check if the pollinator(s) is/are also threatened by forest clearance.

The patchy distribution of *Calyptrochilum aurantiacum* seems surprising and suggests that additional surveys should be made to search for new populations. The discovery of the satellite population in the Republic of the Congo also raised the question whether the Cameroonian and Congolese populations of *C. aurantiacum*, separated by more than 650 km, have different species of pollinators. Seemingly, the intriguing pollinator may be absent in Yaoundé since we have never observed "natural" pollination on the living plants in cultivation.

Notwithstanding the success of our *ex situ* conservation programme, strategies to ensure the long-term survival of *C. aurantiacum* must focus primarily on *in situ* conservation efforts. During the 2017 survey, we found one individual growing on a cocoa tree within a plantation at Ebonji, Cameroon. Since clearance of forest for conversion to agricultural plantations will continue to increase, we performed in September 2017 the transplantation of 45 individuals on three cocoa trees (15 on each) in Ebonji village, in order to see if the species could maintain viable populations in those plantations. If the transplantation tests prove successful, the next step will be to ensure that the pollinator will also survive in this human-transformed habitat.

Conclusion

Morphological and DNA-based evidence led to the transfer of the narrow-endemic *Ossiculum aurantiacum* to the widespread genus *Calyptrochilum* and, thus, recognises the monotypic genus *Ossiculum* as a junior synonym of *Calyptrochilum*. Thanks to recent intensive field trips and laboratory work on central African orchids, new localities of *Calyptrochilum aurantiacum* have been discovered, a situation that contributed to downgrade the IUCN conservation status of the species. *Calyptrochilum aurantiacum* is currently known from five locations in two countries (Cameroon and Republic of the Congo) and is assessed as Endangered [EN B2ab(iii,v)] according to the IUCN Red List Categories and Criteria. Fieldwork within and around the Odzala National Park is required to evaluate the species habitat and the state of the subpopulation there. *In situ* studies on reproductive biology would be necessary for greater efficiency in conservation measures.

Acknowledgments

We express our gratitude to the Mohamed bin Zayed Species Conservation Fund (Grant n°16255698, V. Droissart as PI) and the National Geographic Society (Grant C303-15, V. Droissart as PI) who supported fieldwork and monitoring of the orchid living collection in Cameroon. The U.S. National Science Foundation (Grant 1051547, T. Stévart as PI, G. M. Plunkett as Co-PI) also supported fieldwork and herbarium visits. We are grateful to the curators of the following herbaria BR, BRLU, K, WAG and YA for making their collections available and for facilities kindly provided. The American Orchid Society provided financial support to the work of the first and the last author in Cameroon. We are grateful to the authorities of Higher Teachers' Training College, University of Yaoundé I, to host the Yaoundé shadehouse and for allowing authors to access their facilities. We would like to thank Marie-Noël Djuikouo, Gyslène Kamdem, Charlemagne Nguembou and Lise Zemagho for collecting vouchers and taking photographs of the species in the Yaoundé shadehouse in Cameroon. Finally, we thank João N. M. Farminhão for commenting and improving the English of the manuscript and the two reviewers for their constructive comments on an earlier version of the manuscript.

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RESEARCH ARTICLE

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Vatica najibiana (Dipterocarpaceae), a new species from limestone in Peninsular Malaysia

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Abstract

Vatica najibiana Ummul-Nazrah (Dipterocarpaceae), from the Relai Forest Reserve, Gua Musang, Kelantan and Gua Tanggang, Merapoh, Pahang, is described and illustrated. This species is Endangered and known from small populations restricted to two isolated karst limestone hills. The type locality, Relai Forest Reserve limestone, is currently under threat from encroaching oil palm plantations and ongoing logging, which, if it continues, will threaten the Kelantan population with extinction. The morphology of *V. najibiana* and the similar *V. odorata* subsp. *odorata* and *V. harmandiana* is compared.

Keywords

Dipterocarpaceae, *Vatica*, Kelantan, Pahang, limestone hills, oil palm, logging

Introduction

In Peninsular Malaysia, *Vatica* L., known in Malay as *resak*, includes 32 species (Saw 2002; Ashton and Appanah 2004; El-Taguri and Latiff 2010, 2012; Tan et al. 2014; Chua et al. 2015). It is a genus of understorey and main canopy trees from lowland forest to hill dipterocarp forest but it also occurs in coastal peat swamp and other swampy areas with only *V. harmandiana* Pierre and *V. kanthanensis* Saw restricted to limestone habitats.

In Ashton and Appanah (2004) and Chua et al. (2015), *V. harmandiana* was called *V. cinerea* King that Pooma et al. (2017, p. 670) argued was a synonym of *V. harmandiana*. The genus *Vatica* is distinctly different from other Malayan genera of Dipterocarpaceae. Most *Vatica* species are small or medium-sized trees, unbuttressed with smooth bark and leaves with reticulate tertiary venation. There are two sections in *Vatica*: sect. *Vatica* with equal fruit calyx lobes and sect. *Sunaptea* with unequal fruit calyx lobes. This new *Vatica* species belongs to sect. *Sunaptea*, which now includes ten species.

This new *Vatica* species was discovered on the summit of a karst limestone hill within the Relai Forest Reserve, Gua Musang District, Kelantan, during the biodiversity survey of the flora of five sizeable limestone hills scattered within the FELDA Chiku oil palm plantation (Kiew et al. in prep.). The impetus for the survey was the issuing of a licence to quarry the largest hills named FELDA Chiku 7 and FELDA Chiku 8 to supply limestone to a new cement factory reputed to be the largest in SE Asia (Utusan Online 2015). The aim of the survey was to document the flora of the two hills scheduled for quarrying, which were previously hardly known botanically and to test the assertion by the cement company that protecting another nearby hill, FELDA Chiku 4, would compensate for biodiversity lost from the two larger hills. None of the limestone hills within the FELDA Chiku plantation is legally protected and all are currently under threat from disturbance associated with the oil palm plantation (clearing and burning the limestone forest around the base of the hills, grazing by free ranging cattle, hunting of the protected serow, *Capricornis sumatraensis*, collecting orchids etc.). Due to these threats, one species, *Impatiens chikuensis,* that is a strict endemic known only from FELDA Chiku 5 and 8, faces extinction (Kiew 2016).

The limestone hill flora in Peninsular Malaysia is being exploited and disturbed by quarrying, clearing the surrounding forest for agricultural plantations or burning limestone vegetation during land clearing, disturbance associated with caves, the establishment of temples and resorts, as well as from recreational and tourism activities (Kiew 1997). Kiew et al. 2017 demonstrated that no single hill has more than a fraction of limestone flora and that 192 species are endangered being known from less than five localities limestone hills, for example *Monophyllaea musangensis* A. Weber (Weber 1998), *Gymnostachyum kanthanense* Kiew, *Meiogyne kanthanensis* Ummul-Nazrah & J.P.C.Tan and *Vatica kanthanensis* Saw (Tan et al. 2014), *Impatiens glaricola* Kiew and *Impatiens vinosa* Kiew (Kiew 2016). Therefore, limestone hills are one of the most threatened vegetation types in Peninsular Malaysia and are recognised nationally as Environmentally Sensitive Areas because of their high biodiversity and vulnerability (73 of the 445 hills are the sites of active or former quarries, Liew et al. 2016). In addition, many karsts are still incompletely known botanically meaning that new species await discovery. For example, during the botanical survey of the Chiku limestone (Kiew et al. in prep.), several rare and endangered species were discovered, including this new species.

In determining the identity of the specimens, we discovered that the species had in fact already been collected from Gua Tanggang (a.k.a. Tagang) in Merapoh, Pahang, a limestone hill about 40 km south of the Relai Forest Reserve limestone but that it had been incorrectly identified as *Vatica cinerea* King (now *V. harmandiana*), a species restricted to NW Malaysia (Chua et al. 2010).

Materials and methods

The new *Vatica* species was discovered on a limestone hill (5.024478 N, 102.114360 E, Ktn 50, numbering follows Price 2014) in the Relai Forest Reserve (Ktn 50), Gua Musang District, Kelantan. Herbarium specimens were collected and a photographic record was made. The population was in mature fruit but a few old dried flowers were obtained. The extensive collection of Dipterocarpaceae in the Kepong Herbarium (KEP) was used for comparison and for measurements of similar species and the other specimen of this new taxon (FRI 44774), previously collected from Gua Tanggang (a.k.a. Tagang), Merapoh, Pahang was examined in detail. The description of the new species was compared with similar species in standard texts (Saw 2002; Ashton and Appanah 2004; El-Taguri and Latiff 2010, 2012; Tan et al. 2014; Chua et al. 2015). The description is based on field observations and comparison by using KEP herbarium specimens. The provisional conservation assessment is based on the IUCN Red List Categories and Criteria Version 3.1 (IUCN 2012).

Taxonomy

Vatica najibiana **Ummul-Nazrah, sp. nov.**
urn:lsid:ipni.org:names:77178683-1
Figures 1, 2

Diagnosis. Amongst the Vaticas with a half inferior ovary, it groups with *Vatica harmandiana* and *V. odorata* (Griff.) Symington subsp. *odorata*. *Vatica harmandiana* occurs on limestone hills and rocks but is different in having leaves that are elliptic-lanceolate, leaf base cuneate and nut diameter 7–10 mm as oppose to the obovate-elliptic leaf, leaf base cordate-subcordate and nut diameter of 5–6 mm in *V. najibiana*. *Vatica odorata* subsp. *odorata* is closely similar to the new species but can be separated by its elliptic-oblong leaf, leaf base obtuse, leaf apex acuminate, nut diameter 8–9 mm and occurrence in lowland and hill forest (Table 1).

Type. Peninsular Malaysia. Kelantan, Gua Musang District, Relai Forest Reserve (Ktn 50), 05°02'47.8"N, 102°11'43.6"E, 19 October 2016, Ummul-Nazrah et al. FRI 86369 (holotype KEP!; isotypes K!, SAN!, SING!).

Description. Small tree, 5–7 m tall; bole to 15–17 cm diameter, without buttresses. **Bark** smooth with faint horizontal rings, dark brown with white lichen patches; inner bark pale yellow, exuding clear sap when cut. **Twigs** robust, 3–5 mm diameter, covered with 6–15-armed stellate hairs, 94–169 μm diameter, glabrous when mature, older twigs terete. **Leaves** when young brown rusty beneath, glabrous when mature; petioles 0.8–1.5 cm long, 0.1–0.2 cm wide, densely covered with stellate hairs, caducous when mature, drying dark brown; lamina obovate to elliptic, (3–)5–10.2 × 1.5–5 cm, thickly chartaceous, bullate, green on both surfaces when fresh, base cordate to subcordate, margin entire and recurved, apex acute; midrib prominent on both surfaces; lateral veins (6–)7–10 pairs, prominent below, slightly raised and visible above, ascending to margin; intercostal veins reticulate-scalariform and slightly

Figure 1. *Vatica najibiana*. **A** Plant in its natural habitat **B** Bole **C** Inner bark **D–E** Leafy shoots with infructescences **F** Fruit. (Photographs by K. Imin & A.R. Ummul-Nazrah).

Table 1. Differences between *Vatica najibiana*, *V. odorata* subsp. *odorata* and *V. harmandiana*.

Character	*V. najibiana*	*V. odorata* subsp. *odorata*	*V. harmandiana*
Habit	Small tree to 5–7 m	Tall tree to 24 m	Tree, 15–24 m
Leaves			
Petiole indumentum	Dark brown	Mid brown (reddish-brown)	Pale brown
Lamina shape	Obovate to elliptic	Elliptic to oblong	Elliptic to lanceolate
Lamina size (cm)	(3–)5–10.2 × 1.5–5	8–16 × 2.7–6	5.2–12 × (1.8–)2–5
Lamina base	Cordate to subcordate	Obtuse	Cuneate
Lamina margin	Recurved	Not recurved	Not recurved
Lamina apex	Acute	Acuminate	Blunt to acute
No. of lateral veins (pairs)	(6–)7–10	9–15	7–8
Fruits			
Calyx lobes length (cm)	2.3–3.3 × 0.5–0.8	4–5.5 × 1–1.5	2.6–7 × 1–1.8
Nut Diameter (mm)	5–6	8–9	7–10
Habitat	Limestone only	Lowland and hill forest	Limestone only

conspicuous on both surfaces. **Flowers:** (dry) pedicels with velvety brown stellate hairs; calyx 5-lobed, elliptic, 4–7 × ca. 1 mm, densely covered with stellate hairs on both surfaces, apex acute; petals narrowly elliptic, ca. 6 × 2 mm, glabrous outside,

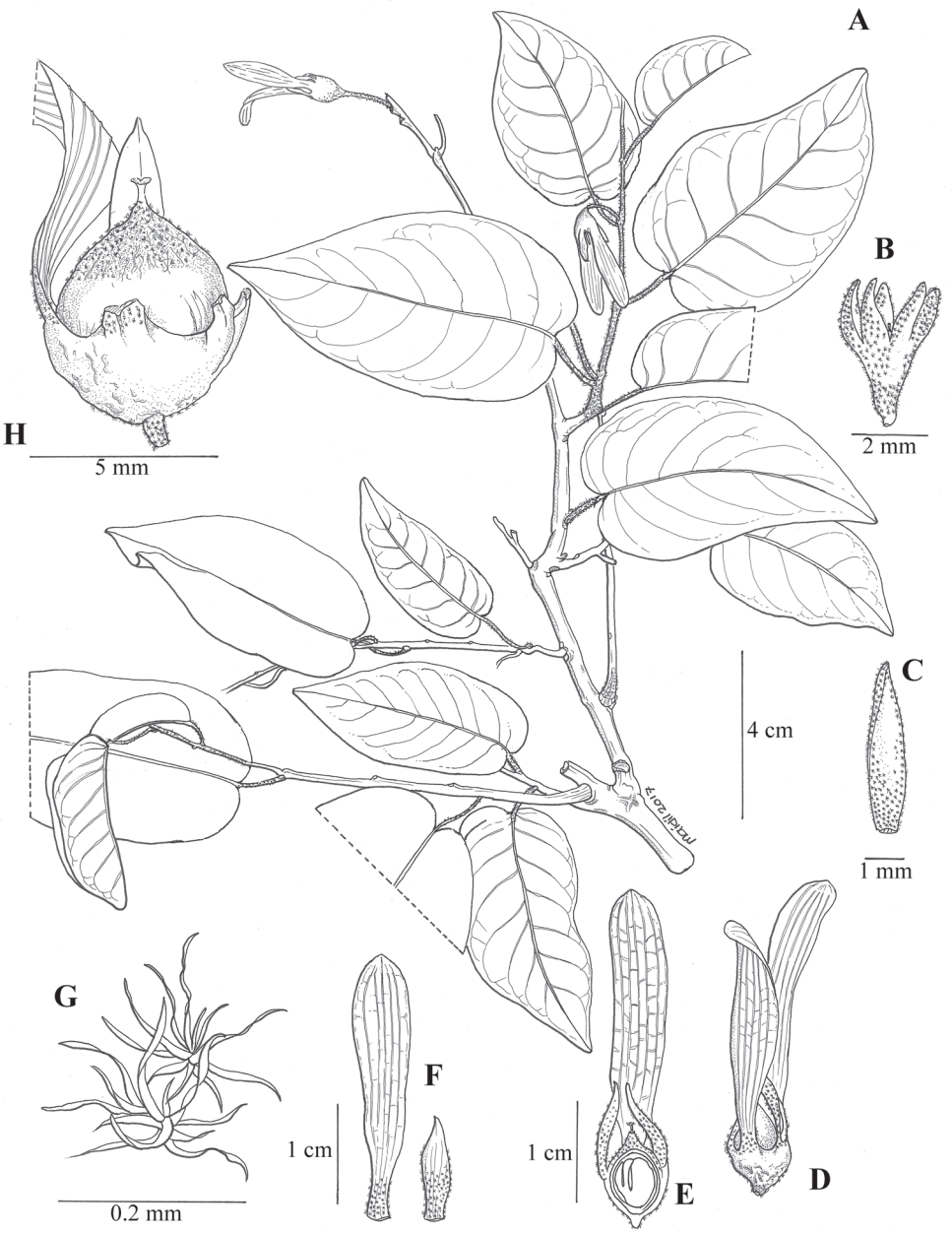

Figure 2. *Vatica najibiana*. **A** Leafy shoot with fruits **B** Calyx **C** Petal **D–E** Fruit **F** Long & short calyx lobes of fruit **G** Stellate hair **H** Fruit nut. (Drawn by N. Mohamad-Aidil from Ummul-Nazrah et al. FRI 86369).

inside from base to tip completely covered with 6–10-armed stellate hairs, 77–120 μm diameter. **Infructescence** axillary, near apex of leafy shoot, ca. 4 cm long, densely covered with rusty stellate hairs, branching once or twice, densely covered with stellate hairs; first branches with 1–7 fruits along axis, nodes 4–5 mm apart. **Fruits:** stalks 1–2

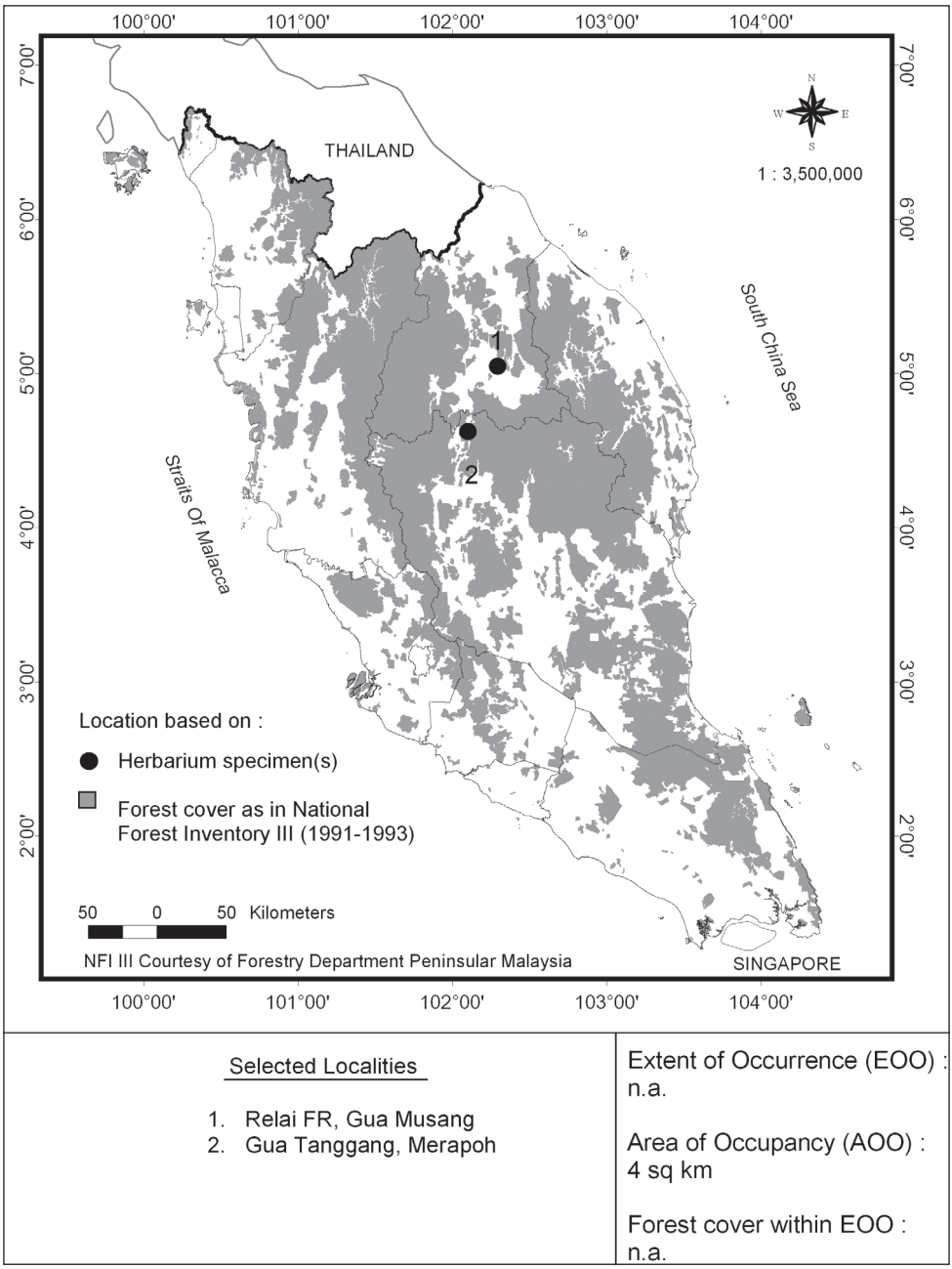

Figure 3. Distribution of *Vatica najibiana* in Peninsular Malaysia.

mm long, ca. 1 mm thick at base, covered with caducous stellate hairs; in life mature calyx red-brown, chartaceous, lobes 5, 2–3 larger than rest, attached to half inferior ovary, above forming a cup, glabrous outside, inner part at base completely covered

with stellate hairs, lobes elliptic, apex rounded with 5 longitudinal prominent veins on the adaxial surface, 2.3–3.3 × 0.5–0.8 cm; shorter lobes 0.8–1 × ca. 0.2 cm; nut ovoid, 5–6 mm diameter, with persistent stigma, densely covered with stellate hairs, half hidden within calyx.

Distribution. Endemic in Peninsular Malaysia, known only from Kelantan (Relai Forest Reserve, Gua Musang) and Pahang (Gua Tanggang, Merapoh).

Etymology. This species is named in honour of the Prime Minister of Malaysia, Dato' Sri Mohd Najib bin Tun Abdul Razak, for his strong interest in nature conservation and protection of the environment.

Provisional conservation status. Endangered B2ab(iii). This species is known from the summit of two isolated karst limestone hills in Relai Forest Reserve, Gua Musang District, Kelantan and Gua Tanggang, Merapoh, Pahang, about 40 km apart (Liew et al. 2016). Together they have an area of occupancy of less than 10 km^2 (Figure 3). The Relai Forest Reserve is classified as a permanent forest reserve but is currently threatened by encroachment by oil palm plantations that pose a high risk of burning to the limestone vegetation, as well as disturbance from ongoing logging in the Sungai Relai Forest Reserve. Gua Tanggang in Merapoh, on the other hand, is situated outside of Taman Negara which means that it is not in a protected area.

Habitat. It is an emergent tree on the rugged summit of karst limestone at 178–520 m altitude growing in rock fissures with a thick layer of leaf litter.

Phenology. Fruiting specimens were collected in Relai Forest Reserve in October and Gua Tanggang in early August; complete flowers not seen but in October, many calyces and a few petals were collected.

Additional specimen examined. Peninsular Malaysia. Pahang, Lipis District, Merapoh, Gua Tanggang, 4.410000N, 102.055000E, 520 m alt., 6 August 1996, Saw et al. FRI 44774 (KEP!)

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RESEARCH ARTICLE

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Coccomyxa antarctica sp. nov. from the Antarctic lichen *Usnea aurantiacoatra*

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Abstract

The single celled green alga *Coccomyxa antarctica* Shunan Cao & Qiming Zhou, **sp. nov.** was isolated from the Antarctic torrential lichen *Usnea aurantiacoatra* (Jacq.) Bory. It is described and illustrated based on a comprehensive study of its morphology, ultrastructure, ecology and phylogeny. *C. antarctica* is a lichenicolous alga which has elongated cells and contains a parietal chloroplast as observed under the microscope. *C. antarctica* is clearly different from other species by phylogenetic analysis (ITS rDNA and SSU rDNA sequences), also it differs from its phylogenetic closely species *C. viridis* by its larger cell size.

Keywords

Lichen epiphyte, morphology, TEM, phylogeny

Introduction

Lichens, the typical symbiosis, generally consist of one fungal partner and its photosynthetic partner alga (usually a green alga or a cyanobacterium). With the development of research techniques, many other eukaryotic (Wilkinson et al. 2015, Spribille et al. 2016) and prokaryotic microbes (Aschenbrenner et al. 2016) have been observed in concurrence with lichen thalli besides the mycobiont and photobiont partners, such as lichenicolous fungi (Edwards et al. 2017, Asplund et al. 2017) and algae (Gustavs et al. 2017).

The green algae of the genus *Coccomyxa* (Trebouxiophyceae, Chlorophyta) are distributed worldwide and can be found in both aquatic and terrestrial habitats, in free living and symbiotic status (Malavasi et al. 2016). The species of *Coccomyxa* can be lichenicolous algae or lichenised photosynthetic partners in lichens (Malavasi et al. 2016). Historically, the taxonomy of this genus has been problematic. Originally a total of 14 free living species, 13 lichenised species and six lichen epiphytic species were summarised by Jagg (1933) based on morphology. Recently, a total of seven species has been distinguished, since the morphological characters of the unicellular green algae *Coccomyxa* vary in different environments and a DNA-based identification approach was proposed by Darienko et al. (2015). Subsequently, an improved method based on phylogenetic and ecological features was used for delimiting the species of this genus and 27 species scenario were recognised (Malavasi et al. 2016). The combination of ecological and DNA sequences data seems to be effective in distinguishing the *Coccomyxa* species.

In this current study, an epiphytic green alga was isolated from the Antarctic lichen *Usnea aurantiacoatra* (Jacq.) Bory. It will be demonstrated that this green alga is new to science based on the comprehensive analysis approach including morphology, ultrastructure, ecology and phylogeny.

Methods

Isolation and culture

During the 30th Chinese National Antarctic Research Expedition (from 1st Feb. 2014 to 15th March 2014), a specimen of Antarctic lichen *U. aurantiacoatra* was collected from Fildes Peninsula, King George Island, (62°12.70'S, 58°55.70'W). The specimen was incubated at 4 °C till the isolation was processed.

An *Usnea aurantiacoatra* specimen (d-B1), kept in the Resource-sharing Platform of Polar Samples which includes samples of Biology, Ice-snow, Rock, Deep-space and Sediment (BIRDS ID 2131C0001ASBM100063), was used to isolate the green alga. One green alga (Ua6) (Freshwater Algae Culture Collection at the Institute of Hydrobiology, FACHB-2140) was isolated by an improved tissue culture procedure: 1. Washing lichen tissues (2–3 pieces, about 5 mm of each) three times in sterile water; 2. Grinding each piece of tissue in a 1.5 ml centrifuge tube by a mini glass pestle; 3. Sifting the fragments through three different screen meshes (hole sizes: 0.35 mm, 0.10 mm and 0.03 mm); 4. Washing the fragments in the mesh whose hole size was 0.03 mm for 5 min with sterile water, repeating three times; 5. Selecting the fragments on the 0.03 mm-mesh (the size of these fragments is between 0.03 mm and 0.10 mm) and then culturing them on PDA and BBM petri-dish medium. All the operations were undertaken under aseptic conditions. The isolations were incubated in an illumination incubator (4 °C, 12 hr light/12 hr dark, natural lighting). The algal cultures were maintained in both PDA and BBM petri-dish medium at 4 °C.

Microscope and transmission electron microscopy (TEM) analysis

Compound microscopes (Nikon Eclipse 80i and Nikon ACT-1 V2.70) were used for morphology observation and photographing the algal cultures.

For transmission electron microscopy (TEM) observation, algal cells were fixed with 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) for 2 h, washed using the same buffer for 15 min and repeated three times, then post-fixed using 1% OsO_4 fixing solution for 3 h and washed using the same phosphate buffer for 15 min, three times. Samples were dehydrated in a graded ethanol series and replaced by propylene oxide. All the procedures above were operated at 4 °C. The samples were embedded using Spurr resin kit (Spi-Chem, USA). The resin was polymerised at 37 °C overnight, 45 °C for 12 h and 60 °C for 48 h. Thin sections (70 nm) were cut with a Leica EM UC6 (Germany) and stained with 3% uranyl acetate and lead citrate. The collections were observed using a JEM1230 (JEOL, Japan) electron microscope at 80–120kV. Micrographs were acquired by an Olympus SIS VELETA CCD camera equipped with iTEM software.

Molecular analysis

Genomic DNA of the green alga was extracted by a modified CTAB method (Cao et al. 2015a). The SSU rDNA was amplified using eukaryote universal primer pairs NS1, NS4; NS3, NS6; NS5, NS8 (White et al. 1990). The ITS rDNA was amplified by the primer pair ITS5, O2 (Cao et al. 2015a). A total volume of 50 μl PCR reaction was selected, the PCR application conditions and products verification following Cao et al. 2015a. Double-stranded PCR products were sequenced with an ABI 3730XL sequencer.

Double-directional sequences data of ITS nrDNA and SSU nrDNA were checked and assembled using the SEQMAN programme within the Lasergene v7.1 software package (DNASTAR Inc.), respectively. The regions of rDNA flanking the ITS region were trimmed off. Preliminary alignment of the sequences obtained in the present study and those retrieved from GenBank (Table 1) was performed using the ClustalW algorithm included in MEGA 5 and then adjusted manually (Tamura et al. 2011). The phylogenetic structure of each alignment was constructed using a Neighbour Joining (NJ) method. The reliability of the inferred trees was tested using bootstrap searches of 1000 resamplings. Altogether, 35 ITS nrDNA and 37 SSU nrDNA sequences, used in the phylogenetic analysis, were retrieved from GenBank (Table 1). The sequence representing the new species was sequenced by the authors and submitted to GenBank (MF465900).

Results

We examined the algal strain (Ua6) isolated from Antarctic lichen *Usnea aurantiacoatra* using both morphological identification and molecular markers. The isolated

Table 1. Sequences used in the present study.

Species	Collection No.	GenBank No.	
		ITS rDNA	SSU rDNA
Clade B**Coccomyxa* sp.	GA5a	AB917140	AB917140
Clade C* *Chlorella saccharophila*	CCAP 211/60		FR865679
Clade D* *Coccomyxa* sp.	CCAP 216/24	FN298927	FN298927
	CCAP 812/2A	HG972992	HG972992
Clade E* *Coccomyxa* sp.	IB-GF-12		KM020052
Clade E* *Coccomyxa subellipsoidea*	CCAP 812/3	HG972972	HG972972
Clade H* *Coccomyxa* sp.	KN-2011-U5	HE586557	
Clade I* *Coccomyxa* sp.	KN-2011-T3	HE586515	HE586515
	KN-2011-T1	HE586550	
Clade J* *Pseudococcomyxa simplex*	CAUP H 103		HE586505
Clade K* *Coccomyxa* sp.	KN-2011-C4	HE586508	HE586508
Clade L* *Monodus* sp.	UTEX B SNO83		HE586506
Clade M* *Monodus* sp.	CR2-4	HE586519	HE586519
Clade N* *Coccomyxa viridis* 3	CAUP H5103	HG973007	HG973007
	SAG 2040	HG973004	HG973004
Coccomyxa actinabiotis	216-25	FR850476	FR850476
Coccomyxa actinabiotis	KN-2011-T4	HE586516	HE586516
Coccomyxa antarctica	**Ua6 (FACHB-2140)**	**MF465900**	**MF465900**
Coccomyxa avernensis	SAG 216-1		HG972999
Coccomyxa avernensis	Wien C19	HG973000	HG973000
Coccomyxa dispar	SAG 49.84	HG972998	HG972998
Coccomyxa elongata	CAUP H5107	HG972981	HG972981
	SAG 216-3b	HG972980	HG972980
Coccomyxa galuniae	CCAP 211/97	FN298928	FN298928
	SAG 2253	HG972996	HG972996
Coccomyxa melkonianii	SCCA048	KU696488	KU696488
Coccomyxa onubensis	ACCV1	HE617183	HE617183
Coccomyxa polymorpha	CAUP H5101	HG972979	HG972979
	KN-2011-T2	HE586514	HE586514
Coccomyxa simplex	CAUP H 102	HE586504	HE586504
	SAG 216-2	HG972989	HG972989
Coccomyxa solorinae	SAG 216-12	HG972987	HG972987
	SAG 216-6	HG972988	HG972988
Coccomyxa subellipsoidea	SAG 216-7	HG972976	HG972976
	Wien C20	HG972975	HG972975
	CAUP H5105	HG972974	
Coccomyxa vinatzeri	ASIB V16	HG972994	HG972994
Coccomyxa viridis	SAG 216-14	HG973002	HG973002
	SAG 216-4	HG973001	HG973001
Elliptochloris bilobata	SAG 245.80	HG972969	HG972969
Hemichloris antarctica	SAG 62.90	HG972970	HG972970

Note: * Clades referred to Malavasi et al. (2016); The information about the new species *Coccomyxa antarctica* is marked in bold.

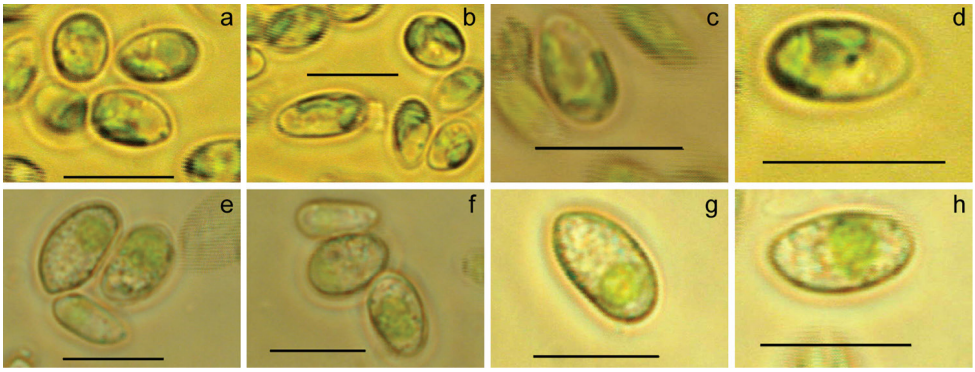

Figure 1. Morphology of *Coccomyxa antarctica* Shunan Cao & Qiming Zhou, sp. nov. **a–d** cultured in BBM medium; e-f cultured in PDA medium. Scale bars: 10 μm.

alga Ua6 was observed with elongated cells (4–7 μm wide and 8–12 μm long), whose cell wall was thin and smooth, each cell contained a parietal chloroplast (Figure 1); no pyrenoid was observed within their chloroplast using transmission electron microscopy (Figure 2). The alga strain Ua6 appeared to have a shorter growth cycle when cultured in PDA medium than that in BBM medium, but no significant morphological differences were observed from the cells cultured in PDA and BBM mediums (Figure 1).

The phylogenetic analysis of both ITS rDNA and SSU rDNA supported that the isolated green alga Ua6 was an undescribed *Coccomyxa* species. For the ITS rDNA, the sequences of *Coccomyxa* clustered as six subgroups. The newly isolated green alga Ua6, *C. viridis*, *C. avernensis*, *Coccomyxa* sp. Clade M, Clade N and Clade KL clustered as a subgroup, was supported with a bootstrap value 100, but the new species Ua6 was clearly different from the other species in this subgroup according to the branch length. For the SSU rDNA, the sequences of *Coccomyxa* clustered as five subgroups. The newly isolated green alga Ua6 also showed a close relationship with *C. viridis*, *C. avernensis*, *Coccomyxa* sp. Clade K, Clade L, Clade M and Clade N as a well-supported subgroup with the bootstrap value 100. Furthermore, the SSU rDNA sequence of Ua6 was clearly distinguished from the other species.

According to the comprehensive study of both morphological and phylogenetic analysis, the isolated single cell green algae Ua6 is a newly reported species and here described as new:

Coccomyxa antarctica **Shunan Cao & Qiming Zhou, sp. nov.**
Figures 1, 2

Holotype. Preparation FACHB-2140, Freshwater Algae Culture Collection, the Institute of Hydrobiology (FACHB-Collection) represented here by Figure 1d.

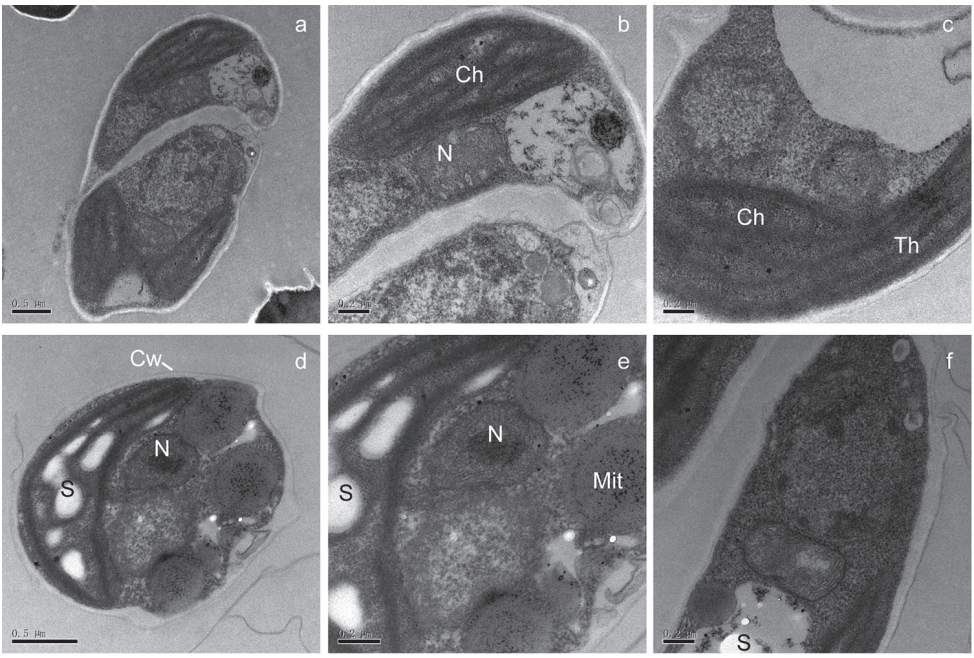

Figure 2. Ultrastructure of *Coccomyxa antarctica* Shunan Cao & Qiming Zhou, sp. nov. **a–c** cultured in BBM medium; d-f cultured in PDA medium. **a, b** mature autosporangium **c, d** Cup-shaped chloroplast **e, f** vegetative cell. Key: Ch: chloroplast; Cw: cell wall; Mit: mitochondria; N: nucleus; S: starch granules; Th: thylakoids. Scale bars: 0.5 μm(**a, d**); 0.2 μm (**b, c, e, f**).

Type locality. Antarctic, Fildes Peninsula, on stone (62°12.70'S, 58°55.70'W), 44 m a.s.l., Isolated from the Antarctic lichen *Usnea aurantiacoatra* (d-B1, BIRDS ID: 2131C0001ASBM100063) on 5th May 2014.

Diagnosis. The vegetative cells are ovoid to ellipsoidal, asymmetrical, 4–7 μm wide and 8–12 μm long; some cells were sub-sphaeroidal in BBM medium, without mucilaginous sheath. Cell wall smooth, double in ultrastructures. Protoplast with single central cell nucleus, filled with lipid droplets. Chloroplast parietal, with starch granules in interthylacoidal spaces, without pyrenoid. Reproductive cells were not observed. It looks morphologically similar to other *Coccomyxa* species but differs from other species of *Coccomyxa* in ITS rDNA (Table 1 & Figure 3a) and SSU rDNA (Table 1, Figure 3b).

Habitat. Epiphytic green alga, living with lichen *Usnea aurantiacoatra* in harsh environments (Antarctic).

Discussion

The morphological and ultrastructure characters indicate that the isolated green alga Ua6 is a *Coccomyxa* species, which is characterised by ovoid to ellipsoidal single cells. The isolated strain Ua6 is morphologically similar to the other *Coccomyxa* species, but

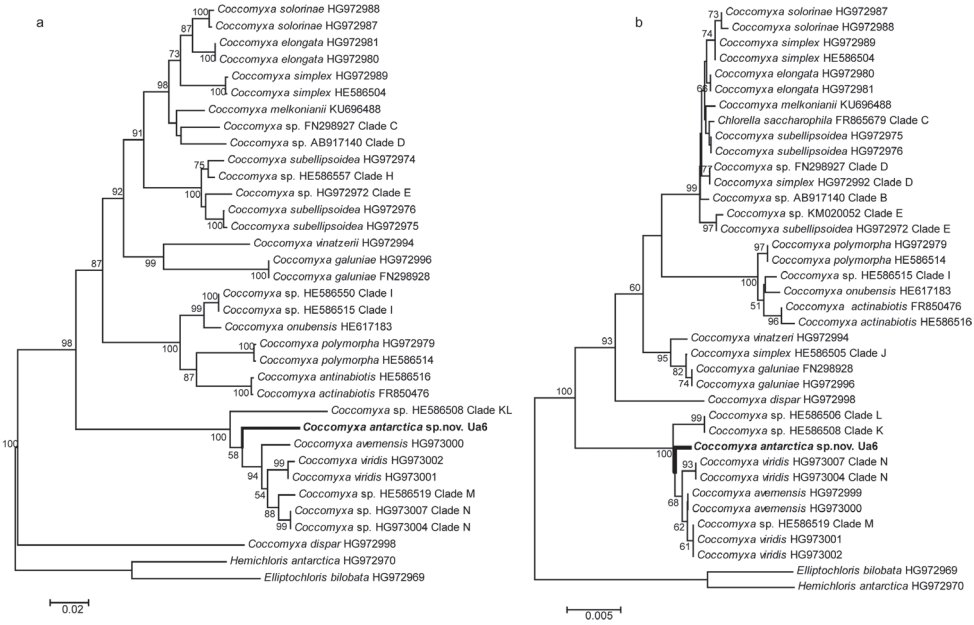

Figure 3. The NJ tree based on ITS rDNA (**a**) and SSU rDNA (**b**) sequences phylogenetic analyses. The sequences obtained by the authors were exhibited in bold font. The clades referred to Malavasi et al. (2016).

different from the phylogenetic closely related species *C. viridis* by its larger cell size (4–7 μm wide and 8–12 μm long vs 1.8–3.6 μm wide and 4.7–8.4 μm long) (Hodač 2015). However, the morphological characters are not stable and non-credible as they change under different environments or culture conditions. For example, the cell shape is significantly dependent on culture conditions (Tsarenko and John 2011) and the mucilaginous sheaths are highly dependent on nutrient availability which is the key trait in separating *Coccomyxa* and *Pseudococcomyxa* (Darienko et al. 2015).

Since the molecular barcode provides a more stable and informative tool in identification and classification of the species of *Coccomyxa* (Darienko et al. 2015, Malavasi et al. 2016), both the ITS rDNA and SSU rDNA phylogenetic analyses were applied in the current study. The results supported the observation that the single cell green alga *Coccomyxa antarctica* sp. nov. is different from the other reported species of *Coccomyxa*, indicating that it is a species new to science.

Furthermore, species of *Coccomyxa* have been reported as photobionts of lichen genera *Baeomyces*, *Dibaeis*, *Icmadophila*, *Lichenomphalia*, *Micarea*, *Multiclavula*, *Nephroma*, *Orceolina*, *Peltigera*, *Placynthiella*, and *Solorina* in earlier studies (Poulsen et al. 2001, Smith et al. 2009, Wirth et al. 2013, Gustavs et al. 2017), but not *Usnea*. The authors' earlier studies also revealed that the photosynthetic partner of the Antarctic lichen *U. aurantiacoatra* was *Trebouxia jamesii* (Hildreth and Ahmadjian) Gärtner (Cao et al. 2015b, Cao et al. 2017); as a result, the isolated green alga *Coccomyxa antarctica* sp. nov. is not lichenised alga, but a lichen epiphytic alga.

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RESEARCH ARTICLE

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Lysimachia tianmaensis (Primulaceae), a new species from Anhui, China

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Abstract

A new species of *Lysimachia* (Primulaceae), *Lysimachia tianmaensis* K. Liu, S.B. Zhou & Ying Wang **sp. nov.**, is described and illustrated from Jinzhai County, Anhui, China. It is endemic to Dabieshan Mountain, China. The new species has yellow flowers and belongs to the subgenus *Lysimachia* section *Nummularia* series *Grammicae*. It is very easily distinguishable from other related species by having alternate leaves with brown patches beneath and an auriculated leaf base.

Keywords

Lysimachia, species nova, China, taxonomy

Introduction

Lysimachia Linnaeus is one of the largest genera of Primulaceae s. l. and it comprises about 200 species, mainly distributed in the temperate and subtropical parts of the northern hemisphere, as well as in some tropical mountain regions (Chen and Hu 1979, Hu and Kelso 1996, Marr and Bohm 1997, Hao et al. 2004, Julius et al. 2016). On the whole, it is almost cosmopolitan, but the greatest concentration of the species occurs in China (with ca. 140 species; Chen et al. 1989, Hu and Kelso 1996). Some

new species in *Lysimachia* are still being found (Peng and Hu 1999, Shao et al. 2004, Shao et al. 2006, Zhang et al. 2006, Yan and Hao 2012, Liu et al. 2014a, 2014b, Estes et al. 2015, Zhou et al. 2015, Baskose et al. 2016, Julius et al. 2016).

In 2007, during the course of checking specimens in the herbaria of Anhui Normal University, a specimen of *Lysimachia* caught the authors' attention. This plant was collected by Shen in 1983 from Jinzhai County, Anhui Province and was not identified. This plant has alternate leaves and an obvious broadly-winged petiole with an auriculate base. It should thus represent an undescribed species, as this character combination is not known from any other species. In 2008–2009, the authors made several botanical expeditions to Tianma Nature Reserve, in Jinzhai County, Anhui Province. Many populations of this plant were found bearing flowers or fruits there. In this paper, this plant and related species were comparatively studied.

Materials and methods

Vouchers of *Lysimachia tianmaensis* were collected from Tianma National Nature Reserve of Anhui. Gross morphology and phenology data were obtained during the field expedition. Descriptions were collected from living plants.

Taxonomy

Lysimachia tianmaensis **K. Liu, S.B. Zhou & Ying Wang, sp. nov.**
urn:lsid:ipni.org:names:77178766-1
Figures 1–3

Type. CHINA. Anhui Province: Jinzhai County, Tianma National Nature Reserve, growing at margins of mountain roads, elevation ca. 1165 m, 1 June 2009 (fl.), Kun Liu 2009042 (holotype: ANUB!; isotypes: ANUB!, IBK!).

Diagnosis. *Lysimachia tianmaensis* is similar to *Lysimachia grammica* Hance in the alternate leaves, but differs by having a larger blade with brown patches beneath, an auriculate leaf base and subcapitate inflorescences.

Description. Herbs perennial, 15–45 cm tall. Stems often many, erect or arcuate at base, terete, simple or short branched, with tangled multicellular hairs. Leaves alternate, occasionally opposite on lower part; petiole 3–25 mm, broadly winged, base auriculate on leaves from middle and lower part of stems and branches. Leaf blades ovate to ovate-elliptic, rarely ovate-lanceolate, 1.5–5.5 × 1.0–3.5 cm, abaxially multicellular hairs, brown patches, adaxially pubescent, base broadly cuneate to subrounded, apex acute to subobtuse; veins 2 or 3 pairs, inconspicuous. Flowers solitary, in axils of apically diminished leaves, often in shortened, nearly capitate inflorescences at apex of stems and branches. Pedicel densely covered with multicellular hairs; lowest pedicels 2–3 cm, gradually reduced in length in upper flowers, recurved in fruit. Calyx lobes

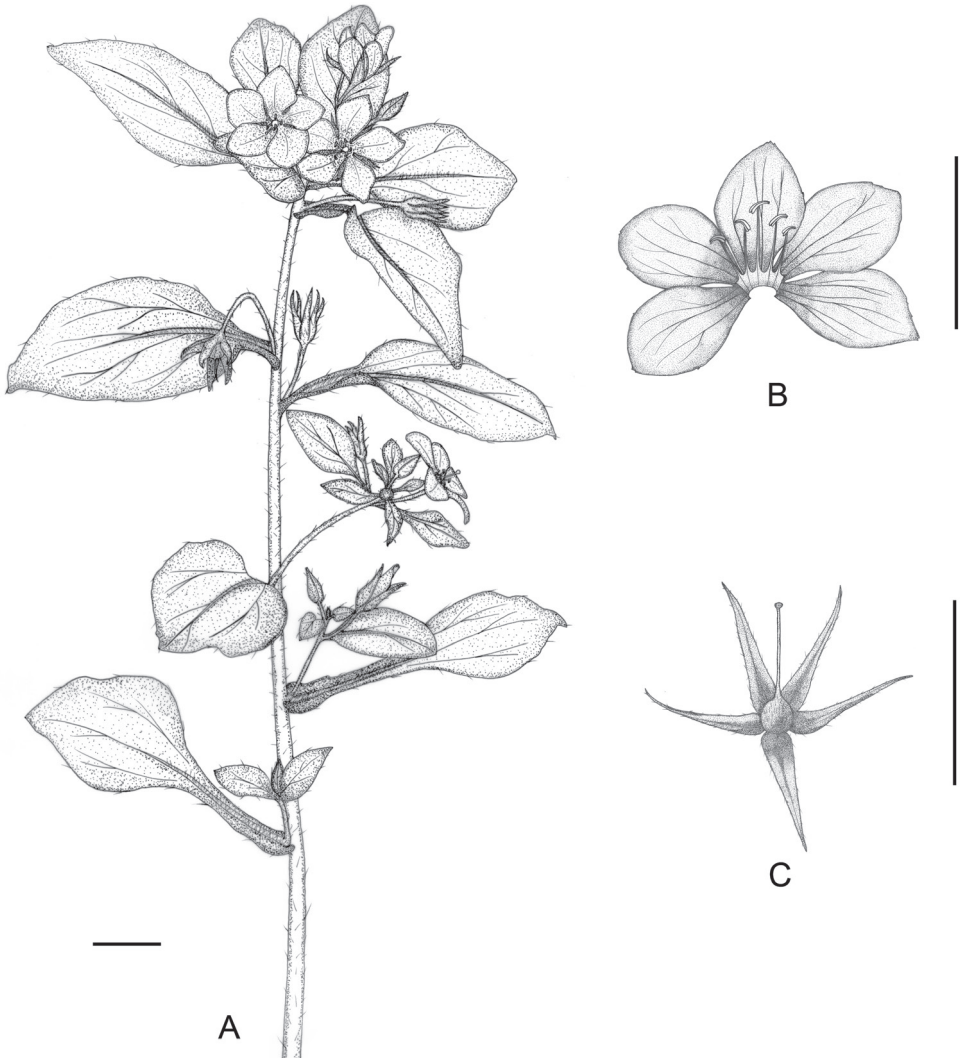

Figure 1. *Lysimachia tianmaensis* sp. nov. (**A**) the upper part in flowering period **B** opened corolla showing stamens **C** pistil and calyx. Scale bars = 1 cm.

ovate-lanceolate, 6–7 × 1–1.4 mm, abaxially sparsely pubescent. Corolla yellow; tube 0.5–1 mm; lobes ovate or rhomboid-ovate, 8–11 × 5–7.5 mm, transparent glandular. Filaments connate basally into a 0.5–1 mm high ring, free parts 2.5–3 mm; anthers dorsifixed, opening by lateral slits. Ovary pubescent; style 5–6 mm. Capsule subglobose, 3.5–5 mm in diam. Fl. Apr–Jun.

Additional collection. CHINA. Anhui Province: Jinzhai County, Tiantangzhai, ca. 650 m, 17 June 2008, *K. Liu 2008056* (ANUB); Jinzhai County, Mazongling mountain, 950 m, 1 June 2009, *K. Liu 2009038* (ANUB); Jinzhai County, Tiantangzhai, ca. 700 m, 1983, *X.S. Sheng 1437* (ANUB); Jinzhai County, Baimazhai,

Figure 2. Holotype sheet of *Lysimachia tianmaensis* sp. nov.

900 m, 18 May 1984, *G. Yao 9004* (NAS); Jinzhai County, Baimazhai, 700 m, 23 May 1984, *G. Yao 9056* (NAS); Jinzhai County, Gubeizhen, 720 m, 4 May 2016, *J.W. Shao ANUB00569* (ANUB).

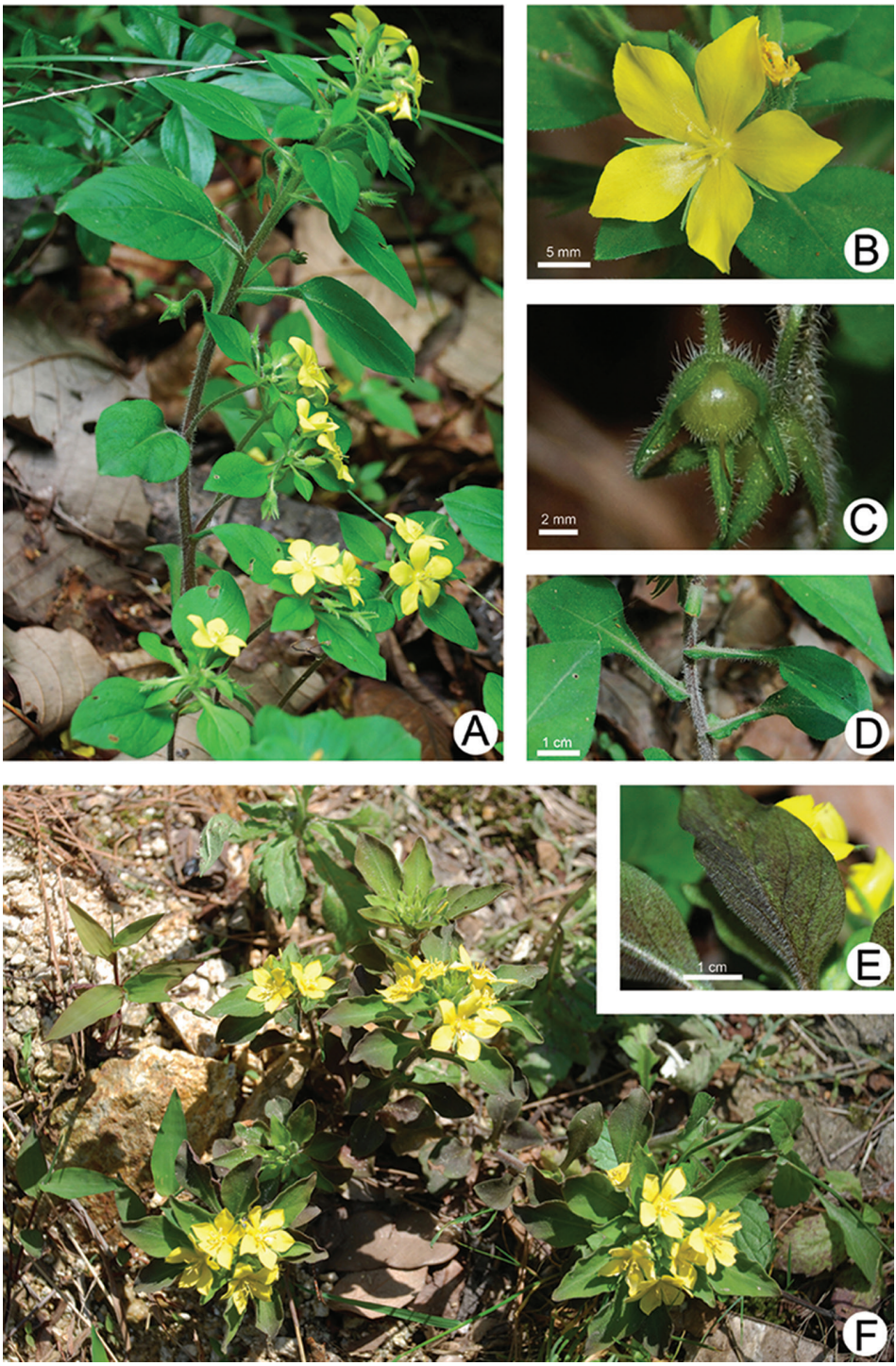

Figure 3. *Lysimachia tianmaensis* sp. nov. **A** plant in flowering **B** flower **C** young fruit **D** leaves showing winged petiole with auriculate base **E** blades showing brown patches abaxially **F** habit in flowering.

Distribution and habitat. *Lysimachia tianmaensis* is endemic to Dabieshan Mt., China (including Jinzhai County, Yingshan County etc.), growing at margins of mountain woodlands, roadsides or under broad-leaved forests at altitudes of 600–1200 m.

Etymology. The epithet "tianmaensis" is derived from the type locality, Tianma National Nature Reserve, Jinzhai Xian, Anhui Province, China.

Vernacular name. China: tian ma guo lu huang.

Phenology. Flowering April–June, fruiting June–August.

Conservation status. A large number of populations of *Lysimachia tianmaensis* were found during the extensive investigation in Tianma National Nature Reserve. This species is also distributed in other areas in Dabieshan Mt. as well as Tianma National Nature Reserve. This species often grows under broad-leaved forests above 600 m. This species is fairly common there and therefore proposed as Least Concern following the IUCN Red List Criteria (IUCN 2016).

Discussion

Lysimachia tianmaensis is quite distinct from all other species in subgenus *Lysimachia*. Its morphological affinity is with *L. grammica*, *L. remota* Petitmengin and *L. pseudohenryi* Pampanini, but it can be easily distinguished by some characters (Table 1). *L. remota*, a member of subgenus *Lysimachia*, section *Nummularia*, series *Deltoideae*, is characterised by opposite leaves with sparsely transparent glandular punctate. *L. pseudohenryi* has opposite leaves and terminal racemes, often nearly capitate, belonging to

Table 1. Diagnostic character differences amongst *Lysimachia tianmaensis*, *L. grammica*, *L. remota* and *L. pseudohenryi*.

Species	*L. tianmaensis*	*L. grammica*	*L. remota*	*L. pseudohenryi*
Source	This study	Hu and Kelso (1996)	Hu and Kelso (1996)	Hu and Kelso (1996)
Leaf	alternate, occasionally opposite on lower part; abaxially brown glandular punctate; base auriculate on middle and lower part of stems and branches	opposite on lower part, alternate on upper part; black glandular stripes	opposite, occasionally alternate on upper part; sparsely transparent glandular punctate	opposite; sparsely transparent glandular
Blade size (cm)	1.5–5.5 × 1.0–3.5	1.3–3.5 × 0.8–2.5	1.5–3.2 × 0.7–2.0	2–8 × 0.8–2.5
Inflorescence	flowers solitary, in axils of apically diminished leaves, often abbreviated, nearly capitate at apex of stems and branches	flowers solitary, in axils of upper leaves	flowers solitary, in axils of upper leaves, or capitate with flowers aggregated near apex of stems	racemes terminal, abbreviated, often nearly capitate
Filament	filaments connate basally into a 0.5–1.0 mm high ring	filaments connate basally into a ca. 0.5 mm high ring	filaments connate basally into a 0.5–1.0 mm high ring	filaments connate basally into a 2–3 mm high tube
Corolla	transparent glandular	brown glandular stripes	transparent glandular	transparent glandular

subgenus *Lysimachia*, section *Nummularia*, series *Phyllocephalae*. Due to the opposite leaves in both *L. remota* and *L. pseudohenryi*, *L. tianmaensis* can be distinguished from them by its alternate leaves with brown patches beneath and auriculate leaf base. Taking into consideration the existence of alternate leaves and the filaments connate into a ring at base, *L. tianmaensis* should be a member of the subgenus *Lysimachia*, section *Nummularia*, series *Grammicae*, according to the classification system of the genus modified by Chen and Hu (1979). Series *Grammicae* is a well-defined group, so far consisting of only one species. *L. grammica* is a widely distributed species with its distribution centre in Anhui, Henan, Hubei, Jiangsu, Jiangxi, S Shaanxi and Zhengjiang and the new species is endemic to Dabieshan Mt. However, the new endemic species rarely, if ever, co-occurs with the widespread *L. grammica* in intermixed populations because of the distinct altitudes for each natural habitat (*L. tianmaensis*: 600–1200 m; *L. grammica* 0–600 m, rarely to 800 m). The new species has a larger lamina with brown patches beneath than that of *L. grammica* with blank glandular striates. Moreover, the leaves of the new species are characterised with an obvious auriculate base. Based on these characters, *L. tianmaensis* can be very readily distinguished from *L. grammica*.

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RESEARCH ARTICLE

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Coelogyne victoria-reginae (Orchidaceae, Epidendroideae, Arethuseae), a new species from Chin State, Myanmar

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Abstract

Coelogyne victoria-reginae, a new species of section *Proliferae*, from Natma Taung (Mt.Victoria) National Park, Chin State, Myanmar, is described and illustrated. It is morphologically similar to *C. prolifera*, but the clustered pseudobulbs, pure brownish- red flowers and column wing with irregular notches at the apex of the new species differ from the other species. A preliminary risk-of-extinction assessment shows that the new species is regarded as EN C2a[i] according to the IUCN Red List Categories and Criteria.

Keywords

NatmaTaung (Mt. Victoria) National Park, risk of extinction assessment, section *Proliferae*, taxonomy

Introduction

Coelogyne Lindley (1821) is characterised by a free, never-saccate lip, with high lateral lobes over the entire length of the hypochile and smooth, papillose, toothed or warty keels on the epichile (Seidenfaden and Wood 1992). This genus comprises over 200 species, distributed from Southeast Asia to the south-western Pacific Islands (Butzin 1992, Clayton 2002, Chen and Clayton 2009). Around 46 species of this genus have been recorded from Myanmar (Kress et al. 2003, Kurzweil and Lwin 2014, Aung et al. 2017).

The Natma Taung (Mt. Victoria) National Park is located in the south-western part of Myanmar. Mount Victoria is the highest mountain in this range and regarded as an ecological refugium, offering a temperate climate that is absent from neighbouring regions (Tanaka et al. 2010a). It is estimated that there are about 2500 vascular plant species on Mt. Victoria (Mill 1995) and a number of endemic and relict species have been found in this area (Hutchinson 1917, Cowley 1982, Ikeda and Ohba 1995, Tanaka et al. 2010b, 2010c, Yukawa et al. 2010). During our field expeditions in this area since 2016, carried out by the Xishuangbanna Tropical Botanical Garden, CAS, in cooperation with the Forest Department, Union of Myanmar Ministry of Forestry, a new species of *Coelogyne* was discovered and is described below. The new species belongs to *Coelogyne* section *Proliferae* (Lindl.) Pfitzer and Kraenzlin (1907).

Materials and method

Morphological descriptions (Stearn 1983) of the new species were carried out based on five living plants and three dried herbarium specimens (HITBC: herbaria of Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Science). Measurements were made using a vernier caliper. Herbarium or fresh specimens of *Coelogyne prolifera* (PE: herbaria of Institute of Botany, the Chinese Academy of Science), *C. schultesii* (CAL: herbaria of Calcuttense), *C. ustulata* (K: herbaria of Royal Botanical Garden, Kew) and *C. ecarinata* (HTIBC) were also examined. The conservation status of the new species was evaluated based on the International Union for Conservation of Nature standard of the criteria C (Small population size and decline): C1 is an observed, estimated or projected continuing decline of at least up to a maximum of 100 years in the future; C2 is an observed, estimated, projected or inferred continuing decline and including at least 1 of the following 3 conditions: a [i] number of mature individuals in each subpopulation; a [ii] percentage (%) of mature individuals in one subpopulation; (b) extreme fluctuations in the number of mature individuals. Here, we have just observed the number of mature individuals in the subpopulation and criteria of C2a [i]is used to evaluate the threatened status, of which the number of mature individual ≤ 50 is CR (critically endangered); ≤ 250 is EN (endangered); ≤ 1000 is VU (vulnerable) (IUCN Standards and Petitions Subcommittee 2017).

Taxonomic treatment

***Coelogyne victoria-reginae* Q.Liu & S.S.Zhou, sp. nov.**
urn:lsid:ipni.org:names:77178924-1
Figs 1, 2

Diagnosis. *Coelogyne victoria-reginae* is closely related to *C. prolifera* by having the elliptic mid-lobe with two lamellae terminating at 2/3 on to mid-lobe, ovate or oblong lateral lobes. However, the new species can be distinguished from the latter by the ovoid pseudobulb and 1.1–1.4 cm apart on rhizome, flower brownish-red, lateral sepals (10–11 ×5.5–6.0 mm) significantly larger than dorsal sepal (7.0–8.0 × 4.5–5.0 mm).

Type. MYANMAR. Chin State. Natma Taung (Mt. Victoria) National Park, subtropical evergreen broad-leaved forest, 2400–2600 m, epiphytic on the upper trunk, 1 May 2017, Qiang Liu, *M17-18* (holotype, HITBC!, isotypes, RAF!).

Description. Epiphytic, rhizome creeping, rigid, 3.5–4.5 mm in diameter, densely covered with leathery scaly sheaths, with rather short internodes. Pseudobulbs 1.1–1.4 cm apart on rhizome, globose or ovoid-oblong 3.1–4.0 × 1.0–1.5 cm. Leaves two on each pseudobulb, terminal, oblong lanceolate, coriaceous, 6.5–8.3 × 2.4–3.1 cm, apex acuminate; petiole 1.0–1.7 cm. Inflorescence arising from top of mature pseudobulbs, up to 21.5–32.3 cm long, 4–6-flowered, far longer than leaves, with many persistent distichous sterile bracts just below rachis and several closely spaced distichous sterile bracts at apex of the rachis. Rachis extending and producing annual sets of imbricate bracts and flowers. Floral bracts lanceolate, almost deciduous at anthesis, ca. 1.2 cm; pedicel and ovary 8.0–10.0 mm. Flowers brownish-red, dorsal sepal triangular-ovate, 7.0–8.0 × 4.5–5.0 mm, acuminate; lateral sepals similar to dorsal sepal,10.0–11.0 × 5.5–6.0 mm. Petals narrowly linear, acuminate, 9.0–10.0 × 1.0 mm; lip 3-lobed, subovate, 10.0–11.0 × 7.0–8.0 mm, callus with 2 conspicuous longitudinal lamellae extending from base of mid-lobe; lateral lobes erect, ovate, 5.0 × 3.0 mm; mid-lobe nearly elliptic, reflexed, ca. 6.0 × 5.0 mm, margin undulate, apex emarginate; column ca. 6.0 mm, apex winged with serration; anther cap coniform; pollinia four, semi-orbicular.

Etymology. The new species is named after Victoria Mountain region, NatmaTaung National Park, Chin State, South-western Myanmar, where it was discovered in a vast area of mountain forest.

Phenology. Flowering occurs in April and May.

Distribution and habitat. *Coelogyne victoria-reginae* is only known from the type locality. It grows as an epiphyte on tree trunks in subtropical evergreen broad-leaved forest, which is dominated by *Lithocarpus xylocarpus* (Kurz) Markg. (Fagaceae).

Conservation status. *Coelogyne victoria-reginae* was collected in the Victoria Mountain, Natma Taung National Park, Chin State, South-western Myanmar. However, only one population, consisting of approximately100 individuals, has been discovered so far in the National Park. Although other populations may be found with further investigation because the area is legally protected under the

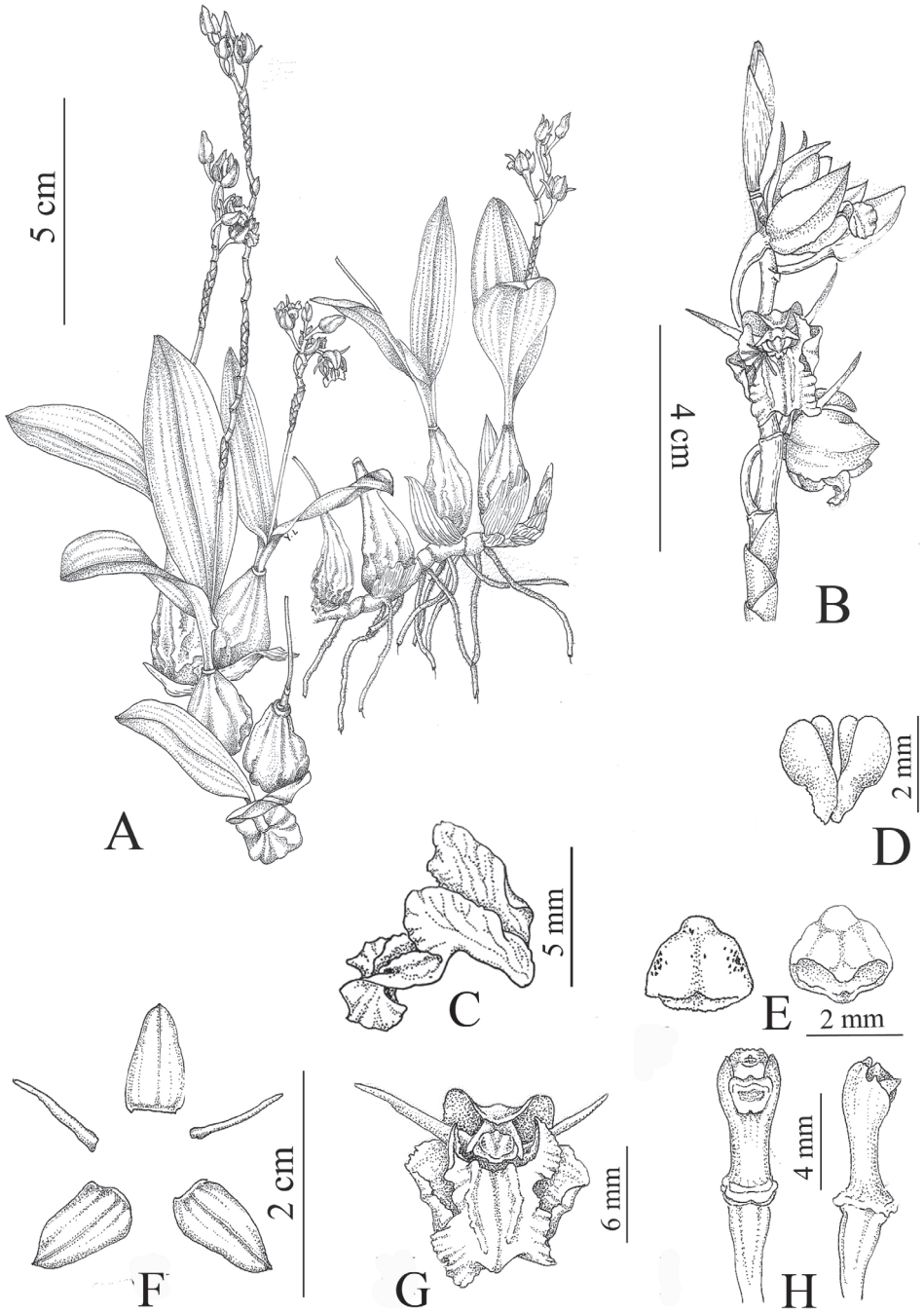

Figure 1. *Coelogyne victoria-reginae*. **A** Plant **B** Inflorescence **C** Lateral view of labellum **D** Pollinarium **E** Abaxial and adaxial anther cap **F** Sepals and petals **G** Front view of flower **H** Front and lateral view of column. All from the type collection (Qiang Liu, *M17-18*) and drawn by Lan Yan.

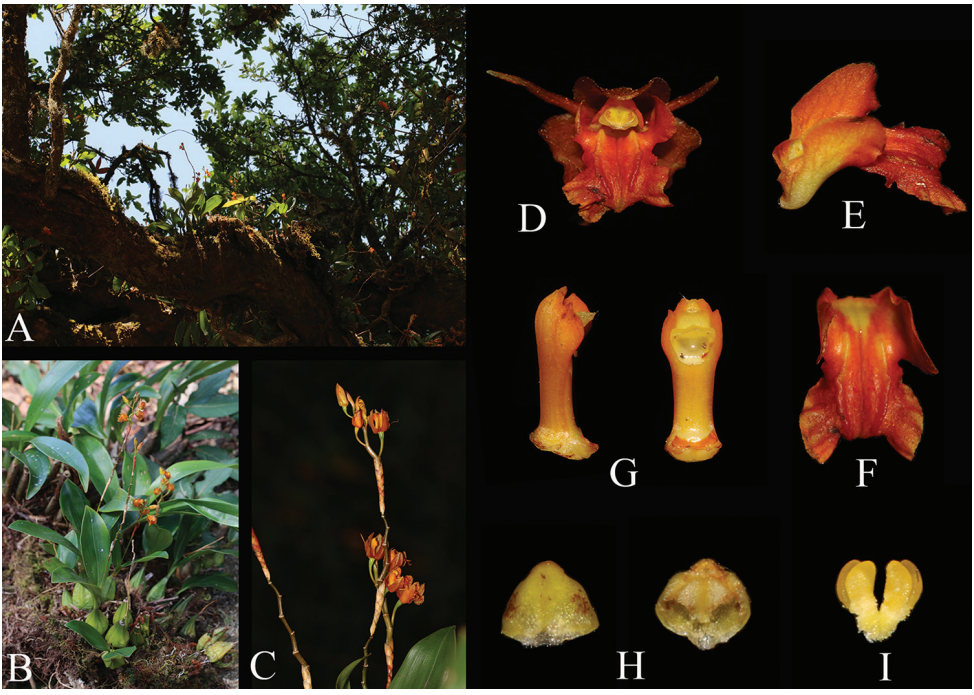

Figure 2. *Coelogyne victoria-reginae*. **A** Habitat **B** Plant **C** Inflorescence **D** Flower **E** Lateral view of labellum **F** Front view of labellum **G** Front and lateral view of column **H** Abaxial and adaxial anther cap. I. Pollinarium (Photographed by Q. Liu)

management of the Myanmar Forest Department, the number of mature individuals of the subpopulation may be less than 250 on account of similar habitat. Thus, the species is here assigned a preliminary status of EN C2a [i]according to the guidelines for using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Subcommittee 2017).

Key to *Coelogyne* sect. Proliferae

1 Lip without lamellae, lateral lobes semi-orbicular in size **2**
– Lip with 2 lamellae, lateral lobes ovate or oblong **3**
2 Flower brownish-red and lip white with a black apex, labellum large (15.0 × 9.0 mm), mid-lobe rotund ..... ***C. ecarinata***
– Flower pale yellow-green, labellum small (6.5 × 6.0), mid-lobe semi- elliptic.... ..... ***C. ustulata***
3 Mid-lobe orbicular or sub-quadrate, with 2 lamellae faint near base of mid-lobe..... ***C. schultesii***
– Mid-lobe nearly elliptic, with 2 lamellae terminating at 2/3 on to mid-lobe **4**

4 Pseudobulb ovoid and 1.1–1.4 cm apart on rhizome, flower brownish-red, lateral sepals (10–11 × 5.5–6.0 mm) significantly larger than dorsal sepal (7.0–8.0 × 4.5–5.0 mm) ..... ***C. victoria-reginae***

– Pseudobulb narrowly ovoid-oblong and 2.5–4.0 cm apart on rhizome, flower green or yellow green, lateral sepals (3.0-3.6×1.2 mm) equal or smaller than dorsal sepal (3.0-4.0 × 3.0 mm) in size..... ***C. prolifera***

Discussion

Coelogyne was established by Lindley in 1821 and is currently divided into 4 subgenera and 19 sections (Gravendeel 2005). Although revisions of several sections have been published in the last decade (Gravendeel and de Vogel 1999, Pelser et al. 2000), a comprehensive infrageneric delimitation combined with descriptions of morphological characters and molecular phylogeny within *Coelogyne* is needed (Gravendeel 2000, Sierra et al. 2000). Morphologically, the inflorescence of this new species with imbricate sterile bracts at the junction of the peduncle and rachis indicate that it belongs to the section *Proliferae* (Pfitzer and Kraenzlin 1907, Seidenfaden 1975, Chen et al. 1999). *Coelogyne victoria-reginae* is similar to *C. prolifera*, *C.ustulata*, *C.schultesii* and *C. ecarinata*, both in vegetative morphology and shape of the flowers. However, the new species differs from *C. ustulata* by its 2 longitudinal lamellae on the mid-lobe and lateral lobes smaller than the mid-lobe (mid-lobe without 2 longitudinal lamellae and lateral lobes significantly larger than mid-lobe in *C. ustulata*) (Seidenfaden 1975, Pedersen et al. 2014). It differs from *C. ecarinata* by its small and tight sterile bracts at the apex of the rachis and a brownish- red lip without a black tip (large and loose sterile bracts at apex of rachis and white lip with black tip in *C. ecarinata*) (Jin and Li 2006, Kurzweil and Lwin 2014). It differs from *C. schultesii* by its brownish-red flowers, lateral sepals (10.0–11.0× 5.5–6.0 mm) larger than dorsal sepal (7.0–8.0 × 4.5–5.0 mm), ovate lateral lobes and size is 5.0 × 3.0 mm, elliptic mid-lobe and size is 6.0 × 5.0 mm, apex of column wing is significantly notched (brownish-yellow to dark brown sometimes light greenish flowers, lateral sepals (12.0-18.0× 4.0-6.0 mm) as large as dorsal sepal (12.0–18.0× 6.0–9.0 mm), oblong lateral lobes and size is 8.0–12.0× 3.0–5.0 mm, orbicular or subquadrate mid-lobe and size is 7.0–10.0 × 8.0–11.0 mm, apex of column wing is entire in *C. schultesii*) (Seidenfaden 1975, Jain and Das 1978, Chen and Clayton 2009). It differs from *C. prolifera* by having globose pseudobulbs, 1.1–1.4 cm apart on the rhizome and conical anther cap (narrowly ovoid-oblong pseudobulb, 2.5–4.0 cm apart on rhizome and subglobose anther cap in *C. prolifera*) (Seidenfaden 1975, Chen and Clayton 2009). Meanwhile, the new species is only growing in the subtropical evergreen broad-leaved forest at 2400–2600 m in South-western Myanmar; *Coelogyne prolifera* is growing in the montane rain forest or subtropical evergreen broad-leaved forest at 1100–2200 m in Southeast Asia; *C. ustulata* is growing in the montane rain forest at 1700–1800 m in Myanmar and Thailand; *C. schultesii* is growing in the subtropical evergreen broad-leaved forest at 1700–2000 m in Bhutan, India, Myanmar, Nepal, Thailand, Vietnam and China; *C. ecarinata* is growing

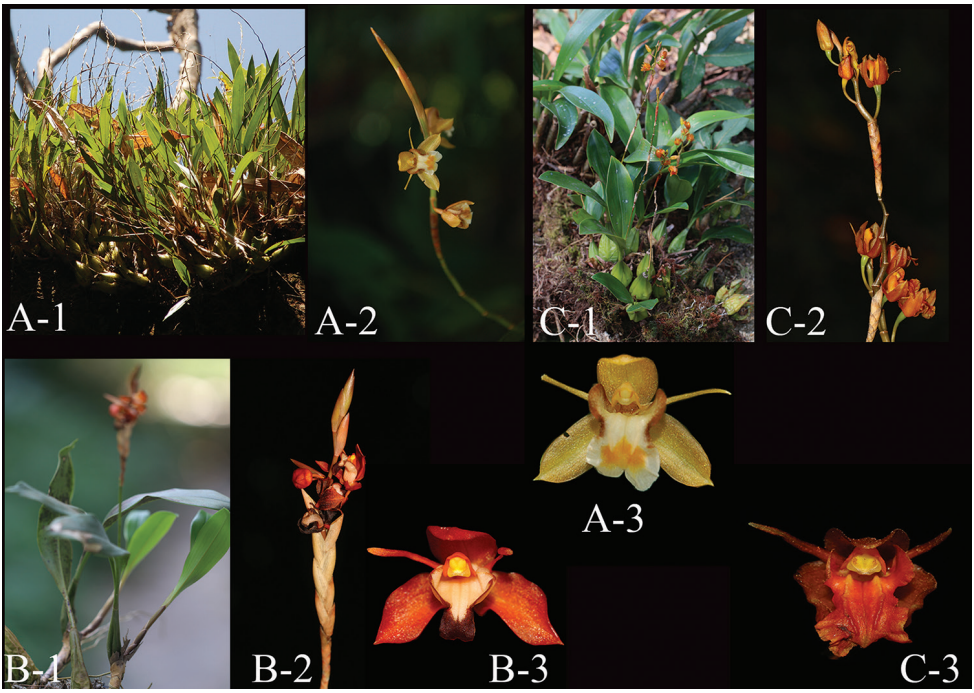

Figure 3. A *Coelogyne schultesii* (**A-1** Plant **A-2** Inflorescence **A-3** Flower) **B** *Coelogyne ecarinata* (**B-1** Plant **B-2** Inflorescence **B-3** Flower) **C** *Coelogyne victoria-reginae* (**C-1** Plant **C-2** Inflorescence **C-3** Flower) (Photographed by Q. Liu).

in the montane rain forest at 1000–2600 m in North of Myanmar and Yunnan of China (Seidenfaden 1975, Jin and Li 2006, Chen and Clayton 2009).

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