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A new natural hybrid of *Iris* (Iridaceae) from Chongqing, China

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Abstract

A newly discovered natural hybrid, *Iris* × *ampliflora* Y.E. Xiao, F.Y. Yu & X.F. Chen (Iridaceae: subgenus *Limniris* section *Lophiris*) from Chongqing, China, is described and illustrated. This hybrid is morphologically similar to *I. japonica* Thunb. and *I. wattii* Baker, but can be distinguished by its giant leaves and large purple flowers. Phylogenetic trees based on cpDNA data support the separation of *I.* × *ampliflora* from other closely related species in the section Lophiris. According to its morphological features, molecular systematic evidence and chromosome data, we speculate that *I.* × *ampliflora* [31 chromosomes] likely is a new hybrid between *I. japonica* [$2n$ = 32] and *I. wattii* [$2n$ = 30].

Keywords

chloroplast DNA, Chongqing, *Iris* × *ampliflora*, Section *Lophiris*

Introduction

Iris L. is the largest genus in family Iridaceae with up to 280 species that are mainly distributed in temperate regions of the Northern Hemisphere (Goldblatt and Manning 2008). The characteristics of iris flowers are petaloid-style branches, two obvious perianth whorls, and floral tubes with nectaries (Goldblatt and Manning 2008). Irises produce showy flowers and are well-known and popular ornamental plants worldwide. To gain more cultivars for gardens, many crosses between *Iris* species have been

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attempted and achieved success by artificial hybridization (Hu and Xiao 2012; Yu et al. 2017). Natural hybrids between sibling species do occur in the genus *Iris*, for example in some species within subgen. *Limniris* in North America (Burke et al. 1998). However, there is no natural hybrid of *Iris* reported in East Asia.

The outer sepals of irises are equipped with raised beards, crests, signal patches, and midveins, which probably have significance for pollination (Sapir et al. 2005; Guan et al. 2009; Xiao et al. 2019). The sepal crest is an important character in the taxonomic delineation of higher ranks of *Iris*. Dykes (1913) first placed six rhizomatous species in sect. *Evansia*. Lawrence (1953) changed sect. *Evansia* to the subsection level. Rodionenko (1987) elevated subsect. *Evansia* to subgen. *Crossiris*. Mathew (1981) elevated subsect. *Evansia* of Lawrence to section level, namely sect. *Lophiris*, placing the section within subgen. *Limniris*. Sect. *Lophiris* contains 11 species, most of which are distributed in Eastern Asia, apart from three species in North America (*I. cristata* Solander, *I. lacustris* Nuttall, and *I. tenuis* S. Watson) (British Iris Society Species Group 1997; Zhao et al. 2000). Mathew's classification was used in this study.

During field work in Chongqing, we found an interesting specimen originally from the Qingyang Town in Fuling District, Chongqing City, China. Our observations show that it is morphologically similar to *I. japonica* Thunb. and *I. wattii* Baker with a yellow and irregularly toothed crest on the outer segments. However, its morphological features differ markedly from those of all known species in sect. *Lophiris* described by Mathew (1981). We have observed the situation concerning seed set since 2014 when the species was initially introduced to the conservation nursery of Shanghai Botanical Garden. It is sterile with no seed production, which indicates its hybrid origin.

This study was undertaken to assess the status and parentage of the new hybrid by morphological surveys, phylogenetic and chromosome data. The Qingyang collection is a large evergreen plant with large attractive flowers, and it can adapt to warm, wet, and full-sun or partly shady environmental conditions. It has potential uses in breeding and landscaping. We formally publish its description here with the aim of better understanding and utilization of the remarkable morphological divergence of *Iris* species in subgenus *Limniris* sect. *Lophiris*.

Materials and methods

Morphological surveys

Living specimens and vouchers of the new hybrid were examined and compared with four species of sect. *Lophiris* (*I. confusa* Sealy, *I. japonica* Thunb., *I. tectorum* Maxim. and *I. wattii* Baker) in Southern China using measurements and descriptions of the main characteristics. Species from the conservation nursery of Shanghai Botanical Garden from mid-March and late-April in 2019 were compared. Eight or ten randomly chosen individuals of each taxon were used for the morphometric surveys (Table 1). Meanwhile, herbarium sheets (CDBI, CSH, IMC, IBSC, LBG, KUN, PE) and type

Table 1. Differences between *Iris* × *ampliflora*, *I. confusa*, *I. tectorum*, *I. wattii* and *I. japonica* in the conservation nursery of Shanghai Botanical Garden.

Species		*I. tectorum* (n = 10)	*I. confusa* (n = 10)	*I. wattii* (n = 8)	*I.* × *ampliflora* (n = 10)	*I. japonica* (n = 10)
Aerial stem Length		No	67.2 ± 19.9a	30.3 ± 9.3b	27.0 ± 2.7b	12.3 ± 2.7c
Leaf	Waxy	No	Yes	No	No	Yes
	Longitudinal veins	Clearly	Clearly	Clearly	Clearly	Clearly
	Texture	Wrinkled	Smooth	Wrinkled	Wrinkled	Smooth
	Length	49.5 ± 3.5c	54.8 ± 3.8b	70.1 ± 9.2a	74.2 ± 6.4a	48.2 ± 2.9c
	Width	2.9 ± 0.2d	5.3 ± 0.6b	4.3 ± 1.0c	6.6 ± 0.8a	3.8 ± 0.7c
Flower	Flowering-stem	1–2 branches	2–4 branches	5–7 branches	7–10 branches	5–12 branches
	Color	Bluish violet	Pale reddish purple	Bluish violet	Violet	Violet / Bluish violet
	Size (in diam.)	9.2 ± 1.1b	5.1 ± 0.4d	7.3 ± 0.4 c	12.5 ± 0.5a	4.8 ± 0.4d
	Crest	White	Yellow	Yellow	Yellow	Yellow
	Anthers	White	White	Yellow	White	White
Chromosome number		28	30	30	31	28, 30, 31, 32, 33, 34, 35, 54 and 55
Distribution		Subtropical and temperate zone of China	Chongqing, Sichuan, Xizang, Yunnan [NW India]	Chongqing, Sichuan, Xizang, Yunnan [NE India, Myanmar].	Chongqing	Subtropical and temperature zone of China [Japan]

Note: n, sample number.

Table 2. Primers used in this study.

Gene name	Primer	Sequence (5' to 3')
*mat*K	*mat*K-3914F	ATCTGGGTTGCTAACTCAATGG
(Wilson 2004)	*mat*K-1235R	GGAGTGGGGTATTAGTATA
	*mat*K-1176F	CTATTCATTCCATTTTTCCT
	*mat*K-*trnK*2R	AACTAGTCGGATGGAGTAG
*ndh*F	*ndh*F-*pair*1	ATGGAACA(GT)ACATAT(CG)AATATGC
(Shaw et al. 2007)	*ndh*F-1201ir	GGAATACCACAAAGAGAAAGTGTACCT
	*ndh*F-972i	GTCTCAATTGGGTTATATTATG
	*ndh*F-2210R	CCCCCTA(CT)ATATTTGATACCTTCTCC

descriptions of *I. confusa*, *I. japonica*, *I. milesii* Baker ex Foster, *I. tectorum* and *I. wattii* were compared with the new hybrid.

Phylogenetic analyses

We collected samples of five species / hybrid (*I. confusa*, *I. japonica*, *I. tectorum*, *I. wattii*, and Qingyang collection) of sect. *Lophiris*, and three species (*I. anguifuga* Y.T. Zhao, *I. henryi* Baker and *I. proantha* Diels) of section *Limniris* and *Iris domestica* (L.) Goldblatt & Mabb. to construct phylogenetic trees based on cpDNA data. The samples of the new hybrid were collected from the type locality. Other iris samples were collected from the conservation nursery of the Shanghai Botanical Garden.

Total genomic DNA was extracted from each sample (30 mg dried leaves) using a DNA Plant Kit (Tiangen, Shanghai, China) according to manufacturer's instructions. The extracted DNAs were dissolved in 100 µl TE buffer for storage. We amplified and sequenced part of the *mat*K gene (Wilson 2004) and *ndh*F gene (Shaw et al. 2007) (Table 2). Each PCR mixture (50 µl) contained ddH_2O, 1 × buffer (Mg^{2+}

free), 2.5 mM $MgCl_2$, 2.5 mM each dNTP, 0.5 μM each primer, 2 U Taq polymerase (Sangon, Shanghai, China), and 20 ng DNA. The PCR reactions were conducted on a Mastercycler pro Thermal Cycler (Eppendorf, Hamburg, Germany). The procedure was performed with initial denaturing for 5 min at 94 °C followed by 35 cycles of 30 sec at 94 °C (denaturing), 30 sec at 50 °C (annealing), and 45 sec at 72 °C (elongation), and final extension for 10 min at 72 °C. After checking products by electrophoresis on a 1.2% agarose gel, the purified products were bi-directionally sequenced by standard methods on the ABI 3731 automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequences of these irises have been deposited in GenBank (see accession numbers in Appendix 1: Table A1. For the cpDNA datasets, the CLUSTALW program combined with manual adjustment was used for multiple alignments of all sequences (Thompson et al. 1994). We used three phylogenetic methods (i.e., Bayesian inference, maximum parsimony, and maximum likelihood) to analyze the alignments. We conducted the Bayesian inference analysis with MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003), and the maximum parsimony and maximum likelihood analyses using PAUP v.4.0b10 (Swofford 2002).

Chromosome number analyses

To determine the chromosome number of the new hybrid from somatic cells. Root tips (1–1.5 cm in length) were collected and washed with distilled water and immersed in 0.002 mol/L 8-hydroxyquinoline with dark pretreatment for 2–2.5 h. These roots then were fixed in Carnoy solution (volume ratio: 95% ethanol: acetic acid = 3:1) at 4 °C for 2–3 h. The fixed roots were dissociated in 5 mol/L hydrochloric acid for 8–10 min and washed with distilled water, then stained with carbol fuchsin and squashed on glass slides. Finally, the samples were observed and photographed using a Motic BA400 optical microscope. More than 20 cells were observed to determine the number of chromosomes for each specimen examined.

Results

Morphological comparisons

The Qingyang collection is morphologically similar to the species of section *Lophiris*, *I. japonica* which has 5–10 branches of the flowering stem, a yellow crest on the outer sepals, and is the most common species of *Iris* in Southern China (Zhao et al. 2000). However, the Qingyang collection has several characteristics that distinguish it from *I. japonica*, *I. confusa*, *I. milesii*, *I. wattii* and *I. tectorum*, including plant larger flowers, larger leaves, and other features as described in Table 1. The flowering stem of this specimen has 7–10 branches and an aerial stem (mean length = 27.0 ± 2.7 cm, n = 10) (Fig. 1). Compared with other species in sect. *Lophiris*, the Qingyang collection has larger leaves (mean length = 74.2 ± 6.4 cm, n = 10; mean

width = 6.6 ± 0.8 cm, n = 10) and larger purple flowers (mean diameter = 12.5 ± 0.5 cm, n = 10) (Fig. 2).

Phylogenetics

Among eight individuals of different species / hybrid, there were 318 variable sites, 187 singleton variable sites, and 131 parsimony informative sites across 4779 bp aligned positions of two cpDNA fragments. There were 22 and 39 mutations between the sequences of *I. japonica* / *I. wattii* and the new hybrid, respectively.

In the molecular tree based on cpDNA data, the sampled new hybrid was resolved as sister to the sample of *I. japonica* (Fig. 3). In Bayesian, maximum parsimony, and maximum likelihood trees based on cpDNA data, this specimen clustered into a clade with *I. japonica* (Fig. 3). The inter-clade sequence difference (*I. japonica* vs. *I. wattii*, 0.11) was 22 times greater than the intra-clade sequence differences (the new hybrid vs. *I. japonica*, 0.05).

Chromosome number

Cytological study indicated that the Qingyang collection has 31 chromosomes (Fig. 4). No evidence of chromosome pairing could be examined and it is unknown if it functions as a diploid.

Taxonomy

***Iris* × *ampliflora* Y.E. Xiao, F.Y. Yu & X.F. Chen, nothosp. nov.**
Figures 1, 2, Appendix 2: Figure A1

Diagnosis. Morphologically similar to *I. japonica*, the new hybrid differs in having 7–10 branches and an aerial stem (25–29 cm compared to 10–15 cm), larger leaves (length and width, 68–80 cm and 6–7 cm compared to 45–51 cm and 3–4.5 cm) and larger purple flowers (diam., 11–13 cm compared to 4–5 cm).

Type. Shanghai Botanical Garden, grown from collection from the Qingyang Town of Fuling District, Chongqing, 14 June 2014, *Y.E. Xiao XYE20140614* (holotype: CSH-0180673!; isotypes: CSH!). A photo of the holotype is shown in Appendix 2: Figure A1.

Description. *Rhizomes* creeping, thick, ca. 1.5–2 cm in diam. *Overall plant* up to 85.4–125.5 cm. *Stem* ascending upright, 24–31 cm. *Leaves* mainly in basal fans, green, broadly sword-shaped, curved, midrib evident, 69.0–82.9 × 5.5–7.5 cm, the basal leaves fibrous. *Flowering stems* with 7–10 branched that arise in the axillary leaves, 1- or 2 leaved subtend the flower on the branch, ca. 50.0 × 4.5 cm. *Spathes* 2 or 3, green, lanceolate, ca. 2 cm, 3- or 4- flowered, seldom 5, apex acuminate. *Flowers* blueish violet, 11.5–12.8 cm in diam.; *pedicel* 1.5–3.0 cm, perianth tube slender, ca. 1.5 cm; outer segments mottled darker around conspicuous, yel-

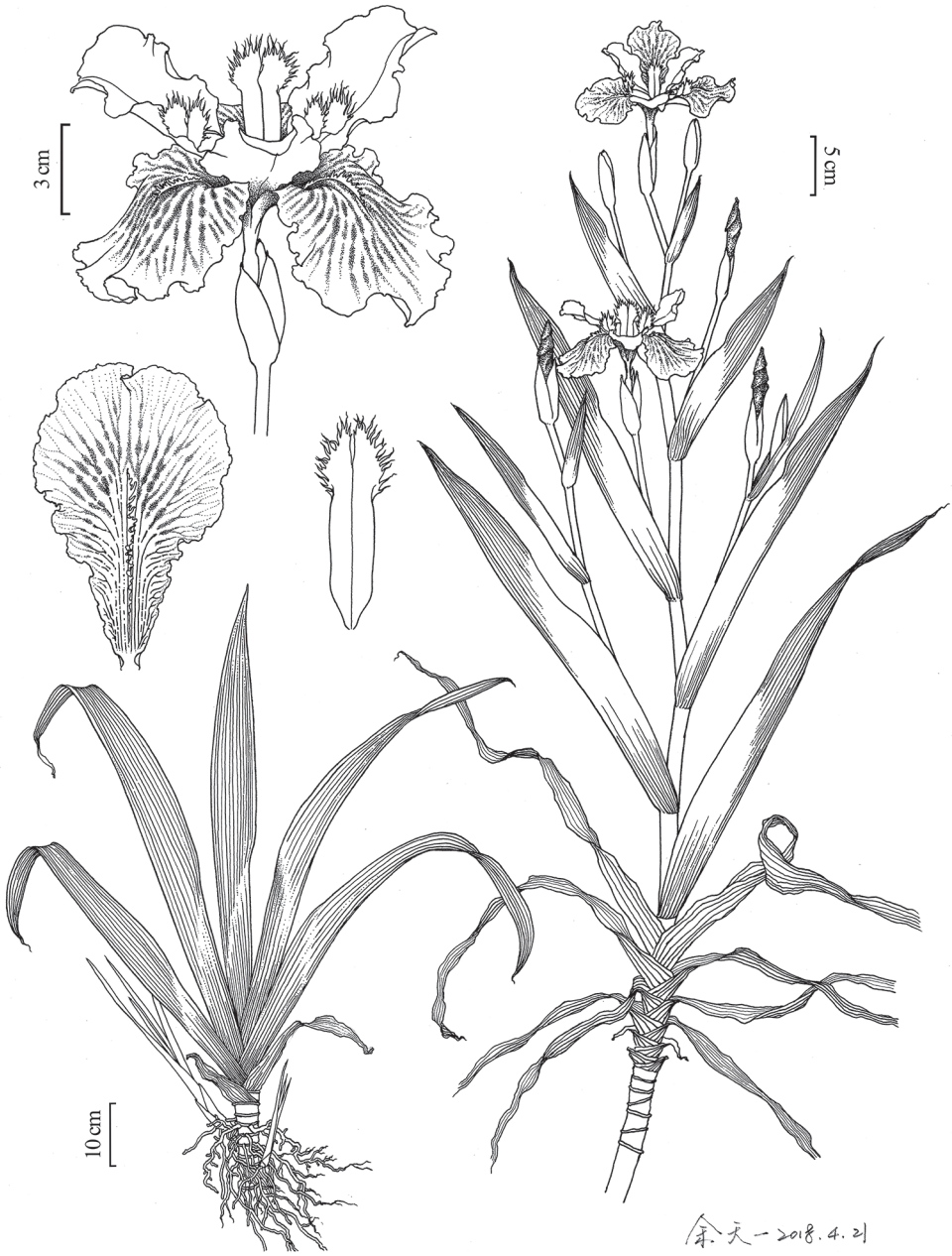

Figure 1. Line drawings of *Iris* × *ampliflora* based on photos and the type specimens (Drawn by Tian-Yi Yu).

low, irregularly toothed crest, broadly ovate, 6.8–7.3 × 5.2–5.5 cm; inner segments spreading horizontally at anthesis, elliptical, 5.5–6.2 × 3.9–4.2 cm. *Stamens* ca. 2 cm; anthers bright white without pollen. *Style branches* pale bluish violet, 4–5 cm,

Figure 2. A *Iris* × *ampliflora* in flower **B** flower anatomy of *Iris* × *ampliflora*.

feathery apex, terminal lobes fimbriate. *Ovary* cylindrical, ca. 2 cm. Flowering season, March–April. Sterile.

Etymology. The new hybrid is named for the large flower.

Distribution and habitat. *Iris* × *ampliflora* was collected from the Qingyang Town, Fuling District, Chongqing, China (29°31'40.8"N, 107°12'54"E). With complicated mountainous topography, the Qingyang Town is located in the range of the Dalou Mountains with an average altitude of 750 m. There were about 10 clones

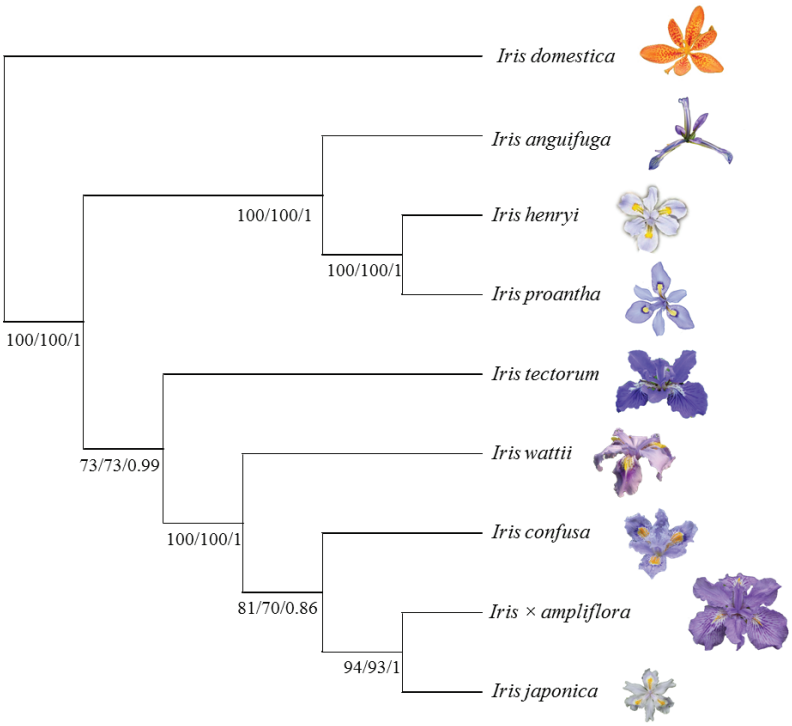

Figure 3. Phylogenetic relationships of *Iris* × *ampliflora* based on two plastid DNA fragments. Numbers around nodes are Bayesian posterior probabilities and bootstrap percentages (PP /BS_{MP}/BS_{ML}). (Pictures of flowers are relatively proportional sizes among different *Iris* species).

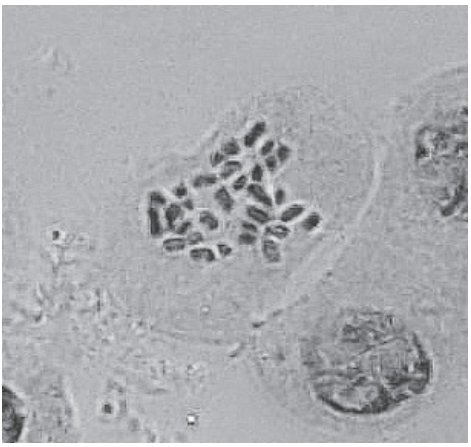

Figure 4. Mitotic metaphase chromosomes of *Iris* × *ampliflora* [number of chromosomes, 31].

each with 6–10 individuals in the population of *I.* × *ampliflora*, covering an area of 200 m^2. Plants of *I.* × *ampliflora* grow well on roadsides of subtropical mixed evergreen deciduous broad-leaved forest in full-sun and partly-shaded environments at an alti-

tude of about 650 m. The lowest and highest temperature of the original site are about -5 °C and 38 °C, respectively.

Phenology and reproductive characteristics. *Iris* × *ampliflora* blooms in March to April in Chongqing, and it blooms in April in Shanghai. It is evergreen. No fruits have been observed, but it can reproduce vegetatively.

Preliminary conservation status. Only one population of *I.* × *ampliflora* was found by our investigation in Qingyang Region and there are risks of disturbance by human activities. *I.* × *ampliflora* is currently cultivated in the conservation nurseries of Shanghai Botanical Garden and the Flower Fragrance Horticulture Limited Company in Chongqing.

Other *Iris* species examined. ***I. japonica*** Chongqing Municipality, WUK0495843; PE01012482; PE01012483; PE01012489; PE01012492; IMC0013795. ***I. confusa*** Chongqing Municipality, CDBI0169691, IMC0013989, IMC0013998; IMC0014000; IMC0014010. ***I. milesii*** Yunnan Province, LBG 00106670; LBG00106671; KUN0360444. ***I. wattii*** Chongqing Municipality, KUN0360622. Mount Emei, Sichuan Province, CSH0086611. Liangshan Prefecture, Sichuan Province, PE01013840. ***I. tectorum***, Chongqing Municipality, IBSC0629040; IBSC0629027; CDBI0169658.

Discussion

With the characters of maternal inheritance in cpDNA genes, *I. japonica* was most likely postulated as maternal parent of the hybrid *I.* × *ampliflora* because these two cluster as sister taxa in a clade in the phylogenetic tree. It is difficult to find the actual maternal parent of *I.* × *ampliflora* because the chromosome number of *I. japonica* is variable, $2n$ = 28, 30, 31, 32, 33, 34, 35, 54 and 55 (British Iris Society Species Group 1997). However, the chromosome number of the paternal parent of the hybrid can be speculated, $2n$ = 34, 31, 30, 29, 28, 27, since *I.* × *ampliflora* has 31 chromosomes. Thus, the possibilities about the parentage of *I.* × *ampliflora* are: *I. japonica* ($2n$ = 34) × *I. tectorum* ($2n$ = 28) or *I. japonica* ($2n$ = 32) × *I. wattii* / *I. confusa* ($2n$ = 30).

Furthermore, the parents of *I.* × *ampliflora* can be deduced according to morphological features. The hybrid has aerial stems (mean length = 27.0 ± 2.7 cm, n = 10). Without an aerial stem, *I. tectorum* cannot be the paternal parent. *Iris wattii* and *I. confusa* both have aerial stems (*I. wattii*, mean length = 67.2 ± 19.9 cm, n = 10; *I. confusa*, mean length = 30.3 ± 9.3 cm, n = 10). However, the leaf surface of *I.* × *ampliflora* has no waxy coat; it is dull and ruffled similar to that of *I. wattii*. The leaf surfaces of *I. confusa* and *I. japonica* have a waxy coat, glossy and smooth. Thus, compared with *I. confusa*, *I. wattii* is possibly more likely to be the paternal parent of *I.* × *ampliflora*.

Though *I. japonica* and *I.* × *ampliflora* are clustered into one clade, there are 22 mutations between the sequences of these two species. Compared with leaves (length 45–51 cm and width 3–4.5 cm) and flowers (diam. 4–5 cm) of *I. japonica*, *I.* × *ampliflora* has larger leaves (length 68–80 cm and width 6–7 cm) and larger purple flowers (diam.

11–13 cm). It cannot be determined which population of *I. japonica* it is derived from for the maternal parent since intraspecific chromosome numbers are variable. Thus, the sequence divergence could not reflect the real evolutionary distance between *I. japonica* and *I.* × *ampliflora*. The evolution of *I.* × *ampliflora* is in need of further study.

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Appendix 1

Table A1. Genbank accession of two chloroplast fragments for nine *Iris* species (Mathew 1981; Goldblatt and Manning 2008) in the study.

Species	Collect location	Collector	Genbank accession	
			***mat*K**	***ndh*F**
I. × *ampliflora*	Shanghai Botanical Garden	Yue-E Xiao	MW203044	MW203053
I. anguifuga		Yue-E Xiao	MW203045	MW203054
I. confusa		Yue-E Xiao	MW203046	MW203055
I. domestica		Yue-E Xiao	MW203043	MW203052
I. henryi		Yue-E Xiao	MW203047	MW203056
I. japonica		Yue-E Xiao	MW203048	MW203057
I. proantha		Yue-E Xiao	MW203049	MW203058
I. tectorum		Yue-E Xiao	MW203050	MW203059
I. wattii		Yue-E Xiao	MW20305	MW203060

Appendix 2

Figure A1. The photo of holotype of *Iris* × *ampliflora*.

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Systematics of Lobelioideae (Campanulaceae): review, phylogenetic and biogeographic analyses

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Abstract

Lobelioideae, the largest subfamily within Campanulaceae, includes 33 genera and approximately1200 species. It is characterized by resupinate flowers with zygomorphic corollas and connate anthers and is widely distributed across the world. The systematics of Lobelioideae has been quite challenging over the years, with different scholars postulating varying theories. To outline major progress and highlight the existing systematic problems in Lobelioideae, we conducted a literature review on this subfamily. Additionally, we conducted phylogenetic and biogeographic analyses for Lobelioideae using plastids and internal transcribed spacer regions. We found that former studies have reached agreement on the southern African origin of Lobelioideae, herbaceous habit and Asian origin of giant lobelioids, the convergent evolution of giant rosette lobelioids, and lastly, the multiple cosmopolitan and independent radiation of lobelioids in Africa, Pacific Basin, and the Hawaiian Islands. Also, *Apetahia* Baill., *Sclerotheca* A.DC., and *Cyanea* Gaudich. are paraphyletic, while *Lobelia* L., *Pratia* Gaudich., *Centropogon* C.Presl, *Siphocampylus* Pohl, and *Isotoma* Lindl. are polyphyletic. The taxonomy of these genera, especially *Lobelia*, is particularly quite frustrating. This calls for further reappraisals using both morphological and molecular data.

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Keywords
Lobelioideae, monophyletic, polyphyletic

Introduction

Lobelioideae, the largest subfamily within Campanulaceae, includes 31 genera and approximately 1200 species (Knox et al. 2008a). They are characterized by resupinate flowers with zygomorphic corollas and connate anthers (Lammers 2011). They are widely distributed across the world, but absent in the Middle East, Arctic, and some sections of the Central Asia region, with half of them native to South America (Lagomarsino et al. 2014). Some species, such as *Lobelia siphilitica* L. (Linnaeus 1753), *Lobelia erinus* L. (Linnaeus 1753), and *Lobelia cardinalis* L. (Linnaeus 1753), are known for their horticultural value (Lagomarsino et al. 2014). Approximately half of the species in this subfamily belong to three Neotropical genera: *Centropogon* C.Presl, (Presl 1836) (approximately 210 species), distributed from southern Mexico to Bolivia and Brazil, with two species in the lesser Antilles; *Burmeistera* H.Karst. and Triana (Karsten and Triana 1857), (approximately 100 species) distributed in Guatemala through northern Peru; and *Siphocampylus* Pohl (Pohl 1827), (approximately 230 species) distributed from Costa Rica to Argentina and Greater Antilles (Lammers 2007a).

Since the 1990s, many researchers have studied the systematics and biogeography of Lobelioideae using morphological and molecular data, for example, Lammers (1990, 1991, 1993), Knox and Kowal (1993), Knox et al. (1993), Lammers et al. (1993), Givnish et al. (1994, 2009, 2013), Givnish (1995, 1998, 2010), Antonelli (2009), Lagomarsino et al. (2014), Chen et al. (2016), and Knox and Li (2017), among others. However, the systematics of Lobelioideae has been full of contradictory conclusions. Almost all recent classifications involving this subfamily, for example, Lammers (1990, 1991, 1993, 2011), Knox and Kowal (1993), Givnish et al. (1994, 2009, 2013), Givnish (1995, 1998, 2010), Antonelli (2009), Lagomarsino et al. (2014), Chen et al. (2016), and Knox and Li (2017), among others, contradict early taxonomic conclusions of Wimmer (1943, 1953, 1968), McVaugh (1949a), and Murata (1995). For instance, Lammers (2011) recommended the need for revision in the genus *Lobelia* L. (Linnaeus 1753). Lammers (2011) claimed that Wimmer (1943,1953), based his classification on a few morphological characters. He also added that Murata (1995) only stated the exemplars for each taxon instead of assigning species to their taxonomic groups. Additionally, both Wimmer (1943,1953) and Murata (1995) violated the International Code of Botanical Nomenclature (ICBN) with their classification (Lammers 2011).

Given these recent studies, there is an emerging need to outline the major progress and the existing systematic and biogeographic problems in the Lobelioideae subfamily. To meet this need, we conducted a literature review, phylogenetic, and biogeographic analyses of this subfamily using almost all available sequences of family Campanulaceae from the GenBank.

Materials and methods

Literature sources

The systematics of Lobelioideae was explored by checking literature works through online libraries and journals. We explored previous works to understand the debates and contentions that had been there previously and the steps that had been taken to solve the contentions. We also wanted to have a general overview of the taxonomic progress with regards to this subfamily (Zuccarini 1832; McVaugh 1941, 1943a, b, 1949a, b, 1955; Wimmer 1943, 1953, 1968; Bowden 1959, 1961; Moeliono and Tuyn 1960; Carlquist 1962, 1969, 1974; Carlquist et al. 1965; Mabberley 1974, 1975; Thulin 1986a, 1991; Thulin et al. 1986b; Phillipson 1989; Ayers 1990; Lammers 1990, 1991, 1993, 2007a, b, 2010, 2011; Murray and Cameron 1990; Harvey 1992; Lammers and Hensold 1992; Lammers et al. 1993; Knox and Kowal 1993; Knox et al. 1993; Cosner et al. 1994; Givnish et al. 1994; Givnish 1995, 1998; Gustaffson and Bremer 1995; Murata 1995; Vieira and Shepherd 1998; Serra et al. 1999; Givnish 2000; Schultheis 2001a, b; Eddie et al. 2003; Givnish et al. 2004; Knox et al. 2004, 2008a, b; Murray et al. 2004; Knox 2005, 2014; Koopman and Ayers 2005; Knox et al. 2006; Antonelli 2008, 2009; Givnish et al. 2009; Haberle et al. 2009; Givnish 2010; Givnish et al. 2013; Crowl et al. 2014; Lagomarsino et al. 2014, 2016; Chen et al. 2016; Crowl et al. 2016; Knox and Li 2017; Uribe-Convers et al. 2017; Hunter 2018).

Taxon sampling

We aimed to include as many of the Lobelioideae species as possible. Nineteen loci were obtained, that is, eighteen plastid gene loci (*atpB-rbcL* spacer, *atpB*, *atpF*, *atpF-atpH* spacer, *atpH*, *matK*, *ndhF*, *psbA-trnH* spacer, *psbA-trnK* spacer, *petD*, *rbcL*, *rpoC1*, *trnL-trnF* spacer, *trnT-trnL* spacer, *trnV-trnK* spacer, *trnK-matK* spacer, *rpl32-ndhF* spacer, *rpl16*) and one nuclear gene, internal transcribed spacer (ITS). These sequences were generated using the NCBI ENTREZ UTILITY (Kans 2020) program (Accessed 1ST April 2020) and double-checked manually at the GenBank database. Additionally, almost all available Campanulaceae complete plastid genomes were manually accessed from the GenBank. The respective plastid regions were extracted using NCBI BLASTN v. 2.9.0+ (Camacho 2018) with default settings. We included nine outgroup taxa to increase the chances of recovering the early branching of Campanulaceae (Knox 2014). The outgroups included: *Abrophyllum ornans* (F.Muell.) Benth. (Bentham and Mueller 1864), *Carpodetus serratus* J.R.Forst. & G.Forst. (Forster 1776), *Corokia cotoneaster* Raoul (Raoul 1846), *Cuttsia viburnea* F.Muell. (Mueller 1865), *Pentaphragma ellipticum* Poulsen (Poulsen 1903), *Phelline lucida* Vieill. ex Bail. (Baillon 1872), *Roussea simplex* Sm. (Smith 1789), *Scaevola* sp. L. (Linnaeus 1771) and *Stylidium adnatum* R.Br. (Brown 1810). Taxa voucher information and GenBank accession numbers are provided in the Suppl. material 1: Lobelioideae data matrix.

Alignment and phylogenetic analyses

All the gene regions were aligned separately using MAFFT v. 7.429 (Katoh and Standley 2013) with an adjust-direction and 1000 maximum iterations options. The alignment of each region was manually checked and taxa with short sequences (≤ 200bp) were edited using GENEIOUS Pro v. 5.6.4 (Kearse et al. 2012). Edited sequences were analyzed using PHYUTILITY v. 2.2.6 (Smith and Dunn 2008) to delete gaps and ambiguous sequences. The indels within the sequences were treated as missing data and they were therefore excluded from the analysis. The *trnF-trnL* spacer region had the highest number of sequences while *atpF* recorded the least (Table 1). Each dataset was analyzed using JMODELTEST v. 2.1.10 (Darriba et al. 2012) to determine the best evolution substitution model (Table 1). Maximum Likelihood (ML) analysis for each of the aligned dataset was done using RAxML v. 8.2.12 (Stamatakis 2014). Datasets with unavailable models were analyzed using the GTRCAT model. Each dataset was analyzed using 100 bootstrap values to measure clade support. After pilot phylogenetic analyses, nine plastid regions, that is, *atpB*, *atpF*, *atpF-atpH* spacer, *atpH*, *matK*, *psbA-trnK* spacer, *petD*, *rbcL*, and *rpoc1*, were selected (Table 1) as they had a better phylogenetic resolution. The nine plastid regions were concatenated to form a combined plastid (cp) dataset and used for ML analyses with the best-selected model. The selection of the best substitution model of the combined dataset did not employ the use of partitioning in this analysis. ITS region was also subjected to ML analyses separately since it formed a tree with a poor resolution when combined with the plastid regions.

Table 1. Gene regions used in this study.

Dataset	Gene region	#Seq.	Total seq. length (bp)	Aligned seq. length (bp)	Models
Plastid region	*atpB*†	453	1,402	1,334	GTR+I+G
	atpB-rbcL	350	809	643	TVM+I+G
	atpF†	126	375	360	GTR+G
	atpF-atpH†	169	605	529	GTR+I+G
	atpH †	134	243	235	TVM+I+G
	matK†	466	872	781	TVM+I+G
	ndhF	153	2,177	2,002	GTR+I+G
	psbA-trnH	279	367	263	GTR+I+G
	psbA-trnK†	136	1,264	1,219	GTR+I+G
	petD†	696	889	818	TVM+I+G
	rbcL†	681	1,131	1,076	TVM+I+G
	rpoc1†	187	621	596	TVM+I+G
	trnL-trnF	701	875	743	GTR+I+G
	trnT-trnL	127	1,191	1,114	TVM+I+G
	trnV-trnK	173	654	600	TVM+I+G
	trnK-matK	374	2361	2,155	TVM+I+G
	rpl32-ndhF	250	698	587	TVM+G
	rpl16	402	901	791	GTR+I+G
	Combined	991	7,402	4,826	TMV+I+G
Nuclear	*ITS*	642	669	471	GTR+I+G

† = gene regions that were concatenated to form combined plastid (cp) dataset.
Seq. = Sequences.
#Seq. = Total number of sequences (including outgroups).

Biogeography analyses

Biogeographic analyses were conducted in MESQUITE v. 3.61 (Maddison and Maddison 2019) using the parsimony ancestral states reconstruction method. The biogeographic regions were divided into Africa (Madagascar, tropical, and southern Africa), America (North, Central, and South America), Asia (tropical and temperate Asia), Australasia (Australia and New Zealand), Mediterranean (northern Africa, Cyprus, Sicily, Sardinia, and Crete) and the Pacific Islands (Hawaii, Kaua'i, French Polynesia, Rarotonga, and the Marquesas Islands) according to Chen et al. (2016). The ancestral regions for the outgroups species, Campanulaceae sp. and *Lobelia* sp. were unclear and therefore were not assigned any value (region), however, the reconstruction method employed was set to consider missing and inapplicable data.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.3xsj3txfw.

Results

We accessed eighteen plastid loci and one nuclear gene region of almost all available Campanulaceae species, out of which, nine plastid regions were selected for the combined plastid region datasets. The combined plastid (cp) region dataset included 981 Campanulaceae species, with 298 species from Lobelioideae, which covered almost all Lobelioideae species available in GenBank (Accessed on 1st April 2020) (Table 2).

The combined plastid dataset had representatives from all genera except *Howellia* A.Gray (Gray 1879), *Heterotoma* Zucc. (Zuccarini 1832), *Ruthiella* Steenis (van Steenis 1965), *Dielsantha* E.Wimm. (Wimmer 1948), *Trimeris* C.Presl (Presl 1836), and *Unigenes* E.Wimm. (Wimmer 1948) (Table 3). The interspecific bootstrap (BS) values were quite distinct. The BS value for the *Clermontia*, *Centropogon*, *Burmeistera*, and *Siphocampylus* clades recorded the least BS values. The combined plastid (cp) dataset showcased a better phylogram with a higher sampled taxon and a clearer resolution (Fig. 1) than the nuclear gene phylogeny.

Discussion

Agreements on previous debates

Many scholars have expressed their insights with the existing systematics of the Lobelioideae genera (Lammers 2007a; Givnish 2010; Chen et al. 2016; Knox and Li 2017). The uncertainty in circumscription among different lineages in Lobelioideae has been a

Table 2. List of genera used in this study.

Genus	No. of accepted species	No. of species in this study	References
Apetahia Bail.	4	3	(Baillon 1882)
Brighamia A.Gray	2	1	(Gray 1867)
Burmeistera H.Karst. and Triana	103	28	(Karsten and Triana 1857)
Centropogon C.Presl	215	41	(Presl 1836)
Clermontia Gaudich.	33	18	(Gaudichaud-Beaupré 1829)
Cyanea Gaudich.	85	6	(Gaudichaud-Beaupré 1829)
Delissea Gaudich.	16	1	(Gaudichaud-Beaupré 1829)
Dialypetalum Benth.	6	2	(Bentham 1876)
Diastatea Scheidw.	7	1	(Scheidweiler 1841)
Downingia Torr.	15	5	(Torrey 1857)
Grammatotheca C.Presl	1	1	(Presl 1836)
Hippobroma G.Don	1	1	(Don 1834)
Hypsela C.Presl	–	1	(Presl 1836)
Isotoma Lindl.	13	7	(Lindley 1826)
Legenere McVaugh	1	2	(McVaugh 1943a)
Lithotoma E.B.Knox	–	1	(Knox 2014)
Lobelia L.	437	117	(Linnaeus 1753)
Lysipomia Kunth	35	3	(Kunth 1818)
Monopsis Salisb.	18	5	(Salisbury 1817)
Palmerella A.Gray	2	1	(Gray 1876)
Porterella Torr.	1	1	(Hayden 1872)
Pratia Gaudich.	–	4	(Gaudichaud-Beaupré 1829)
Sclerotheca A.DC.	6	8	(Candolle 1839)
Siphocampylus Pohl	235	32	(Pohl 1827)
Solenopsis C.Presl	7	4	(Presl 1836)
Trematolobelia Zahlbr. ex Rock	8	2	(Rock 1913)
Wimmerella Serra, M.B.Crespo and Lammers	10	2	(Serra et al 1999)

– No accepted species available only synonyms.

result of rapid diversification and divergence of this subfamily approximately 20 million years ago (Knox and Li 2017). After extensive literature search and reviews, we found three main areas that were previously in contention: South African origin of Lobelioideae (Mabberley 1975; Knox and Li 2017), herbaceous habit, and Asian origin of giant lobelioids (Carlquist 1962; Mabberley 1974; Chen et al. 2016), and the convergent evolution of giant rosette (perennial monocarpic herbs mostly occurring in alpine and subalpine bogs) lobelioids (Antonelli 2009; Givnish 2010). Currently, agreements regarding these contentions appear to have been reached and are in accord with our analyses.

The geographical origin of the Lobelioideae had been a point of contention, with different scholars having varying biogeographic theories. Mabberley (1975) suggested a South American origin of lobelioids. Mabberley (1975) postulated that the South American pachycaul lobelioids gave rise to plants, which spread to Chile and the Caribbean (*Lobelia* § *Tylomium* (C.Presl) Benth. (Bentham 1876)), Hawaii (*Trematolobelia*) and Brazil (*Lobelia* § *Rhynchopetalum* (Fresen.) Benth. (Bentham 1876)). He added that the rise of winged seeds in Hawaii permitted the inter-island spread of lobelioids and in Brazil, it allowed the *Lobelia* § *Rhynchopetalum* to travel to Africa. However, Knox et al. (1993, 2008a) stated that the South American species are mixed assemblage, possibly involving pantropical dispersal events. Knox et al. (2006), Chen

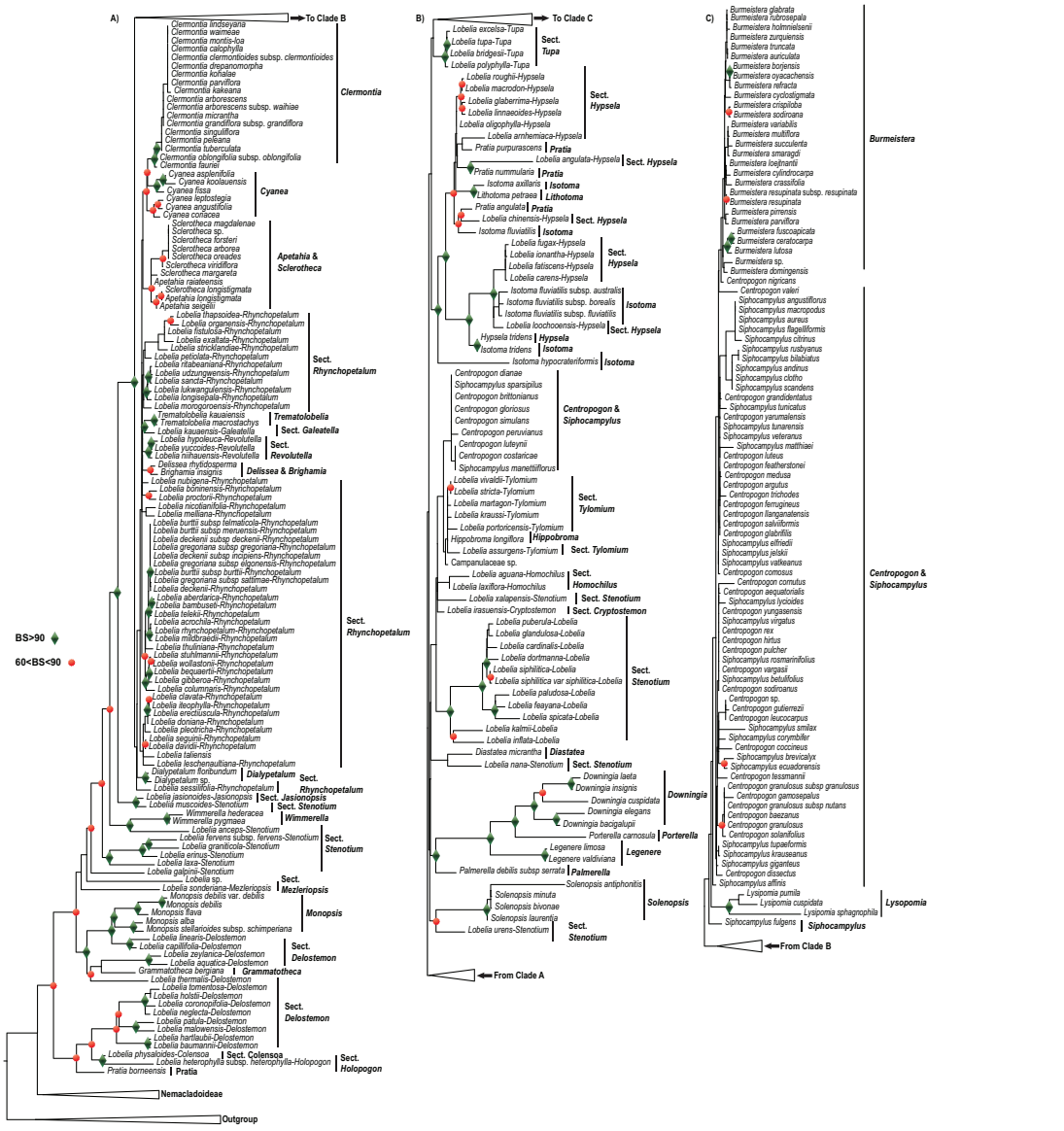

Figure 1. Phylogeny, genera, and bootstrap values of Lobelioideae using combined plastid (cp) regions dataset.

et al. (2016), and Knox and Li (2017) concluded Lobelioideae originated from South Africa and underwent multiple cosmopolitan radiation events. Our results supported the 'Out of Africa' hypothesis and multiple cosmopolitan radiations of Lobelioideae, which corroborated Antonelli (2009), Chen et al. (2016), and Knox and Li (2017).

The ancestral habit type and origin of the giant Lobelioids have been in the limelight for years. Carlquist (1962, 1969), using wood anatomy, suggested an herbaceous

Table 3. Classification and biogeography of Lobelioideae (Campanulaceae).

Genus	Sections (§)	No. of Species		Monophyletic				Ancestral region		
(Crowl 2016)	(Lammers 2011)	(Lammers 2011)	Current	Lammers (2011)	Antonelli (2008)	Chen (2016)	Current	Lammers (2011)	Chen (2016)	Current
Lobelia	*Holopogon*	14	1	Yes	No	–	–	Australia	Australasia	Australasia
	Colensoa	1	1	–	No	–	–	New Zealand	Australasia	Australasia
	Delostemon	44	14	Yes	–	No	No	S. Africa, T. Africa, S.E Asia	Africa	Africa, Asia, America
	Mezleriopsis	7	1	Yes	No	–	–	Africa	Africa	Africa
	Stenotium	144	10	Yes	Yes	No	No	Africa (Tropical and Southern), Med, America (North and South), S.E Asia	Africa, Med, America	Africa, Med, America
	Jasionopsis	1	1	Yes	–	–	–	Africa	Africa	Africa
	Rhynchopetalum	61	48	Yes	No	No	No	S.E Asia, T. Africa, S. America, Asian Islands	Asia, P. Islands, Africa, America	Asia, America, Africa
	Revolutella	9	3	Yes	–	Yes	Yes (H)	Hawaii	P. Islands	P. Islands
	Lobelia	22	11	Yes	No	No	Yes (H)	N. America	America	America
	Cryptostemon	9	1	Yes	–	Yes	–	America	America	America
	Homochilus	5	2	Yes	Yes	–	Yes (L)	America	America	America
	Tylomium	38	6	Yes	–	No	No	N. America	America	America
	Hypsela	43	13	Yes	No	No	No	S.E Asia, Australasia, S. America	America, Australasia, Asian Islands, Asia	Australasia, Asia, America
	Tupa	4	4	Yes	Yes	Yes	Yes (H)	S. America	America	America
	Galeatella	5	1	Yes	–	Yes	–	Hawaii	–	–
	Plagiobotrys	1	–	Yes	–	–	–	Malesia	–	–
	Trimeris	1	–	Yes	–	–	–	St. Helena	–	–
	Speirema	5	–	Yes	–	–	–	S.E Asia	–	
Pratia	–	13	4	–	–	–	No	–	–	Australasia
Grammatotheca	–	1	1	–	–	–	–	–	Africa	Africa
Monopsis	–	20	5	–	–	Yes	Yes (H)	–	Africa	Africa
Wimmerella	–	10	2	–	–	–	Yes (H)	–	Africa	Africa
Dialypetalum	–	6	2	–	–	–	Yes (H)	–	–	Africa
Delissea	–	10	1	–	–	Yes	–	–	P. Islands	P. Islands
Brighamia	–	2	1	–	–	Yes	–	–	P. Islands	P. Islands
Trematolobelia	–	4	2	–	–	Yes	Yes (H)	–	P. Islands	P. Islands
Apetahia	–	4	2			No	No	–	P. Islands	P. Islands
Sclerotheca	–	10	8	–	–	Yes	No	–	P. Islands	P. Islands
Cyanea	–	70	6	–	Yes	No	Yes (H)	–	P. Islands	P. Islands
Clermontia	–	22	18	–	–	No	Yes (H)	–	P. Islands	P. Islands

Genus	Sections (§)	No. of Species		Monophyletic				Ancestral region		
(Crowl 2016)	(Lammers 2011)	(Lammers 2011)	Current	Lammers (2011)	Antonelli (2008)	Chen (2016)	Current	Lammers (2011)	Chen (2016)	Current
Solenopsis	–	10	4	–	–	Yes	Yes (H)	–	Europe	Mediterranean
Downingia	–	13	5	–	Yes	Yes	Yes (H)	–	America	America
Legenere	–	2	2	–	–	–	Yes (H)	–	America	America
Palmerella	–	2	1	–	–	–	–	–	America	America
Porterella	–	1	1	–	–	–	–	–	America	America
Diastatea	–	6	1	–	–	–	–	–	America	America
Hippobroma	–	1	1	–	–	–	–	–	America	America
Isotoma	–	12	7	–	–	No	No	–	Australasia	Australasia
Hypsela	–	1	1	–	–	–	–	–	–	Australasia
Lithotoma	–	1	1	–	–	–	–	–	–	Australasia
Lysipomia	–	40	3	–	Yes	Yes	Yes (H)	–	America	America
Siphocampylus	–	220	32	–	Yes	No	No	–	America	America
Burmeistera	–	102	28	–	Yes	Yes	Yes (L)	–	America	America
Centropogon	–	49	41	–	Yes	No	No	–	America	America
Howellia	–	1	–	–	–	–	–	–	America	–
Heterotoma	–	1	–	–	–	–	–	–	–	–
Ruthiella	–	4	–	–	–	–	–	–	–	–
Trimeris	–	1	–	–	–	–	–	–	–	–
Unigenes	–	1	–	–	–	–	–	–	–	–
Dielsantha	–	1	–	–	–	–	–	–	–	–

–, unknown or uncertain.

L = Bootstrap value <60. M = 60 ≤ BS < 90. H = BS ≥90.

P. Islands = Pacific Islands (Hawaii, French Polynesia, Rarotonga, and the Marquesas Islands). Med = Mediterranean (N. Africa, Cyprus, Sicily, Sardinia, and Crete). Australasia = (Australia and N. Zealand). Asia = (Tropical and Temperate Asia). Africa = (Madagascar, Tropical, and S. Africa). America = (North, Central, and South America).

origin of giant lobelioids. However, Mabberley (1974, 1975) challenged the above sentiment and suggested that the herbaceous species of lobelioids have been derived from large, thick-stemmed ancestors (*Lobelia* § *Rhynchopetalum* and *Lobelia* § *Tylomium*). He added that the herbaceous habit of lobelioids is an advanced character. Knox et al. (1993), using cpDNA restriction sites and inversions, supported Carlquist's (1962) hypotheses of herbaceous ancestry. Givnish (2000), based on molecular phylogenetic analysis, showed that the ancestor of the Hawaiian lobelioids was most likely woody, corroborating Mabberley's (1974, 1975) proposals. Also, Givnish et al. (2009) hypothesized that an Asian group – represented by the placeholder *Lobelia nicotianifolia* Roth (Roth 1821) – might have the ancestral stock from which both Pacific and African giant lobelioids had evolved. Most recently, Chen et al. (2016) confirmed (1) the herbaceous habit of lobelioids ancestors, and (2) the Asian origin of giant lobelioids. Knox and Li (2017) corroborated Chen et al. (2016) and added that extant Hawaiian/Pacific and Brazilian/African giant lobelioids are derived from herbaceous giant lobelioids (Knox and Li 2017). Our results corroborated that of Chen et al. (2016) and Knox and Li (2017), in which, herbaceous ancestry of giant rosette lobelioids was well illustrated.

The Hawaiian lobelioids form a remarkable clade, encompassing more species than any other plant clade restricted to a single oceanic island or archipelago, and their geographic source has been hotly debated (Givnish et al. 1994, 2009; Givnish 1995). They have long been viewed as one of the most spectacular cases of adaptive radiation in plants on oceanic islands (Carlquist et al. 1965; Carlquist 1974; Lammers 1990; Givnish et al. 1994, 2004; Givnish 1995; Givnish 1998; Givnish and Montgomery 2014). Wimmer (1953) and Mabberley (1974, 1975) postulated that fleshy-fruited genera are a product of a single colonization event while capsular-fruited taxa are products of more than one colonization event. However, Givnish (2000), using molecular phylogenetic analysis, illustrated that Hawaiian lobelioids are instead a product of a single immigration event. Antonelli (2009) suggested that the Hawaiian and African giant lobelioids appeared to have evolved from a single common ancestor. However, Givnish (2010) refuted those claims and argued that the giant rosette lobelioids are an exemplar of convergent evolution rather than single common ancestry. Our analysis corroborated one of Givnish's (2010) illustrations on lobelioids' convergent evolution theory, that is, compared to the rest of lobelioids species, only a minority number of species (*Lobelia* § *Rhynchopetalum*) have the giant rosette growth form adapted to alpine or mountain conditions, with non-rosette species forming the remainder of the clade. These 'rosette-species' are embedded within the non-rosette species (Fig. 1), a clear indication that indeed the giant rosette lobelioids are a result of convergent evolution. Knox and Li (2017) also used maximum-likelihood analyses of whole plastomes to conclude that the giant African lobelioids (including some descendants in South America) were sister to the Pacific giant lobeliads as a whole and with *Lobelia boninensis* Koidz. (Nakai 1920), from the Bonin Islands, then *Apetahia*/*Sclerotheca* from the Society Islands and the Cook Islands forming a sister to the Hawaiian lobelioids, and then all of them forming a sister to some Asian giant lobelioids, corroborating the proposal by Givnish et al. (2004). Our combined plastid data ties together *Delissea-Brighamia*,

Trematolobelia, *Lobelia* § *Galeatella*, and *Lobelia* § *Revolutella*, all from Hawaii, closely related to giant African and South American lobelioids.

In addition, Knox and Li (2017) summarized the cosmopolitan radiation of lobelioids in four out-of-Africa dispersal scenarios. (1) The biogeographic pattern of South African species relative to lobelioids elsewhere in the world maps Lobelioideae ancestry to the modern-day Western Cape Province (Knox and Li 2017). *Lobelia anceps* L.f. (Linnaeus 1782), for instance, originated from South Africa and subsequently dispersed to many other southern hemisphere sites, including New Zealand (Knox et al. 2006). Madagascar acted as a stepping-stone to eastern Asia where the robust, herbaceous, hemicryptophyte growth form evolved from (Knox and Li 2017). (2) The Amphi-tropical dispersal from the Western Cape to the Mediterranean region did occur and with rapid subsequent dispersal to the North America region (Knox and Li 2017). (3) The dispersal from South Africa to South America stood the greatest likelihood of success if the initial colonization occurred at a similar latitude with a similar habitat (Knox and Li 2017). The circumscription of *Lobelia xongorolana* E.Wimm. (Wimmer 1935) (Endemic in Angola) as the sister lineage to the Brazilian species (Mabberley 1974), would implicate Angola as a stepping-stone in dispersal to Brazil, whereas a true sister-species relationship with *Lobelia stricklandiae* would suggest that dispersal to Brazil originated from East Africa and that dispersal to Angola was a separate event (Knox and Li 2017). (4) Successful colonization of Australia from South Africa also would have been favored by latitudinal and habitat similarity. The Western Australia endemic *Isotoma hypocrateriformis* Druce (Druce 1917), is sister to the remaining species in this predominantly Australasian clade that subsequently diversified in most Australian habitats, dispersed on three separate occasions to New Zealand (Knox et al. 2008b; Heenan et al. 2008), and dispersed twice to eastern Asia (Knox and Li 2017).

Phylogeny and biogeography of lobelioideae

Lobelioideae consisted of up to 31 genera (Knox et al. 2008a). However, through our extensive literature review, we found a total of 33 currently documented genera (Table 3). We sampled 27 out of the 33 Lobelioideae genera in our combined plastid (cp) dataset (for easy understanding, subsequent discussion part is based on the combined (cp) plastid tree (Fig. 1; Fig. 2) and the ITS region tree. Our analyses found ten monophyletic Lobelioideae genera, that is, *Monopsis*, *Wimmerella*, *Dialypetalum*, *Clermontia*, *Solenopsis*, *Legenere*, *Downingia*, *Burmeistera*, *Lysipomia*, and *Trematolobelia*, three paraphyletic genera, that is, *Apetahia*, *Sclerotheca*, and *Cyanea*, and lastly, five polyphyletic genera, that is, *Lobelia*, *Pratia*, *Centropogon*, *Siphocampylus*, and *Isotoma*. *Grammatotheca*, *Delissea*, *Brighamia*, *Palmerella*, *Porterella*, *Diastatea*, *Hypsela*, *Hippobroma*, and *Lithotoma* had only one representative in each genus. *Pratia borneensis* Hemsl. (Hemsley 1886), *Lobelia physaloides* A.Cunn. (Cunningham 1838), *Lobelia heterophylla* subsp. *heterophylla*, and some members of the *Lobelia* § *Delostemon* (E.Wimm.) J.Murata (Murata 1995) formed the basal group of Lobelioideae with a BS value of 83 (Fig. 1).

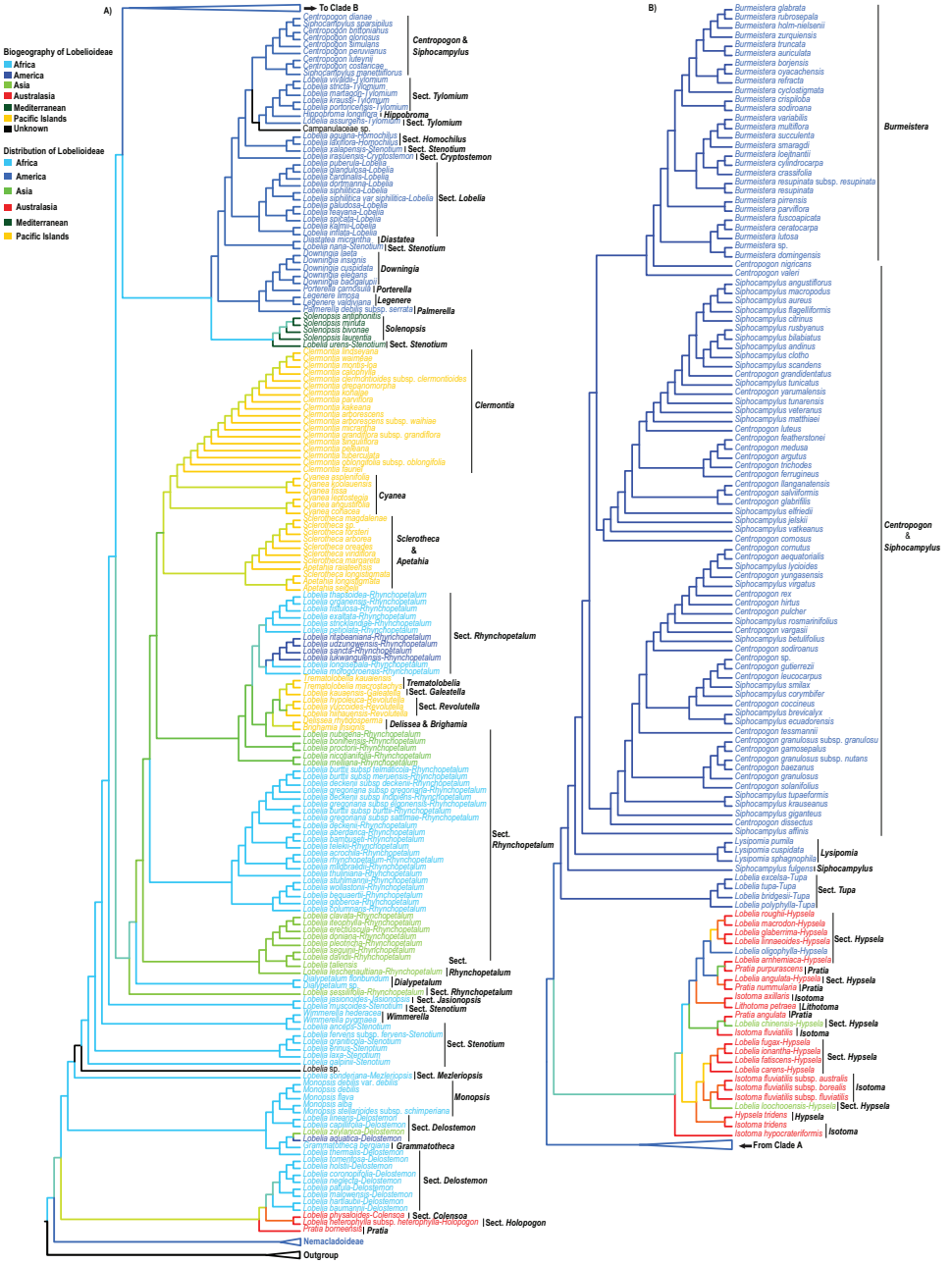

Figure 2. Biogeography of Lobelioideae combined plastid (cp) region datasets using parsimony ancestral state reconstruction. Taxa color coding represents the geographical distribution of the species.

Lobelioideae originated from Africa and this corroborated Knox et al. (2006) and Knox and Li (2017). Nemacladoideae forms a sister to the Lobelioideae group. This group is endemic to North America. However, their position and endemicity in North

America do not affect the African origin of Lobelioideae (Fig. 2). Knox et al. (2006) stated that the removal and/or inclusion of *Cyphia* clade (Endemic to Africa) as the sister to Lobelioideae would not interfere with the African origin of this subfamily, and this corroborated with our biogeographic results. Besides, it is also evident that multiple dispersal events occurred in this subfamily. The basal group consisted of species with their ancestral region in Australasia and Africa. The African group nested some species from Asia, America, and the Pacific Islands. This depicted possibilities of long-distance dispersal and diversification events in some species.

The following is a discussion of specific genera within the Lobelioideae subfamily. The order of the discussion is according to the positioning of the genera in the phylogram, starting from the basal position (Fig. 1). ***Pratia*** is polyphyletic. *Pratia borneensis* is a sister (BS = 83) to a clade formed by *Lobelia physaloides*, *L. heterophylla* subsp. *heterophylla* and seven members from the *Lobelia* § *Delostemon*. *Pratia angulata* Hook.f. (Hooker 1844), forms a clade with *Lobelia chinensis* Lour. (de Loureiro 1790) with a BS value of 86. *Pratia nummularia* A.Braun & Asch. (Braun 1861), on the other hand forms a clade with *Lobelia angulata* with a BS value of 100, while *Pratia purpurascens* (R.Br.) E.Wimm. (Wimmer 1953), forms a clade with *Lobelia arnhemiaca* E.Wimm. (Wimmer 1948) with a BS value of 61 (Fig. 1). Biogeography: Murray et al. (2004) described New Zealand as the ancestral region of *Pratia*. This was also echoed by Knox et al. (2008b) in their work on the phylogenetic position of *Lobelia glaberrima* Heenan (Heenan et al. 2008), in New Zealand. Our analysis placed this genus in Australasia as the ancestral region (Fig. 2).

Grammatotheca has only one species, *Grammatotheca bergiana* C.Presl (Presl 1836). It is nested within some members of the *Lobelia* § *Delostemon* with a BS value of 62 (Fig. 1). Our results confirmed that of Antonelli (2009) and Chen et al. (2016). Biogeography: Knox et al. (2006) indicated South Africa as the origin of this genus. Chen et al. (2016) also placed *Grammatotheca* in Africa. This genus is embedded within *Lobelia* § *Delostemon* clade which has its ancestral region in Africa. Knox et al. (2006) stated that holopogonoid *Lobelia* gave rise to this particular genus which diversified in South Africa and was later introduced to Australia via hay shipped with cattle from South Africa. Our results corroborate the above-mentioned studies and placed this genus in Africa as its ancestral area (Fig. 2).

Monopsis forms a monophyletic group with a BS value of 91. It forms a clade with members of the *Lobelia* § *Delostemon* with a BS value of 100 (Fig. 1). This result corroborates that of Chen et al. (2016). Biogeography: Phillipson (1989) indicated Cape Province, South Africa as the ancestral region of *Monopsis*. Knox et al. (2006) also indicated South Africa as the ancestral region of this genus. Our result placed this genus in Africa as its ancestral region (Fig. 2).

Wimmerella forms a clade with a BS value of 100. They form a sister to *Lobelia anceps* L.f. (Linnaeus 1782) with a BS value of 100 (Fig. 1). This result corroborates that of Knox and Li (2017) and Chen et al. (2016). Biogeography: Knox et al. (2006) placed this genus in Western Cape, South Africa as its ancestral region. Chen et al. (2016) and Knox and Li (2017) corroborated Knox et al. (2006) results. Our analysis corroborates the above-mentioned studies and placed this particular genus in Africa (Fig. 2).

Delissea and ***Brighamia*** form a clade with a BS value of 86, a result similar to that of Murata (1995), Antonelli (2008), Givnish et al. (2009), Chen et al. (2016), and Knox and Li (2017). Biogeography: These genera were placed in Kaua'i or some older island as their ancestral region (Givnish et al. 1994, 2004, 2009; Givnish 1995). Our results place these genera in the Pacific Islands as their ancestral area which corroborates both Givnish et al. (2004) and Knox and Li (2017).

Trematolobelia forms a clade with a BS value of 99 (Chen et al. 2016) (Fig. 1). This clade forms a sister to *Lobelia kauaensis* (A.Gray) A.Heller (Heller 1897), with a BS value of 90. Biogeography: Givnish (1998) placed *Trematolobelia* on Kaua`i as its ancestral area. Givnish et al. (2009) indicated the Hawaiian archipelago as the ancestral region of this particular genus. Our analysis placed this genus in the Pacific Islands as its ancestral area which corroborates the above-mentioned studies (Fig. 2).

Apetahia and ***Sclerotheca*** form a clade with a BS value of 64. This result confirms that of Givnish et al. (2009) and Chen et al. (2016). We sampled only three *Apetahia* and eight *Sclerotheca* species. The low BS value might have been a result of incomplete sampling in these two genera. *Sclerotheca margaretae* F.Br. (Brown 1935), *Sclerotheca viridiflora* Cheeseman (Cheeseman 1903), *Sclerotheca oreades* E.Wimm. (Wimmer 1948), *Sclerotheca arborea* DC. (Candolle 1839), *Sclerotheca forsteri* Drake (Del Castillo 1892), and *Sclerotheca magdalenae* J.Florence (Florence 1996) form a clade with a BS value of 60. Biogeography: Chen et al. (2016) placed these genera in French Polynesia as their ancestral area. Knox and Li (2017) indicated the ancestral location of *Apetahia longistigmata* (F.Br.) E.Wimm. (Wimmer 1948) to be in Marquesas and *S. viridiflora* to be in Rarotonga, both in the South Pacific Islands. Our results placed the genera in the Pacific Islands as their ancestral area which concur with the above-mentioned studies.

Cyanea forms a paraphyletic group (Fig. 1). This result corroborates with Antonelli (2009), Chen et al. (2016), and Hunter (2018). *Cyanea aspleniifolia* Hillebr. (Hillebrand 1888), *Cyanea koolauensis* Lammers, Givnish and Sytsma (Lammers et al. 1993), and *Cyanea fissa* Hillebr. (Hillebrand 1888) form a clade with a BS value of 98 that is sister to *Clermontia* (BS=77) (Fig. 1). ***Clermontia*** forms a clade (Givnish et al. 2009, 2013; Chen et al. 2016) with a BS value of 90 (Fig. 1). More broadly, Hunter (2018) used phylogenomic data from hundreds of single-copy nuclear genes and whole plastomes to infer that most of *Clermontia* are sister to the purple-fruited clade of *Cyanea* (see Givnish et al. 1994; Givnish 1995), with the orange-fruited clade of *Cyanea* sister to both. Biogeography: Givnish et al. (1994, 2009, 2013) placed the origins of both *Clermontia* and *Cyanea* on Kaua`i or some older island. Chen et al. (2016) and Knox and Li (2017) placed these two genera in the Hawaiian Islands as their ancestral region. Our results placed the genera in the Pacific Islands as their ancestral area, corroborating the above-mentioned studies (Fig. 2).

Solenopsis is monophyletic with a BS=100. *Lobelia urens* L. (Linnaeus 1753) (*Lobelia* § *Stenotium* (C.Presl) Lammers (Lammers 2011)) formed a sister to *Solenopsis* with the BS value of 87 (Fig. 1). Our results corroborate that of Knox and Li (2017). Biogeography: Crespo et al. (1998) indicated the Mediterranean as the ancestral region

of this genus. Knox and Li (2017) also indicated the Mediterranean as the ancestral area for *Solenopsis*. Our result corroborates that of Crespo et al. (1998) and Knox and Li (2017) and places this genus in the Mediterranean region as its ancestral region (Fig. 2).

Downingia formed a monophyletic clade with a BS value of 96. ***Porterella*** is sister to *Downingia* with a BS value of 98. This result corroborates Chen et al. (2016). ***Legenere*** *limosa* (Greene) McVaugh (McVaugh 1943a), and *Legenere valdiviana* (Phil.) E.Wimm. (Wimmer 1953) form a clade (BS=100), which is a sister to *Downingia* and *Porterella*. ***Palmerella*** forms a sister to *Downingia*, *Porterella*, and *Legenere* with a BS of 90 (Fig. 1). Biogeography: McVaugh (1941) indicated western North America as the ancestral area of *Downingia*. Schultheis (2001a, b) corroborated McVaugh (1941). Chen et al. (2016) placed *Downingia*, *Porterella*, and *Legenere* in North America. Our analysis places the genera in North America as their ancestral region (Fig. 2).

Diastatea is clustered with *Lobelia nana* Kunth (Kunth et al. 1976) with a BS value of 62 (Fig. 1). However, our phylogenetic results contradicted that of Chen et al. (2016) that appeared to form a clade with *Solenopsis*. *Diastatea* was differentiated from genus *Lobelia* by two main features: a superior ovary, and a persistent corolla lacking a dorsal fissure (McVaugh 1940). Albeit these characters have been used to separate the two genera, some species in the *Lobelia* § *Stenotium* (featured by partially inferior to the superior ovary) possess the same characteristics e.g. *Lobelia xalapensis* Kunth (Kunth et al. 1976), *L. nana*, and *Lobelia diastateoides* McVaugh (McVaugh 1940). Biogeography: Knox et al. (2008a) placed the genus in South America as the ancestral area. Chen et al. (2016) also indicated America as the ancestral area of *Diastatea*. Our biogeographic results corroborate both Knox et al. (2008a) and Chen et al. (2016) and place the genus in South America as its ancestral area.

Hippobroma is monotypic and is nested within members of the *Lobelia* § *Tylomium* (Fig. 1). This result corroborates Chen et al. (2016). Biogeography: Knox and Li (2017) indicated Mexico as the ancestral region of *Hippobroma*. The ancestral area of § *Tylomium*, which nestles this genus, is in North America (Lammers 2011). This genus might have resulted following morphological diversification. This corroborates Chen et al. (2016) that placed the genus in Central America. Our result places the genus in Central America as its ancestral area which corroborates the above-mentioned studies.

Isotoma is polyphyletic. *Isotoma hypocrateriformis* Druce (Druce 1917), is sister to *P. angulata*, *P. nummularia*, *P. purpurascens*, ***Hypsela***, ***Lithotoma***, and some *Lobelia* species belonging to the *Lobelia* § *Hypsela* (C.Presl) Lammers (Lammers 2011), with a BS value of 96. All these genera are from the Australasian region except for *Lobelia loochooensis* Koidz. (Koidzumi 1929), and *L. chinensis* that are from Southeast Asia and *Lobelia oligophylla* (Wedd.) Lammers (Lammers 1999), from South America. *Isotoma tridens* (E.Wimm.) Lammers (Lammers 1999), forms a clade with *Hypsela tridens* E.Wimm. (Wimmer 1943) with a BS value of 100. *Isotoma fluviatilis* F.Muell. ex Benth. (Bentham and Mueller 1869) is sister to *L. chinensis* and *P. angulata* with a BS value of 81. *Isotoma axillaris* Lindl. (Lindley 1826) forms a clade with *Lobelia petraea* with a BS value of 91 (Fig. 1). According to Givnish et al. (2009), *Isotoma* formed a sister to the tropical American taxa. Our results corroborate that of Chen et al. (2016).

Biogeography: Bussell et al. (2002) and Chen et al. (2016) placed *Isotoma* in Australia as its ancestral region. Knox and Li (2017) placed *Hypsela* and *Lithotoma* in Australasia as their ancestral region. Our results place *Isotoma*, *Lithotoma*, and *Hypsela* in Australasia as their ancestral area which corresponds to the above-mentioned studies (Fig. 2).

Lysipomia *pumila* (Wedd.) E.Wimm. (Wimmer 1937), *Lysipomia cuspidata* McVaugh (McVaugh 1955), and *Lysipomia sphagnophila* Griseb. (Lechler 1857) forms a clade with a BS value of 100 (Fig. 1). This result is consistent with that of Antonelli (2008) and Chen et al. (2016). Biogeography: McVaugh (1955), in his revision of *Lysipomia*, indicated South America as the ancestral area of this particular genus. Knox and Li (2017) stated diversification in S. America generated *Lysipomia*. Our results placed *Lysipomia* in South America as its ancestral region which corroborates McVaugh (1955) (Fig. 2).

Siphocampylus and ***Centropogon*** are polyphyletic and intercalates with each other, albeit their statistical support values are low (<50) (Fig. 1). *Centropogon dianae* Lammers (Lammers 1998), *Siphocampylus sparsipilus* E.Wimm. (Wimmer 1924), *Centropogon brittonianus* Zahlbr. (Zahlbruckner 1897), *Centropogon gloriosus* Zahlbr. (Zahlbruckner 1897), *Centropogon simulans* Lammers (Lammers 1998), *Centropogon peruvianus* (E.Wimm.) McVaugh (McVaugh 1949a), *Centropogon luteynii* Wilbur (Wilbur 1977), *Centropogon costaricae* (Vatke) McVaugh (McVaugh 1943a), and *Siphocampylus manettiiflorus* Hook. (Hooker 1848) forms an early clade with the members of the *Lobelia* § *Tylomium*. *Centropogon nigricans* Zahlbr. (Zahlbruckner 1915) is sister to the *Burmeistera* clade (Fig. 1). ***Burmeistera*** forms a clade with a low support value (BS=43) (Fig. 1). The low BS values for *Burmeistera* and *Siphocampylus* may be due to inadequate taxon sampling (Uribe-Convers et al. 2017). Biogeography: The ancestral areas for *Centropogon*, *Burmeistera*, and *Siphocampylus* are in South America (Antonelli 2009; Knox and Li 2017; Uribe-Convers et al. 2017). Our analysis placed these genera in S. America which corroborates the above-mentioned (Fig. 2).

Sections within genus *Lobelia*

Lobelia is the 'core genus' among members of the Lobelioideae group (Knox et al. 2006). Lammers (2011) classified this genus into eighteen sections based on morphological characteristics. Our analysis included fifteen out of the eighteen sections: four monophyletic, five polyphyletic, one paraphyletic and five had only one representative each. The unsampled sections were *Speirema* (Hook.f. and Thomson) Lammers (Lammers 2010), *Trimeris* (C.Presl) A.DC. (Candolle 1839), and *Plagiobotrys* Lammers (Lammers 2010), (Table 2).

Lobelia § ***Holopogon*** Benth. (Bentham and Mueller 1869). This section had only one out of fourteen species (Lammers 2011) sampled in a combined plastid dataset, that is, *Lobelia heterophylla* subsp. *heterophylla*. It occurred at the basal position of the phylogram and formed a clade with *L. physaloides* with a BS value of 92 (Fig. 1). Our analysis corroborated that of Antonelli (2008) and Knox et al. (2006). Biogeography: Lammers (2011) indicated Australia as the ancestral area of *L. heterophylla* subsp.

heterophylla. Our biogeographic results corroborate the above-mentioned studies and place this species in Australasia as its ancestral area (Fig. 2).

Lobelia* § *Colensoa (Hook.f.) J.Murata (Murata 1995). Only one species was sampled in the combined plastid dataset. According to Lammers (2011), this section is monotypic. *Lobelia physaloides* forms a sister clade with *L. heterophylla* subsp. *heterophylla* (§ *Holopogon*) with a bootstrap value of 92 (Fig. 1). In the ITS phylogram, it is embedded between the *Lobelia* § *Stenotium* and *Lobelia* § *Delostemon*. Biogeography: Lammers (2011) placed this section in New Zealand's North Island as the ancestral location. Our results place this section in Australasia as its ancestral region which corroborates Lammers (2011).

Lobelia* § *Delostemon. This section is paraphyletic. We sampled fourteen out of forty-four species recorded by Lammers (2011) in our combined plastid dataset. *Lobelia baumannii* Engl. (Engler 1894), *Lobelia hartlaubi* Buchenau (Buchenau 1881), *Lobelia malowensis* E.Wimm. (Wimmer 1948), *Lobelia patula* L.f. (Linnaeus 1782), *Lobelia neglecta* Roem. and Schult. (Roemer and Schultes 1819), *Lobelia coronopifolia* L. (Linnaeus 1753), *Lobelia holstii* Engl. (Engler 1894), and *Lobelia tomentosa* L.f. (Linnaeus 1782) form a clade with a BS = 84. *Lobelia thermalis* Thunb. (Thunberg 1794) is sister to a clade of *Lobelia aquatica* Cham. (Chamisso 1833) and *Lobelia zeylanica* L. (Linnaeus 1753) with a BS value of 88. However, this clade is intercalated by *Grammatotheca*. *Lobelia capillifolia* A.DC. (Candolle 1839) and *Lobelia linearis* Thunb. (Thunberg 1794) form a clade with a BS value of 97 and is sister to *Monopsis* with a BS value of 100 (Fig. 1). Our result corroborates that of Chen et al. (2016), Antonelli (2008), and Knox et al. (2006). Antonelli (2008) clustered *Grammatotheca* and *L. aquatica* together and further indicated the similarities between them, that is, both are slender annual herbs and have smaller dorsal corolla lobes. These similarities are also observed in *Monopsis debilis* (L.f.) C.Presl (Presl 1836), (Phillipson 1986). Lammers (2011) described plants in the *Lobelia* § *Delostemon* as perennial with prostrate, decumbent, and ascending stems, sessile or petiolate leaves, bilabiate corolla, and capsular fruit. These features are also found in *Monopsis alba* Phillipson (Phillipson 1986), *Monopsis simplex* (L.) E.Wimm. (Wimmer 1948), and *Monopsis stellarioides* Urb. (Urban 1881), (Phillipson 1986). Antonelli (2008) suggested that if a cladistic approach of classification were to be observed strictly, then *Grammatotheca* and *Monopsis* would have been placed under this section. A suggestion that is highly supported by our analysis. Biogeography: Our analysis indicates Africa as the ancestral area of this section. However, it embeds two species; *L. aquatica* and *L. zeylanica* which were placed in South America and Southeast Asia respectively. Our results corroborated that of Antonelli (2009) and Lammers (2011).

Lobelia* § *Mezleriopsis Lammers (Lammers 2011). This section had only one out of seven species (Lammers 2011) sampled in the combined plastid dataset. *Lobelia sonderiana* (Kuntze) Lammers (Lammers 1999) forms a sister to the remaining members of the Lobelioideae group except for *Grammatotheca*, *Monopsis*, *Lobelia* § *Delostemon*, *Lobelia* § *Colensoa*, *Lobelia* § *Holopogon*, and *P. borneensis* (BS = 56) (Fig. 1). This result corroborates Antonelli (2008). Biogeography: Lammers (2011)

indicated the ancestral area of this section to be in South Africa with *L. sonderiana* extending up to Kenya. This corroborates with our results and places Africa as the ancestral region of this section (Fig. 2).

Lobelia* § *Stenotium. We sampled ten species out of a hundred and forty-four proposed by Lammers (2011) in our combined plastid dataset. This section is polyphyletic. *Lobelia laxa* McOwan (MacOwan 1890), *L. erinus*, *Lobelia graniticola* E.Wimm. (Wimmer 1948), and *Lobelia fervens* Thunb. (Thunberg 1794) formed a clade with a BS value of 100. *Lobelia anceps* (BS = 100) is sister to a clade of *Wimmerella pygmaea* (Thunb.) Serra M.B. Crespo and Lammers (Serra et al 1999) and *Wimmerella hederacea* (Sond.) Serra and Lammers (Serra et al 1999). *Lobelia muscoides* Cham. (Chamisso 1833) forms a clade with *Lobelia jasionoides* (A.DC.) E.Wimm. (Wimmer 1943) with a BS value of 100. *Lobelia urens* forms a sister to *Solenopsis* with a BS value of 87. *Lobelia nana* and *Diastatea* form a clade with a BS value of 62 (Fig. 1). According to our phylogenetic analysis, this section appears to be polyphyletic, a suggestion that corroborates Antonelli (2008) and Knox et al. (2006). More so, *Solenopsis*, just like *Wimmerella*, has corolla completely fused (Knox et al. 2006). Lammers (2011) suggested the inclusion of *Wimmerella* in this section. A suggestion that is well supported by our phylogenetic analysis. Biogeography: Our analysis placed the ancestral area of this section in Africa. However, *L. nana* and *L. xalapensis* have their ancestral areas in South America while *L. urens* has its ancestral region in the Mediterranean. Our results corroborated that of Lammers (2011).

Lobelia* § *Jasionopsis Lammers (Lammers 2011). Only one sample was analyzed in our combined plastid dataset. This section is monotypic. The sampled species included *L. jasionoides* which form a clade with *L. muscoides* (§ *Stenotium*) with a BS value of 100 (Fig. 1). This corroborates Knox and Li (2017). Biogeography: Lammers (2011) described this species as endemic to the Cape provinces of South Africa. Chen et al. (2016) placed it in Africa as its ancestral area. Knox and Li (2017) corroborated both analyses. Our result places Africa as its ancestral region (Fig. 2) which corroborates the above-mentioned studies. The close relationship between *L. jasionoides* and *L. muscoides*, both statistically (BS = 100) and geographically (both in S. Africa), suggest a biphyletic nature of this section and/or the inclusion of *L. jasionoides* in *Lobelia* § *Stenotium*.

Lobelia* § *Rhynchopetalum (Giant Lobelioids/Rosettes). We sampled forty-eight out of the sixty-one species (Lammers 2011) in our combined plastid dataset. This section is polyphyletic. *Dialypetalum*, *Brighamia*, *Delissea*, *Trematolobelia*, and *Lobelia* § *Revolutella* E.Wimm. (Wimmer 1948) are all embedded within this section. *Lobelia sessilifolia* Lamb. (Lambert 1811) is sister to the members of this section however with a low support value of 26 (Fig. 1). This corroborates Chen et al. (2016), Crowl et al. (2016), and Knox and Li (2017) that the ancestor to giants lobelioids might have its ancestral region in S.E Asia. Biogeography: Lammers (2011) described this section as almost pantropical with species in three disjunct areas, that is, Southeast Asia, tropical Africa, and South America. According to our results, species with S.E. Asia as their ancestral region formed the basal group of this section. *Lobelia sessilifolia* forms a sister to the members of this section. Our analysis placed this taxon in S.E. Asia as its ancestral

area, corroborating Chen et al. (2016) that the ancestor of the giant lobelioids could have been from S.E Asia. Our result corroborates that of Lammers (2011), Chen et al. (2016), and Knox and Li (2017) (Fig. 2).

Lobelia* § *Revolutella. We sampled three species out of nine (Lammers 2011): *Lobelia niihauensis* St.John (John 1931), *Lobelia yuccoides* Hillebr. (Hillebrand 1888), and *Lobelia hypoleuca* Hillebr. (Hillebrand 1888). They form a clade with a BS value of 100 (Fig. 1). This corroborates Givnish's (1998), Antonelli's (2008), Givnish et al.'s (2009), and Chen et al.'s (2016) results. Biogeography: Lammers (2011) and Chen et al. (2016) indicated the Hawaii archipelago as the ancestral area of sampled members of this section. A more detailed phylogenomic analysis by Hunter (2018) placed the origin of § *Revolutella* in Kaua`i. Our analysis corroborates the above-mentioned studies and places this section in the Pacific Islands as its ancestral region (Fig. 2).

Lobelia* § *Galeatella E.Wimm. (Wimmer 1948). In this section, we sampled one species, that is, *L. kauaensis*. Lammers (2011) included five species in this section, however, *L. kauaensis* was not amongst those included. Lammers (2007b) indicated that this species is a hybrid of natural taxa (nothotaxon). It forms a sister to *Trematolobelia* with a BS value of 90. Our results corroborate with that of Chen et al. (2016). Biogeography: Lammers (2007b, 2011) and Hunter (2018) stated that this section has its ancestral region in the Hawaiian archipelago. This corroborates with our results as it places this section in the Pacific Islands as its ancestral region.

Lobelia* § *Lobelia. Eleven out of twenty-two species (Lammers 2011) were sampled in our combined plastid dataset. This section is monophyletic. *Lobelia inflata* L. (Linnaeus 1753), and *Lobelia kalmii* L. (Linnaeus 1753) form a clade with a BS value of 68 and form sister to members of this section with a BS value of 96 (Fig. 1). This result corroborates Antonelli (2008). Biogeography: Lammers (2011) indicated North America as the ancestral location of this section. Our analysis concurs with Lammers (2011) and places North America as the ancestral area of this section (Fig. 2).

Lobelia* § *Cryptostemon (E.Wimm.) J.Murata (Murata 1995). We sampled two out of nine species included by Lammers (2011) in this section. The combined plastid dataset included *Lobelia irasuensis* Planch. & Oerst. (Planchon and Oersted 1857), whereas *Lobelia fenestralis* Cav. (Cavanilles 1791) was included in the ITS dataset. *Lobelia irasuensis* forms a clade with *Lobelia divaricata* Hook. and Arn. (Hooker et al. 1838) with a BS=96 (Fig. 1) while *L. fenestralis* forms a clade with *Lobelia laxiflora* Kunth (Kunth and Bonpland 1820) with a BS = 59. Biogeography: Chen et al. (2016) placed *L. irasuensis* in Central America as its ancestral region, which corroborated Antonelli (2009). Our analysis places this section in Central America as the ancestral region which corroborates with the above-mentioned studies (Fig. 2).

Lobelia* § *Homochilus DC. (Candolle 1839). We sampled two out of five species (Lammers 2011). *Lobelia laxiflora* Kunth (Kunth and Bonpland 1820) and *Lobelia aguana* E.Wimm. (Wimmer 1935) form a clade with a BS value of 48 (Fig. 1). Biogeography: Givnish et al. (2009) and Chen et al. (2016) indicated the ancestral area of these two species to be in Central America. Our results corroborate the above-mentioned and places this section in Central America.

Lobelia* § *Tylomium. Six out of thirty-eight species were sampled. This section is paraphyletic. *Hippobroma longiflora* (L.) G.Don (Don 1834) is nested within this section. *Lobelia portoricensis* Urb. (Urban et al 1899), *Lobelia kraussii* Graham (Graham 1830), *Lobelia martagon* Hitchc. (Hitchcock 1893), *Lobelia stricta* Sw. (Swartz 1788), and *Lobelia vivaldii* form a clade (Fig. 1). Biogeography: *Lobelia assurgens* L. (Linnaeus 1759), *L. portoricensis*, *L. martagon*, and *L. vivaldii* were placed in the Greater Antilles as their ancestral region while *L. kraussi* and *L. stricta* were placed in the Lesser Antilles as their ancestral region (Lammers 2011). Chen et al. (2016) indicated Central America as their ancestral area which corroborated our results.

Lobelia* § *Hypsela. Thirteen out of forty-three species within this section were sampled. This section is polyphyletic. It is intercalated with *Isotoma*, *Hypsela*, *Pratia*, and *Lithotoma* species. *Lobelia fugax* Heenan, Courtney & P.N.Johnson (Heenan et al 2008), *Lobelia ionantha* Heenan (Heenan et al 2008), *Lobelia fatiscens* Heenan (Heenan et al 2008), and *Lobelia carens* Heenan (Heenan et al 2008) form a clade with a BS value 98. *Lobelia roughii* Hook.f. (Hooker 1864), *Lobelia linnaeoides* Petrie (Petrie 1890), *Lobelia macrodon* (Hook.f.) Lammers (Lammers 1998), *Lobelia glaberrima* Heenan (Heenan et al 2008), and *L. oligophylla* also form a clade with a BS value of 70. *Lobelia arnhemiaca* forms a clade with *P. purpurascens* with a BS value of 61. *Lobelia oligophylla* is sister to *L. angulata*, *L. roughii*, *L. macrodon*, *L. glaberrima*, and *L. linnaeoides* (Fig. 1). This result corroborates that of Antonelli (2008). In the ITS region dataset, *Pratia*, *Isotoma*, *Hypsela*, and *L. chinensis* form a clade with a BS=100. Lammers (2011) indicated the chromosome number of this section as $2n$=12, 14, 28, 42, 56, 70, 77, 84, 91 and 140. *Pratia* also shows these same chromosome number variations, consistent with it being an exemplar of interspecific hybridization. *Pratia angulata* is $2n$=70 while *P. perpusilla* is $2n$=42, the hybrids between these two species have $2n$=77, 91, and 140 chromosome numbers reported (Murray et al. 2004). According to Knox et al. (2008b), *Isotoma* was distinguished by floral fusion with adnate filaments, *Pratia* on the other hand was classified using berry fruits, and *Hypsela* was differentiated by having both floral fusion and berry fruits. These features are similar to those used by Lammers (2011) used to describe the *Lobelia* § *Hypsela*. More so, Lammers (2011) suggested the inclusion of *Isotoma* in this section, a suggestion that is well supported by our phylogenetic analysis. Our analysis proposes the inclusion of *Hypsela*, *Pratia*, and *Lithotoma* in this section too. Biogeography: Lammers (2011) described this section as Amphi-pacific with a majority of the species in the southern hemisphere. Our analysis placed this section in Australasia as their ancestral region although *L. loochooensis* and *L. chinensis* were placed in Asia as their ancestral region. *Lobelia oligophylla*, on the other hand, has its ancestral region in South America. Our analysis corroborated that of Lammers (2011) (Fig. 2).

Lobelia* § *Tupa (G.Don) Benth. (Bentham 1876). We sampled all four members of this section in our combined plastid dataset. The sampled species included *Lobelia polyphylla* Hook. & Arn. (Hooker and Arnott 1830), *Lobelia bridgesii* Hook. & Arn. (Hooker and Arnott 1830), *Lobelia tupa* L. (Linnaeus 1753), and *Lobelia excelsa* Bonpl. (Bonpland et al. 1816), and form a monophyletic group with a BS value of 99 (Fig. 1). So far, this corroborates Lammers and Hensold (1992) and Antonelli

(2008) that species belonging to this section might be monophyletic due to the uniform occurrence of an unusual chromosome number (2*n*=42). Biogeography: The ancestral area of this section is Chile (Lammers 2011). Chen et al. (2016) corroborated Lammers's (2011) results. Our result is consistent with both of them and places South America as the ancestral region of this section.

Conclusion

In this study, we conducted a literature review and phylogenetic analyses on Lobelioideae. We found that previous studies have currently reached an agreement on the southern African origin of Lobelioideae, herbaceous habit, and Asian origin of giant lobelioids, and lastly, the convergent evolution of giant rosette lobelioids. We also found that several genera, such as *Lobelia*, are polyphyletic and their systematics is particularly frustrating, which calls for further reappraisals using both morphological and molecular data. More so, taxon sampling and sequencing of some genera such as *Centropogon*, *Burmeistera*, *Siphocampylus*, and *Clermontia* are quite minimal. The phylogenetic analyses in this paper were based primarily on 18 plastid loci; the resolution and support provided by ITS were weak. Future advances in Lobelioideae phylogenetics should include phylogenomic approaches based on hundreds of single-copy nuclear genes and flanking regions, and direct assessment of possible hybridization, incomplete lineage sorting, or other forms of reticulate evolution, to investigate extensively the classification of Lobelioideae.

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Supplementary material 1

Lobelioideae data matrix

Authors: Samuel Paul Kagame, Andrew W. Gichira, Lingyun Chen, Qingfeng Wang

Data type: sequence data

Explanation note: Sequence GenBank ID and voucher information of all the sequences used in the study. Sheet 1. Linked data table for sequences and voucher information used in the study. heet 2. Lobelioideae taxa in the combined plastid dataset. Sheet 3. References of sequences used in the study.

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Link: https://doi.org/10.3897/phytokeys.174.59555.suppl1

Supplementary material 2

Figure S1. Phylogeny of Campanulaceae family with bootstrap values using combined cp dataset

Authors: Samuel Paul Kagame, Andrew W. Gichira, Lingyun Chen, Qingfeng Wang

Data type: Phylogenetic

Explanation note: This file contains the bootstrap values for the whole campanulaceae family generated from combined chloroplast dataset using maximum likelihood analysis.

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Link: https://doi.org/10.3897/phytokeys.174.59555.suppl2

Supplementary material 3

Figure S2. Phylogeny of Campanulaceae family with bootstrap values using ITS dataset

Authors: Samuel Paul Kagame, Andrew W. Gichira, Lingyun Chen, Qingfeng Wang

Data type: Phylogenetic

Explanation note: Phylogenetic tree of whole campanulaceae family with boostrap values generated from Internal Transcribed Spacer (ITS) regions using maximum likelihood analysis.

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Supplementary material 4

Figure S3. Phylogeny, genera and bootstrap values of Lobelioideae using ITS dataset

Authors: Samuel Paul Kagame, Andrew W. Gichira, Lingyun Chen, Qingfeng Wang

Data type: Phylogenetic image

Explanation note: Phylogenetic tree image indicating the genera classification and bootstrap values of lobelioideae subfamily trimmed from ITS campanulaceae tree.

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Link: https://doi.org/10.3897/phytokeys.174.59555.suppl4

Supplementary material 5

Figure S4. Phylogeny of Lobelioideae with bootstrap values using combined plastid dataset

Authors: Samuel Paul Kagame, Andrew W. Gichira, Lingyun Chen, Qingfeng Wang

Data type: Phylogenetic

Explanation note: Phylogenetic tree of lobelioideae subfamily trimmed from combined plastid campanulaceae tree.

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Taxonomic revision of *Ceropegia* sect. *Huernia* (Asclepiadoideae, Apocynaceae) in Saudi Arabia with three new combinations

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Abstract

This study provides a taxonomic revision for *Ceropegia* sect. *Huernia* in the flora of Saudi Arabia. Forty-six quantitative and qualitative morphological characters were analysed using principal component analysis (PCA), principal coordinates analysis (PCoA) and the unweighted pairs group using mean average (UPGMA) to separate and help delimit taxa. We propose to reduce the number of species reported in Saudi Arabia from 11 to four: *C. khalidbinsultanii* **comb. nov.**, *C. laevis*, *C. lodarensis* and *C. macrocarpa*. This study also suggested reducing two names to varietal level under *C. lodarensis* (var. *foetida* **comb. nov.** and var. *rubrosticta* **comb. nov.**). A key to the species, detailed morphological descriptions, illustrations, distribution maps, ecology, etymology and preliminary conservation assessments are provided that follow IUCN criteria.

Keywords

Arid plants, flora of Saudi Arabia, *Huernia*, stapeliads, stem-succulent

Introduction

The stapeliads, essentially stem-succulent leafless members of the tribe Ceropegieae (Asclepiadoideae, Apocynaceae), comprise 357 species placed in 31 genera. All of these were reduced to sections of *Ceropegia* after a broad circumscription of the genus, based

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on a recent molecular study (Bruyns et al. 2017). The stapeliads are widely distributed in semi-arid to arid areas of the Old World from southern Africa north-eastwards to India and Myanmar (Bruyns 2005).Their flowers are amongst the most beautiful of the dicotyledons, as well as amongst the most complex with almost all of them scented of carrion or bad fish and similar pungent odours (Pillans 1920; Court 2000). They exhibit an extraordinarily wide range of floral shapes and sizes and a wide range of complicated structures in the centre of the flower that are associated with the pollination process (Bruyns 2005). The flowers are specialised exclusively for fly pollination and this diversity appears to have arisen in response to the wide range of sizes of flies that are present in the region, combined with the wide spectrum of geological and topographical niches in the area (Bruyns 2005).

Ceropegia sect. *Huernia* (R.Br.) Bruyns (formerly the genus *Huernia* R.Br.) has the widest distribution of all stapeliads, extending from west of Al-Madinah in Saudi Arabia, north of the Tropic of Cancer, to near Cape Town in South Africa, to the south of the Tropic of Capricorn (Plowes and McCoy 2003). As can be expected in a section with a range this large, *Huernia* has a great number of species and subspecies, with over 54 species currently recognised, making it the most diverse section in the stapeliads (Bruyns et al. 2017). It is distinguished from other angled-stemmed stapeliads by the leaf-rudiments without stipular denticles; corona very rarely raised above the base of the tube on a stipe, outer series spreading at the base of the tube and often partially fused to it, tube often with an annular thickening around the mouth, but not entirely formed by an annulus (Bruyns 2014). Plants of *Huernia* have almost identical stems, so that the species cannot always be identified accurately without flowers. Flowers also sometimes show a variety of forms within a single species, such as *H. humilis* and *H. thuretii* from South Africa (Bruyns 2005). Consequently, the number of species approved for *Huernia* has varied widely over time: 45 for White and Sloane (1937), 64 for Leach (1988), 49 for Bruyns (2005) and a little over 54 currently.

In Saudi Arabia, sect. *Huernia* is restricted to the mountainous area of the western and south-western part of the country (the mountains of Sarat and Hejaz) (Collenette 2000). The famous plant collector, Mrs I. S. Collenette, was the first to collect *Huernia* from Saudi Arabia. Between 1972 and 1998, she collected several apparently undescribed species of *Huernia* with relatively large papillate flowers (Plowes 2014). These specimens have been deposited at the Royal Botanic Gardens herbaria at Kew (K) and Edinburgh (E). Only one species had previously been described by Field, in 1980, in the course of naming her collections at Kew; this was *H. saudi-arabica* D.V.Field (Field 1980). Somewhat later, in 1985, Collenette published her first book on Saudi Arabian plants, *An Illustrated Guide to the Flowers of Saudi Arabia*. She recorded two species, *H. lodarensis* Lavranos and *H. saudi-arabica* and four unnamed species (Collenette 1985). In her subsequent publications (Collenette 1998, 1999, 2000), three species were recognised: *H. arabica* N.E.Br., *H. laevis* J.R.I.Wood and *H. saudi-arabica*. Some of the five species which lacked names have an affinity to *H. boleana* M.G.Gilbert and *H. lodarensis*. Soon after, Al-Hemaid published the name *H. haddaica* for the specimen *Collenette 5944* from Al-Hadda (Al-Hemaid 2001), but

this name was not validly published (Goyder and Al-Hemaid 2009). Tom A. McCoy collected a similar plant in 1999 from Khamis Mushait, which was described in 2003 as *H. khalidbinsultanii* Plowes & McCoy (Plowes and McCoy 2003). Just two years later, in 2005, Bruyns reduced the number of *Huernia* species in north-eastern Africa and Arabia to 14 species. In his treatment, he considered *H. saudi-arabica* and *H. khalidbinsultanii* to be synonyms of *H. lodarensis* and *H. arabica* to be a synonym of *H. penzigii* N.E.Br. (Bruyns 2005). However, this was not accepted by Plowes who published seven new names for Collenette's other Saudi Arabian collections of *Huernia* that lacked names (Plowes 2012; Plowes 2014). Thus, from the taxonomic perspective of Plowes, *Huernia* in Saudi Arabia was represented by 11 species *H. anagaynensis* Plowes, *H. arabica*, *H. asirensis* Plowes, *H. collenetteae* Plowes, *H. decaloba* Plowes, *H. foetida* Plowes, *H. khalidbinsultanii*, *H. laevis*, *H. radhwana* Plowes, *H. rubrosticta* Plowes and *H. saudi-arabica*.

Plowes' classification of Saudi Arabian *Huernia* needs further investigation. His taxonomic treatment of several taxa was based on a single photo (e.g. *H. decaloba*) or a single specimen (e.g. *H. anagaynensis*, *H. radhwana*, *H. foetida*, *H. khalidbinsultanii*). Furthermore, morphological characters used by Plowes are not strong enough for delimiting species within sect. *Huernia*. For example, corolla tube size and its exterior colour, the number of flowers in the inflorescence and flower odour were used as diagnostic characters to separate *H. anagaynensis*, *H.radhwana* and *H. asirensis*. Observations of the first author have shown that such characters are not constant in this complex group. In addition, Plowes' description of the species is not sufficiently detailed and is not even clearly enough illustrated for one to distinguish between these closely-related species. Moreover, it is unclear how the Saudi Huernias are distinguished from closely-allied species, such as *H. boleana* and *H. lodarensis*. There is, therefore, a need for much more sampling and detailed examination before a conclusive taxonomic statement on Saudi Arabian *Huernia* can be made.

Remarkably, sect. *Huernia* has received little taxonomic attention in Saudi Arabia, other than Plowes' work. Taxonomic revision of this plant group in Saudi Arabia is urgently needed. Plants of sect. *Huernia* are commonly used for diabetes treatments in traditional medicine in the western and south-western regions of Saudi Arabia (Hamam et al. 2018). Ongoing investigations on the medicinal value of Saudi Huernias have been performed (Ali et al. 1984; Mossa and Abdul Hameed 1991; Almehdar et al. 2012; Alzahrani et al. 2015; El Sayed et al. 2018, 2020; Hamam et al. 2018). However, in some recent studies (e.g. Alzahrani et al. 2015; Hamam et al. 2018), the species of sect. *Huernia* investigated was identified as *Huernia* sp. nov. aff. *boleana* according to Collenette (1999). The precision and usefulness of medicinal investigations on sect. *Huernia* of Saudi Arabia will be increased by a detailed taxonomic treatment. It will also enable studies on their conservation status to be made, as it would appear that some of them are being severely threatened by overgrazing, infrastructure and housing development (Abulfatih and Nasher 1988; Collenette 2000; Plowes 2012).

The objectives of the present study are: 1) to revise *Ceropegia* sect. *Huernia* in the flora of Saudi Arabia, 2) to examine morphological characters in detail and try to find

new ones that can be used in the classification of the section in Saudi Arabia, 3) to investigate the relationship between Saudi Arabian *Huernia* and other allied species in the Arabian Peninsula and 4) to provide a diagnostic key for the species in Saudi Arabia. This will enable us to test whether the taxonomic treatment in Plowes (2012) was justified.

Material and methods

Taxon sampling

Twenty individuals of *H. asirensis* and *H. collenetteae* were sampled from the Ash Shafa area in Al-Taif Province, western Saudi Arabia (21°3.6583'N, 40°20.1917'E) during several expeditions to the area between September 2010 and May 2011. Specimens were preserved for each collection in a mixture of Formalin, Glycerol and Water (in the ration 2:1:20). Herbarium specimens were then made from this preserved material as described in Leach (1995). Voucher specimens were deposited in the herbarium of Umm Al-Qura University (UQU, proposed abbreviation). Pickled and dried specimens of *Huernia* from Saudi Arabia and the Arabian Peninsula, generally, were examined at the herbaria at Kew (K) and Edinburgh (E).

Morphological characteristics

The morphological characters were examined and recorded from the available specimens (one *H. anagaynensis*, 12 *H. asirensis*, 10 *H. collenetteae*, one *H. foetida*, one *H. laevis*, one *H. radhwana*, one *H. rubrosticta* and one *H. saudi-arabica*). Since some characters are difficult to interpret in dry specimens, dried specimens of *H. collenetteae*, *H. rubrosticta* and *H. saudi-arabica* are excluded from the morphometric analysis. For species where material was unavailable, such as *H. arabica* and *H. khalidbinsultanii* and the closely-related species from Ethiopia and the Arabian Peninsula *H. boleana* and *H. lodarensis*, measurements and character-states have been extracted from the relevant literature (Gilbert 1975; Leach 1976; Field 1980; Albers and Meve 2002; Plowes and McCoy 2003; Plowes 2012; Plowes 2014). Height of the plant, odour and colour of flowers were immediately documented in the field. Floral characters were examined using a NOVEX AP-8 binuclear microscope. Pollinia, inner corona and apices of papillae were examined using a XSZ-107BN compound optical microscope. Quantitative morphological characteristics were measured using a ruler; Suppl. material 1: Appendix 1 illustrates how the plant parts were measured. Initially, 69 characters were recorded, but 23 proved invariant leaving 46 (19 quantitative and 27 qualitative) for the analysis (Table 1, 2). The data were entered into an Excel spreadsheet and were later transformed into a format suitable for morphometric analysis. These morphological characteristics were used as the basis for our taxonomic revision for the species of sect. *Huernia* in Saudi Arabia. The features are richly illustrated using ibisPaint X ver.6.4.3 for Android, which allows visual comparison of the species.

Data analysis

Qualitative characters were coded as multi-state, for example (1. cream, 2. shiny creamy-yellow, 3. white). Quantitative variables were standardised using the R studio version (2017) scale balance function to remove bias due to size alone, following Katapally and Muhajarine (2014). The standardised data were analysed with R studio package Factor Analysis of Mixed Data (FAMD) version 1.2.3; this method included principal component analysis (PCA), used here to extract relevant information from high-dimensional datasets. Cluster analysis including principle coordinates analysis (PCoA) and unweighted pairs group using mean average (UPGMA) were carried out using the statistical software Minitab ver.18.1.1.0 (Minitab, Inc., State College, PA).

IUCN Preliminary Conservation Status

To assess the conservation status of each taxon, the guidelines for the IUCN Red List Categories and Criteria version 13 (IUCN Standards and Petitions Subcommittee 2017) and the guidelines for the Application of the IUCN Red List Criteria at the regional and national levels version 4.0 (IUCN 2012) were followed. Current threats and point distribution data were gathered from field observations and from the available scientific literature. These distributional data were then input into the GeoCAT software (Bachman et al. 2011), which, in turn, calculated two main spatial metrics: the Extent of Occurrence (EOO) and Area of Occupancy (AOO). If the EOO were less than the AOO, the EOO was set equal to the AOO to ensure consistency with the definition of the AOO as an area within the EOO following the IUCN guidelines (IUCN Standards and Petitions Subcommittee 2017). Criterion B was only used for the species assessment due to data availability. Distribution maps were created using ArcGIS Online (Esri, 'Topography').

Data resources

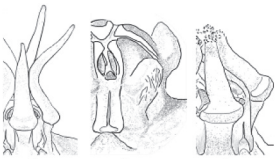
The data underpinning the analyses reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility: https://doi.org/10.15468/6n2rgz.

Table 1. Nineteen quantitative morphological characters used in morphometric analysis of *Ceropegia* sect. *Huernia* in Saudi Arabia. All measured in mm.

No.	Character	No.	Character
1	length of branches	11	diam. of corolla tube at mouth
2	length of tubercles on branches	12	length of papillae in throat of corolla (max.)
3	width of base of tubercles	13	thickness of papillae at base
4	number of flowers per inflorescence	14	length of intermediate lobes
5	length of pedicel	15	length of corolla lobe
6	diam. of pedicel	16	width of corolla lobe at base
7	length of sepals	17	diam. of outer corona
8	width at base of sepals	18	length of inner corona
9	diam. of corolla	19	width of Inner corona at base
10	length of corolla tube		

Table 2. Twenty-seven qualitative morphological characters and character states used in morphometric analysis of *Ceropegia* sect. *Huernia* in Saudi Arabia.

No.	Characters	Character state
1	stem grooves between tubercle rows	1. deep 2. shallow
2	flower smell	1. no bad smell 2. faint or no bad smell 3. very foetid
3	flowers opening	1. successively 2. simultaneously
4	pedicel tapering towards the point of flower attachment	1. not tapering 2. slightly tapering 3. conspicuously tapering
5	pedicel growth direction	1. spreading and holding flower facing horizontally 2. ascending holding flower facing upwards
6	corolla shape	1. shallow bowl shape 2. broadly funnel-shaped, margin weakly bulging like an annulus 3. tubular-campanulate 4. campanulate
7	corolla lobe apex groove	1. absent 2. present, but not deep (concave) 3. present and deep (channel)
8	corolla inside surface texture	1. tube base smooth, tube throat and lobes papillate 2. glabrous with very short papillae at apices of lobes
9	corolla tube	1. cylindrical 2. pentagonal
10	shape of papillae inside corolla	1. very small, wart-like 2. slender (hair-like) 3. conical, compressed 4. cylindrical or slightly compressed
11	corolla inside (background colour)	1. cream 2. shiny creamy-yellow 3. white
12	corolla inside (colour pattern)	1. purple 2. brownish-red (maroon)
13	corolla exterior (colour pattern)	1. dark spots especially on the lower portion of corolla tube 2. pale spots uniformly scattered 3. dark spots on the upper half 4. no spots
14	corolla tube interior (colour pattern)	1. uniform colour (purplish-red) 2. shiny irregular broad streaks 3. concentric broken lines and dashes 4. concentric short dashes 5. uniform colour (cream) 6. dots
15	corolla lobes colour	1. uniform colour (purplish-red) 2. shiny irregular broad streaks 3. irregular shaped fine short lines and dashes 4. dots 5. deep coloured areas concentrated between the lobes; apex is streaked with irregular short lines and dashes 6. irregular shaped short lines and dashes
16	corolla lobes spreading	1. ascending 2. reflexed 3. slightly spreading 4. spreading with recurved apices
17	corolla lobe shape	1. deltoid-acute 2. deltoid-acuminate 3. deltoid-caudate

No.	Characters	Character state
18	outer corona colour	1. blackish-maroon 2. cream at the base of the lobes then gradually turning maroon towards blackish-maroon apex
19	outer corona shape	1. discrete, with 5 lobes 2. disc
20	outer corona lobe shape	1. subquadrate 4 3 2 1 2. rectangular 3. short and broad rounded lobes 4. no distinct lobes
21	outer corona lobe apex	1. shallowly bifid 2. slightly crenate 3. crenate 4. emarginate 5. slightly emarginate 6. mucronate 7. bifid 8. dentate
22	outer corona fleshy tubercle	1. present 2. absent
23	inner corona shape	1. exceeding anthers and meeting in centre 2. shorter than anthers 3. adpressed to anthers in their lower half then rising up connivent and then diverging towards apices
24	inner corona dorsal gibbosity	 3 2 1 1. broadened transversely and conspicuously gibbous 2. ascending obtuse and conspicuously gibbous 3. inflated transversely not conspicuously gibbous
25	inner corona apices	1. obtuse 2. acute 3. taper to fine, slender points
26	inner corona colour	1. maroon 2. purple with cream at base 3. ivory white with a few maroon spots at the tips 4. ivory white 5. purplish-black with cream at base
27	inner corona apex texture	1. bristly 2. smooth 3. minutely scabrous

Results

The first two axes of PCA accounted for 74.7% of the overall variation (Fig. 1). Screen plot Eigenvalues for identification of principal components and Boxplot showing differences in morphological characters can be seen in the supplementary information Suppl. material 1: Appendices 2 and 3, respectively.

Cluster analysis by UPGMA of quantitative and qualitative data indicated the presence of four clearly-distinguished groups: Group 1, with *H. arabica* and *H. laevis*

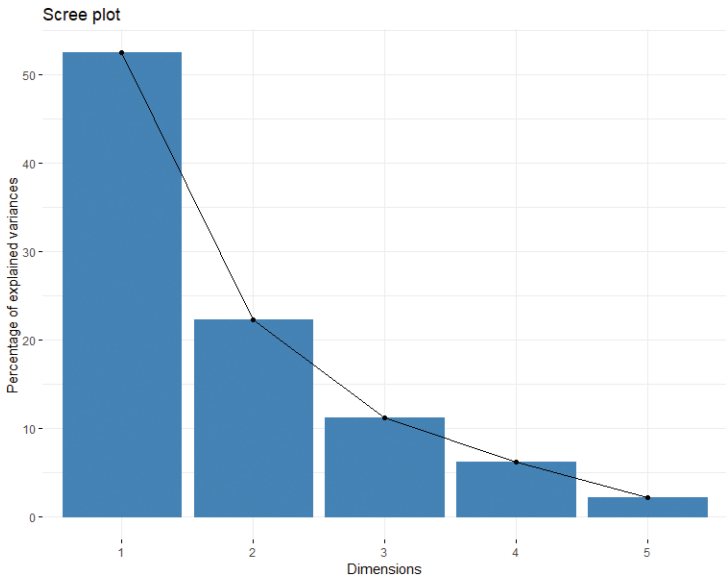


Figure 1. The proportion of variance retained by the different dimensions (axes), in PCA.

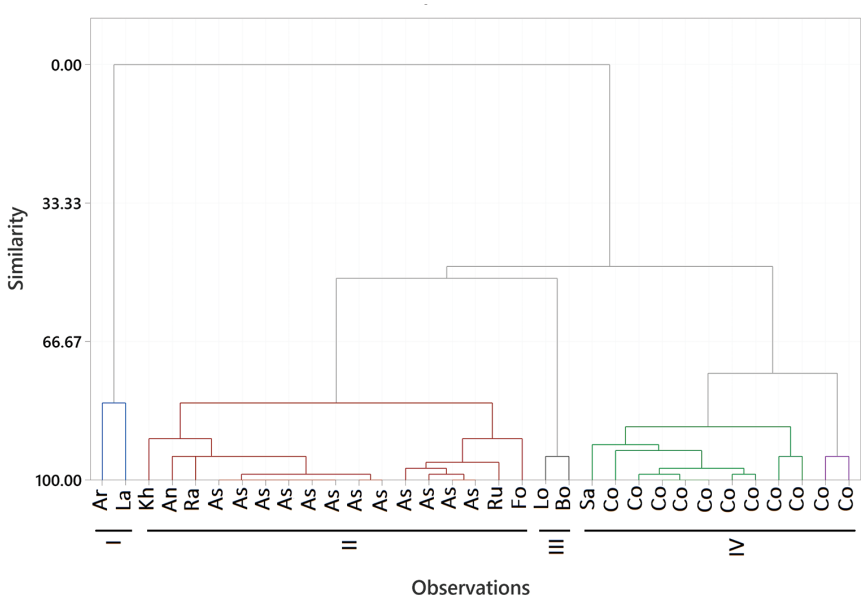


Figure 2. Unweighted pair-group method with arithmetic mean (UPGMA) phenogram resulting from cluster analysis. Explanations: Ar-*Huernia arabica*, La- *H. laevis*, Kh- *H. khalidbinsultanii*, Ra- *H. radhwana*, An- *H. anagaynensis*, As- *H. asirensis*, Ru- *H. rubrosticta*, Fo- *H. foetida*, Lo- *H. lodarensis*, Bo- *H. boleana*, Sa- *H. saudi-arabica*, Co- *H. collenetteae*.

is the furthest away from all other groups; Group 2, consisting of *H.asirensis*, *H. anagaynensis*, *H. khalidbinsultanii*, *H. radhwana*, *H. foetida* and *H. rubrosticta*; Group 3, with *H. lodarensis* and *H. boleana*; and Group 4, with *H. collenetteae* and *H. saudi-arabica* (Fig. 2).

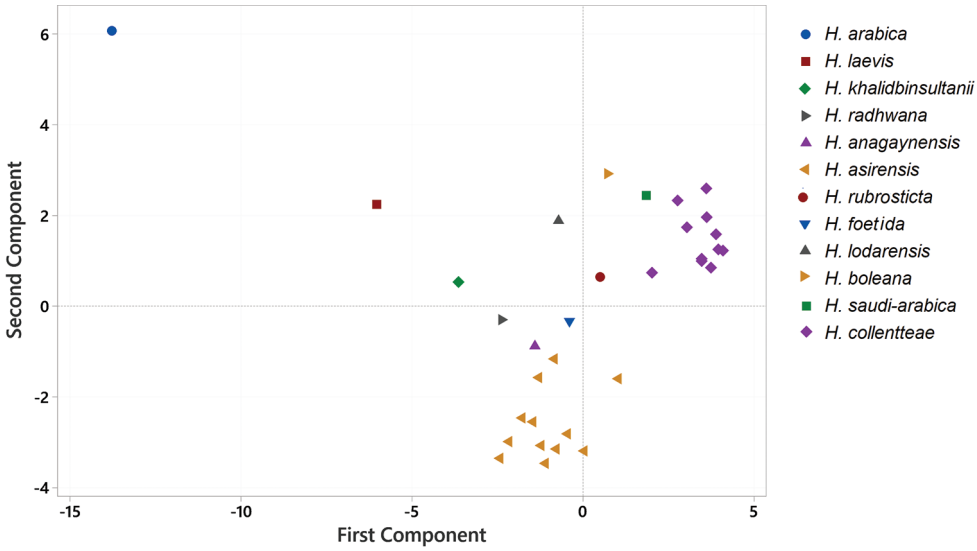


Figure 3. PCoA representation of morphological data of accessions of sect. *Huernia*. Principal Component axis 1 and 2.

PCoA separated 33 specimens into four distinct groups, corresponding largely to those obtained by UPGMA. Group 1 and 2 represent *H. arabica* and *H. laevis*, respectively, which were well-separated from the others. Accessions of *H. asirensis* were clustered together in one group and weakly separated from *H. anagaynensis*, *H. radhwana*, *H. foetida* and *H. khalidbinsultanii*. Accessions of *Huernia collenetteae* and *H. saudi-arabica* were grouped in one cluster in the positive axes and weakly separated from individuals of *H. rubrosticta*, *H. lodarensis* and *H. boleana* (Fig. 3).

The most important characters, contributing to the separation of the groups, were corolla characteristics (corolla shape, corolla tube diam. and shape, corolla lobes spreading, corolla colour patterns and papillae shape) and corona characteristics (outer corona shape, diam. and lobe shape; inner corona length, apex and dorsal gibbosity) Table 3.

Discussion

In the experience of the present authors, sect. *Huernia* is a difficult group in the flora of Saudi Arabia and it has not received adequate attention. Perhaps the most comprehensive account is Plowes (2012). However, the diagnostic characters that were used by Plowes are questionable. In this study, multivariate analysis of 46 quantitative and qualitative morphological characters was conducted. Analyses by PCA, PCoA and UPGMA were used to determine the characters that were useful in the taxonomy of species of sect. *Huernia* in Saudi Arabia. Vegetative characters, such as habit, the number of angles into which the tubercles are arranged along the branches and leaf-rudiments are extremely variable across sec. *Huernia*, especially amongst the southern African

Table 3. Eigenvalues in two principal components (PC1 and PC2) of value relative to 46 morphological characters of sect. *Huernia* in Saudi Arabia.

Characters	PC1	PC2
width of base of tubercles (mm)	0.078	-0.038
length of branches (mm)	0.003	-0.107
length of tubercles on branches (mm)	0.050	0.254
stem grooves between tubercle rows	0.132	-0.208
flower smell	-0.079	-0.317
number of flowers per inflorescence	0.014	-0.023
flowers opening	-0.025	-0.261
length of pedicel (mm)	0.171	0.053
diam. of pedicel (mm)	0.162	0.019
pedicel tapering toward the point of flower attachment	0.139	-0.077
pedicel growth direction	0.194	0.268
length of sepals (mm)	0.141	-0.202
width at base of sepals (mm)	0.125	-0.043
corolla shape	-0.252	0.009
corolla lobe apex groove	0.036	0.027
corolla inside surface texture	-0.085	0.072
corolla tube	0.160	-0.215
shape of papillae inside corolla	0.241	0.092
thickness of papillae at base	0.160	0.074
corolla inside (background colour)	0.171	0.142
corolla inside (colour pattern)	-0.108	0.011
corolla exterior (colour pattern)	0.076	-0.224
corolla tube interior (colour pattern)	0.158	0.153
corolla lobes colour	0.075	-0.158
corolla lobes spreading	0.172	0.011
width of base of tubercles (mm)	0.128	0.229
corolla lobe shape	0.194	0.110
diam. of corolla	0.089	0.022
length of corolla tube (mm)	0.198	-0.139
diam. of corolla tube at mouth (mm)	0.209	-0.045
length of intermediate lobe (mm)	0.156	0.004
length of corolla lobe (mm)	0.186	-0.085
width of corolla lobe at base (mm)	0.131	-0.077
outer corona colour	0.043	0.056
outer corona shape	-0.031	-0.237
outer corona lobe shape	0.091	-0.259
outer corona lobe apex	0.164	0.058
outer corona fleshy tubercle	-0.039	-0.075
diam. of outer corona (mm)	0.216	0.080
inner corona shape	0.219	-0.183
length of inner corona (mm)	0.244	0.054
width of inner corona at base (mm)	0.173	-0.029
inner corona dorsal gibbosity	0.255	0.071
inner corona base end	0.214	-0.210
inner corona apices	0.031	-0.265
inner corona apex texture	0.100	-0.072

species (Bruyns 2005). However, these characters were valueless in the taxonomy of Arabian members, due to their considerable similarity between species. Therefore, the species were differentiated mainly on the basis of their floral characters.

In the multivariate analysis, accessions of *H. radhwana* (Fig. 6D), *H. asirensis* (Fig. 6F), *H. anagaynensis* (Fig. 6G) and *H. khalidbinsultanii* (Fig. 6H) grouped into one

large cluster (Figs 2, 3). Plowes (2012) distinguished between those species by: 1) size of the corolla tube, 2) colour of the exterior of the corolla, 3) the number of flowers per inflorescence and the succession of their opening and 4) the odour of the flowers. A careful examination of the type specimens suggests that they are all samples of a single species and these characters have all proved to be unreliable in our experience. The first point is not of any value since the size of the corolla tube in *H. radhwana*, *H. anagaynensis* and *H. khalidbinsultanii* is easily accommodated within the known range of *H. asirensis*. Variation found in specimens of sect. *Huernia* from Wadi Thee Gazal has also demonstrated the invalidity of the second point. In the case of the third, Plowes distinguished *H. radhwana* from other species in the group by its solitary flowers. This is encountered often in *H. asirensis*, where several specimens were found to have few flowers (2–4) that opened in succession. In respect of the fourth point, we do not consider that the odour of the flower is a prominent character. We observed that the foetid odour in flowers of *H. asirensis* becomes faint or vanishes completely after all pollinia were removed. In addition, this character is not considered diagnostic, as it is impossible to observe in preserved specimens. Thus, our results paper suggest that Plowes' names for these species with slender papillae and tubular-campanulate flowers should all be included as synonyms under *H. khalidbinsultanii*, since it is the first valid name from this group.

In 2005, Bruyns reduced *H. khalidbinsultanii* to a synonym under *H. lodarensis*; here, the two taxa are differentiated according to the shape of the papillae and the corolla (see the key in the next section). In sect. *Huernia*, the shape of the papillae provides an important character when it is combined with other characters, such as inner corona and the shape of the corolla is the most important character indicative of the relationship between species (Leach 1983). Thus, this study suggests that *H. khalidbinsultanii* must be maintained.

Huernia collenetteae and *H. saudi-arabica* accessions overlapped in one cluster in both the UPGMA (Fig. 3) and PCoA (Fig. 3) analyses. Close examination of the relevant specimens shows that characters of *H. saudi-arabica* are accommodated within the variation range of *H. collenetteae*. The most noticeable character in Plowes (2012), which can be used to distinguish them, is patterns of streaking on the inside of the corolla (see figs 1–6 in Plowes 2012). Inside, the corolla tube in *H. saudi-arabica* (Jabal Sawdah population) is uniformly coloured with purple and with deeply coloured areas concentrated between the lobes; sometimes the inside of the corolla is entirely purple (Fig. 10 D, H, K). However, this pattern has also been seen in the population of *H. collenetteae* from Ash Shafa region (from 379.87 km north of Jabal Sawdah) (Fig. 10E, I; *S.A. Alharbi S4B*), but with concentric broken maroon lines and stripes in the corolla tube instead of a uniform maroon colour. Colours vary greatly in the flowers of widespread species of sect. *Huernia*, such as that recorded in *H. thuretii* and *H. hallii* from South Africa (Bruyns 2005). Thus, separating these two entities (*H. collenetteae* and *H. saudi-arabica*) into distinct species is inconsistent and this study will handle all data obtained from specimens of *H. collenetteae* as *H. saudi-arabica* in the subsequent discussion.

Huernia saudi-arabica (Fig. 10D), described from a single specimen *Collenette* 549, was related by its author to *H. lodarensis* and *H. boleana* (Field 1980). Distinctive features

given are: 1) the corolla is slightly larger; 2) the corolla lobes have a more conspicuous papillose, frill-like margin; 3) the inner surface of the attenuate lobe-tip is covered with short, but even-sized, papillae; 4) the outer corona is distinctly 5-lobed, but, unlike *H. lodarensis*, each lobe is considerably wider than long and narrows towards the bifid tip rather than being somewhat parallel-sided; and 5) the inner corona-lobes are smooth and more acute towards the tips (fig. 1 in Field 1980). The floral measurements given by Field for *H. lodarensis* and *H. boleana* are easily accommodated within the known range of *H. saudi-arabica*. In the case of the third point, Field stated that "in *H. lodarensis*, the indumentum is a mixture of a few papillae and low tubercles" (fig. 1K in Field 1980). This feature can clearly be seen in a number of samples of *H. saudi-arabica*. In the case of the last two points, the corona lobes turned out to be far more variable than suspected and the range, that was observed, was found in specimens collected at a single locality. Here, it was found that the outer corona lobes range from rectangular to subquadrate or rarely fused entirely to form a disc (e.g. *Alharbi S5B*). Likewise, the tips of the inner coronal lobes vary from smooth to minutely scabrous. Consequently, our results support Bruyns' (2005) opinion that *H. saudi-arabica* should treated as a synonym under *H. lodarensis* (Fig. 10A).

The numerical analysis carried out in this study did not resolve the relationship between *H. lodarensis*, *H. boleana*, *H. foetida* and *H. rubrosticta* and the other species. This is probably due to the low number of specimens included in the analysis and the incomplete nature of the data obtained from literature for *H. lodarensis* and *H. boleana*. However, a thorough examination of *H. foetida* (Fig. 12) and *H. rubrosticta* (Fig. 14B) type specimens reveal that they are very close to *H. lodarensis*. In view of the unique streaking patterns on the interior of the corolla that were not observed in any specimens of *H. lodarensis*, these taxa are described here as varieties under *H. lodarensis*. Nevertheless, many more samples and additional taxonomic work are considered necessary to either confirm or modify this treatment.

The delimitation of *H. arabica* (Fig. 4) is a matter of long debate. While Plowes (2014) considered the taxon as an accepted species, Albers and Meve (2002) and Bruyns (2005) considered it to be synonymous under *H. macrocarpa* and *H. penzigii*, respectively. On the other hand, Berger (1910) and White and Sloane (1937) considered the taxon to be a variety under *H. penzigii* and *H. macrocarpa*, respectively. As not enough samples were available for this study to decide whether the species with uniformly purplish-maroon flowers (*H. arabica*, *H. macrocarpa* and *H. penzigii*) are synonymous or distinct species, Albers and Meve (2002) were followed. They treated *H. arabica* and *H. penzigii* as synonymous under *H. macrocarpa*.

In order to know the extent of variability within taxa, examining as many samples as possible is crucial. Our results show that the major weakness of previous taxonomic accounts of the Arabian members of sect. *Huernia*, particularly in Saudi Arabia, was caused by the fact that the taxa were described from single or only very small numbers of plants. This led to the recognition of many unnatural taxa, as seen in some of Plowes' (2012, 2014) names. The 'folk concept' of species (Cronquist 1988), in which groups are formed intuitively by individuals 'essentially similar' and referred to as species, are found in the taxonomy of most succulent plants and largely held sway amongst the stapeliads

(Bruyns 2005). This can clearly be seen in Leach's (1988) taxonomic revision of *Huernia*. As he saw relatively few specimens, this led him to recognise too many taxa (64) and his classifications turned out to have little predictive value (Bruyns 2005). Thus, dense sampling of sect. *Huernia* or of stapeliads in general is essential, especially when describing new species or assessing the status of species. Members of sect. *Huernia* in Saudi Arabia still need attention and, with the aid of modern molecular methods, it seems that their complexity can be mastered. Our results suggest reducing the number of names reported in Saudi Arabia from 11 to four species: *H. khalidbinsultanii*, *H. laevis* and *H. lodarensis* (including three proposed varieties: var. *lodarensis*, var. *foetida* and var. *rubrosticta*) and *H. macrocarpa*.

Based on recent phylogenetic reconstructions in the Ceropegieae, the species of *Huernia* were transferred to *Ceropegia*, where they were placed under sect. *Huernia* and over 50 new combinations were made (Bruyns et al. 2017). However, Plowes' names of Saudi Huernias are still not transferred yet. Therefore, we propose three new combinations in *Ceropegia* sect. *Huernia*. Those are: *C. khalidbinsultanii* comb. nov., *C. lodarensis* var. *foetida* comb. nov. and *C. lodarensis* var. *rubrosticta* comb. nov.

Taxonomic treatment

***Ceropegia* sect. *Huernia* (R.Br.) Bruyns, S. African J. Bot. 112: 423 (2017).**

≡ *Huernia* R.Br., Mem. Wern. Nat. Hist. Soc.: 22 (1810). Lectotype
Huernia campanulata (Masson) Haw. (designated by White and Sloane (1937))
= *Ceropegia clavigera* (Jacq.) Bruyns.

Diagnostic features. Perennial leafless dwarf succulent herb, mat-forming rarely rhizomatous, sometimes prostrate or pendulous succulent. ***Branches*** glabrous, smooth, 4- to 16-angled. ***Leaves*** reduced mainly to soft point without stipular structures. The leaf-rudiments are borne on a raised tubercle which is a much swollen leaf-base. These ***tubercles*** are arranged in rows along the branch and joined towards their bases into angles along the branch with a groove between vertical rows of tubercles. ***Inflorescence*** glabrous, usually only one per branch, arising mainly in lower half of branch between tubercles, 1–10 flowered. ***Corolla*** urceolate to campanulate to subrotate, shallowly lobed. ***Staminal corona*** in two well-separated series, inner pressed to backs of anthers mostly exceeding them and meeting in centre, often with prominent transversely-rounded dorsal projections. Outer spreading along base of tube, discrete to fused into spreading disc with fleshy tubercle beneath guide-rail obscuring entrance to small nectarial cavity. ***Anthers*** horizontal on top of style-head, margins shrinking back to expose pollinia, rectangular. ***Pollinium*** ellipsoidal, longer than broad, insertion-crest exactly along outer edge, caudicle attached with broad cupular pad to base. ***Follicles*** erect, terete-fusiform, obclavate, slender, consisting of two horns diverging at 30–60°, longitudinally mottled with narrow broken purple stripes, glabrous, smooth (Bruyns 2005, 2014; Bruyns et al. 2017).

Key for *Ceropegia* sect. *Huernia* in Saudi Arabia

1 Corolla inside glabrous or covered with papillae ≤ 1 mm long; inner corona lobes not or shortly exceeding anthers, not tapering to a fine point **2**

– Corolla inside densely covered with papillae > 1 mm long; inner corona lobes much exceeding anthers, tapering to a fine point **3**

2 Corolla bowl-shaped, papillate, uniformly purplish-maroon without annulus around mouth of tube..... ***C. macrocarpa***

– Corolla funnel-shaped, glabrous, shiny with irregular broad red streaks on a yellowish background with an annulus-like structure around mouth of tube... ..... ***C. laevis***

3 Tubercles on branches up to 16 mm long; corolla covered with slender (hair-like) papillae, tubular-campanulate, lobes ascending .... ***C. khalidbinsultanii***

– Tubercles on branches up to 12 mm long; corolla covered with conical compressed papillae, campanulate, lobes spreading or reflexed..... ***C. lodarensis***

1. *Ceropegia macrocarpa* (Sprenger) Bruyns, S. African J. Bot. 112: 424 (2017)
urn:lsid:ipni.org:names:77215098-1
Figs 4, 5; Map 1

≡ *Huernia macrocarpa* Sprenger, Cat. Dammann & Co. 59: 4 (1892) Type: Eritrea • Penzig s.n. (K epitype).
= *Huernia macrocarpa* var. *arabica* (N. E. Brown) A. C. White & B. Sloane (1937)
= *Huernia macrocarpa* var. *penzigii* (N. E. Brown) A. C. White & B. Sloane (1937)
= *Huernia macrocarpa* var. *schweinfurthii* (A. Berger) A. C. White & B. Sloane (1937)
= *Huernia penzigii* N. E. Brown (1892)
= *Huernia penzigii* var. *arabica* (N. E. Brown) A. Berger (1910)
= *Huernia penzigii* var. *schimperi* A. Berger (1910)
= *Huernia penzigii* var. *schweinfurthii* A. Berger (1910)

Description. Dwarf succulent forming dense clump. ***Branches*** 60 mm long, non-rhizomatous, erect, decumbent, grey-green mottled with purple-red; tubercles up to 10 mm long (including leaf-rudiment), conical, spreading, laterally flattened and joined into 5 angles along branch, each tipped with a soft slender acuminate caducous leaf-rudiment. ***Inflorescence*** usually only 1 per branch, arising in lower half of branch, each bearing 2–3 flowers developing mainly successively, flowers with no unpleasant smell; ***pedicel*** spreading and holding flower facing horizontally. ***Corolla*** 15 mm diam., shallow bowl shape; outside smooth, cream-speckled with maroon, with 1 heavy (+ 2 lighter) raised longitudinal veins running from lobes to base of tube; inside uniformly coloured with purplish-red, covered except in lower third of tube with very small wart-like papillae; ***tube*** cupular; ***lobes*** ascending, deltoid, acuminate. ***Corona*** without basal stipe; ***outer lobes*** spreading on base of tube and fused partially to it, discrete to 5-lobed

Map 1. Distribution of *Ceropegia macrocarpa* in Saudi Arabia.

Figure 4. *Ceropegia macrocarpa*, Jabal Melhan, 20 km E Al Mighlaf, Yemen, ex JRI Wood 1202, sub DP7571. Reproduced from Plowes (2014).

with each lobe subquadrate emarginate or slightly crenate, blackish-maroon; ***inner lobes*** maroon, adpressed to backs of anthers exceeding them and meeting in centre, dorsiventrally flattened around laterally broadened base becoming terete above and tapering gradually to obtuse bristly apex, a transversely conspicuously gibbous, broadened at the base with an acute end.

Distribution in Saudi Arabia. Rare, known only from Asir between Abha and Jabal Sawdah, SW Saudi Arabia (Chaudhary 2001).

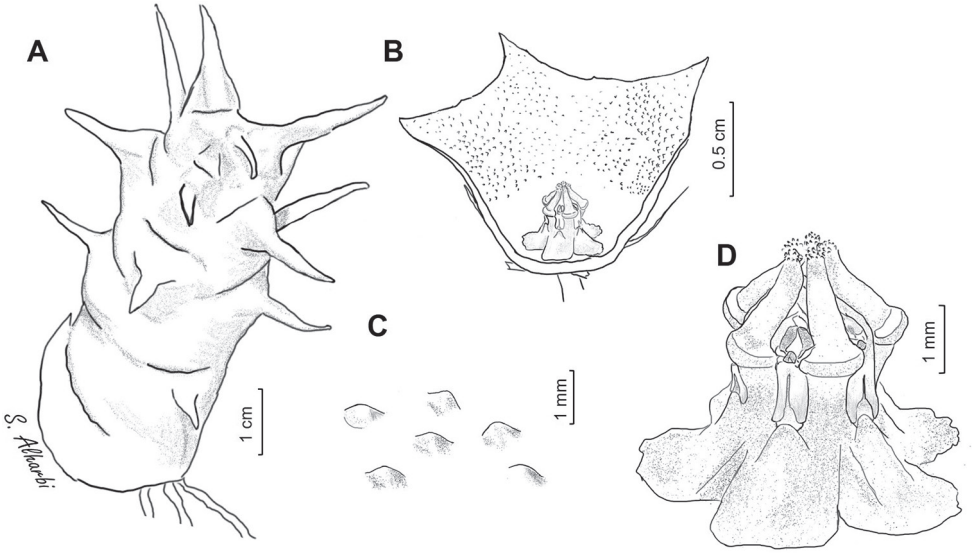


Figure 5. *Ceropegia macrocarpa* **A** branch **B** side view of dissected flower **C** papillae inside corolla in mouth of tube **D** side view of gynostegium. Drawn from photo of ex JRI Wood 1202, sub DP7571, Jabal Melhan, 20 km E Al Mighlaf, Yemen.

General distribution. Somaliland, Sudan, Eritrea, Ethiopia, South west Arabian Peninsula (Saudi Arabia, Yemen) (Albers and Meve 2002).

Habitat and ecology. Growing amongst granitic rocks and scattered shrubs on a steep hillside at 2700 m alt. (Collenette 1999).

Diagnosis. This species can be easily distinguished from other members of sect. *Huernia* in Saudi Arabia by the small maroon bowl-shaped flowers.

Etymology. Macrocarpus (Greek) 'makros', large; and 'karpos', fruit (Eggli and Newton 2004).

Preliminary conservation status. The species is known only from one location near Sawda Mountain; the estimated EOO and AOO of 8 km^2 would place the species in the Critically Endangered (CR) status. However, little is known about the size of the population and possible threats. Therefore, Data Deficient (DD) is assigned to this species.

Additional specimens examined. Ethiopia, *Gilbert 2945* (E [fl in spirit])

2. *Ceropegia khalidbinsultanii* (Plowes & McCoy) Alharbi & Al-Qthanin, comb. nov.
urn:lsid:ipni.org:names:77215099-1
Figs 6, 7; Map 2

≡ *Huernia khalidbinsultanii* Plowes & McCoy, Cact. Succ. J. (Los Angeles) 75(1): 19 (2003). Type: Saudi Arabia – Asir • *T.A. McCoy 2446* (holotype: MO; isotypes

P & SRGH); 25 km SW of Khamis Mushayt; 18°4.0906'N, 42°43.8908'E; alt. 2100 m; 15 Jan 1999.

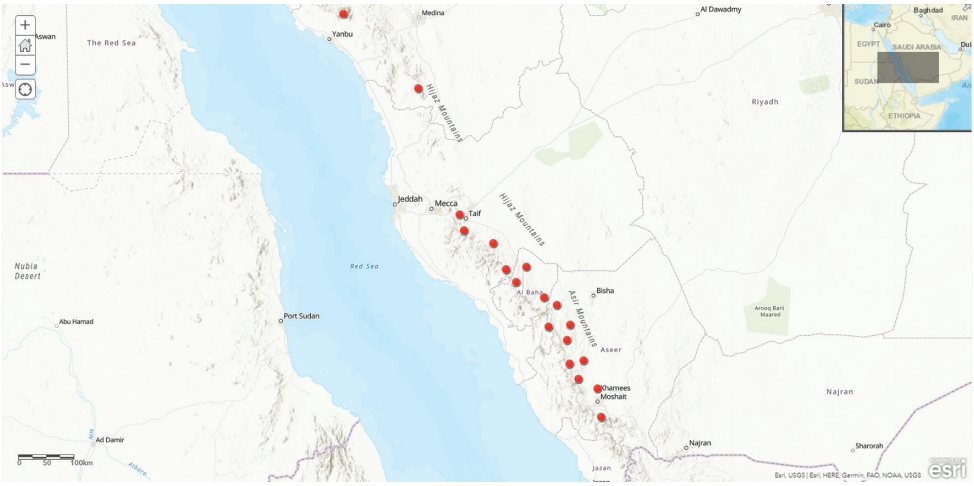
= *Huernia asirensis* Plowes, Asklepios 114: 7 (2012), syn. nov. Type: Saudi Arabia – Tanumah • *I.S. Collenette 2655* (Holotype: K!, [fl in spirit: 44279.000]); 12 km S. of An Numas on Taif to Abha Road;18°56.1481'N, 42°11.2139'E; alt.1800 m; 10 May 1981.

= *Huernia radhwana* Plowes, Asklepios 114: 10 (2012), syn. nov. Type: Saudi Arabia – Jabal Radhwa • *I.S. Collenette 5944* (Holotype: K!, [fl in spirit: 51187.000]); 75 km NE Yanbu; 24°32.3717'N, 38°20.4741'E; alt. 1750 m; 01 Feb 1987.

= *Huernia anagaynensis* Plowes, Asklepios 114: 7 (2012), syn. nov. Type: Saudi Arabia – Jabal Anagayn • *I.S. Collenette 5970* (Holotype: K!, [fl in spirit: 50937.000]); 95 km south of Madinah; 23°21.5747'N, 39°34.9766'E; alt. 1371 m; 06 Dec1986.

Huernia haddaica Al-Hemaid (*nom. inval.* Art 37.2), Saudi J. Biol. Sci. 8: 168 (2001).

Description. Dwarf succulent forming dense clump. ***Branches*** 50–100 mm long, non-rhizomatous, decumbent, grey-green mottled with purple-red; tubercles 7–16 mm long (including leaf-rudiment), 1.5–5 mm broad at base, conical, spreading, laterally flattened and joined into 5 angles along stem, each tipped with a soft slender acuminate caducous leaf- rudiment. ***Inflorescence*** usually only 1 per branch, arising in lower half of branch, each bearing 1–7 flowers developing mainly simultaneously or in gradual succession from short peduncle, with several filiform bracts without lateral teeth, flowers with mainly very foetid odour, rarely faint or no unpleasant smell; ***pedicel*** 5–21.5 mm long, 1–2 mm thick, spreading and holding flower facing horizontally, tapering sometimes toward the point of flower attachment; ***sepals*** 10–18 mm long, 1–2 mm broad at base, attenuate. ***Corolla*** 27–47 mm diam., tubular-campanulate to campanulate; outside smooth, white to creamy-white or cream speckled with pale maroon spots uniformly scattered, sometimes spots become darker especially on the lower or upper half of corolla tube, with 1 heavy



Map 2. Distribution of *Ceropegia khalidbinsultanii*.

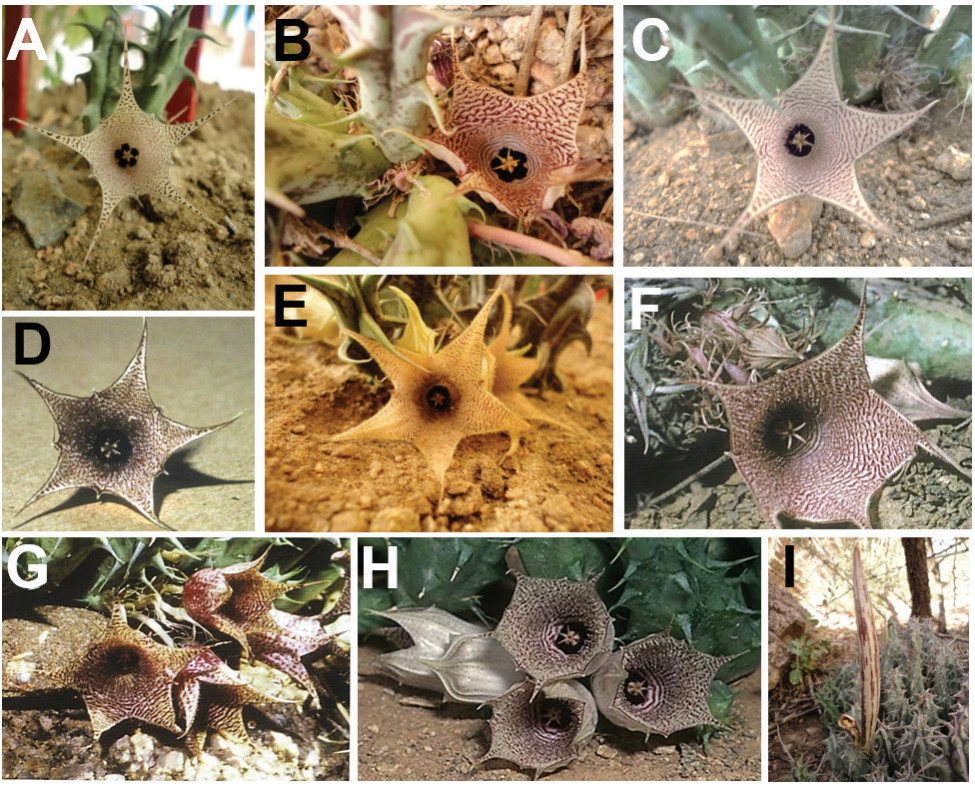


Figure 6. *Ceropegia khalidbinsultanii* **A** *Alharbi S00* (*H. asirensis*) **B** *Alharbi S10b* (*H. asirensis*) **C** *Alharbi S4a* (*H. asirensis*) **D** *Collenette 5944*, Jabal Radhwa, (*H. radhwana*, Type) **E** *Alharbi S16a* (*H. asirensis*) **F** *Collenette 1309*, Al-Baha, (*H. asirensis*, Type) **G** *Collenette 5970*, Jabal Anagyan (*H. anagaynensis*, Type) **H** ex *Tom McCoy KSA129 sub DP8384*, 25 km SW of Khamis Mushayt, (*H. khalidbinsultanii*, Type) **I** follicles, *Alharbi S14a*. (**A–C, E, I**) photo by the first author from Wadi Thee Gazal, Ash Shafa; (**D, F, G**) reproduced from Plowes (2012)); (**H**) received from D. Plowes in 2011.

(+ 2 lighter) raised longitudinal veins running from lobes to base of tube; inside creamy-white to cream with irregular-shaped narrow short maroon lines and dashes changing to narrow concentric broken lines in lower half of tube, covered except in lower third of tube with slender (hair-like) papillae densely crowded around mouth of tube (up to 3 mm long and 0.75 mm an base in tube mouth), each tipped by minute apical acuminata bristle; ***tube*** 7–13 mm long, 9–14 mm broad at mouth, pentagonal; ***lobes*** 13–18 mm long, 8–12 mm broad at base, ascending to slightly spreading, narrowly deltoid and usually longer than wide, attenuate usually concave or form channel above, ***intermediate lobes*** 1–2.5 mm long. ***Corona*** without basal stipe; ***outer lobes*** (4.5–8 mm diam.) spreading on base of tube and fused partially to it; fused together into disc with crenate margin to a slightly disc-like with rounded to subquadrate short and broad lobes, rarely discrete to 5-lobed with each lobe subquadrate mucronate, blackish maroon; ***inner lobes*** 3–5.5 mm long, 0.5–1.5 mm at base, ivory white sometimes mottled with a few maroon spots at the

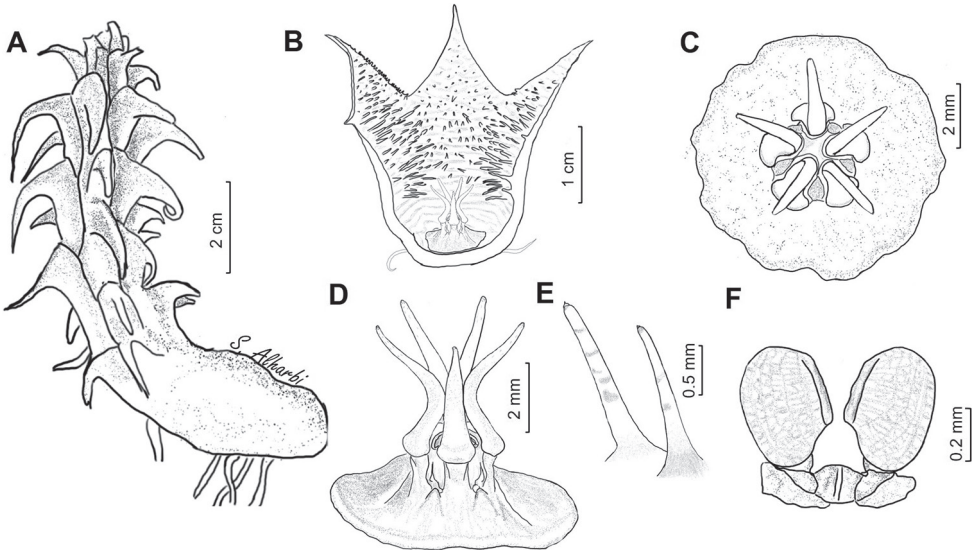


Figure 7. *Ceropegia khalidbinsultanii* **A** branch **B** side view of dissected flower **C** face view of gynostegium **D** side view of gynostegium **E** papillae inside corolla in mouth of tube **F** pollinarium. Drawn from *S.A. Alharbi S4a*, Wadi Thee Gazal, Ash Shafa.

tips, adpressed to anthers in their lower half then rising up connivent and then diverging towards apices, dorsiventrally flattened around laterally-broadened base becoming terete above and tapering gradually to a slender fine minutely-scabrous apex, at base with inflated transversal dorsal gibbosity with rounded to acute end. ***Pollinia*** 0.1–0.7 mm long.

Distribution in Saudi Arabia. Scattered over a wide area, extending from Khamis Mushait in SW of the country to Jabal Radhwa, 75 km north of Yanbu in the Western Region.

General distribution. Probably endemic to SW Arabian Peninsula, known so far from Saudi Arabia only.

Habitat and ecology. Growing on granitic outcrops often under shrubs, from 1800–2100 m alt. Flowering Dec.-May

Diagnosis. *Ceropegia khalidbinsultanii* is best distinguished from the closely-related *C. lodarensis* by longer tubercles (up to 16 mm) on the branches and the smaller, white to creamy-white tubular-campanulate corolla streaked with narrow maroon lines, slender (hairy) papillae and a very foetid odour.

Notes. The foetid odour of the flower becomes weak or completely vanishes after all pollinia have been removed from the flower.

Etymology. Khalidbinsultanii for Prince Khalid bin Sultan bin Abdulaziz M., a former Saudi Deputy Minister of Defence (Plowes and McCoy 2003).

Preliminary conservation status. Near Threatened (NT) has been assigned to *Ceropegia khalidbinsultanii*, based on the species' EOO of 41,490 km^2 and AOO of 2,012 km^2 and the current threats of habitat transformation (roads and housing construction), population fragmentation and tourism.

Additional specimens examined. Saudi Arabia – Al-Taif • *S.A. Alharbi S3a* (UQU); Wadi Thee Gazal, Ash Shafa; 21°5.5702'N, 40°21.785'E; alt. 2057 m; 23 Jan 2011; *S.A. Alharbi S4a* (UQU); same data as for preceding; 1 Jan 2011; *S.A. Alharbi S6a* (UQU); same data as for preceding;10 Jan 2011; *S.A. Alharbi S7a* (UQU); same data as for preceding; 9 Dec 2010; *S.A. Alharbi S8a* (UQU); same data as for preceding; 19 Jan 2011; *S.A. Alharbi S16a* (UQU); same data as for preceding; 8 Jan 2011; *S.A. Alharbi S16A* (UQU); same data as for preceding; 30 Dec 2010; *S.A. Alharbi S13a* (UQU); same data as for preceding; 9 Dec 2010; *S.A. Alharbi S14a* (UQU); same data as for preceding; 17 Dec 2010; *S.A. Alharbi S00* (UQU); 9 Dec 2010; *S.A. Alharbi S10b* (UQU); same data as for preceding; 21°5.4656'N, 40°21.7937'E; 9 Dec 2010.

3. *Ceropegia laevis* (J.R.I. Wood) Bruyns, S. African J. Bot. 112: 424 (2017)

Figs 8, 9; Map 3

≡ *Huernia laevis* J.R.I. Wood, Kew Bull. 39:128 (1984).

Type. Yemen • *J.R.I. Wood 3037* (***holotype***: K [46740.000]); Jebel Marran, Khawlan As Sham; 16°49.2672'N, 43°24.7619'E; alt. 1400 m; 31 Oct 1979.

Description. Dwarf succulent forming dense clump. ***Branches*** non-rhizomatous, up to 80 mm long, erect, decumbent, grey-green mottled with purple or red; tubercles 3–5 mm long, 1 mm broad at base, conical, spreading, laterally flattened and joined towards base into 5 angles along branch, abruptly narrowing into fine spreading slender acuminate tooth. ***Inflorescences*** 1–2 per branch, each of 2–5 flowers developing in gradual succession on short peduncle with few narrow filiform bracts; ***pedicel*** 15 mm long, spreading and holding flower facing horizontally; flowers with no scent; *sepals* 15 mm long, 3 mm broad at base, narrowly ovate attenuate.

Map 3. Distribution of *Ceropegia laevis* in Saudi Arabia.

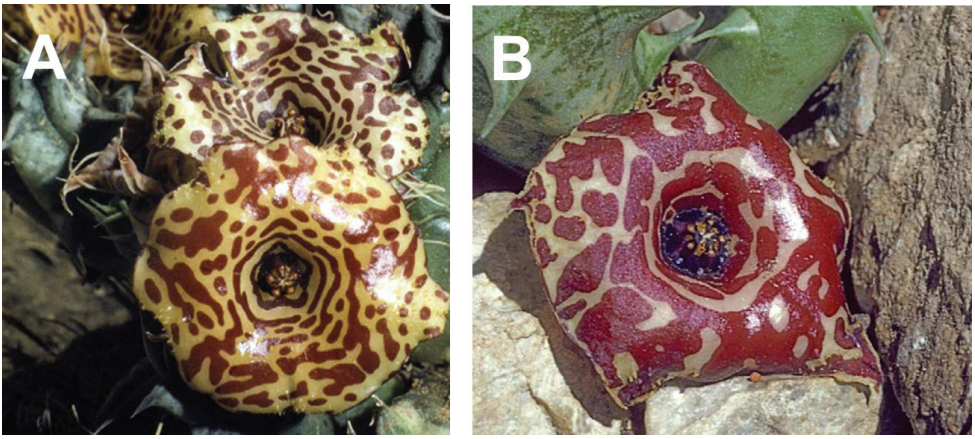


Figure 8. *Ceropegia laevis*, Jabal Qahar, 90 km NE of Baysh, Jazan, Saudi Arabia **A** *Collenette 8177* **B** ex *S Collenette 8180* sub *DP8296*. Reproduced from Plowes (2014).

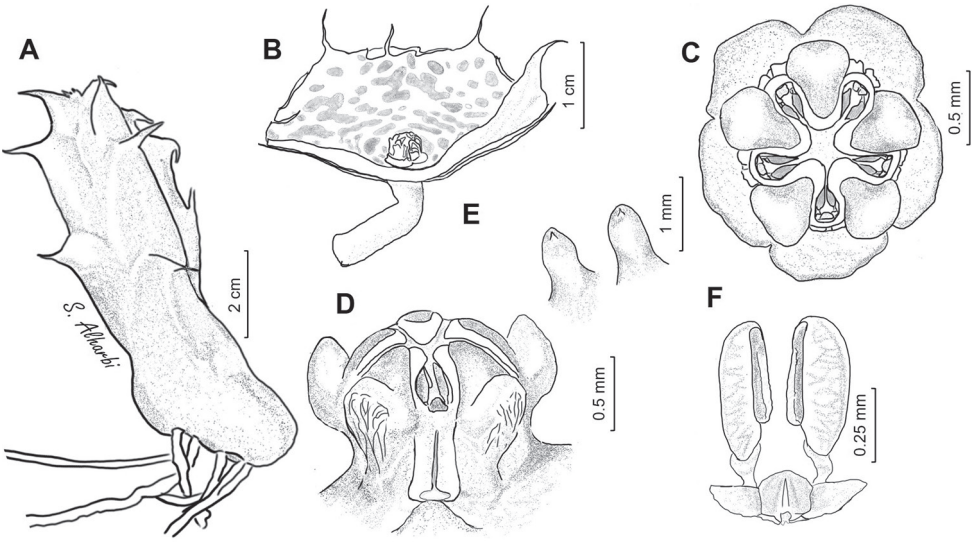


Figure 9. *Ceropegia laevis* **A** branch **B** side view of dissected flower **C** face view of gynostegium **D** side view of gynostegium **E** papillae inside corolla in lobe tip **F** pollinarium. Drawn from *Collenette 8180*, Jabal Qahar.

Corolla 32 mm diam., broadly funnel-shaped, margin weakly bulging like an annulus; outside smooth, pale cream with 1 heavy (+ 2–4 lighter) raised longitudinal veins running down each lobe; inside shiny creamy-yellow, marked with shiny irregular broad maroon streaks and scrolls, smooth with few low conical papillae (wart-like) at corolla lobes apices each with minute apical bristle; *tube* 6 mm long, 10 mm broad at mouth, cupular; ***lobes*** 10 mm long, 14 mm broad at base, reflexed, deltoid-acuminate, ***intermediate lobes*** 1 mm long. ***Corona*** without basal stipe; ***outer lobes*** (discrete 5 lobes), 4 mm diam., subquadrate, emarginate to shallowly bifid, spread-

ing on base of tube and fused to it towards base, blackish-maroon; *inner lobes* 1 mm long, purple with cream at base, adpressed to backs of anthers and shorter than them, dorsiventrally flattened with ascending obtuse conspicuous gibbous at base, tapering to small smooth acute apex.

Distribution in Saudi Arabia. Jabal Al Qahar, 90 km NE of Baysh, Jazan, SW Saudi Arabia (Chaudhary 2001).

General distribution. Probably endemic to SW Arabian Peninsula, known so far from Saudi Arabia and Yemen (Chaudhary 2001).

Habitat and ecology. Growing amongst limestones amongst *Juniperus* at 1828–2000 m alt. (Collenette 1999). Flowering: mainly September–May

Preliminary conservation status. *Ceropegia laevis* should be considered as Nationally Endangered (EN), according to the IUCN Red List criteria. The species is known from only one location, its EOO and AOO (104.00 km^2) would both qualify as Endangered. Its habitat is not part of any protected area and its continuing decline is projected because of anthropogenic activities in the area.

Diagnosis. *Ceropegia laevis* can easily be distinguished from most other species of sect. *Huernia* in Saudi Arabia by the glabrous shiny yellow background colour of the inside of the corolla, which has an annulus-like area around the mouth of the tube.

Etymology. Laevis (Latin) smooth, flat; for the glabrous corolla (Eggli and Newton 2004).

Specimens examined. Saudi Arabia – Jazan • *I.S. Collenette 8180* (K [fl in spirit: 57656.000]); Jabal Qahar; 17°42.0367'N, 42°51.1983'E; alt. 2000 m; 20 Apr 1992.

4. *Ceropegia lodarensis* (Lavranos) Bruyns, S. African J. Bot. 112: 424 (2017).

Description. Dwarf succulent forming dense clump. ***Branches*** 30–100 mm long, non-rhizomatous, erect to decumbent, grey-green mottled with purple-red; tubercles 4–10 mm long (including leaf-rudiment), 1.5–5 mm broad at base, conical, spreading, laterally flattened and joined into 5 angles along branch, each tipped with a soft slender acuminate caducous leaf-rudiment. ***Inflorescence*** arising in lower half of branch, usually 1 per branch, each bearing 2–10 flowers developing in gradual succession from short peduncle sometimes 3 flowers developing simultaneously, bracts filiform without lateral teeth, flowers with no foetid odour, rarely with faint unpleasant smell; ***pedicel*** 8–20 mm long, 1–2.5 mm thick, ascending holding flower facing upwards, tapering sometimes toward the point of flower attachment; ***sepals*** 8–18 mm long, 1–2.5 mm broad at base, attenuate. ***Corolla*** 30–50 mm diam., campanulate; outside smooth, cream-speckled with pale maroon spots uniformly scattered or concentrated on the upper half of corolla tube sometimes spots become darker especially on the upper half, with 1 heavy (+ 2–4 lighter) raised longitudinal veins running from lobes to base of tube; inside cream with irregular-shaped short maroon lines and dashes changing to concentric broken lines in lower half of tube or uniformly coloured with purplish-red, sometimes deep coloured

areas concentrated between the lobes or corolla entirely uniformly coloured with purplish-red; corolla covered, except in lower third of tube with compressed conical papillae densely crowded and reaching maximum size around mouth of tube (up to 3 mm long and 1.2 mm base width), each tipped by minute apical acuminata bristle; ***tube*** 7.5–15.5 mm long, 11–22 mm broad at mouth, pentagonal; ***lobes*** 9–22.8 mm long, 9–14.25 mm broad at base, spreading with recurved apex or sometimes reflexed, deltoid, caudate to acute or acuminate rarely attenuate usually concave or form channel at tip, ***intermediate lobes*** 1.5–4 mm long. ***Corona*** without basal stipe; ***outer lobes*** (5–10 mm diam.) spreading on base of tube and fused partially to it, discrete to 5 lobes with each lobe rectangular rarely subquadrate crenate, dentate, mucronate, emerginate or bifid blackish-maroon; ***inner lobes*** 3–6 mm long, 1–1.5 mm at base, ivory white sometimes mottled with a few maroon spots at the tips or marked entirely with small purple spots adpressed to anthers in their lower half, then rising up connivent and then diverging towards apices, dorsiventrally flattened around laterally-broadened base becoming terete above and tapering gradually to a slender fine minutely-scabrous or smooth apex, at base with slightly inflated transversal dorsal gibbosity with rounded to truncate end, sometimes a conspicuous acute humb appearing in the staminal tube under corona base, rarely hook-like appendages grow from both sides of the base meeting above the guard-rails. ***Pollinia*** 0.7–0.8 mm long.

Key to three varieties of *Ceropegia lodarensis*

1 Branches not stout, up to 100 mm long; corolla up to 50 mm diam., marked inside with irregular short maroon lines and dashes or rarely dotted; outer corona mostly consisting of five distinct rectangular lobes..... **2**

– Branches stout, up to 60 mm long; corolla up to 30 mm diam., marked inside with maroon rounded spots or dashes; outer corona disc or disc-like .... ***C. lodarensis* var. *rubrosticta***

2 Corolla tube inside with concentric broken lines or uniformly coloured with purplish-red, lobes marked with irregular-shaped short maroon lines and dashes sometimes deep coloured areas concentrated between the lobes or corolla entirely uniformly coloured with purplish-red ..... ***C. lodarensis* var. *lodarensis***

– Corolla tube inside with concentric short dashes, corolla lobes marked with small maroon dots ..... ***C. lodarensis* var. *foetida***

4.1 *Ceropegia lodarensis* (Lavranos) Bruyns var. *lodarensis*

Figs 10, 11; Map 4

≡ *Huernia lodarensis* Lavranos, J. S. African Bot. 30: 87 (1964). Type: Yemen – Lodar (Lawdar) • *J.J.Lavranos 1900* (holotype: K [fl in spirit: 24982.000]); 13°52.6751'N, 45°51.7598'E; alt. 900 m; 19 Aug 1962.

Map 4. Distribution of *Ceropegia lodarensis* var. *lodarensis* in Saudi Arabia.

= *Huernia collenetteae* Plowes, Asklepios 114: 8 (2012). syn. nov. Type: Saudi Arabia – Asir • *I.S. Collenette 1176* (clonotype: SRGH [DP6868]); between Abha and Jabal Sawdah; 18°14.425'N, 42°25.2244'E; alt. 2650 m.

= *Huernia saudi-arabica* D.V.Field, Kew Bull. 35(4): 754 (1981). Type: Saudi Arabia – Asir • *I.S. Collenette 549* (holotype: k! [K000911103]); between Abha and Jabal Sawdah, 12 km NW Abha; 18°15.7389'N, 42°23.1535'E; alt. 2650 m.; 31 Mar 1978.

Description. ***Branches*** 30–90 mm long; tubercles 4–10 mm long (including leaf-rudiment), 1.5–5 mm broad at base. ***Inflorescence*** bearing 2–10 flowers developing in gradual succession from short peduncle, sometimes 3 flowers developing simultaneously, flowers with no foetid odour, rarely with faint unpleasant smell; ***pedicel*** 8–20 mm long, 1–2.5 mm thick, ascending holding flower facing upwards, tapering sometimes towards the point of flower attachment; *sepals* 8–18 mm long, 1–2.5 mm broad at base, attenuate. ***Corolla*** 30–50 mm diam., campanulate; outside smooth, cream-speckled with pale maroon spots uniformly scattered or concentrated on the upper half of corolla tube, sometimes spots become darker especially on the upper half; inside cream with irregular-shaped short maroon lines and dashes changing to concentric broken lines in lower half of tube or uniformly coloured with purplish-red, sometimes deep coloured areas concentrated between the lobes or corolla entirely uniformly coloured with purplish-red; ***papillae*** up to 3 mm long and 1.2 mm base width; ***tube*** 7.5–15.5 mm long, 11–22 mm broad at mouth, pentagonal; ***lobes*** 9–22.8 mm long, 9–14.25 mm broad at base, spreading to spreading with recurved apex or sometimes reflexed, deltoid, caudate to acute or acuminate, concave at tip, ***intermediate lobes*** 1.5–4 mm long. ***Outer corona lobes*** (5–10 mm diam.) discrete to 5 rectangular lobes; ***inner lobes*** 3–6 mm long, 1–1.5 mm at base.

Figure 10. *Ceropegia lodarensis* var. *lodarensis* **A** ex *J Lavranos 1789*, sub *DP3604*, Yemen, (*H. lodarensis*, Type) **B** *Alharbi S6B* (*H. collenetteae*) **C** *Alharbi S9B* (*H. collenetteae*) **D** ex *Collenette 549* sub *DP6865*, Jabal Al Sawdah, (*H. saudi-arabica*, Type) **E** *Alharbi S2B* (*H. collenetteae*) **F** *Alharbi S18a* (*H. collenetteae*) **G** *Collenette 2227*, Al-Hadda, (*H. collenetteae*) **H** ex *Collenette 8232* sub *DP8126*, (*H. saudi-arabica*) **I** *Alharbi S4B* (*H. collenetteae*) **J** ex *Collenette 1176* sub *DP6868*, Jabal Al Sawdah, (*H. collenetteae*, Type) **K** maroon uniform colour of corolla tube in ex *Collenette* sub *DP6594*, Abha, (*H. saudi-arabica*) **L** concentric broken maroon lines of corolla tube in *Alharbi S6B* (*H. collenetteae*). (**A**) reproduced from Plowes (2014); (**D, G, H, J, K**) reproduced from Plowes (2012); (**B, C, E, F, I, L**) photo by the first author from Wadi Thee Gazal, Ash Shafa.

Distribution in Saudi Arabia. Scattered over a wide area, extending from Al Habala in SW of the country to Al-Hadda in Al-Taif in the Western Region.

General distribution. Arabian Peninsula (Saudi Arabia and Yemen) and Africa (Ethiopia; *Bruyns*, *P.V. 8432*, E; http://data.rbge.org.uk/herb/E00995868)

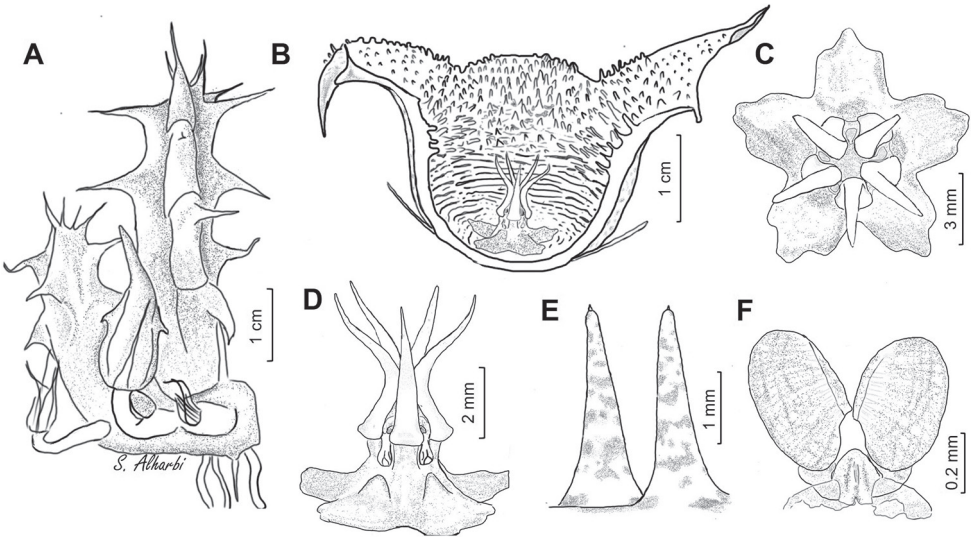


Figure 11. *Ceropegia lodarensis* var. *lodarensis* **A** branch **B** side view of dissected flower **C** face view of gynostegium **D** side view of gynostegium **E** papillae inside corolla in mouth of tube **F** pollinarium. Drawn from (**A**) *S.A. Alharbi S2B*; (**B–F**) *S.A. Alharbi S6B*, Wadi Thee Gazal, Ash Shafa.

Habitat and ecology. It occurs at 900–2650 m alt. in granitic outcrops mainly under shrubs. Flowering: mostly Aug-May

Diagnosis. *Ceropegia lodarensis* var. *lodarensis* is most similar to *C. khalidbinsultanii*, but differs in having a larger campanulate corolla with compressed conical papillae (up to 1.2 mm broad at base), sometimes uniformly coloured with purplish-red, flower with no or only faint bad smell and has shorter tubercles on the branches.

Etymology. Lodarensis for the occurrence at Lodar (Lawdar) in Yemen (Eggli and Newton 2004).

Preliminary conservation status. *Ceropegia lodarensis* var. *lodarensis* should be assessed as Near Threatened (NT) in Saudi Arabia due to species' AOO of 3,900 km^2 and EOO of 12,509.959 km^2 and the current threats of tourism, overgrazing, infrastructure and housing development.

Additional specimens examined. Saudi Arabia – Asir • *I.S. Collenette 1280* (k [fl in spirit: 44272.000] & E); Al Habala, 50 km SE of Abha; 18°1.6787'N, 42°51.3655'E; alt. 2384 m; 06 Apr1979.

Saudi Arabia – Al-Baha • *I.S. Collenette 7785* (k [fl in spirit: 57339.000]); Jabal Shada, SW of Al Baha; 19°50.9947'N, 41°19.0693'E; alt.1933 m; 07 Apr1991; *I.S. Collenette 8267* (K [fl in spirit: 59350.000]); same data as for preceding; 15 Sep1992.

Saudi Arabia – Al-Taif • *I.S. Collenette 815* (K!, herbarium specimen); Wadi Ammak near Al Hadda; 21°20.9808'N, 40°17.7485'E; alt. 2100 m; *I.S. Collenette 2227* (K [fl in spirit: 44371.000, 53692.000]); Al-Hada; 21°20.8387'N, 40°17.152'E; alt. 2000 m; 1981; *I.S. Collenette 2633* (K [fl in spirit:45473.000, 45894.000, herb. material sub DP6599 & sub Leach 17652]); SW of Al Hadda, off Taif to Abha Road; 21°18.4696'N, 40°22.1371'E; alt. 2100 m; 07 May 1981; *I.S. Collenette 5780* (K [fl in spirit: 35856.000]);

Between Al Hadda and Harithi; 21°5.7571'N, 40°55.0155'E; alt. 1620 m; 23 Mar1986; *S.A. Alharbi S1b* (UQU); Wadi Thee Gazal, Ash Shafa; 21°5.4656'N, 40°21.7937'E; alt. 2057 m; 29 Dec 2010; *S.A. Alharbi S2b* (UQU); same data as for preceding; 09 Dec 2010; *S.A. Alharbi S3b* (UQU); same data as for preceding; 23 Jan 2011; *S.A. Alharbi S4b* (UQU); same data as for preceding; 29 Dec 2010; *S.A. Alharbi S5b* (UQU); same data as for preceding; 23 Nov 2010; *S.A. Alharbi S6b* (UQU); same data as for preceding; 02 Oct 2011; *S.A. Alharbi S9b* (UQU); same data as for preceding; 23 Nov 2010; *S.A. Alharbi S9a* (UQU); same data as for preceding; 05 Jan 2011; *S.A. Alharbi S18a* (UQU); same data as for preceding; 21°5.5702'N, 40°21.785'E; 17 Dec 2010.

I.S. Collenette 1523 (E [fl in spirit]); *A.J. Bntler AJB 13* (E [fl in spirit]).

4.2 *Ceropegia lodarensis* (Lavranos) Bruyns var. *foetida* (Plowes) Alharbi & Al-Qthanin, comb. nov.

urn:lsid:ipni.org:names:77215100-1
Figs 12, 13; Map 5

≡ *Huernia foetida* Plowes, Asklepios 114: 9 (2012).

Type. Saudi Arabia – Jazan • *I.S. Collenette 3743* (holotype: K! [fl in spirit: 38892.000]); Jabal Fayfa, 80 km NE Jazan; 17°14.5296'N, 43°4.9368'E; alt. 1550 m; 31 Jul 1982.

Description. ***Branches*** up to 100 mm long; tubercles 7 mm long (including leaf-rudiment), 1.5 mm broad at base. ***Inflorescence*** bearing up to 4 flowers developing in gradual succession, flowers with very foetid odour; ***pedicel*** spreading and holding flower facing horizontally, tapering towards the point of flower attachment. ***Corolla*** 40 mm diam., campanulate; outside smooth, cream; inside cream with ma-

Map 5. Distribution of *Ceropegia lodarensis* var. *foetida*.

roon dots changing to concentric short dashes in lower half of tube covered, except in lower third of tube with compressed conical papillae densely crowded and reach maximum size around mouth of tube (up to 1 mm long and 0.5 mm broad at base); ***tube*** 11 mm long, 10 mm broad at mouth, pentagonal; ***lobes*** spreading, deltoid, attenuate with deep groove at tip, ***intermediate lobes*** 1 mm long. ***Outer corona lobes*** 8 mm diam., five discrete rectangular lobes; ***inner lobes*** 3 mm long, 1 mm at base.

Distribution in Saudi Arabia. Rare, known so far from Jabal Fayfa, 80 km NE Jazan, SW of the country (Plowes 2012).

General distribution. Probably endemic to SW Arabian Peninsula known so far from Saudi Arabia.

Habitat and ecology. Occurs in granitic outcrops at 1550 m alt (Collenette 1999).

Diagnosis. Clearly distinct by its campanulate corolla that is dotted with maroon inside and has a very foetid odour when it opens.

Figure 12. *Ceropegia lodarensis* var. *foetida* (*H. foetida*) Jabal Fayfa, Type. Reproduced from Plowes (2012).

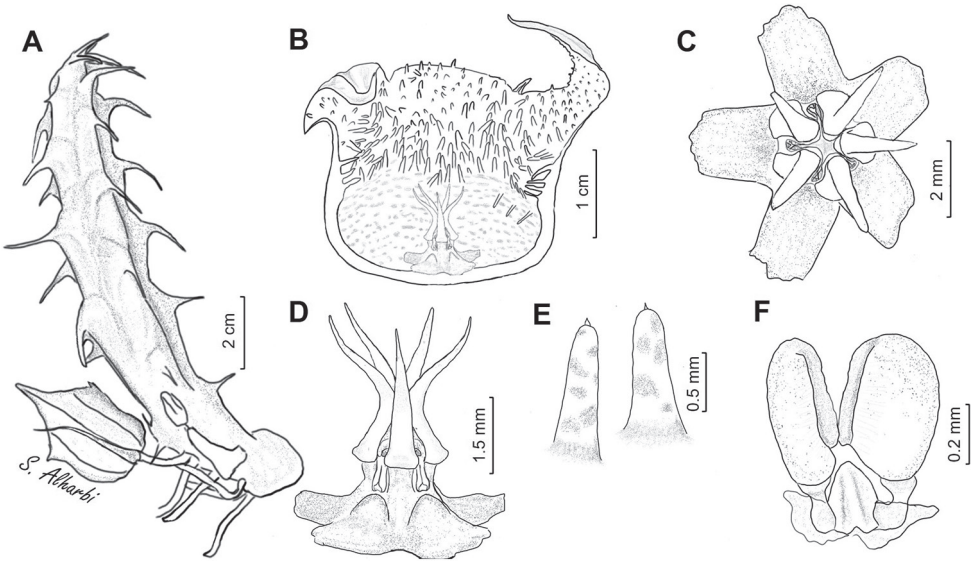


Figure 13. *Ceropegia lodarensis* var. *foetida* **A** branch **B** side view of dissected flower **C** face view of gynostegium **D** side view of gynostegium **E** papillae inside corolla in mouth of tube **F** pollinarium. Drawn from *Collenette 3743*, Jabal Fayfa.

Etymology. Foetidus (Latin) smelly, for the strong, unpleasant smell of flowers.

Preliminary conservation status. *Ceropegia lodarensis* var. *foetida* is estimated to have an EOO of 80.173 km^2 (which would place the species in Critically Endangered, CR) and AOO of 88 km^2 (which would place it in EN). The size of its populations and current threats are not well-known, but populations in mountainous areas in Saudi Arabia are likely impacted by agriculture, overgrazing, development and tourism. Therefore, var. *foetida* should be considered Data Deficient (DD).

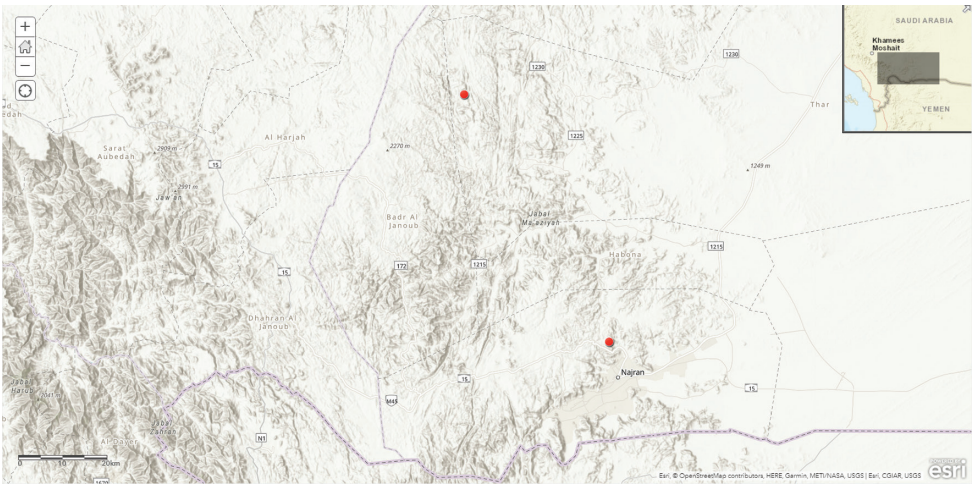
4.3 *Ceropegia lodarensis* (Lavranos) Bruyns var. *rubrosticta* (Plowes) Alharbi & Al-Qthanin, comb. nov.

urn:lsid:ipni.org:names:77215101-1
Figs 14, 15; Map 6

≡ *Huernia rubrosticta* Plowes, Asklepios 114: 11 (2012).

Type. Saudi Arabia – Najran • *I.S. Collenette 1482* (holotype: k! herb. material); Jabal Manfah, 24 km NE Najran; 17°36.9386'N, 44°12.3742'E; alt. 1700 m; 30 Apr 1979.

Description. ***Branches*** 30–60 mm long, stout; tubercles 5.5–6 mm long (including leaf-rudiment), 2–3 mm broad at base. ***Inflorescence*** bearing 6 flowers developing in gradual succession from short peduncle, flowers with faint unpleasant smell; ***pedicel*** 11 mm long, 2 mm thick, ascending holding flower facing upwards, tapering sometimes towards the point of flower attachment; ***sepals*** 9.5 mm long, 2 mm broad at base, attenuate.



Map 6. Distribution of *Ceropegia lodarensis* var. *rubrosticta*.

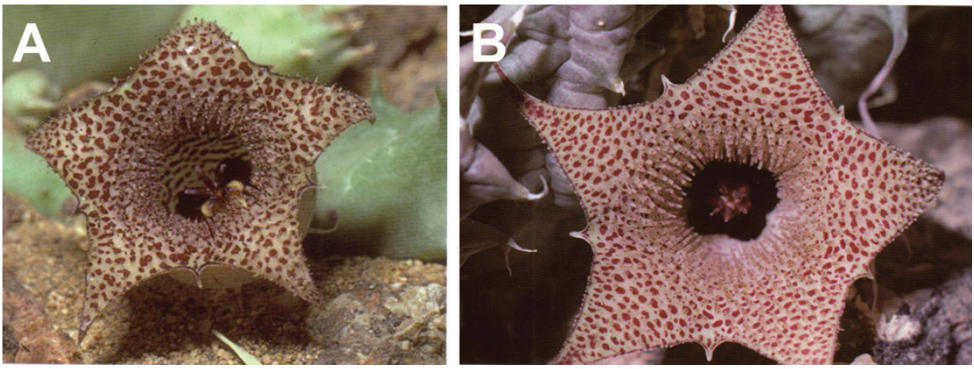


Figure 14. *Ceropegia lodarensis* var. *rubrosticta* (*H. rubrosticta*) **A** ex *S Collenette* s.n. sub *DP7639*, Al Jawshan, 70 km NW Najran (Type) **B** *Collenette 1482*, Jabal Manfah, 24 km NE Najran. Reproduced from Plowes (2012).

Corolla 32 mm diam., campanulate; outside smooth, cream-speckled with pale maroon spots on the upper half of corolla tube; inside cream with rounded maroon spots or dashes changing to concentric broken lines in lower half of tube; ***papillae*** up to 1.5 mm long and 0.75 mm broad at base; ***tube*** 10 mm long, 12 mm broad at mouth, pentagonal; ***lobes*** 8 mm long, 7.5 mm broad at base, spreading with recurved apex, deltoid, acute concave at tip, ***intermediate lobes*** 1.5 mm long. ***Outer corona lobes*** 5 mm diam. fused into disc or a slightly disc-like with short subquadrate crenate; ***inner lobes*** 3 mm long, 1 mm at base.

Distribution in Saudi Arabia. Rare, known only from Najran Region, SW Saudi Arabia (Plowes 2012).

General distribution. Probably endemic to SW Arabian Peninsula, known so far only from Saudi Arabia

Habitat and ecology. Concentrated amongst rounded granitic boulders at 500–1700 m alt. (Collenette 1999). Flowering: March-April.

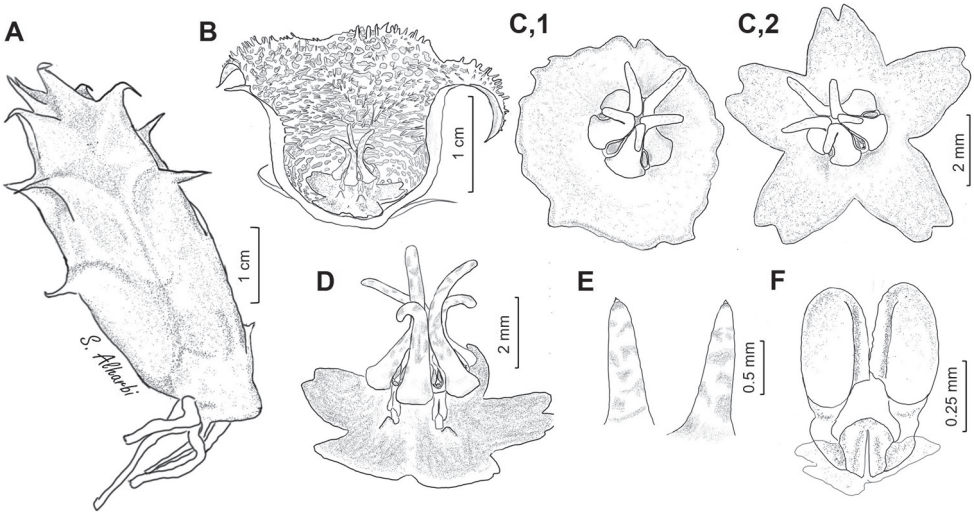


Figure 15. *Ceropegia lodarensis* var. *rubrosticta* **A** branch **B** side view of dissected flower **C** face view of gynostegium **D** side view of gynostegium **E** papillae inside corolla in mouth of tube **F** pollinarium. Drawn from (**A, B2, C**) *DP7639*, Al Jawshan, 70 km NW Najran; (**B1, D, E**) *Collenette 6059*.

Diagnosis. The variety is most similar to the Ethiopian endemic *Huernia boleana*, from which it can be separated with flowers by the much more campanulate corolla that is wider than the long, pentagonal tube, the more conical papillae and the shorter inner coronal lobes (ca. 3 mm compared to ca. 6 mm in *H. boleana*) and with habit and habitat that is erect to decumbent amongst granitic rocks compared to *H. boleana* that is erect, pendulous or prostrate in basalt or sandstone.

It can be easily distinguished from the other varieties of *C. lodarensis* proposed here by the stout branches, flowers with more evenly-rounded spots inside that are more red in colour and by the more slender papillae.

Preliminary conservation status. *Ceropegia lodarensis* var. *rubrosticta* has an estimated EOO of 97.188 km^2 (which would place the species in CR) and AOO of 20 km^2 (which would place it in EN). The size of populations and current threats are little known. Therefore, *C. lodarensis* var. *rubrosticta* should be considered Data Deficient (DD).

Additional specimens examined. Saudi Arabia – Najran • *I.S. Collenette 6059* (K [fl in spirit: 51184.000]); Al Jawshan, 70 km NW Najran; 18°8.4287'N, 43°51.2486'E; alt. 1520 m.; 07 Mar 1987.

Acknowledgements

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Supplementary material 1

Appendices 1–3

Authors: Samah A. Alharbi, Rahmah N. Al-Qthanin

Data type: statistical data

Explanation note: Illustration shows how the plant parts were measured, Screen plot eigenvalues for identification of principal components and boxplot showing differences in morphological characters of *Huernia* species.

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doi: 10.3897/phytokeys.174.62059
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RESEARCH ARTICLE

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Lilium leichtlinii subsp. *maximowiczii* (Regel) J.Compton (Liliaceae): a new combination for Maximowicz's orange lily

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Abstract

The newly-proposed *Lilium leichtlinii* subsp. *maximowiczii* (Maxim.) J.Compton recognises the wide distribution of Maximowicz's lily and provides long-term stability of the name. Lectotypes are designated for the names *Lilium leichtlinii* Hook.f., *L. maximowiczii* Regel, *L. maximowiczii* var. *tigrinum* Regel, *L. pseudotigrinum* Carrière and *L. tigrinum* var. *lishmannii* T.Moore.

Keywords

Lilium leichtlinii, nomenclature, taxonomic conspectus, typification

Introduction

Lilium leichtlinii Hook. f. was first described by Joseph Dalton Hooker of Kew. His description of this elegant lily was based on a citron-yellow flowered plant with strongly recurving perianth segments spotted with dark brownish-purple as shown clearly in the accompanying painting by Walter Hood Fitch (Hooker 1867: t. 5673).

Lilium leichtlinii is endemic to a disparate range of localities in Honshu and the Ryukyu Islands, Japan (Hayashi 2016: 113). It is in all probablility a recessive expression of a widespread orange flowered species that occurs in China, Korea, Japan and along the southern seaboard of the Russian Far East. The orange flowered lily has been given a number of names at the ranks of species, varietas and forma which are discussed below.

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Morphologically, there is little to segregate the yellow and orange flowered lilies that belong in *L. leichtlinii* other than the colour of their floral organs. Both have floccose hairs on their pedicels, buds and on the perianth segments at the apices and median lines externally.

The purpose of this paper is to establish the name *L. leichtlinii* subsp. *maximowiczii* (Regel) J.Compton. Currently, it is widely known and accepted worldwide under the varietal name *L. leichtlinii* var. *maximowiczii* (Regel) Baker (Baker 1871) and is recognized under that name in both the horticultural literature (e.g. Haw 1986: 116); floristic publications (e.g. Hayashi 2016: 113) and in molecular phylogenies (e.g. Kim, Lim and Kim 2019: 2.1). At the rank of variety, however, it is predated by *L. maximowiczii* var. *tigrinum* Regel (Regel 1870). Under the rules of the ICN (Turland et al. 2018), names only have priority within their own rank and *L. maximowiczii* var. *tigrinum* still has priority even though it is attached to a superfluous, but valid, species name (see ICN Art. 11.2 ex. 4). Furthermore, additional use of the epithet *L. leichtlinii* var. *tigrinum* (Regel) G.Nicholson (1885), as cited in, for example, Ohwi (Ohwi 1984: 297), would inevitably engender much confusion with the morphologically-similar species *L. lancifolium* Thunb. (Thunberg 1794: 333) which was consistently and widely-known under the synonym *L. tigrinum* Ker Gawl. (Ker 1809) for more than two centuries and is still universally referred to today as the "Tiger lily".

Lilium lancifolium is readily distinguished from *L. leichtlinii* by the production of dark purple bulbils formed in the leaf axils along the inflorescence axis. These are not found on the inflorescence axis in *L. leichtlinii*. Moreover, *L. leichtlinii* has bulbs which frequently send out underground stolons which produce axillary bulbils, a habit that does not occur in *L. lancifolium*. There is, however, an additional element of possible confusion between *L. lancifolium* and *L. leichtlinii*, the rare occurrence in Kyushu of the yellow flowered tiger lily *L. lancifolium* var. *flaviflorum* Makino (Makino 1933). This variety can also be distinguished from *L. leichtlinii* by the presence of purple stem bulbils produced in the leaf axils.

In recent phylogenetic studies, based on plastid and nuclear DNA sequence data, *L. lancifolium* has been shown to belong on a clade with *L. maculatum* and *L. pensylvanicum*, whereas *L. leichtlinii* has been shown to belong with *L. callosum* and *L. concolor* (Dubouzet and Shinoda 1999; Givnish et al. 2020).

Typification of Leichtlin's yellow lily

This lily was sent from Japan during the decade of Japanese history known as 'bakumatsu' or 'end of the curtain'. Japan had finally ended its three centuries of isolationist 'sakoku' or 'locked in' period under the Tokugawa shoguns and opened up to trade with foreign nations under the government of the re-instated Meiji emperor. Little in the way of lily introductions from Japan to Europe or North America had occurred since von Siebold's employment by the Dutch in Japan from 1823 to 1829. Siebold's activities were limited almost exclusively to the surrounding countryside around Nagasaki on Kyushu Island and specifically to the little artificial Island of Dejima (Compton and Thijsse 2013).

Siebold had been responsible for sending back many good garden-worthy plants from Japan and was particularly fond of the Japanese lilies. He included a number of them in his Prix Courants and Catalogues Raisonnés from plants he cultivated in his Leiden garden. He encountered this lily on his second visit to Japan from 1859 to 1862 when he returned to Japan as a trade envoy for the Dutch Government during the bakumatsu. Although Siebold did not give this yellow flowered lily a Latin name, he had it painted in 1861 on fine Japanese paper by Shimizu Higashiya under the Japanese name Kihirato yuri (Siebold's Florilegium vol. 1b, Pl. 299; vol. 2 no. 830). The painting, which is kept in the Russian Academy of Sciences Library in St. Petersburg, was annotated in pencil '*Lilium* spec. nov.' by Siebold and on the verso bottom left in pencil by Maximowicz '*Lilium testaceum*'. The latter annotation is probably Maximowicz's identification of it as the lily described by Lindley (1842: 51; Lindley 1843 t. 11) as the "Yellow Japan Lily" under the name *L. testaceum* Lindl. This curious lily was in cultivation at the nursery of William Rollison & Sons of Upper Tooting near London and is now known to be a hybrid of two European species (Sterling 2017: 202).

Sir Joseph Dalton Hooker described *Lilium leichtlinii* after it had been introduced to England in a batch of bulbs of *L. auratum* Lindl., which itself had only been described as a new species five years earlier. The bulbs had been sent from Japan to the nursery of James Veitch & Sons of Chelsea. Hooker does not say who had sent the bulbs, but the shipment could have been arranged through a local supplier by John Gould Veitch who was in Japan from 1861–1862 from where he is recorded to have sent back bulbs of *L. auratum* (Veitch 1906: 50).

Hooker named the yellow flowered species after Maximilian Leichtlin (1831–1910), a keen horticulturist and bulb enthusiast who corresponded with other like-minded people including lily monographer Henry John Elwes (see below). Another of Leichtlin's correspondents was John Gilbert Baker who had joined the herbarium and library staff at Kew in 1866. Max Leichtlin, born in Karlsruhe in southwest Germany, had worked for two years in the Van Houtte nursery near Ghent before founding his own private botanic garden in Baden-Baden, Germany. There he grew many bulbous plants, including Hooker's recently described *L. leichtlinii* and another he recorded in his list of cultivated plants as *L. leichtlinii* var. *major* (Leichtlin 1873: 10). The latter plant, although undescribed by him, might refer to the taxon described in the same year as *L. leichtlinii* [unranked] *majus* G.F.Wilson (Wilson 1873: 371) and later as *L. leichtlinii* f. *majus* G.Nicholson, who stated that this yellow-flowered, purple-black spotted form was luxuriant and attained a height of 5 ft [1.52 m] and that it had been introduced from Japan in 1872 (Nicholson 1885: 270).

There is a sheet at K which is partitioned into three different sections. On the right hand section with the barcode K-000464728, the upper right hand portion consists of a pedicel and three leaves along with a dissected flower in a herbarium capsule and bears a label with "Lilium leichtlinii Hk fil. Hort. Barr July 30 1872". Below that is a cut out illustration in pencil of two bulbs, the drawing bearing the legend "2 & 3 Lilium leichtlinii nat. size. From bulbs cult. by Colchester Bulb Company. Comm. F. Burbidge February 1877". The Barr specimen and the Burbidge drawing are both added on to this sheet after Hooker's protologue and are, therefore, not original material. The history of these

two additions on the sheet may be of minor interest. Peter Barr was a daffodil specialist who, with his business partner Edward Sugden, owned a shop in King Street, St. James's, London, England from 1861. *Lilium leichtlinii* was listed as a new entrant in their 1871 catalogue for the very expensive price of "ten shillings and sixpence" [equivalent to ca. 600 GBP or 830 USD today]. Frederick William Burbidge was employed at Kew principally as a draughtsman from 1868 to 1870. He then became a plant collector in Borneo for James Veitch's nursery. Although Burbidge communicated the bulb sketches to Kew (probably to Joseph Hooker) in 1877, it is possible that he drew them earlier.

The whole left hand portion of the sheet with a stamp for Herbarium Hookerianum 1867 on it and with the modern barcode K-000464729 is taken up with two stems, one with a single flower and has a label with "Lilium leichtlinii? Fl. July 5673. Bot. Mag. From Mr Fitch, Accpt. Veitch 1867. Japan". There is also an accompanying letter from the Royal Exotic Nursery, King's Road, Chelsea, London dated 24 July 1867 from Veitch which includes the following information: "Dear Mr Hooker, Amongst the imported *Lilium auratum* roots, which came home last winter we have found one now in bloom which seems to us quite different from any other kind we have seen, in fact, more like a yellow Turk's Cap lily. We send you the flower by bearer and should be glad to know if you consider it new and worth figuring". The annotation on the label with the number "5673", "Bot. Mag.", "Fl. July" and "from Mr Fitch" are direct references to the illustration by Walter Hood Fitch that accompanied Hooker's protologue dated 1 November 1867 in *Curtis's Botanical Magazine* (Hooker 1867: t. 5673). In his protologue, Hooker mentions that the lily was "communicated to me in July of the present year". There are, therefore, two elements that accompany the protologue, the sheet at K and the illustration in the Bot. Mag. The portion of the sheet barcoded K-000464729 is undoubtedly original material and is eligible to be the lectotype, if not the holotype for the name (Art. 9.3, Turland et al. 2018). http://specimens.kew.org/herbarium/K000464729

Naming and typification of the orange lily

The first publication of *Lillium leichtlinii* with orange rather than yellow flowers was, in fact, on exactly the same day i.e. 1 November 1867 as the publication of the yellow-flowered species named *L. leichtlinii* by Joseph Hooker (J. McNeill pers. com.). Precedence for the use of *L. leichtlinii* (Art. 11.5 of the ICN), however, was provided by Baker who chose *L. pseudotigrinum* as a synonym of *L. leichtlinii* var. *maximowiczii* (Regel) Baker (Baker 1871: 1422).

Lilium pseudotigrinum Carrière was named by Elie-Abel Carrière, a Parisian horticulturist as the false tiger lily (Carrière 1867: 411). He named it specifically in contrast to *Lilium lancifolium* Thunb. which was known at that time under the later synonym *L. tigrinum* Ker Gawl. Morphologically *L. pseudotigrinum* has all the characters that equate it to *L. leichtlinii*, but with orange rather than yellow flowers and it has, consequently, been combined within that species at various ranks.

Carrière included a painting by F. Yerna in his protologue of *L. pseudotigrinum* (Carrière 1867: t. "*Lilium pseudotigrinum*"). Yerna's illustration was painted from a

plant cultivated in the Muséum [national d'histoire naturelle] de Paris, also known as the Jardin des Plantes. Carrière stated that the plant had been introduced from China, but he did not mention who had collected it or its precise locality. There were a number of French botanists who collected seeds and bulbs in eastern China and sent them back to Carrière in Paris in the early 1860s, following the Treaty of Tientsin [Tianjin] in 1858. One such was Gabriel Eugène Simon (1829–1896), a French diplomat who travelled extensively in Hebei and Jilin Provinces where the lily occurs in the wild and who is known to have sent seedlings of *Prunus simonii* (Decne) Carrière back to the Paris Muséum in the 1860s. Yerna's illustration clearly shows the flowers of the orange flowered *L. leichtlinii* with their dark brown speckling and anthers with brown pollen. As there are no garden records for the living collections at P for that time and, as there is no evidence of any herbarium specimen in P that could refer to Carrière's plant (Florent Martos pers. comm.), Yerna's illustration is chosen here as the lectotype for the name.

A year later, Eduard August von Regel also described the orange flowered *L. leichtlinii* as *L. maximowiczii* from one of Maximowicz's collections in Japan (Regel 1868a: 26). Regel described the lily in the supplement to the 1866 Index Seminum of the Imperial Botanic Garden in St. Petersburg, published in 1868, in which he mentioned the distinctive scarlet-orange sulcus or groove in the perianth segments and their being dotted with dark purple from their centres towards the base. Later that same year, Regel included another description of *L. maximowiczii* with an illustration in *Gartenflora*, the journal he edited and had founded in 1852 (Regel 1868b: 322, t. 596). The illustration was painted from a plant introduced from a garden in Japan collected by Carl Johann Maximowicz between 1860 and 1864 and then cultivated in the Imperial Botanical Garden, St. Petersburg.

Amongst the collections in the Herbarium at St. Petersburg (LE) is a fine watercolour on paper of this orange flowered lily annotated in pencil above the lily "9 Lil maximowiczii Rgl" and in ink "Aka hirado yuri" by an unknown hand (Fig. 1). Below on the left is also written in pencil "misit Tokuda 1889". The sender is very likely to have been the botanist Shôzô Tokuda who had participated in the International Congress of Botany and Gardening in St Petersburg in 1884 and while there, had helped Maximowicz clarify the Japanese localities of several of his collections (Grabovskaya-Borodina 2016: 63). The artist of the illustration is unknown and the work is undated; however, as Tokuda sent the illustration to Maximowicz in 1889, it cannot be material that could have been the basis for either of the illustrations in *Gartenflora* referring to either *L. maximowiczii* or *L. maximowiczii* var. *tigrinum* (Regel 1868b; 1870).

There are also eight sheets in LE labelled *Lilium leichtlinii* that were collected in Japan near Yokohama in 1862 by C. J. Maximowicz, five of them bearing the annotations 'cult.' or 'cultivatus'. These all have the clear paleness on drying of the yellow flowered species described by Hooker (1867) and are all probably of cultivated origin. In addition, there are six collections by Maximowicz labelled *Lilium maximowiczii* and a further eight collections similarly labelled that were collected by Maximowicz's Japanese assistant Sugawa Tschonoski [Chonosuke] all collected between 1862 and 1864 in Japan. Regel specifically mentioned that *L. maximowiczii* was based on material cultivated in the Imperial Botanic Garden in St. Petersburg and therefore, none of these specimens can be considered as type material of *L. maximowiczii*.

Figure 1. Watercolour by an unknown Japanese artist entitled "Aka hirado yuri" depicting *Lilium leichtlinii* subsp. *maximowiczii* (Regel) J.Compton.

There are however, three sheets annotated by Regel "L. maximowiczii" with the printed label 'Ex horto bot. Petropolitano'. One is dated 68.6 with "teste Rgl" [according to Regel] indicating that it was gathered in June 1868. The sheet consists of a few scattered tepals, some floral dissections and some leaves (LE-01072026). Another is dated 67.7 indicating

July 1867 with the addition of "vv Rgl" [vidi vivit], stating that Regel had seen the living plant (LE-01072027), the stem having a single open flower and three basal leaves. The third with a flowerless stem and a herbarium capsule with some seeds in it, has the additional annotations "fl. Punctato, 6. x. 71 v.v Maxim." (LE-01072028). This last specimen is dated 6 October 1871. Although it was seen by Maximowicz, it was collected after the dates of the two publications and is, therefore, not original material. The other two, however, can be considered as original material. Bearing in mind that Regel specifically mentioned material that was cultivated in the St Petersburg Garden in his protologue and, in consideration of the time needed then for the process of publication, it would be wise to choose the earlier of these specimens gathered from the Garden in 1867 (LE-01072027), as the lectotype for the name (Fig. 2). The other specimen can be considered to be a paratype.

Two years later, another of Maximowicz's collections from Japan flowered in the Imperial Garden and was figured again by Regel in *Gartenflora*. This he called *L. maximowiczii* var. *tigrinum* Regel. It was distinguished from his earlier species description by being more upright, having narrower leaves and flowers with perianth segments that recurved only at the tip and with blackish-purple speckling (Regel 1870: 290). In all respects, these can be considered to be merely minor variations of what he had already described. As far as selecting material for typification purposes, there is no direct link to any of the herbarium specimens in LE that Regel saw and/or annotated that links them with the varietal name. The illustration in *Gartenflora* (Regel 1870: t. 664), therefore, must be considered as the only original material available as the lectotype for that name.

Lilium tigrinum var. *lishmannii* T.Moore was described briefly under the name *L. lishmanni* by R. D. [Richard Dean] in *Florist and Pomologist* 1872: 259 where it was awarded a First Class Certificate at the Royal Horticultural Society's meeting in South Kensington, London on 24 August of that year. It was said to "represent a fine variety of the tigrinum type, with large dull-red flowers profusely spotted with black in a very distinct manner" and that it had originated from Mr T. R. Tuffnell of Uxbridge (R.D. 1872: 259). As Dean described it as a variety that was not attached to any species, it is not considered to be validly published (Art. 11.4 and Art. 24.1) and is, in any case, merely a dark-flowered horticultural variant of *L. leichtlinii* subsp. *maximowiczii*.

The following year, the editor of *Florist and Pomologist* and curator of the Society of Apothecaries Garden in Chelsea [Chelsea Physic Garden], Thomas Moore, formally recognised this lily with an accompanying illustration and a full description as a variety of the tiger lily (Moore 1873: 16). The illustration plate 2 shows a stem with tawny orange-red flowers with dark blackish spotting. Clearly shown are also the papillose margins of the orange nectaries. This illustration was painted from Tuffnell's plant, cited by Dean the year before who had received the plant in 1871 from Mr. Lishmann in Japan. Moore clearly believed that this lily actually belonged to the Tiger lily which was then almost universally known as *L. tigrinum*, [now *L. lancifolium*] which also occurs in Japan. The stem in the illustration, however, is green and scabrid, not dark purple and there are no stem bulbils present. It is clearly a dark orange-red flowered variant of *L. leichtlinii*.

Between March 1877 and May 1880, Henry John Elwes produced his magnificent monograph of the genus *Lilium* (Elwes 1877–1880). In that work, he included the yellow-flowered *Lilium leichtlinii* as plate 39 in part three of his published monograph for

Figure 2. Lectotype sheet LE-01072027 of *Lilium leichtlinii* subsp. *maximowiczii* (Regel) J.Compton.

August 1877, with a plate by Walter Hood Fitch. In the text, he attempted to distinguish between *L. leichtlinii* and what he later portrayed as *L. maximowiczii*. Elwes included a short table given to him by Maximowicz consisting of two columns showing the distinguishing diagnostic characters of the two lilies. Elwes also added that these characters were, in his opinion, not very stable and he believed that *L. maximowiczii* should be regarded as a variety of *L. leichtlinii*, following Baker's earlier recombination (Baker 1871).

Elwes later included an illustration of *L. maximowiczii* as plate 40 in part six of his monograph for January 1879 (Elwes 1877–1880). The plate painted by Walter Hood Fitch, included four examples of the variation he had seen within the orange lily. These comprised *L. maximowiczii* with orange flowers and paler reverse; *L. maximowiczii* var. *bakeri* Elwes, with dark reddish-orange perianth segments speckled with dark purple and pale orange on the reverse; *L. maximowiczii* var. *regeli* Elwes, with dark reddish perianth segments and red streaking, pale orange on the reverse and *L. pseudotigrinum* orange with red speckles and a yellow reverse. These all clearly belong within the circumscription of the subspecies proposed here. It is clear too that Elwes's recognition of var. *bakeri* and var. *regelii* are merely floristic colour variants and are here best regarded as horticultural cultivars 'Bakeri' and 'Elwesii'.

Taxonomic Conspectus

***Lilium leichtlinii* Hook.f., Bot. Mag. 93 t. 5673 (1867).**

Lectotype. Designated here: Japan, herbarium Hookerianum 1867 "from Mr Veitch, Japan, received 1867" fl? July. 5673. Bot. Mag." [K-000464729] (K, lecto.!)

Key to subspecies of *Lilium leichtlinii*

1 Flowers with perianth segments yellow, filaments and style pale yellow, Japan ..... **subsp. *leichtlinii***

2 Flowers with perianth segments orange to dark brownish-red, filaments and style pale pinkish-orange, China, Japan, Korea, Russia..... **subsp. *maximowiczii***

Lilium leichtlinii* subsp. *leichtlinii

= *Lilium leichtlinii* [unranked] *majus* G.F.Wilson, J. Hort. Cottage Gard. n.s. 25: 371 (1873)
≡ *Lilium leichtlinii* var. *majus* (G.F.Wilson) Baker, J. Linn. Soc., Bot. 1874: 248 (1874)
≡ *Lilium leichtlinii* f. *majus* (G.F.Wilson) G.Nicholson, Ill. Dict. Gard. 2: 270 (1885).

Note. Although Nicholson refers to the various varieties of lilies in his introduction to his entry on *Lilium*, he distinctly states in his description of *L. leichtlinii majus* "this is a luxuriant form" (Nicholson 1885: 270).

Description. ***Bulb*** subglobose to globose 2–4 × 2–4 cm forming short subterranean stolons with bulbils, scales white, ovate, thick; ***stem*** 40–180 cm, green, slightly scabrid or floccose- tomentose, especially on upper inflorescence axis; ***leaves*** scattered, sessile, linear, 8–16 × 0.5–1.2 cm, glabrous or slightly white floccose, especially beneath, margins tomentose when young; ***inflorescence*** 1–5 flowered, ***pedicels*** 10–18 cm long, glabrous to slightly floccose; ***flowers*** pendulous, citron-yellow, lightly to heavily dotted with purplish-brown speckles from the middle portion of the tepal to the base, rarely covered in purplish-brown streaks, not fragrant, tepals pubescent at apex and below, strongly recurved 5–8 × 1–2 cm lanceolate, apex subacute, nectaries yellow, margins raised, papillose; ***stamens*** glabrous, filaments yellow, spreading outwards, anthers brown, pollen yellow; ***Style*** pale yellow, upwards curving, stigma reddish-brown, lobes short; ***capsule*** ellipsoid 3–5 cm long.

Distribution. Japan, Honshu (Akika Pref., Shizuoka Pref.); Ryukyu Islands (Amami-o-shima).

Ecology. Growing in open rich marshy meadows, along stream margins and in sandy terrain amongst low scrub, sea level to 1300 m of elevation. Flowering in July and August. Germination epigeal.

Illustration. https://rhslilygroup.org/2019/wp-content/uploads/2018/11/L-leichtlinii-lechtlinii.jpeg

Lilium leichtlinii **subsp.** ***maximowiczii*** **(Regel) J.Compton, comb. et stat. nov.**
urn:lsid:ipni.org:names:77215715-1

Basionym: *Lilium maximowiczii* Regel, Ind. Sem. Hort. Petrop. 1866 Suppl. Annot. Bot. VI: 26 (1868a). Lectotype designated here (Fig. 2): ex Japan cult. Hort. Petrop. July 1867. *C.J.Maximowicz s.n.* 1862–1864 [LE-01072027] (LE, lecto.!); Syntype: ex Japan cult. Hort. Petrop. July 1867. *C.J.Maximowicz s.n.* 1862–1864 [LE-01072026] (LE, syn.!)

≡ *Lilium leichtlinii* var. *maximowiczii* (Regel) Baker, Gard. Chron. 1871(2): 1422 (1871)

= *Lilium pseudotigrinum* Carrière, Rev. Hort. 1867: 411 (1867). Lectotype designated here: Cult. (P) ex China without collector [Icon] Rev. Hort. 1867: t. *L. pseudotigrinum*.

≡ *Lilium leichtlinii* var. *pseudotigrinum* (Carrière) Baker, J. Roy. Hort. Soc. n.s. 4(13): 47 (1873)

≡ *Lilium maximowiczii* var. *pseudotigrinum* (Carrière) Elwes, Monogr. *Lilium* (1879)

≡ *Lilium leichtlinii* f. *pseudotigrinum* (Carrière) Hara & Kitam., Acta Phytotax. Geobot. 36(1–3): 93. (1985)

= *Lilium maximowiczii* var. *tigrinum* Regel, Gartenflora 19: 290, t. 664, fig. 4 (1870) Lectotype designated here: [Icon] Gartenflora 19 t. 664, fig. 4 (1870).

≡ *Lilium leichtlinii* var. *tigrinum* (Regel) G.Nicholson, Ill. Dict. Gard. 2: 271 (1885)

– *Lilium maximowiczii* f. *tigrinum* Leichtlin, Pflanzen-Sammlung des Leichtlin'schen Gartens in Baden-Baden (1873: 11) nom. nud.

= *Lilium tigrinum* var. *lishmannii* T.Moore, Florist & Pomol. 1873: 16, tab.2 (1873). Lectotype designated here: [Icon] Florist & Pomol. 1873: tab.2 (1873).
– *L. tigrinum* var. *jocundum* Tilton, Bailey Standard Cycl. Hort. 2: 1870 (1933) nom. nud.

Diagnosis. Differing from subsp. *leichtlinii* in the following characters: ***bulb*** with stolons that can extend to 2 m; ***stem*** green, purple streaked or spotted (vs. stem green unspotted); ***leaves*** linear or lanceolate to 1.6 cm wide (vs. 0.5–1.2 cm wide); ***Inflorescence*** with (2) –5–12 flowers, ***flowers*** light orange or reddish-orange with dark reddish-brown speckling, rarely reddish-brown streaking (vs. yellow), nectaries with orange-red papillae marginally (vs. yellow); stamens with pale orange filaments (vs. pale yellow), anthers brown, pollen reddish-orange (vs. yellowish-brown); ***style*** pale orange (vs. pale yellow).

Distribution. China (Hebei, Jilin, Liaoning, Shaanxi); Japan, Honshu, Kyushu, northern Ryukyu Islands, Shikoku; North Korea (North Hamgyeong); Russian Far East (Primorskiy Kray); South Korea (Chungchungbook, Gangwon, Gyunggi).

Ecology. Growing in open rich marshy meadows, along stream margins and in sandy terrain amongst low scrub, sea level to 1300 m. of elevation. Flowering in July and August. Germination epigeal. It is worth noting that both diploid and triploid plants have been found in Korea and that plants have been observed to produce subterranean stolons that extended to as much as 2 m in length (Kim et al. 2016: 104).

Illustration. https://rhslilygroup.org/2019/wp-content/uploads/2018/11/L-leichtlinii-maximoviczii.jpeg

Conclusion

The yellow flowered *Lilium leichtlinii* has been known for centuries in Japan as the ki-hirato yuri or 'lily of the sun' and the orange flowered as the 'ko-oni yuri', the latter being known under a range of local names in Chinese, Korean and Russian. Naming of the orange lily is compounded, in this case, by the fact that the yellow "species" is almost certainly a recessive variant restricted to a few isolated populations in Japan. The orange-red flowered subspecies represents a much more widespread species. The nomenclatural problem of the varietal epithet *L. leichtlinii* var. *maximowiczii* being superseded by *L. maximowiczii* var. *tigrinum* (see ICN Art. 11.2 ex. 4, Turland et al. 2018) is resolved by the recognition of the orange flowered taxon as *L. leichtlinii* subsp. *maximowiczii*.

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Hoya pyrifolia (Apocynaceae), a new species from south-western Yunnan, China

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Abstract

Hoya pyrifolia, a new species of Apocynaceae from Yunnan Province, China, is described and illustrated. Results from phylogenetic analyses, based on combined DNA fragments of the nuclear ribosomal external transcribed spacer (ETS), intergeneric transcribed spacer (ITS) and three plastid DNA fragments (*matK*, *psbA-trnH* and *trnT-trnL*), showed that the new species was nested within a clade, including *Hoya* species distributed in the subtropical foothills of the Himalayas and the Tibet-Sichuan Plateau. Morphologically, the new species can be distinguished from its close relatives by its pyriform and slightly pubescent leaves, as well as the 4-flowered inflorescences.

Keywords

Apocynaceae, Asclepiodoideae, China, *Hoya*, taxonomy

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Introduction

Hoya R.Br., the wax plants, is a large genus circumscribed within the tribe Marsdenieae, subfamily Asclepiodoideae of Apocynaceae (Wanntorp et al. 2014). It includes over 300 species mainly distributed in the tropical and subtropical regions of Asia, Oceania and the Pacific Islands (Wanntorp et al. 2014), with ca. 40 species recorded in China (Li et al. 1995; Huang et al. 2020a). Several infrageneric classification systems of the genus have been proposed by different authors (Hooker 1885; Schlechter 1916; Burton 1995), but none of them was supported in phylogenetic analyses (Wanntorp et al. 2006; Wanntorp et al. 2014; Huang et al. 2020a).

During a field investigation in Yingjiang Hsien, Yunnan Province, China, in the summer of 2018, one of the authors (E.F. Huang) discovered one population of a *Hoya* species, which obviously is different from congeneric taxa recorded in China and adjacent countries. Later, a specimen representing the same species was collected again from another locality (viz. Longling Hsien) in south-western Yunnan. Detailed morphological comparison and specimen examination for all the *Hoya* species recorded in China and adjacent regions showed that the species is new to science, thus it is formally described and illustrated here as a new species. The phylogenetic position of the new species is studied based on analyses of a combined matrix including five DNA fragments from both plastid and nuclear genomes.

Materials and methods

Morphological study

Specimens of *Hoya* deposited in the herbaria CDBI, GH, HNWP, IBSC, KUN, P and PE were studied carefully in the present study. Field investigations of Chinese *Hoya* species were also conducted in recent years. Morphological characters of leaves, inflorescences and flowers of relevant species were photographed and measured. Herbarium abbreviations cited in the present study follow the Index Herbariorum (Thiers 2013 onwards).

Phylogenetic study

To study the phylogenetic position of the new species within the genus *Hoya*, a phylogenetic study of the genus was performed, based on combined DNA fragments of the nuclear ribosomal external transcribed spacer (ETS), intergeneric transcribed spacer (ITS) and three plastid DNA regions (*matK*, *psbA-trnH* and *trnT-trnL*), following Huang et al. (2020a). Total genomic DNA of the new species was extracted from silica gel-dried leaves (voucher specimen: *E.F. Huang 1905009*, PE) using a Plant Genomic DNA Kit (Biomed, Shenzhen, China). Detailed information of primers of relevant DNA fragments used in Polymerase Chain Reaction (PCR) amplification and sequencing, as well as the procedures of PCR, can be found in Huang et al. (2020a). Based on morphological traits, the

new species studied here seems to belong to clade I in Wanntorp et al. (2014). Thus, other species belonging to this clade sampled in previous studies (Wanntorp et al. 2014; Huang et al. 2020a) were included in our study, as well as representatives of other major clades of the genus. A species from the genus *Marsdenia* R.Br. was selected as outgroup, based on the phylogenetic framework reported in previous studies (Wanntorp et al. 2014). Detailed information of all species sampled and sequences used are available in Appendix 1.

Sequences were aligned using MAFFT 7.221 (Katoh and Standley 2013) and then three major datasets were constructed: the cpDNA dataset (including *matK*, *psbA-trnH* and *trnT-trnL*), the nrDNA dataset (including ETS and ITS) and the combined dataset including the five DNA fragments (ETS, ITS, *matK*, *psbA-trnH* and *trnT-trnL*). The three datasets were analysed with Bayesian Inference (BI) and Maximum Likelihood (ML). Detailed information about the parameter setting in BI and ML analyses is given in Huang et al. (2020b). The models of nucleotide substitution of each fragment used here were selected under the Akaike Information Criterion (AIC) using jModelTest v. 3.7 (Posada 2008): GTR+Γ for ETS, TIM1+Γ for ITS, TPM1uf+I+Γ for *matK*, TrN+Γ for *psbA-trnH* and TPM1uf+Γ for *trnT-trnL*.

Results and discussion

The cpDNA dataset, the nrDNA dataset and the combined dataset contained 2482, 1393 and 3875 characters, respectively. Some major clades within the genus *Hoya* were recovered in the BI and ML analyses of the three datasets (Figures 1–2), but phylogenetic relationships amongst these major clades were inconsistent. However, conflicting phylogenetic nodes were all poorly supported [bootstrap support (BS) in ML analysis < 50% and/or posterior probabilities (PP) in BI analysis < 0.50] (Figures 1–2).

Results from both BI and ML analyses of the three major datasets all showed that the new species studied here formed a clade (marked in blue in Figures 1, 2) with eight other *Hoya* species, viz. *H. bella* Hook., *H. chinghungensis* (Tsiang & P.T. Li) M.G. Gilbert, P.T. Li & W.D. Stevens, *H. edeni* King ex Hook. f., *H. engleriana* Hosseus, *H. lanceolata* Wall. ex D.Don, *H. linearis* Wall. ex D. Don, *H. longicalyx* Wang Hui & E.F.Huang and *H. thailandica* Thaithong. This clade is in accordance with clade I circumscribed in Wanntorp et al. (2014) and it is strongly supported (BSs = 100%, PPs = 1.00) here in all analyses except in the ML analysis, based on the cpDNA dataset (BS = 51%). The *Hoya* species in this clade are mainly restricted to the subtropical foothills of the Himalayas and the Tibet-Sichuan Plateau (Wanntorp et al. 2014). Morphologically, species included in this clade usually have small leaves (no longer than 3 cm in length), flat-topped pseudumbels and non-persistent peduncles (own observation), except the two earliest divergent species *H. thailandica* and *H. edeni* that have large leaves and subhemispherical umbels. The new species is most closely related with. *H. chinghungensis*, *H. engleriana*, *H. lanceolata* and *H. longicalyx* (BSs = 84%, PPs = 1.00) in the analyses of both the nrDNA dataset (Figure 1a) and the combined dataset (Figure 2), but relationships amongst these species were not resolved or poorly supported.

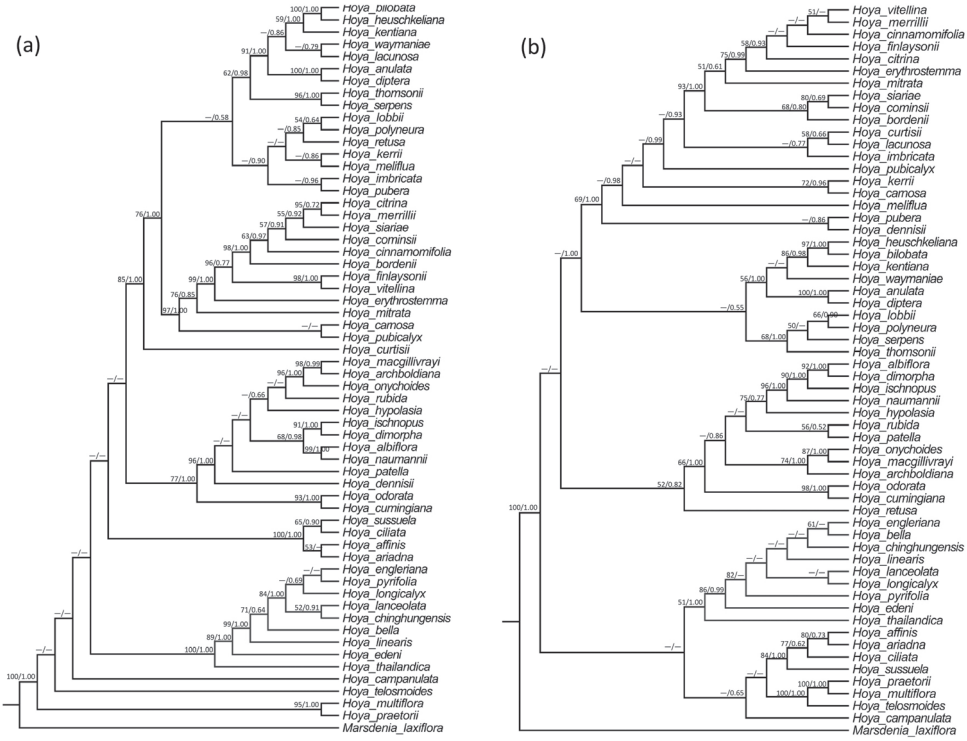

Figure 1. Maximum Likelihood (ML) tree of *Hoya* species inferred from the nrDNA (**a** including ETS and ITS) and cpDNA (**b** including *matK*, *psbA-trnH* and *trnT-trnL*) datasets. Bootstrap (BS) values ≥ 50% in ML analysis and posterior probability (PP) ≥ 0.50 in Bayesian Inference (BI) are indicated on the left and right of slanting bars above a phylogenetic node, respectively. Dashes denote that the phylogenetic node was not supported, the BS value is < 50% in the ML analysis or PP < 0.50 in the BI analysis.

Morphologically, the new species is similar to *H. engleriana* and *H. longicalyx*. However, it can be easily distinguished from the latter two species by a series of morphological traits (Figures 3, 4), such as its pyriform leaves that are 10–14 mm long (Figure 4A, B, K), with rounded or truncate leaf apex (Figure 4K) and mid-vein invisible adaxially and obscure abaxially (Figure 4K), the 4-flowered inflorescences (Figure 4A, B) with 8–10 mm long peduncles, the ca. 4 mm long calyx lobes (Figure 4H), the triangular corolla (Figure 4E), the rose-coloured corona (Figure 4C–E, J) and the oblong and ca. 0.6 mm long pollinia (Figure 4G). In contrast, *H. engleriana* is characterised by its narrowly-oblong leaves that are 20–25 mm long (Figure 5B) with usually acute or obtuse leaf apex (Figure 5B) and mid-vein evident on both surfaces (Figure 5B), the 5–7-flowered inflorescences (Figure 5J), the 1.5–2 mm long calyx lobes (Huang et al. 2020a) and the narrowly-oblong to oblong-triangular corolla (Figure 5J); *H. longicalyx* is characterised by its ovate-lanceolate leaves that are 15–20 mm long (Figure 5F) with

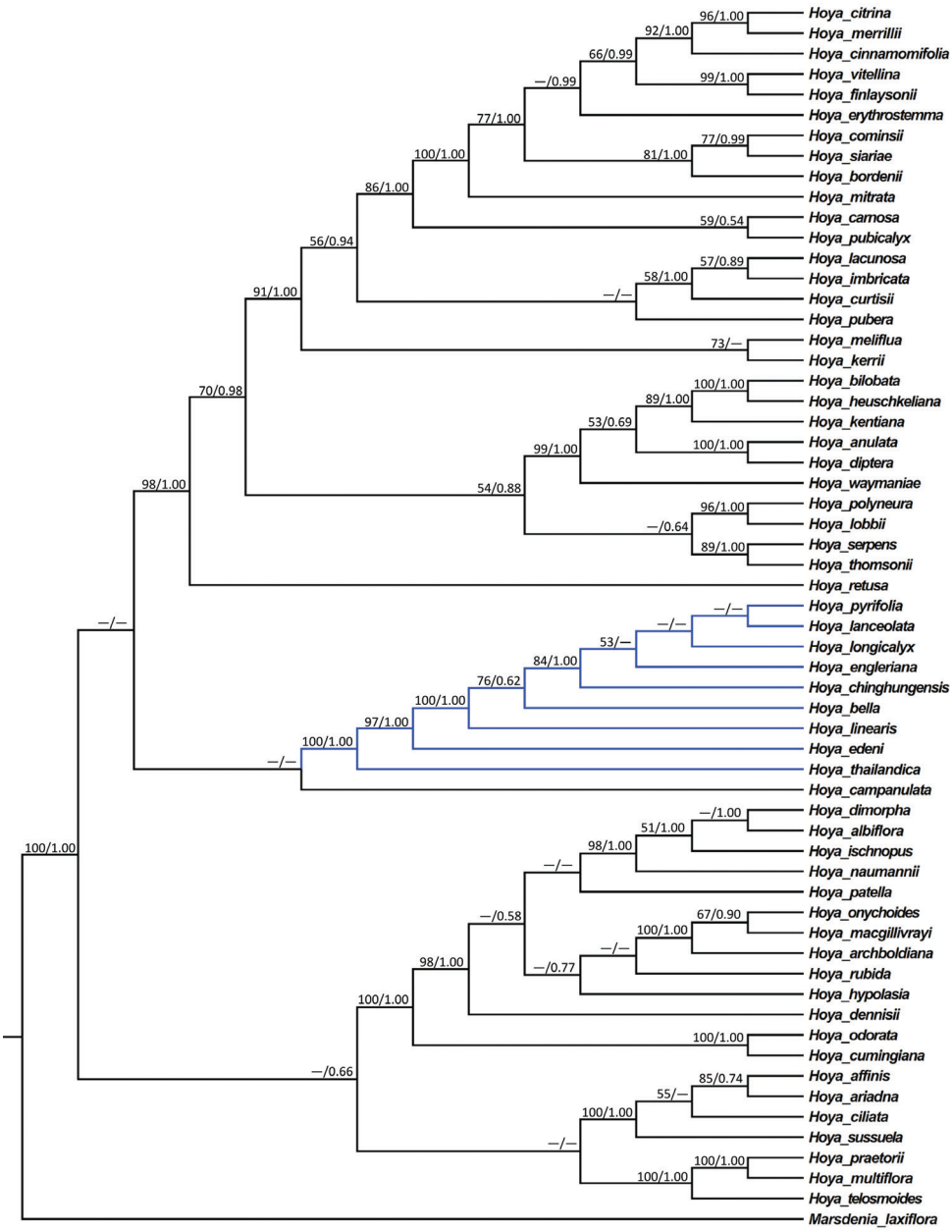

Figure 2. Maximum Likelihood (ML) tree of *Hoya* species inferred from the combined dataset of five DNA regions (ETS, ITS, *matK*, *psbA-trnH* and *trnT-trnL*). Bootstrap (BS) values ≥ 50% in ML analysis and posterior probability (PP) ≥ 0.50 in Bayesian Inference (BI) are indicated on the left and right of slanting bars above a phylogenetic node, respectively. Dashes denote that the phylogenetic node was not supported, the BS value is < 50% in the ML analysis or PP < 0.50 in the BI analysis.

Table 1. Morphological comparison between *Hoya pyrifolia* E.F.Huang, its closest relatives and morphologically-similar species.

Taxa	Leaf	Inflorescence	Corona	Calyx lobes	Pollinia
H. chinghungensis	Ovate to broadly ovate, 10–13 mm × 7–10 mm; pubescent on both surfaces when young; base rounded to truncate, apex rounded to obtuse or acuminate; midvein evident on both surfaces	4–5-flowered; peduncle ca. 8 mm long	Rose-colored	1.5–2 mm long	Unknown
H. dickasoniana	Elliptic or ovate, 9–19 mm × 5–10 mm; glabrous; base broadly cuneate to rounded, apex obtuse; midvein evident on both surfaces	3–6-flowered peduncle ca. 5 mm long	Unknown	2–2.5 mm long	Oblong; ca. 1 mm long;
H. engleriana	Narrowly oblong, 20–25 mm × ca. 5 mm; pubescent on both surfaces when young; base cuneate, apex usually obtuse with mucro; midvein evident abaxially	5–7-flowered; peduncle ca. 6 mm	Rose-colored	1.5–2 mm long	Unknown
H. kingdonwardii	Elliptic to slightly elliptic-lanceolate, 10–17 mm × 5–10 mm; glabrous; base cuneate and apex acuminate; midvein evident on both surfaces	3–4-flowered; peduncle 7–10 mm long	Unknown	ca. 3 mm long	Oblong; ca. 0.8 mm long
H. lanceolata	Lanceolate, ca. 25 mm × 15 mm; based cuneate to narrowly cuneate, apex acuminate; sparsely pubescent on both surfaces when young; midvein evident on both surfaces;	7–12-flowered; peduncle ca. 12 mm	Rose-colored	Unknown	Unknown
H. longicalyx	Ovate-lanceolate, 15–20 mm × ca. 10 mm; base rounded, apex acuminate; slightly pubescent; midvein depressed adaxially, raised abaxially;	3–4-flowered; peduncle ca. 5 mm long	Whitish	5–7 mm long	Clavate; 0.55–0.6 mm long, narrowing towards the base;
H. pyrifolia	Pyriform, 10–14 mm × 4–7 mm; slightly pubescent adaxially, glabrous abaxially; base obtuse or rounded, apex rounded or truncate; midvein absent adaxially, obscure abaxially	4-flowered; peduncle 8–10 mm long	Rose-colored	ca. 4 mm long	Oblong; ca. 0.6 mm long

acuminate leaf apex (Figure 5F) and mid-vein depressed adaxially and raised abaxially (Figure 5F), the ca. 5 mm long peduncles (Huang et al. 2020a), the 5–7 mm long calyx lobes (Huang et al. 2020a; Figure 5M), the whitish corona (Figure 5L) and the clavate pollinia narrowing towards the base (Figure 5N).

The new species is also similar to *H. dickasoniana* P.T.Li and *H. kingdonwardii* P.T.Li in morphology. The two latter species were described from Myanmar, but are not included in the phylogenetic analyses due to lack of DNA material. According to the protologues and holotypes of these two Burmese endemic species, the new species studied here can be distinguished from them by its opposite leaf arrangement, pyriform and slightly pubescent leaves (Figure 4A, B, K) with obtuse or rounded leaf base and rounded to truncate apex (Figure 4K), 4-flowered inflorescences (Figure 4A, B) with 8–10 mm long peduncles and pollinia that are ca. 0.6 mm long (Figure 4G). In contrast, *H. dickasoniana* is characterised by its leaf arrangement which is opposite or in whorls of 3–4 (Figure 5C, D), the leaves which are elliptic to ovate and glabrous on both surfaces (Figure 5C, D) with leaf base broadly cuneate to rounded and apex obtuse (Figure 5C, D), the inflorescence with up to 6 flowers (Figure 5H) and ca. 5 mm long peduncles (Li 1994; Figure 5H) and the ca. 1 mm long pollinia (Li 1994), while *H. kingdonwardii* is characterised by its leaves that are elliptic to slightly elliptic-lanceolate and glabrous on both surfaces (Li 1994; Figure 5I) with cuneate base and acuminate apex (Figure 5I) and mid-vein evident on both surfaces (Figure 5I) and the ca. 0.8 mm long pollinia (Li 1994).

Detailed information about the morphological comparison between the new species and its close relatives are given in Table 1.

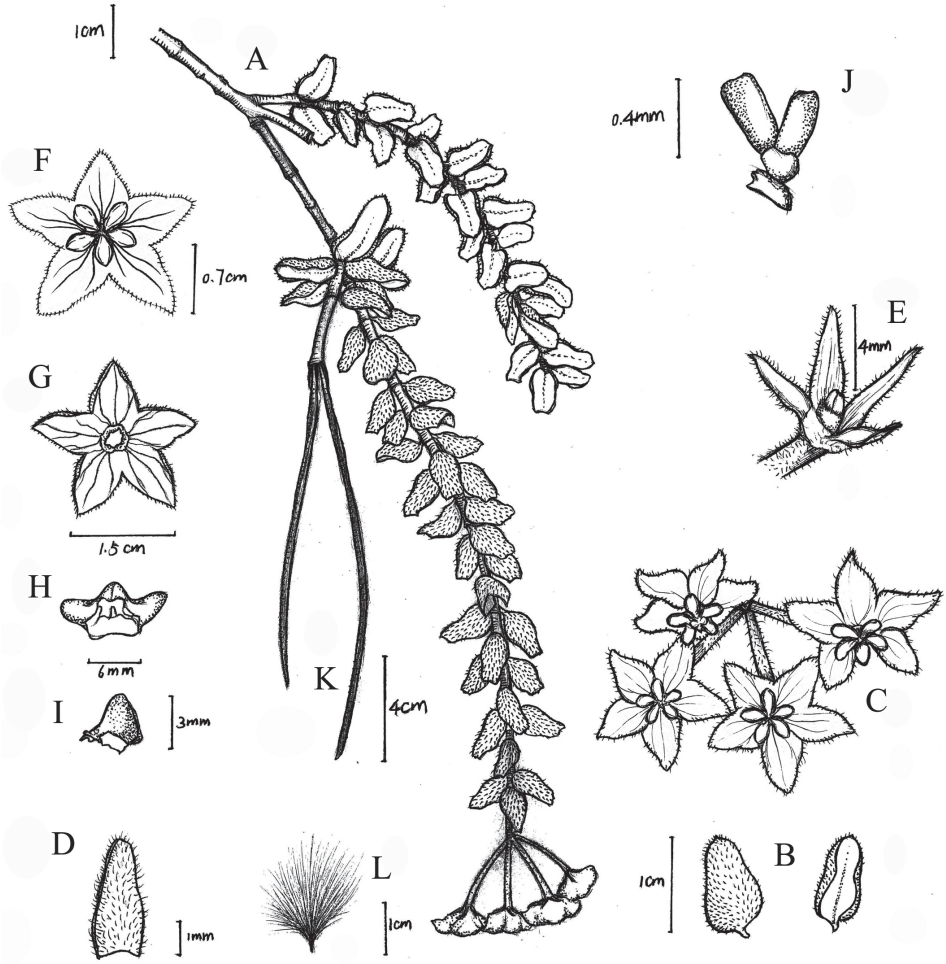

Figure 3. *Hoya pyrifolia* E.F. Huang **A** habit **B** leaf **C** inflorescence **D** bracteole **E** calyx lobes **F** corolla, adaxial side **G** corolla, abaxial side **H** corona **I** corona lobe **J** pollinarium **K** fruit **L** seed. Drawn by Y.J. Chen.

Taxonomic treatment

Hoya pyrifolia **E.F. Huang, sp. nov.**
urn:lsid:ipni.org:names:77215716-1
Figures 3, 4

Diagnosis. The species is morphologically most similar to *H. engleriana*, but differs by its leaves which are pyriform and 10–14 mm long (vs. narrowly oblong and 20–25 mm long), its 4-flowered inflorescences (vs. 5–7-flowered), its calyx lobes ca. 4 mm long (vs. 1.5–2 mm long) and the triangular corolla (vs. narrowly oblong to oblong-triangular).

Type. CHINA. Yunnan Province, Yingjiang Hsien, Sudian Village, Mulonghe River, epiphytic on trunk in mid-montane evergreen forest, 25°9'38"N, 97°53'20"N, at an elevation of 1865 m, 13 August 2019, E.F. *Huang 1905009* (Holotype: PE!; isotypes: PE!).

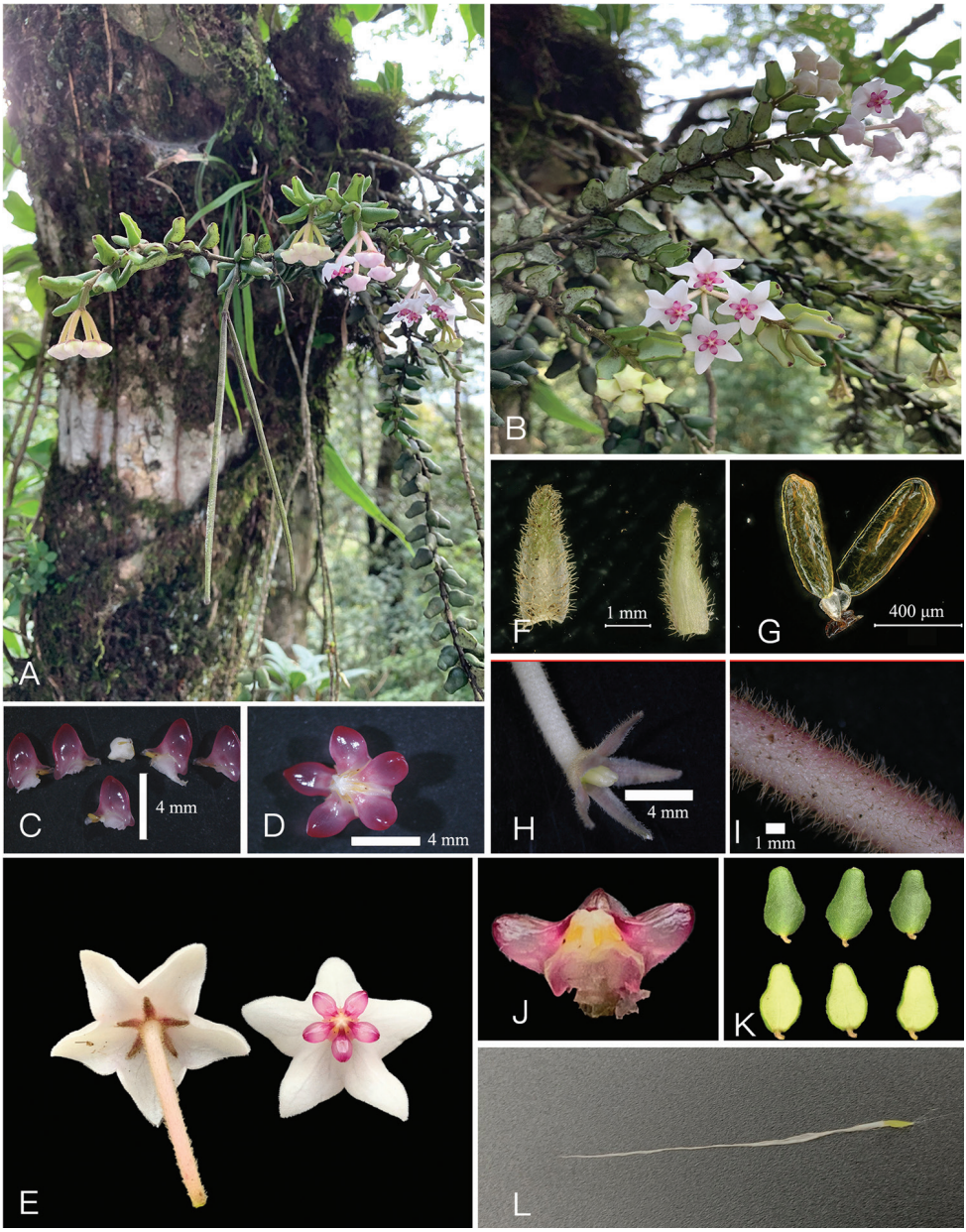

Figure 4. *Hoya pyrifolia* E.F. Huang **A** habit showing inflorescences and mature follicles **B** branch and inflorescence **C** corona lobes **D** corona top view **E** flower **F** bracteoles **G** pollinarium **H** pedicel and calyx lobes **I** part of Pedicel **J** corona side view **K** leaves **L** seed.

Description. Epiphytic shrubs. Stems up to 60 cm in length, 3–4 mm in diam., branching mainly near base, branches pubescent, internodes shorter than leaves. Leaves opposite, pyriform, 10–14 × 4–7 mm, fleshy, slightly pubescent and dark green adaxi-

Figure 5. Leaves and inflorescences of *Hoya* species **A, G** *H. chinghungensis* (Y. Tsiang & P.T. Li) M.G. Gilbert, P.T. Li & W.D. Stevens **B, J** *H. engleriana* Hosseus; **C–D, H** *H. dickasoniana* P.T. Li **E, K** *H. lanceolata* Wall. ex D. Don; **F, L–N** *H. longicalyx* W. Hui & E.F. Huang **I** *H. kingdonwardii* P.T. Li.

ally, glabrous and greyish-green abaxially, base obtuse or rounded, apex rounded or truncate, margin entire and reflexed; mid-vein invisible adaxially, obscure abaxially, lateral veins invisible on both surfaces; petioles ca. 2 mm long. Inflorescences termi-

nal pseudumbels, flat-topped, 4-flowered, pendent; peduncle shorter than pedicels, 8–10 mm long, light green; bracteoles 2 at each pedicel base, linear, 4 × 1 mm; pedicels 1.3–1.5 cm long, light pink to yellow-green, pubescent; calyx lobes pinkish, narrowly triangular to linear, 4 × 1 mm, margin entire; corolla white, flat to slightly incurved, 1.5–1.7 cm in diam., lobes triangular-ovate, ca. 7 mm wide, apex acute; corona rose-coloured, ca. 6 mm in diam., ca. 3 × 3 mm, scales 5, fleshy, translucent, ovate-triangular; pollinia oblong, ca. 0.6 × 0.2 mm, base and apex truncate, caudicula attached at the centre of the retinaculum. Ovaries 2, attached to each other below centre, free higher up, oblong, ca. 2 mm long, ca. 1 mm wide, yellowish-white, pubescent. Follicles linear, 10–12 cm long, pubescent. Seeds linear-oblong, ca. 2.0 × 0.2 mm, coma 2.8–3.0 cm.

Distribution and habitat. The species is endemic to Gaoligong Mountain, distributed in Longling and Yingjiang Counties in Yunnan Province. It is an epiphyte on tree trunks in the mid-montane evergreen forests at an elevation from 1850 m to 2150 m.

Etymology. *Hoya pyrifolia* is named for its pyriform leaf, which is a significant feature that can be used to distinguish the species from its close relatives.

Other specimen examined. CHINA. Yunnan Province, Longling Hsien, Gaoligongshan National Forest Park, 4°50'3"N, 98°45'48"E, at an elevation of 2146 m, 26 August 2019, *E.F. Huang 201908260012* (IBSC).

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Appendix 1

GenBank accession numbers for samples used in this study (—, missing data; *, newly-generated sequences). GenBank accession numbers are given for ETS, ITS, *matK*, *psbA-trnH* and *trnT-trnL*.

Hoya affinis Hemsl., HE794222, DQ334481, HQ327536, HQ327615, HE794732; ***H. albiflora*** Zipp. ex Blume, HE794249, DQ334493, HQ327567, HQ327647, HE794761; ***H. anulata*** Schltr., HE794280, DQ334485, HQ327584, HQ327663, HE794794; ***H. archboldiana*** C.Norman, HE794258, HQ327513, HQ327566, HQ327646, HE794770; ***H. ariadna*** Decne., HE794223, DQ334502, HQ327535, HQ327614, HE794733; ***H. bella*** Hook., HE794215, HQ327518, HQ327581, HQ327660, HE794725; ***H. bilobata*** Schltr., HE794296, HE794381, HQ327599, HQ327678, HE794811; ***H. bordenii*** Schltr., HE794344, HQ327527, HQ327607, HQ327686, HE794862; ***H. campanulata*** Blume, HE794242, HE794387, MH598655, HE794484, HE794754; ***H. carnosa*** (L.f.) R.Br., HE794320, DQ334460, HQ327586, HQ327665, HE794837; ***H. chinghungensis*** (Y.Tsiang & P.T.Li) M.G.Gilbert, P.T.Li & W.D.Stevens, MN089474, MN107851, MT300095, MK361041, MT300101; ***H. ciliata*** Elmer ex C.M.Burton, HE794224, DQ334512, HQ327537, HQ327616, HE794734; ***H. cinnamomifolia*** Hook., HE794328, HQ327510, HQ327562, HQ327642, HE794845; ***H. citrina*** Ridl., HE794329, HQ327507, HQ327558, HQ327638, HE794846; ***H. cominsii*** Hemsl., HE794356, HQ327515, HQ327577, HQ327656, HE794873; ***H. cumingiana*** Decne., HE794275, HQ32752, HQ327594, HQ327673, HE794788; ***H. curtisii*** King &

Gamble, HE794279, DQ334479, HQ327608, HQ327687, HE794792; ***H. dennisii*** P.I.Forst. & Liddle, HE794252, HQ327524, HQ327543, HQ327622, HE794764; ***H. dimorpha*** F.M.Bailey, HE794259, HQ327512, HQ327565, HQ327645, HE794771; ***H. diptera*** Seem., HE794285, HQ327519, HQ327583, HQ327662, HE794798; ***H. edeni*** King ex Hook.f., HE794216, DQ334476, HQ327582, HQ327661, HE794726; ***H. engleriana*** Hosseus, MT300093, MG649233, MT300096, —, MT300102; ***H. erythrostemma*** Kerr, HE794325, HQ327511, HQ327563, HQ327643, HE794842; ***H. finlaysonii*** Wight, HE794333, HQ327506, HQ327557, HQ327637, HE794850; ***H. heuschkeliana*** Kloppenb., HE794299, DQ334416, HQ327542, HQ327621, E794780; ***H. imbricata*** Decne., HE794314, DQ334480, HQ327605, HQ327684, HE794831; ***H. ischnopus*** Schltr., HE794260, HE794411, MH598707, HE794507, HE794772; ***H. kentiana*** C.M.Burton, HE794300, DQ334424, HQ327598, HQ327677, HE794815; ***H. kerrii*** Craib, HE794318, DQ334458, HQ327545, HQ327624, HE794835; ***H. lacunosa*** Blume, HE794310, DQ334499, HQ327601, HQ327680, HE794827; ***H. lanceolata*** Wall. ex D.Don, MT300094, —, MT300097, MT300099, MT300103; ***H. linearis*** Wall. ex D.Don, HE794218, HQ327517, MH598727, HQ327659, HE794728; ***H. lobbii*** Hook.f., HE794365, HE794420, MH598726, HE794516, HE794882; ***H. longicalyx*** Wang Hui & E.F.Huang, MN268319, MN116005, MT300098, MT300100, MT300104; ***H. macgillivrayi*** F.M.Bailey, HE794262, DQ334488, HQ327568, HQ327648, HE794774; ***H. meliflua*** Merr., HE794315, DQ334429, HQ327571, HQ327651, HE794832; ***H. merrillii*** Schltr., HE794349, HQ327505, HQ327555, HQ327635, HE794866; ***H. mitrata*** Kerr, HE794309, DQ334500, HQ327604, HQ327683, HE794826; ***H. multiflora*** Blume, HE794238, DQ334487, HQ327550, HQ327629, HE794750; ***H. naumannii*** Schltr., HE794265, HE794434, MH598742, HE794529, HE794777; ***H. odorata*** Schltr., HE794277, HQ327504, HQ327554, HQ327634, HE794790; ***H. onychoides*** P.I.Forst., Liddle & I.Liddle, HE794248, HE794439, MH598748, HE794533, HE794760; ***H. patella*** Schltr., HE794255, DQ334498, HQ327553, HQ327633, HE794767; ***H. polyneura*** Hook.f., HE794366, HQ327509, HQ327560, HQ327640, HE794883; ***H. praetorii*** Miq., HE794237, HQ327520, HQ327585, HQ327664, HE794749; ***H. puber*** Blume, HE794287, HQ327526, HQ327544, HQ327623, HE794800; ***H. pubicalyx*** Merr., HE794321, DQ334447, HQ327588, HQ327666, HE794838; ***H. pyrifolia*** E.F.Huang, MW066464*, MW035791*, MW066465*, MW066466*, MW066467*; ***H. retusa*** Dalzell, HE794364, DQ334457, MH598775, HQ327632, HE794881; ***H. rubida*** Schltr., HE794266, HE794453, MH598777, HE794545, HE794778; ***H. serpens*** Hook.f., HE794368 DQ334482, HQ327575, HQ327654, HE794885; ***H. siariae*** Kloppenb., HE794355, HE794459, JX120795, HE799701, HE794870; ***H. sussuela*** Merr., HE794233, HE794464, MH598784, HE794559, HE794743; ***H. telosmoides*** Omlor, HE794240, DQ334486, HQ327592, HQ327671, HE794752; ***H. thailandica*** Thaithong, HE794219, HE794466, —, HE794561, HE794729; ***H. thomsonii*** Hook.f., HE794369, HQ327502, HQ327546, HQ327625, HE794886; ***H. vitellina*** Blume, HE794342, HQ327508, HQ327559, HQ327639, HE794859; ***H. waymaniae*** Kloppenb., HE794244, HQ327522, HQ327596, HQ327675, HE794756; ***Marsdenia laxiflora*** Donn.Sm., MK214583, LR794776, MH748870, —, LR794694.

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Three new species of *Uvariodendron* (Annonaceae) from coastal East Africa in Kenya and Tanzania

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Abstract

East Africa is a hotspot of biodiversity with many endemic plant species. We describe three new species of the genus *Uvariodendron* (Annonaceae) from the coastal forests of Kenya and Tanzania. *Uvariodendron mbagoi* Dagallier & Couvreur, **sp. nov.** is endemic to Tanzania and unique within the genus by its strong bergamot scent and its tomentose fruits having regular tufts of higher hair density. *Uvariodendron dzomboense* Dagallier, W.R.Q. Luke & Couvreur, **sp. nov.** is endemic to Dzombo Hill in Kenya and is rendered distinct by its small leaves and very densely pubescent carpels. *Uvariodendron schmidtii* W.R.Q. Luke, Dagallier & Couvreur, **sp. nov.** is endemic to Shimba Hills in Kenya and differs by its small flowers and fused sepals forming a ring. Following IUCN criteria we assessed *U. mbagoi* and *U. dzomboense* as endangered (EN) while *U. schmidtii* is assessed as Vulnerable (VU). We also propose a new combination: *Polyceratocarpus oligocarpus* (Verdc.) Dagallier, **comb. nov.** The description of these three new species underlines the richness in endemics in East Africa and that new discoveries might arise from further botanical exploration of this region.

Keywords

Annonaceae, bergamot, Dzombo Hill, endemic, IUCN conservation status, Shimba Hills

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Introduction

East Africa is one of the richest regions in terms of biodiversity across the continent (Myers et al. 2000; Linder 2001). Recently, this region has been described as acting both as a "cradle" (i.e. promoting lineage divergence) and as a "museum" of diversity (i.e. maintaining old lineages), due to its topographical heterogeneity (Dagallier et al. 2020). East Africa harbors an incredible number of endemic species, particularly in the Eastern Arc Mountains and in coastal forests (Burgess et al. 1998, 2007; Küper et al. 2004). Despite the completion of the Flora of East Africa series (Beentje 2015), East Africa still needs further botanical exploration (Sosef et al. 2017). Indeed, from animals (Huber and Warui 2012) to plants (Poulsen and Lock 1997; Friis et al. 2015), new taxa continue to be described.

Annonaceae is a pantropical family of trees, shrubs and lianas. It is the most species rich family within the order Magnoliales, with ca. 2400 recognized species (Chatrou et al. 2012). In East Africa, several new species have been described for the region following the publication of the Flora of Tropical East Africa (Vollesen 1980; Verdcourt and Mwasumbi 1988; Johnson et al. 1999; Deroin and W.R.Q. Luke 2005; Couvreur et al. 2006; Couvreur and W.R.Q. Luke 2010; Marshall et al. 2016; Johnson et al. 2017; Gosline et al. 2019).

The genus *Uvariodendron* contains a total of 14 species restricted to tropical Africa (Fries 1930; Le Thomas 1967, 1969; Verdcourt 1969, 1986). It belongs to the Monodoreae tribe (Chatrou et al. 2012) and was inferred to be the sister genus to the *Uvariopsis* – *Monocyclanthus* clade based on molecular data (Couvreur et al. 2008; Guo et al. 2017). Like many Annonaceae species, *Uvariodendron* species are trees with simple hair indumentum and palgiotropic branches on an orthotropic axis. Their leaves are distichous, simple and entire, with the midrib sunken above, raised below, the secondary veins weakly brochidodromous to brochidodromous and the tertiary veins reticulate. They have hermaphroditic flowers with one whorl of three valvate to imbricate sepals and two whorls of three free and valvate petals. *Uvariodendron* species don't have a single synapomorphy that can differentiate them from other Annonaceae at first sight, but they can be recognized by the combination of the several characters presented hereafter. The inflorescence is axillary or on the trunk, composed of one to three sessile flowers or with a short pedicel (generally less than 5 cm). The sepals are smaller than, and morphologically different to, the petals. The outer and inner petals are subequal in length, from 10 to 40 mm at anthesis; the outer petals are valvate all along the margin whereas inner petals are valvate only at the apex. The stamens are numerous (more than 200), with linear anthers and truncate connective. The carpels are free, linear, with a coiled stigma. The monocarps are sessile or subsessile and cylindrical.

Here we describe three new species of *Uvariodendron*, from coastal forests in Kenya and Tanzania. We also transfer the species known as *Uvariodendron oligocarpum* Verdc. within the genus *Polyceratocarpus* Engl. & Diels as *Polyceratocarpus oligocarpus* (Verdc.) Dagallier. This brings the number of *Uvariodendron* species up to nine for East-Africa, and 17 for the genus as a whole. A key to East-African *Uvariodendron* species is also presented.

Material and methods

We examined all the 35 herbarium specimens cited in the results. Among them, we measured 12 specimens for *Uvariodendron mbagoi* (three of which were also examined and measured as living individuals), three herbarium specimens for *Uvariodendron dzomboense*, and four herbarium specimens for *Uvariodendron schmidtii*. Herbarium specimens came from B, DSM, EA, K, MPU, MO, P, and WAG. The three new species are morphologically close to *Uvariodendron kirkii* Verdc., one of the other *Uvariodendron* species occurring in East Africa. In order to ease the discrimination between the species, we present a comparison table of the most discriminant characters between the four species (Table 1). The data for *Uvariodendron kirkii* is taken from Verdcourt (1971) and from more than 50 specimens examined in the above-mentioned herbaria.

For morphological descriptions, we followed the terminology developed by Hickey et al. (1999) and by the Systematics Association Committee for Descriptive Biological Terminology (1962) for leaf and plane shapes, by Payne (1978) for the indumentum, and by Harris and Harris (2001) for the other terms.

The identification key was built with the help of Xper^3 comparison tools (http://www.xper3.fr/, Vignes Lebbe et al. 2016).

To make a preliminary conservation status assessment for each species, we calculated the extent of occurrence (EOO) and the area of occupancy (AOO) using the ConR package (Dauby et al. 2017). When calculation of EOO and AOO was impossible due to imprecision of coordinates, as for *Uvariodendron dzomboense* and for *Uvariodendron schmidtii*, we calculated the area of the locality in which they occur (respectively the forested part of Dzombo Hill and the Longomwagandi forest) based on Google satellite images with the surface calculation tool in QGIS v. 2.18.17 (QGIS Development Team 2016). We then assigned a preliminary conservation status following IUCN Red List Categories and Criteria Version 3.1 (IUCN 2012).

The distribution map was plotted using *ggmap* (https://CRAN.R-project.org/package=ggmap) package in R (R Core Team 2016). Data on protected areas was taken from Protected Planet (https://www.protectedplanet.net/, accessed June 2018).

Results

***Uvariodendron mbagoi* Dagallier & Couvreur, sp. nov.**
urn:lsid:ipni.org:names:77215717-1
Fig. 1

Type. Tanzania – Tanga • L.-P.M.J. Dagallier 39 (holotype: MPU (MPU1375316), isotypes: DSM, K, MO, MPU (MPU1375317), P, WAG); Handeni District, Kwedijela forest, ca. 8 km from Kwamsisi village; 5°54'50.12"S, 38°36'12.35"E; alt. 156 m; 13 Nov. 2019.

Diagnosis. Differs from other *Uvariodendron* species by its stiff greyish–green leaves with slightly revolute margins, the strong bergamot scent (the citrusy smell of

Table 1. Comparison of the main characters used to discriminate the described species with *Uvariondendron kirkii*. In bold: character unique to the species.

Species	*U. kirkii*	*U. dzomboense*	*U. mbagoi*	*U. schmidtii*
Scent	none reported	none reported	**strong, bergamot**	none reported
Lamina length (mm)	86–210	65–132	76–157	159–188
Leaves margins	flat	slightly revolute	slightly revolute	flat
Pedicel length (mm)	5–28	8–30	**0–0.6**	10– 15
Sepals	free, valvate to imbricate	fused at base	free, imbricate	**fused, forming a ring**
Petals length (mm)	12–39	16–18	unknown on mature flower	**10–12**
Number of carpels	7–20	**50–75**	12–16	< 10

Citrus bergamia Risso, between lemon and orange scent) of crushed leaves and bark, its globose flower buds easily falling off and its tomentose fruits having regular tufts of higher hair density. Differs from *Uvariodendron kirkii* by having smaller leaves when looking at the greater leaves (157 mm maximum vs. 210 mm maximum) (Table 1).

Description. Tree or shrub 3–6 m tall, 5–10 cm in diameter at breast height (d.b.h.), slash with strong bergamot smell (the citrusy smell of *Citrus bergamia* Risso); young branches sparsely pubescent to glabrous, old branches glabrous. Leaves distichous, simple, entire, margins slightly revolute, stiff, greyish–green. Petiole 3–6.5 mm long, 1.2–3 mm in diameter, young petiole sparsely pubescent to glabrous, old petiole glabrous. Leaf lamina 76–157 mm long, 31–59 mm wide, length:width ratio 2.2–3.5, narrowly elliptic to elliptic to narrowly obovate, between coriaceous and cartilaginous, apex acute to shortly acuminate, acumen 5–10 mm long, base acute to slightly decurrent (sometimes cuneate), above glabrous, below sparsely pubescent to glabrous when young, glabrous when old; mid rib sunken above, raised below, above glabrous when young and old, below sparsely pubescent to glabrous when young, glabrous when old; secondary veins 10–14 pairs, weakly brochidodromous, indistinct to slightly impressed above, slightly raised to raised below, inter–secondary veins absent; tertiary veins reticulate. Inflorescence borne on trunk or old branches, of 1–2 (3) flowers. Flower sessile or subsessile, pedicel 0–0.6 mm long, 2 mm in diameter. Flowers actinomorphic, hermaphroditic, buds globose 5–9 mm in diameter, velutinous, falling off very easily. Only flower buds and old fallen flowers seen. Bracts 2–5, at base of the pedicel, upper bract 5–8 mm long, 10–15 mm wide, appressed, enclosing bud, pubescent outside, glabrous inside. Sepals 3, ca. 7–8 mm long, ca. 7–12 mm wide (measures taken from bud), imbricate, enclosing the petals in bud, velutinous outside, glabrous inside. Outer petals 3, ca. 4 mm long, ca. 4 mm wide (measures taken from bud). Inner petals 3, ca. 5 mm long, ca. 5 mm wide (measures taken from bud), shortly velutinous outside, glabrous inside. Stamens more than 400, mature length unknown, anthers linear, connective truncate. Carpels 12–16, ca. 1.5 mm long, ca. 1 mm wide (measures taken from old flower), velutinous, stigma coiled. Fruiting pedicel 0–6 mm long, ca. 4 mm in diameter, pubescent. Monocarps 1–7, 20–50 mm long, 10–12 mm wide, length:width ratio 2–4.5, cylindrical, generally curved, showing constrictions and longitudinally ridged, green–grey, tomentose with regular tufts of higher hair density, shortly stipitate,

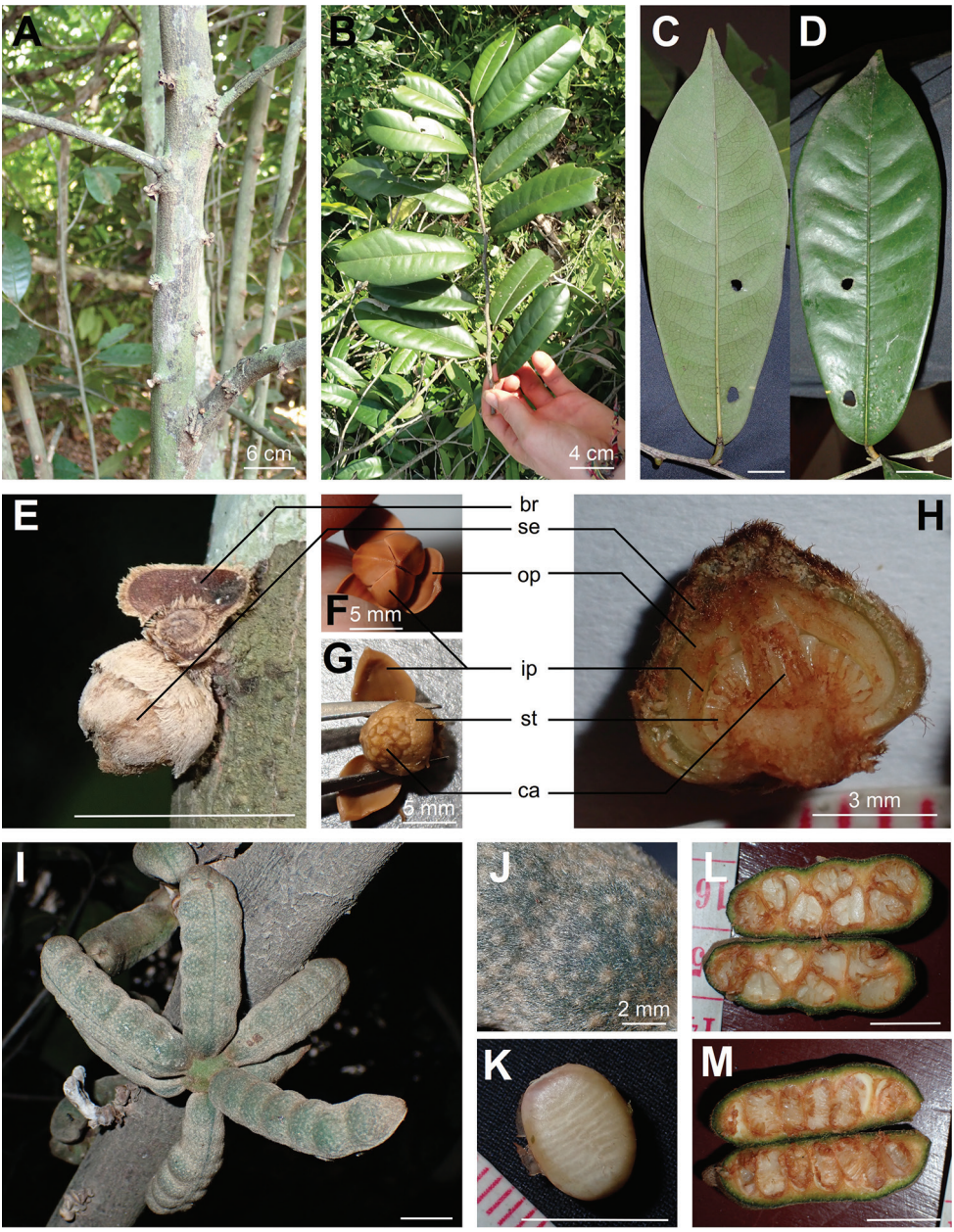

Figure 1. *Uvariodendron mbagoi* **A** trunk with flower buds **B** young branch **C, D** entire leaf: **C** lower side **D** upper side **E–H** pre-anthetic flower bud: **E** on trunk (bottom) and fallen flower bud (top) **F** seen from top with sepals removed **G** seen from top with outer petals removed **H** longitudinal section; *br* bract, *ca* carpel, *ip* inner petal, *op* outer petal, *se* sepal, *st* stamen **I–M** Fruit: **I** entire with 7 monocarps **J** indumentum **K** seed **L** tangential cut **M** longitudinal cut. Photos by L.-P. M.J. Dagallier from the specimens U. Bloesch s.n. (**F, G**), L.-P.M.J. Dagallier 39 (**B, E, H, J, L**), 40 (**A, C, D**) and 50 (**I**). Scale bars: 10 mm unless stated.

stipe 0–1.5 mm long, 5 mm wide, tomentose. Seeds 4–17 per monocarp, uniseriate to biseriate, 8–8.5 mm long, 5.5–6 mm wide, glabrous.

Habitat. Closed evergreen forest dominated by *Scorodophloeus fischeri*, on coral rag soil. Altitude: 90–340 meters.

Distribution. Endemic to Tanzania; only known from seven locations: Kimboza Forest, Msata Hill, Kwedijela forest, Kwedivikilo sacred forest, Mkwaja Ranch, Mkulumuzi river, and Hale (Fig. 2).

Conservation status. This species is known from 11 records in seven locations. The current occurrence of the species in Mkulumuzi river and Hale is really unlikely given that these are now (sub)urban areas and that these records date back, respectively, more than 30 years and over a century. Changes in traditional practices and exploitation of traditionally protected forests had been observed more than 20 years ago (Mwihomeke et al. 1998). This indicates that the current occurrence of the species in Kwedivikilo sacred forest is uncertain. Kwedijela forest is a locality under deforestation pressure with local crops slowly gaining ground (L–P.M.J. Dagallier and F. Mbago, field observations). However, the occurrences of the species in Kwedijela forest are 500 meters from the limit of Saadani National Park (SNP), so the species is likely to occur within the SNP where the protection is strict. The only record of this species occurring within a protected area is in Kimboza Forest Reserve, which has been threatened by encroachment, logging and invasion by the exotic *Cedrela odorata* L. (Hall and Rodgers 1986, Patrick 2008).

For the reasons explained above, we removed the occurrences in Mkulumuzi river and Hale from the calculations of extent of occurrence (EOO) and the area of occupancy (AOO). Considering the five remaining localities, the EOO is 3867 km^2 and AOO is 20 km^2. Following IUCN criterion B (IUCN 2012), *Uvariodendron mbagoi* is therefore assigned a preliminary status of Endangered EN B1ab(i,ii,iii,iv)+2ab(i,ii,iii,iv).

Vernacular names. Zigua (or Chizigua) language: Mchenene, Msenene (C.M. Kisena 3039), Mkenene (T.L.P. Couvreur 3, L–P.M.J. Dagallier 39, F. Mbago 3323).

Uses. The bark is used as a spice for meat meals and for tea.

Additional field notes. Foodplant of *Graphium kirbyi* (Papilionidae) (T.C.E. Congdon 532).

Etymology. Named after Mr. Frank Mbago, curator of the Dar es–Salaam University herbarium (DSM), to whom we owe the discovery of this species, and in honor of his botanic knowledge and fieldwork expertise in Tanzania, in particular of Annonaceae. He is also co-discoverer of the endemic Tanzanian genus *Mwasumbia* (Couvreur et al. 2009).

Paratypes. Tanzania – Morogoro • L.-P.M.J. Dagallier 50 (DSM, K, MO, MPU (MPU1379109), P, WAG); Morogoro Rural District, Kimboza forest; 7°01'18.38"S, 37°48'32.13"E; alt. 267 m; 15 Nov. 2019. – Pwani • U. Bloesch s.n. (WAG (WAG.1549674; WAG.1418750), Kwedijela Coastal Forest, T3; 5°55'00"S, 38°36'00"E; 18 Sep. 2004. • T.L.P. Couvreur 3 (DSM, WAG); Bagamoyo District, Mazizi hill, on road between Chilinze and Wami River; 6°22'14.4"S, 38°21'51"E; alt. 100 m; 09 Nov. 2006. • L.-P.M.J. Dagallier 1 (DSM, K, MO, MPU (MPU1379043,

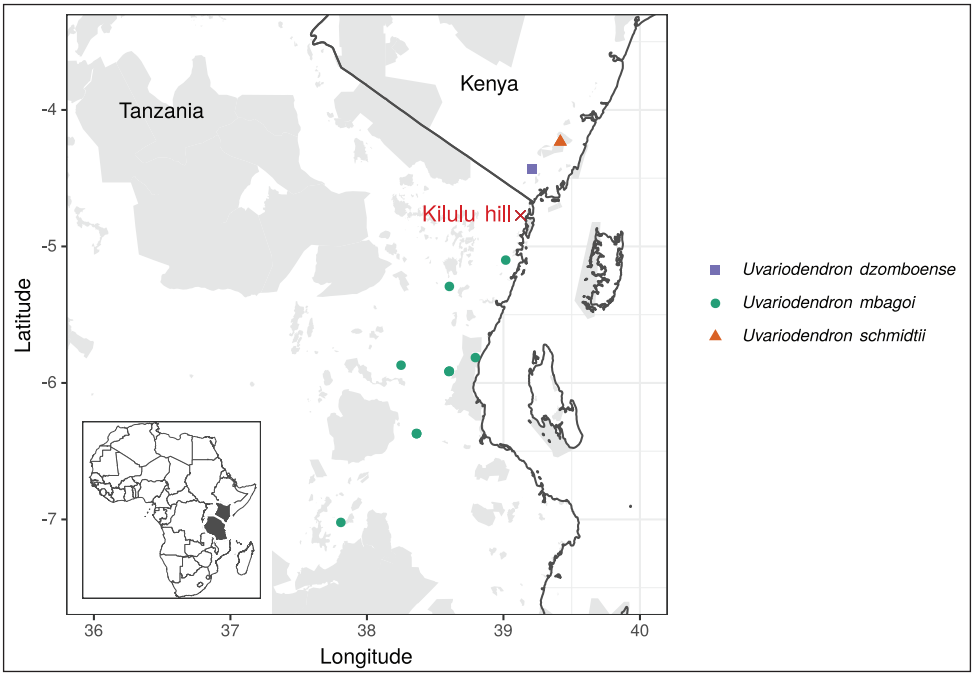

Figure 2. Map of the distribution of the three new *Uvariodendron* species. Protected areas are represented in grey shades (see Material and Methods for details). The red cross indicates Kilulu hill, where *Uvariodendron dzomboense* was expected to occur but was not found.

MPU1379066), P, WAG), Msata Hill, 30 km North of Chalinze; 6°22'17.78"S, 38°21'49.97"E; alt. 317 m; 06 Nov. 2019. – Tanga • T.C.E. Congdon 532 (K); Pangani District, Mkwaja Ranch; 5°48'50.76"S, 38°47'40.92"E; alt. 90 m; 04 Dec. 1998. • L.-P.M.J. Dagallier 40 (DSM, K, MO, MPU (MPU1379099), P, WAG); Handeni District, Kwedijela forest, ~8 km Kwamsisi village; 5°54'50.77"S, 38°36'13.27"E; alt. 155 m; 13 Nov. 2019. • W.D. Hawthorne 1420A (K); Tanga District, Mkulumuzi river, karst river valley, Steinbruch reserve; 5°06'00"S, 39°01'00.12"E; 12 Aug. 1982. • C.M. Kisena 3039 (MO); Handeni District, Collected from Kwedivikilo sacred forest near Manga Village; 5°06'00"S, 30°37'00"E; 17 Nov. 1997. • F.M. Mbago 3323 (DSM, K); Handeni District, Kwedijela forest, ~8 km Kwamsisi village; 5°54'50.77"S, 38°36'13.27"E; 07 Oct. 2004. • G.A. Peter 52283 (B, WAG, K), Inseln des Pangani bei Hale; 5°17'34.8"S, 38°36'14.06"E; alt. 340 m; 31 Jan. 1915.

Discussion. *Uvariodendron mbagoi* is unique within *Uvariodendron* for the strong bergamot (*Citrus bergamia* Risso) scent of the crushed leaves and bark. This scent is between lemon and orange scent. Other African Annonaceae species present strong scents. For example, *Uvariodendron anisatum* Verdcourt (Verdcourt 1955) presents an aniseed scent, and *Uvariodendron molundense* (Diels) R.E.Fries *var. citrata* Le Thomas (Le Thomas 1969) and *Uvariopsis citrata* Couvreur & Niangadouma (Couvreur and

Niangadouma 2016), present a lemon scent. However, no bergamot scent has been reported so far in Annonaceae.

The globose flower buds of this species easily fall off. Only flower buds were observed for this species, thus it is hard to infer the size of mature flowers. In the description above, the measures on the sepals and the petals are based on the dissection of the biggest flower bud of U. Bloesch s.n., and the carpel measurements were based on an old flower of T.C.E. Congdon 532 which has lost sepals and petals.

The fruiting specimens observed were collected from September to December. Collecting this species earlier in the year might permit the observation of flowers at anthesis.

Uvariodendron dzomboense **Dagallier, W.R.Q. Luke & Couvreur, sp. nov.**
urn:lsid:ipni.org:names:77215718-1
Fig. 3

Type. Kenya – Coast • S.A. Robertson et al. Mrima Dzombo Expedition 207 (holotype: K, isotypes: EA, MO, WAG), Kaya Dzombo Hill; 4°25'48"S, 39°12'36"E; alt. 300 m; 07 Feb. 1989.

Diagnosis. This species differs from other *Uvariodendron* species by its 50–75 carpels that are densely pubescent and its leaves smaller than 150 mm in length and narrowly elliptic to elliptic. It differs from *U. kirkii* by its smaller leaves (132 mm maximum versus 210 mm maximum) and higher number of carpels (50–75 versus 7–20) (Table 1).

Description. Tree 4–7 m tall, d.b.h. unknown, young branches sparsely pubescent to glabrous, old branches glabrous. Leaf bud 'eragrostiform', composed of 5, distichous, longitudinally folded, velutinous scales. Leaves distichous, simple, entire, pinnately veined. Petiole 3–4 mm long, 1–1.5 mm in diameter, slightly pubescent to glabrous. Lamina 65–132 mm long, 20–45 mm wide, length:width ratio 2.9–3.6, narrowly elliptic to elliptic, coriaceous, apex attenuate, base acute to slightly decurrent, above glabrous, below glabrous when young and old; midrib sunken above, raised below, above glabrous when young and old, below slightly pubescent to glabrous when young, glabrous when old; secondary veins 12–13 pairs, weak brochidodromous; tertiary veins reticulate. Inflorescence borne on trunk or old branches, 1-flowered. Flowering pedicel 8–30 mm long, 2–2.5 mm in diameter, densely pubescent. Flowers actinomorphic, hermaphroditic, buds spherical 4–4.5 mm in diameter, sparsely pubescent. Bracts 6 at base of the pedicel in flower bud, 1 on mature flower in the lower half of the pedicel, 5–6 mm long, 5–8 mm wide, pubescent to shortly pubescent outside, glabrous inside. Sepals 3, 5–7 mm long, 4.5–7 mm wide, fused at base, pubescent to shortly pubescent outside, glabrous inside. Outer petals 3, ca. 16 mm long, ca. 9 mm wide, shortly velutinous outside, glabrous inside, color unknown. Inner petals 3, ca. 18 mm long, 8 mm wide, shortly velutinous outside, glabrous inside, color unknown. Stamens more than 700, 2 mm long, 0.5 mm wide, anthers linear, connective truncate. Carpels 50–75, ca. 2 mm long, ca. 1–1.5 mm wide, densely pubescent. Stigma not seen. Fruiting pedicel ca. 14 mm long, ca. 4 mm in diameter, pubescent. Monocarps (unripe?) ca. 35, ca.

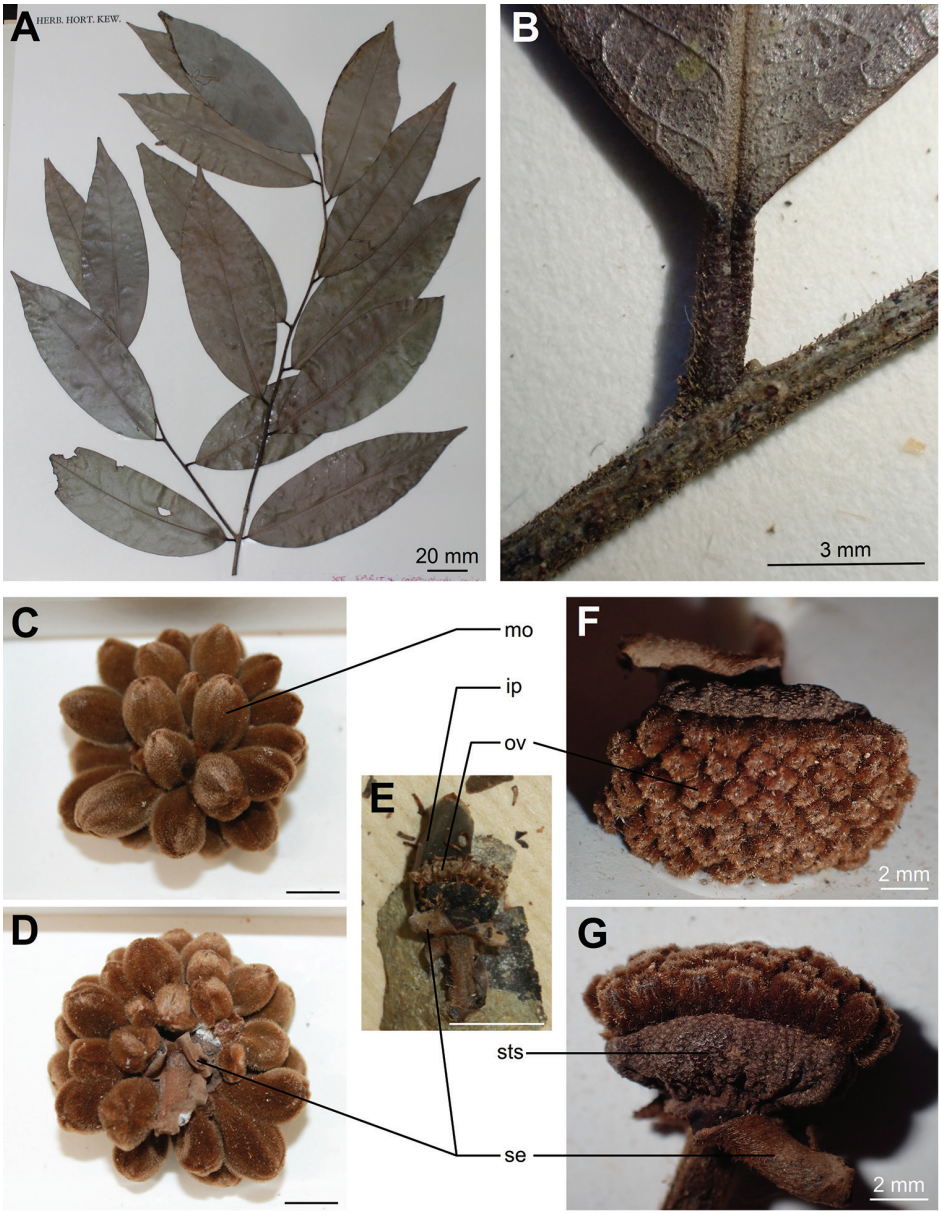

Figure 3. *Uvariodendron dzomboense* **A** young branch with leaves **B** petiole and young branch detail **C, D** young fruit: **C** apical view **D** from the side **E–G** old flower: **E** entire on trunk **F** from top with details of ovaries **G** close-up. *ip* inner petal, *mo* monocarp, *ov* ovary, *se* sepal, *sts* stamen scars. Photos by L.-P. M.J. Dagallier from the specimens W.R.Q. Luke 1654 (**F, G**), W.R.Q. Luke 7443 (**B**) and S.A. Robertson MDE 207 (**A, C, D, F**). Scale bars: 10 mm unless stated.

15 mm long, ca. 10 mm wide, length:width ratio ca. 1.5, ovoid, sessile, densely pubescent, golden brown. Seeds (unripe?) ca. 5 per monocarp, uniseriate, ca. 4.5 mm long, ca. 1 mm wide, glabrous.

Distribution. Endemic to Kenya, only known from Dzombo Hill (Kaya Dzombo) (Fig. 2).

Habitat. Moist semi–deciduous forest on igneous intrusion.

Conservation status. This species is known from five collections from a single location. Literature found on the Dzombo Hill forest reports a surface of 2.95 km^2 (Critical Ecosystem Partnership Fund 2005). We calculated a surface of 5.31 km^2 for the forested part of the hill (see Material and Methods for details). EOO and AOO are thus estimated at less than 5.40 km^2. The Kaya Dzombo forest is gazetted as a sacred forest under the National Museums protection as a National Monument. However, the forest suffers from local logging for timber, poles and firewood, and has been impacted by fire on several occasions (W.R.Q. Luke, personal observations). Following IUCN criterion B (IUCN 2012), *Uvariodendron dzomboense* is therefore assigned a preliminary status of Endangered EN B1ab(iii)+2ab(iii).

Etymology. The specific epithet comes from the Dzombo Hill where the species is endemic.

Paratypes. Kenya – Coast • W.R.Q. Luke 1654 (EA (EA000008806), K); Kwale District, Dzombo Forest Reserve; 4°25'48"S, 39°12'36"E; alt. 270 m; 06 Jan. 1989. • W.R.Q. Luke et al. 2884 (EA, K); Kwale District, Dzombo Forest Reserve; 4°25'48"S, 39°12'36"E; alt. 270 m; 04 Oct. 1991. • W.R.Q. Luke et al. 3370 (EA); Kwale District, Dzombo Forest Reserve; 4°25'48"S, 39°12'36"E; alt. 270 m; 12 Nov. 1992. • W.R.Q. Luke & M. Pakia 7443 (K, EA (EA000008810)); Kwale District, Dzombo; 4°25'48"S, 39°12'36"E; alt. 270 m; 28 Jun. 2001.

Discussion. This species is known as "*Uvariodendron sp. nov. 1 of CFS*" in the annotated checklist of the coastal forests of Kenya (Ngumbau et al. 2020).

The only fruit known from this species (Robertson S.A. et al. MDE 207) presents ca. 35 ovoid monocarps. These are densely pubescent and have small seeds compared to other *Uvariodendron* species (4.5 mm long vs. 8–20 mm long). However, it is unclear whether this observed fruit is ripe or not. Further collections could bring more information. This species also presents an 'eragrostiform' leaf–bud (see discussion of *Uvariodendron schmidtii* below).

During a field trip in Tanzania in November 2019, we explored the forest of Kilulu hill (TANZANIA- Tanga, 4°46'22"S, 39°07'30"E, see Fig. 2). Kilulu hill is ca. 40 kilometers as the crow flies south from Dzombo hill were *U. dzomboense* occurs. We expected to find *U. dzomboense* there but our quest on every slope of the hill was unsuccessful. This indicates that the dispersal distance of *U. dzomboense* might be very short.

Uvariodendron schmidtii W.R.Q. Luke, Dagallier & Couvreur, sp. nov.

urn:lsid:ipni.org:names:77215719-1
Figure 4

Type. Kenya – Coast • W.R.Q. Luke 3087 (holotype: EA (EA000008814), isotypes: K, MO, US); Kwale District, Shimba hills, Longomagandi; 4°14'00"S, 39°25'00"E; alt. 380 m; 20 Apr. 1992.

Diagnosis. This species differs from other *Uvariodendron* species by its flowers that are small (petals < 13 mm long), velutinous, on a 10–15 mm long pedicel, with fused sepals forming a ring around the fruit pedicel, and fewer than 10 carpels. It differs from *U. kirkii* by its smaller petals (< 13 mm versus more than 15 mm) and its sepals fused in a ring (versus free and valvate to imbricate) (Table 1).

Description. Tree 10–12 m tall, d.b.h. unknown, young branches sparsely pubescent to glabrate, old branches glabrous. Leaf bud 'eragrostiform', composed of 5–7, ca. 10 mm long, 10 mm wide distichous, longitudinally folded, velutinous scales. Leaves distichous, simple, entire. Petiole 4.5–7 mm long, 1.5–2 mm in diameter, glabrate to sparsely puberulent. Lamina 159–188 mm long, 49–71 mm wide, length:width ratio 2.4–3.3, narrowly elliptic to elliptic, coriaceous, apex attenuate to acuminate, base acute to decurrent, above glabrous, below sparsely pubescent to glabrate when young, glabrous when old; midrib sunken above, raised below, above glabrous when young and old, below pubescent to glabrous when young, glabrous when old; secondary veins 10–14 pairs, weakly brochidodromous to brochidodromous; tertiary veins reticulate. Inflorescence borne on trunk and branches, 1–2 flowers. Flower pedicel 10–15 mm long, 2.5 mm in diameter, densely velutinous. Flowers actinomorphic, hermaphroditic, buds spherical, 6–7 mm in diameter, velutinous. Bracts 1–3, 1 at base of the pedicel, 1–2 between the 20–70% of the length of the pedicel, ca. 5 mm long, ca. 10 mm wide, velutinous outside, glabrous inside. Sepals 3, 5.5–7 mm long, 7–9 mm wide, fused on ca. 50% of the length, forming a ring around fruit pedicel, densely velutinous to velutinous outside, glabrous inside. Outer petals 3, 11–12 mm long, 9–11 mm wide, densely velutinous to velutinous outside, glabrous inside, brown outside, cream with purple streak at base inside. Inner petals 3, ca. 10 mm long, 8–9 mm wide, connivent at apex on ca. 50% of the length, densely velutinous to velutinous outside, glabrous inside, brown-orange with margins cream and purple at base outside, cream with purple streak at base inside. Stamens more than 500, length and shape unknown. Carpels ca. 7, ca. 1.5 mm long, ca. 1 mm wide, velutinous. Stigma not seen. Fruiting pedicel ca. 16 mm long, ca. 2.5 mm in diameter, pubescent. Monocarps 3–5, ca. 32 mm long, ca. 20 mm wide, length:width ratio ca. 1.6, rounded to ellipsoid with a longitudinal ridge, sessile, sparsely pubescent, green turning orange. Seeds not seen.

Distribution. Endemic to Kenya; only known from the Longomwagandi forest (also found spelled "Longomagandi" or "Longo-Magandi" in the literature) in the Shimba Hills National Reserve, in Kenya (Fig. 2).

Habitat. Lowland forest on ridge with *Antiaris*, *Milicia*, *Lovoa*, *Celtis*, *Quassia*, *Hymenaea*, *Julbernardia*, *Diospyros*, *Memecylon*, and many Rubiaceae shrubs in understorey.

Conservation status. This species is known from seven collections from a single location. Literature for the Shimba Hills forest reserve reports a surface between 0.22 km^2 (Schmidt 1992) and 1.50 km^2 (Cheek 2003) for Longomwagandi forest. We calculated a surface of 1.30 km^2 (see Material and methods for details). EOO and AOO are thus estimated at less than 1.50 km^2. Following IUCN criterion B, this would place the species in the "Critically Endangered CR" category. However, given that the occurrences are in the Shimba Hills National Reserve, no decline is observed or projected in EOO and AOO. The future of *Uvariodendron schmidtii* relies on the

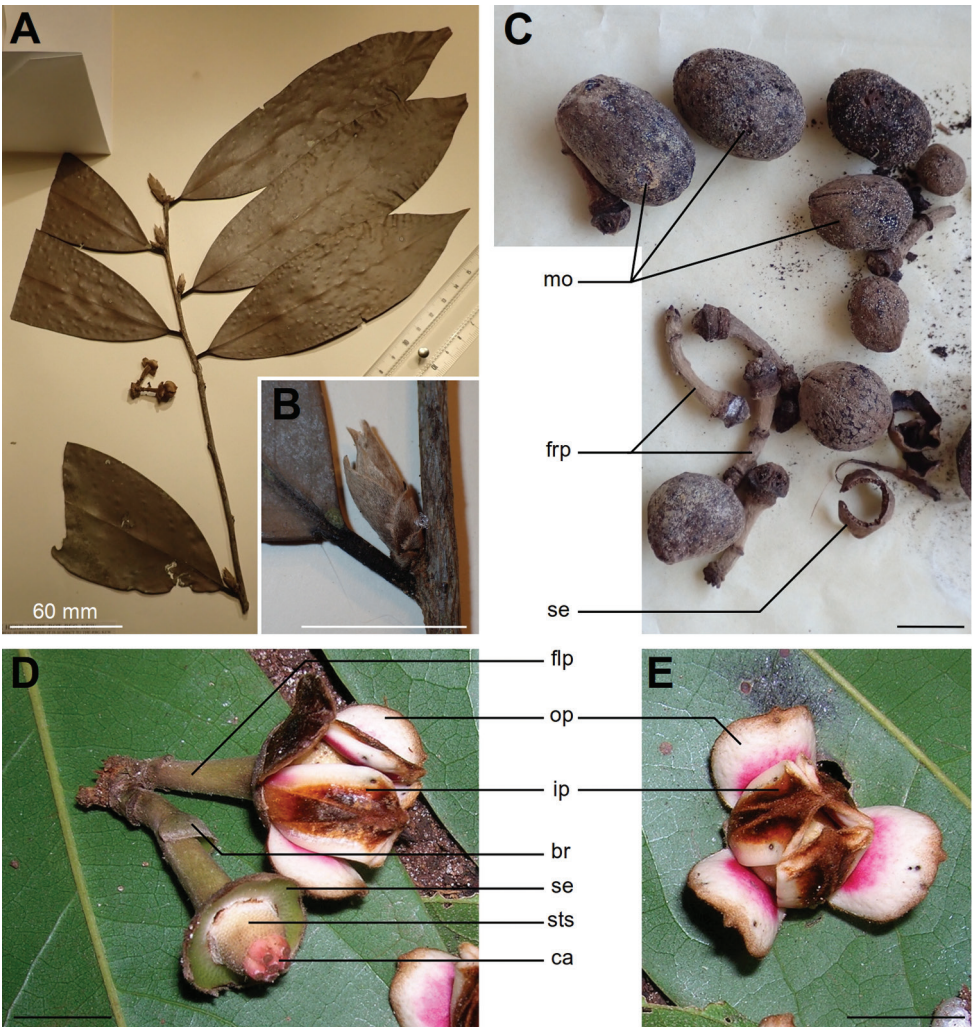

Figure 4. *Uvariodendron schmidtii* **A** young branch with leaves **B** eragrostiform axillary bud **C** detached monocarps and pedicels **D** two-flowered inflorescence **E** flower, apical view. *br* bract, *ca* carpel, *flp* flower pedicel, *frp* fruit pedicel, *ip* inner petal, *mo* monocarp, *op* outer petal, *se* sepals ring, *sts* stamen scars. Photos by L.-P. M.J. Dagallier from the specimens W.R.Q. Luke 3087 (**C**) and W.R.Q Luke 4717 (**A, B**), and by W.R.Q. Luke from a living individual (**D, E**). Scale bars: 10 mm unless stated.

future of Shimba Hills National Reserve. Given that the species occurs in a single locality with a very restricted AOO (less than 20 km^2), it is "prone to the effects of human activities or stochastic events within a very short time period in an uncertain future, and is thus capable of becoming Critically Endangered or even Extinct in a very short time period" (IUCN 2012). Following IUCN criterion D, it can be therefore assigned a preliminary status of Vulnerable VU.

Copious seedlings are found below parent trees, but few survive to maturity. Many of these "wildlings" were moved to the Base Titanium indigenous tree nursery and have been out-planted there as part of their mine rehabilitation program.

Vernacular name. Mbebeneka in Kidigo language (R. Schmidt 788).

Etymology. This species is named after Robert Schmidt, a PhD student studying the ecology of the Shimba Hills National Reserve who first collected it in September 1988 and brought it to the attention of W.R.Q. Luke.

Paratypes. Kenya – Coast • W.R.Q. Luke & S.A. Robertson 2737 (EA, K, MO, US); Kwale District, Shimba hills, Longomagandi; 4°14'00"S, 39°25'00"E; alt. 390 m; 18 Mar. 1991. • W.R.Q. Luke 2919 (EA (EA000008817), K, MO, US); Kwale District, Shimba hills, Longomagandi; 4°14'00"S, 39°25'00"E; alt. 390 m; 15 Oct. 1991. • W.R.Q. Luke 4717 (P (P02084012), Ukunda); Kwale District, Shimba hills, Longomwagandi; 4°14'00"S, 39°25'00"E; alt. 380 m; 12 Sep. 1997. • W.R.Q. Luke 11676 (EA, K, MO, US); Kwale District, Shimba hills, Longomagandi; 4°14'00"S, 39°25'00"E; alt. 380 m; 30 Dec. 2006. • S.A. Robertson 7556 (EA, K, WAG (WAG0129164)); Kwale District, Shimba Hills, Longomagandi; 4°14'00"S, 39°25'00"E; alt. 450 m; 04 Jun. 2005. • R. Schmidt 788 (EA); Kwale District, Shimba Hills, Longomagandi; 07 Sep. 1988.

Discussion. This species shows 'eragrostiform' leaf–buds, a feature described in *Uvariodendron gorgonis* Verdcourt (Verdcourt 1969) and *Uvariodendron dzomboense* Dagallier, W.R.Q. Luke & Couvreur (this publication). This structure is composed of several (5–7 in *U. schmidtii* and *U. dzomboense*, 6–12 in *U. gorgonis*) distichous and densely pubescent scales that might be a protection for the apical meristem against drought or herbivores. The adjective 'eragrostiform' refers to the genus *Eragrostis* (Poaceae) that has a peculiar form of flattened spikelet composed of compact and clustered florets. Even though this feature is striking, it seems hard to use it as a diagnostic character. Similar apical buds are also found in other Annonaceae species such as *Monodora minor* Engler & Diels (Couvreur 2009) or in *Uvariodendron usambarense* Fries and *Uvariodendron giganteum* (Engler) Fries.

***Polyceratocarpus oligocarpus* (Verdc.) Dagallier, comb. nov.**
urn:lsid:ipni.org:names:77215720-1

Uvariodendron oligocarpum Verdcourt, Kew Bulletin 41(2): 289, 1986.

Type. Tanzania – Tanga • J. Lovett 259 (holotype: K (K000198888)); Lushoto District, Ambangulu, West Usambara; alt. 1300 m; 2 Mar. 1984.

We examined 11 specimens (including the type specimen) of *Uvariodendron oligocarpum* Verdc. and found they have percurrent tertiary venation and pitted seeds. These characteristics are typical of the genus *Polyceratocarpus* Engl. & Diels (Couvreur et al. 2009, 2012). Moreover, the fertile specimens observed have the

combination of the following characters: outer petals ca. 35 mm long, 3 to 6 carpels, and 2 to 4 cylindrical and straight to slightly curved monocarps. This combination precludes these specimens from being identified as one of the two other species known from East Africa to date: *Polyceratocarpus scheffleri* Engl. & Diels that has "at least 20 [and] strongly curved" monocarps (Verdcourt 1971), and *Polyceratocarpus askhambryan-iringae* A.R. Marshall & D.M. Johnson that has outer petals 10–16 mm long (Marshall et al. 2016). Based on the above characters, they also cannot be included in any other accepted species from Central or West Africa that all have petals shorter than 25 mm long: *Polyceratocarpus angustifolius* Paiva and *P. germanii* Boutique, *P. gossweileri* (Excell) Paiva, *P. laurifolius* Paiva, *P. microtrichus* (Engl. & Diels) Ghesq. ex Pellegr., *P. parviflorus* Ghesq., and *P. pellegrinii* Le Thomas (Pellegrin 1949, Boutique 1951, Le Thomas 1965, Paiva 1966). Thus, this species initially described as *Uvariodendron oligocarpum* Verdc. is here combined as *Polyceratocarpus oligocarpus* (Verdc.) Dagallier.

Other specimen examined. Tanzania – Tanga • A. Borhidi 86249 (K); Muheza District, East Usambaras Mts., Kwamkoro F.R. SE of Kwamkoro Tea Estate; alt. 1030 m; 28 Oct. 1986. • A. Borhidi 87241 (K); Muheza District, East Usambaras Mts., Kwamkoro F.R. bordering Kwamsambia F.R; alt. 990 m; 05 May. 1987. • L.-P.M.J. Dagallier 63 (DSM, K, MPU (MPU1379122), P, WAG); Korogwe District, East Usambaras, Ambangulu, top of the mountain above the tea plantations; 5°04'13.00"S, 38°24'31.00"E; alt. 1320 m; 20 Nov. 2019. • A.R. Marshall 1457 (K, MO); Lushoto District, Ambangulu – PSP19, Ambangulu Tea Estate Forest, West Usambara Mountains; 5°4'20.69"S, 38°24'24.21"E; alt. 1294 m; 22 Mar. 2008. • A.R. Marshall 1695 (K); Lushoto District, Ambangulu – PSP19, Ambangulu Tea Estate Forest, West Usambara Mountains; 5°4'20.69"S, 38°24'24.21"E; alt. 1294 m; 24 Mar. 2008. • F.M. Mbago 3586 (DSM); Lushoto District, Balangai forest near Tea estate; 4°56'41.24"S, 38°26'42.10"E; alt. 1505 m; 28 Jul. 2012. • F.M. Mbago 3760 (DSM), Korogwe Kunga Forest Mavimo Kwemtonto; 3°20'37.15"S, 37°19'46.06"E; alt. 898 m; 23 Nov. 2016. • C.K. Ruffo 1730 (K); Muheza District, Kwamkoro F.R; alt. 950 m; 28 Oct. 1986. • C.K. Ruffo 1747 (K); Muheza District, Kwamkoro F.R; alt. 1000 m; 31 Jan. 1987. • C.K. Ruffo 1835 (K); Muheza District, Kwamkoro F.R; alt. 1050 m; 18 Sep. 1986.

Key to the East African species of *Uvariodendron*

1 Longest leaf lamina equal to or longer than 35 cm long **2**

– Longest leaf lamina shorter than 35 cm long **4**

2 Number of secondary veins pairs equal to or less than 20; fruit monocarps less than 10 mm wide and with a length:width ratio over 5, stipe 5–11 mm long... ..... ***U. gorgonis* (pro parte)**

– Number of secondary veins pairs more than 20; fruit monocarps more than 13 mm wide and with a length:width ratio below 4, stipe less than 6 mm long **3**

3 Young branches sparsely pubescent to glabrous; leaf lamina oblong to obovate, base rounded to subcordate..... ***U. usambarense***

– Young branches pilose covered with long soft hair quickly falling off; leaf lamina obovate, base acute..... ***U. magnificum***

4 Greatest leaf lamina equal to or shorter than 16 cm long, margins slightly revolute..... **5**

– Greatest leaf lamina longer than 16 cm long, margins flat..... **6**

5 Bark and crushed leaves emitting a strong bergamot scent; flower and fruits (sub)sessile, pedicel less than 6 mm long; carpels 12 to 16; monocarps cylindrical, green-grey, tomentose with regular tufts of higher hair density..... ..... ***U. mbagoi***

– Bark and crushed leaves not emitting a bergamot scent; flower and fruits pedicel 8–30 mm long (but flower buds sessile); carpels 50 to 75; monocarps ovoid, golden-brown, densely pubescent..... ***U. dzomboense***

6 Leaf lamina up to 40 cm long, base rounded to acute; carpels 40 to 50; monocarp length:width ratio over 5..... ***U. gorgonis*** **(pro parte)**

– Leaf lamina up to 32 cm long, base acute to decurrent; carpels up to 40; monocarp length:width ratio below 4..... **7**

7 Leaf lamina apex attenuate to acuminate; flower pedicel equal to or less than 15 mm; sepals fused at base over more than 20% of their length..... **8**

– Leaf lamina apex acute to attenuate; flower pedicel equal to or more than (5)-10 mm; sepals connivent or fused at base over less than 10% of their length.... **9**

8 Bark of trunk and branch peeling off, reddish; leaf lamina length:width ratio equal to or more than 3.4; petals 31–36 mm long, carpels 29 to 40..... ..... ***U. pycnophyllum***

– Bark of trunk and branch not peeling off, greyish; leaf lamina length:width ratio equal to or less than 3.3; petals 10–12 mm long, carpels fewer than 10 ... . ***U. schmidtii***

9 Plant emitting a strong anise scent; longest leaves up to 32 cm; flower pedicel 15–65 mm long; fruit monocarps 38–70 mm long, ripe fresh fruit dark blue-black..... ***U. anisatum***

– Plant not emitting anise scent; longest leaves up to 22 cm; flower pedicel (5)10–30 mm long; fruit monocarps 23–36 mm long, ripe fresh fruit dull-orange..... ***U. kirkii***

Discussion

The three new species described here (*Uvariodendron mbagoi* Dagallier & Couvreur, *Uvariodendron dzomboense* Dagallier, W.R.Q. Luke & Couvreur and *Uvariodendron schmidtii* W.R.Q. Luke, Dagallier & Couvreur) occur in the coastal forests of Kenya and Tanzania. Due to their restricted ranges, they are all threatened following our IUCN preliminary conservation status assessments. Endemism is high in East Africa for plants

and animals (Burgess et al. 1998, 2007), and single-location endemic species are known there (e.g. Scharff 1992, Couvreur et al. 2009, Gosline et al. 2019). The discovery of these three new narrowly endemic species supports this long-standing observation. This also shows that botanically this region is still not fully known despite the publication of the complete flora of East Africa (Beentje 2015). There are still new plant species that have been collected and await description (see e.g. "sp. nov." for several genera in Ngumbau et al. 2020).

The new combination and the three new species described here bring the number of East African *Uvariodendron* species to nine across East Africa, and to 17 across Africa. The described species show characters that have never been found in the family, such as the strong bergamot scent of *Uvariodendron mbagoi* or in the genus, such as the very densely pubescent carpels and monocarps of *U. dzomboense*.

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Chroesthes (Acanthaceae) in Peninsular Malaysia, including a new species from Kelantan and a new record from Terengganu

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Abstract

Chroesthes is a small genus that includes three species from Peninsular Malaysia: *Chroesthes faizaltahiriana* Siti-Munirah **sp. nov.**, *C. lanceolata* (T. Anderson) B.Hansen and *C. longifolia* (Wight) B.Hansen. *Chroesthes faizaltahiriana*, recently discovered in the State of Kelantan, is described and illustrated. This species is similar to the common species *C. longifolia*, but is distinguished mainly by its inflorescence type, calyx shape and its flowers being bright orange instead of dark purple internally. *Chroesthes lanceolata* is a new record for Peninsular Malaysia and has only been collected once. Following the IUCN Red List Categories and Criteria, these three species are assessed (national scale assessment) as Critically Endangered (*C. faizaltahiriana* and *C. lanceolata*) and Least Concern (*C. longifolia*).

Keywords

Conservation status, revision, taxonomy

Introduction

The small genus *Chroesthes* Benoist (1927), Acanthaceae, currently consists of three species distributed in South China, Indo-China and Malaysia (Hansen 1983; Govaerts 1999; POWO 2019). In the current classification of the family Ranunculaceae, it is placed within the putative members of tribe Barlerieae (Scotland and Vollesen 2000; McDade et al. 2008; Champluvier and Darbyshire 2012). This genus can be distinguished from other genera of Acanthaceae by the structure of the calyx and corolla, the

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presence of bicalcarate anthers, i.e. anther thecae spurred at the base or awned anther thecae and are diagnostic within Barlerieae, for which quincuncial aestivation is diagnostic and seeds are shortly pubescent (Hansen 1983; Hu et al. 2011; Darbyshire et al. 2019). The three species of *Chroesthes* are distributed as follows: *Chroesthes lanceolata* (T. Anderson) B.Hansen occurs in Myanmar, N Thailand, N Laos, N Vietnam SW China (Yunnan) and Peninsular Malaysia (Hansen 1983; Ummul-Nazrah et al. 2011); *Chroesthes bracteata* (J.B. Imlay) B.Hansen occurs in SE Thailand (Hansen 1983); and *Chroesthes longifolia* (Wight) B.Hansen is endemic in Peninsular Malaysia (Hansen 1983).

The Forest Research Institute Malaysia (FRIM) launched the Peninsular Malaysia Flora Project (FPM) in 2005, which aims to provide a more complete and up-to-date account of the national flora (Middleton et al. 2019). To achieve this goal, the FRIM flora team continues to conduct vegetation surveys throughout Peninsular Malaysia, especially in unexplored and ecologically-significant areas. Through this fieldwork, the number of species encountered within the Flora region has increased. A few new species are discovered every year and published in journals. For example, many new fairy lantern species (*Thismia*) have been described in recent years, including *Thismia kelantanensis* Siti-Munirah, *T. domei* Siti-Munirah and *T. terengganuensis* Siti-Munirah (Siti-Munirah 2018; Siti-Munirah and Dome 2019). Moreover, new gesneriad species have also been described, including *Codonoboea norakhirrudiniana* Kiew, *C. rheophytica* Kiew, *C. sallehuddiniana* Lim (Kiew and Lim 2019) and, most recently, *Codonoboea kenaboiensis* Syahida-Emiza, Sam & Siti-Munirah and *C. ruthiae* Syahida-Emiza, Sam & Siti-Munirah have been described (Syahida-Emiza et al. 2020).

During a recent botanical survey at Berangkat Forest Reserve (FR), Kelantan, an upright shrub with an unusual bright orange corolla colour was encountered growing in a patch of forest, under shade beside an old logging road. Its morphological characteristics, including its stamen type, indicated that it belongs to the genus *Chroesthes*, but is unmatched amongst the three species currently recognised. This new species is described here as *Chroesthes faizaltahiriana* Siti-Munirah, which brings the total species for *Chroesthes* in the world to four. It is also an additional endemic species in Peninsular Malaysia. With this recent discovery, an account of the genus *Chroesthes* in Peninsular Malaysia is provided, including the key to *Chroesthes* of the world; however, only the Malaysian species are considered in the remainder of the treatment.

Materials and methods

Specimens of *Chroesthes* species from Peninsular Malaysia, held in the herbaria at Kepong Herbarium (**KEP**) and Singapore Botanic Gardens Herbarium (**SING**), were examined. All cited specimens were observed by the author. The study of the new species was based on material collected by the author on 26 February 2020 from the only populations found during a botanical trip to Berangkat FR, Gua Musang, Kelantan. The specimens were pressed as herbarium specimens and an inflorescence was also preserved as a spirit collection. Morphological characters were studied using a stereomicroscope

and high-resolution macro photography. Measurements were taken from living plants and herbarium material. The specimen details were compared with original drawings and descriptions given in the protologues for each species of the genus *Chroesthes* and also with information gathered from the relevant literature (Wight 1850; Clarke 1908; Ridley 1923; Hansen 1983, Hu et al. 2011). The conservation status assessments were made with reference to the Categories and Criteria of IUCN (2019).

Taxonomy

***Chroesthes* Benoist**

Bull. Mus. Natl. Hist. Nat. 33: 107. 1927, in Fl. Gen. I.C. 4: 684. 1935; Hansen, Nordic J. Bot. 3: 209. 1983; Hu, J.C., Deng, Y.F., Daniel, T. & Wood, J.R.I. Acanthaceae. In: Wu, Z.Y., Revan, P. & Hong, D.Y. (Eds.) Flora of China 19: 472. 2011.

Description. Shrubs; cystoliths present. ***Leaves*** opposite, petiolate; leaf blade margin entire; subisophyllous or anisophyllous. ***Inflorescences*** terminal thyrses (the thyrses are branched (then paniculiform) or unbranched (then racemoid); bracts and bracteoles greenish. ***Calyx*** unequally five-lobed: posterior lobe largest, two lateral lobes smaller than two anterior lobes. ***Corolla*** tube basally cylindrical, expanded distally into a throat; limb two-lipped, upper lip two-lobed, lower lip three-lobed; lobes quincuncial in bud; four stamens, connate to the corolla, not connate to one another, posterior pair shorter than anterior pair, inserted at the base of the corolla throat; anthers bithecous; thecae parallel, inserted at different heights, dorsally pubescent, base of each theca spurred; ovary with two ovules per locule; style basally sparsely pubescent; stigma capitate. ***Capsule*** stipe absent or barely present; retinacula present. ***Seeds*** compressed, brownish, shortly pubescent.

Distribution. China, Laos, Malaysia, Myanmar, Thailand, Vietnam. Three species in Malaysia.

Ecology. Lowland dipterocarp forest to upper hill dipterocarp forest.

Key to species of *Chroesthes*

1 Bracts approximately half the length of the calyx ..... ***C. lanceolata***
– Bracts approximately as long as the calyx **2**
2 All calyx lobes narrow, linear..... *C. bracteata* (only in Thailand)
– Posterior and anterior calyx lobes elliptic to elliptic-lanceolate, the lateral lobes linear..... **3**
3 Inflorescence terminal branched; corolla entirely dark purple to purplish-red or sometimes white externally ..... ***C. longifolia***
– Inflorescence terminal unbranched; corolla yellow to dark orange..... ***C. faizaltahiriana***

Chroesthes faizaltahiriana **Siti-Munirah, sp. nov.**
urn:lsid:ipni.org:names:77215721-1
Figs 1–3

Diagnosis. *Chroesthes faizaltahiriana* most closely resembles *C. longifolia*; however, it differs in its inflorescence type, the presence of a terminal raceme not branching (vs. terminal raceme branching) and posterior lobe size ratio 1:4 (vs. 1:2) and corolla length 4.5–5.5 cm (vs. 2–3 cm) and in the corolla tube and lobes being entirely bright yellow to dark orange (vs. entirely dark purple, purplish-red or occasionally white externally) and other significant characters (see Table 1).

Type. Malaysia. Peninsular Malaysia: Kelantan, Gua Musang Distr., Berangkat FR, ca. 822 m alt., 26 Feb 2020, *Siti-Munirah, FRI 91215* (holotype KEP!, barcode KEP 280001).

Description. Shrubs 0.7–1 m high. ***Stems*** terete, erect, not branched, surface glabrous, diameter ca. 2 mm, swollen at nodes. ***Leaves*** opposite; petiole 0.2–1 cm long; straight or twisted (makes the leaf arrangement look decussate); leaf blades elliptic, lanceolate to oblanceolate, 4.5–19 × 1.5–5.5 cm, both surfaces glabrous, lateral veins ca. 8–10 on each side of mid-vein, base attenuate to cuneate, margin entire, apex acute to acuminate. ***Inflorescence*** a terminal raceme (unbranched), up to 13 cm long; flowers secund (one bract at each node being sterile, the other bracts subtending each flower), ca. 10-flowered; glandular-pubescent on most parts; peduncles ca. 1 cm; bracts narrowly lanceolate, 20–30 × 2–5 mm, apex acute, glandular-pubescent, conspicuously 1-nerved; bracteoles narrowly lanceolate, 10–15 × 1–2 mm; pedicel short, ca. 1–2 mm long. ***Calyx*** 2–3 cm long, posterior lobe lanceolate, 30 × 7 mm, 3–5-nerved, apex acuminate; two anterior lobes, elliptic-lanceolate, 22 × 2–2.5 mm, 1-nerved, apex acuminate; two lateral lobes, linear-lanceolate, 20 × 1 mm, 1-nerved; all glandular-pubescent on both sides; all greenish. ***Corolla*** bilabiate, orange-yellow, ca. 4.5–5.5 cm long; outer surface pale yellow, glandular-pubescent; inner surface bright orange-yellow to orange with dark orange spots (or stripes), glabrous; tube with cylindrical basal portion ca. 2 cm long, expanded throat ca. 1.5 cm long; upper lip shortly two-lobed (8–9 mm long), lower lip deeply three-lobed (8–10 mm long). Stamens 4, didynamous, included in the throat, inserted at the base of the inflated part of the corolla, longer pair with filaments ca. 1.5 cm long, shorter pair with filaments ca. 1.2 cm long, all filaments with sparse glandular trichomes on the surface; anther thecae ca. 1–2 mm long, basal spur pointed, surface cover with simple trichomes. Pistil whitish-green; ovary ovoid, 1.5–2 mm long, apex pubescent; style ca. 3.2 cm long, pilose below, glabrous above; stigma subcapitate, minutely bilobed. Fruit not known.

Distribution. Endemic to Peninsular Malaysia, Kelantan. Currently known only from the type collection from Berangkat FR, 5°07'55.5"N, 101°55'28.5"E (Map 1).

Ecology. *Chroesthes faizaltahiriana* is found in upper hill dipterocarp forests under shade at 822 m elevation. It was found flowering in February in patches of unlogged forest beside a logging road (Fig. 4).

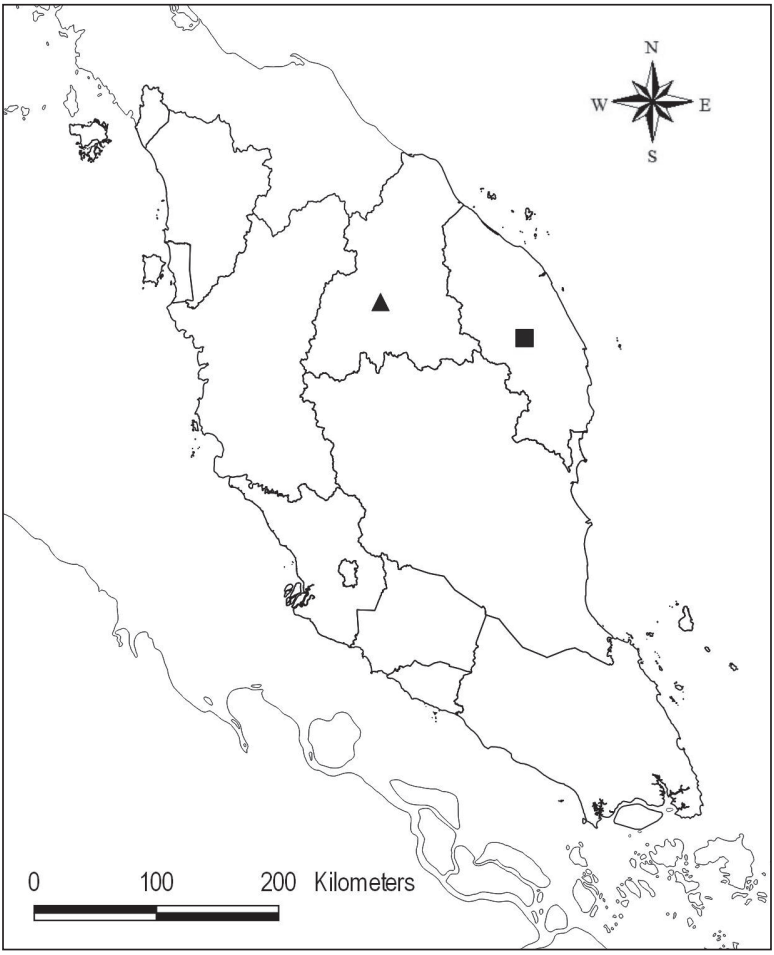
Table 1. Morphological comparison between *Chroesthes faizaltahiriana* and *C. longifolia*.

Character	*C. faizaltahiriana*	*C. longifolia*
Habit		
Height (m)	0.7–1	1–2.5
Stem	Unbranched	Branching
Inflorescence		
Type	Terminal raceme not branching (single)	Terminal raceme always branching (always two)
Length (cm)	8–13	up to 25
Flowers		
Bract (mm)	20–30 × 2–5	15–20 × 4.5–6
Shape	Narrowly lanceolate	Lanceolate
Bracteoles (mm)	10–15 × 1–2	10 × 3
Calyx length (cm)	2–3	1.6–1.8
Posterior lobe (mm)	30 × 7	18 × 8–9
Anterior lobe (mm)	22 × 2–2.5	16 × 5–6
Lateral lobe (mm)	20 × 1	10–12 × 0.5
Calyx colour	Completely always greenish	Green or green to purplish (especially purplish at apex)
Total corolla length (cm)	4.5–5.5	2–3
Corolla colour	Entirely yellow to dark orange: pale yellow externally, bright orangish-yellow with darker spots internally	Entirely dark purple to purplish-red or sometimes white externally
Filament length (cm)		
Longer pair	1.5	1
Shorter pair	1.2	0.8

Etymology. *Chroesthes faizaltahiriana* is dedicated to Mohd Faizal Mat Tahir (known as Faizal Tahir), the husband of Siti-Munirah for his strong support in many ways towards the author's botanical work.

Conservation status. Critically Endangered B2 ab(ii,iii). Following the IUCN Red List Categories and Criteria (IUCN 2019), this species is assessed as critically endangered because it is only known from one locality and AOO is less than 10 km^2. Furthermore, about eight individuals were observed (all at the flowering stage). The collection locality is within the forest reserve, in an area of disturbed forest near a logging road at an elevation of 822 m. The status of the population is uncertain because the active selective logging activity within the forest reserve is ongoing. It is certainly not within a Totally Protected Area. During the 3-day visit to Berangkat FR, no other populations of *C. faizaltahiriana* were found. A further survey is needed to obtain more information that could estimate and determine the population size of this species.

Notes. Based on the general morphology of this plant, *C. faizaltahiriana* is close to *C. longifolia*, which was previously the only known species of *Chroesthes* in Peninsular Malaysia. However, a detailed comparison has shown that its inflorescence type is entirely different (Fig. 5, Table 1). Additionally, its phytogeography is also different when compared to *C. faizaltahiriana*, which is endemic to Gunung Berangkat (Kelantan) in upper hill dipterocarp forest (822 m a.s.l.), while *C. longifolia* is a widely distributed species inhabiting lowland dipterocarp forest. Based on herbarium specimen collections of *C. longifolia* at Kepong Herbarium (KEP) and the Singapore Herbarium (SING), it has been recorded in Kedah, Perak, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Melaka and Johor. *C. faizaltahiriana* is a distinctive



Map 1. Distribution of *Chroesthes faizaltahiriana* (▲) and *C. lanceolata* (■) in Peninsular Malaysia.

species amongst *Chroesthes* due to its flower colour. It is the only *Chroesthes* species with bright orange flowers internally. The flowers of *C. longifolia* are entirely dark purple to deep purplish-red (occasionally white externally), while the flowers of *C. lanceolata* are white, spotted pink to purple and those of *C. bracteata* are reported as pale pink, spotted with red.

Chroesthes lanceolata **(T. Anderson) B. Hansen**

Fig. 6

Chroesthes lanceolata (T. Anderson) B. Hansen, Nordic J. Bot. 3: 209. 1983; Hu, J.C., Deng, Y.F., Daniel, T. & Wood, J.R.I. Acanthaceae. In: Wu, Z.Y., Revan, P. & Hong, D.Y. (Eds.) Flora of China 19: 472. 2011. *Basionym*: *Asystasia lanceolata* T.

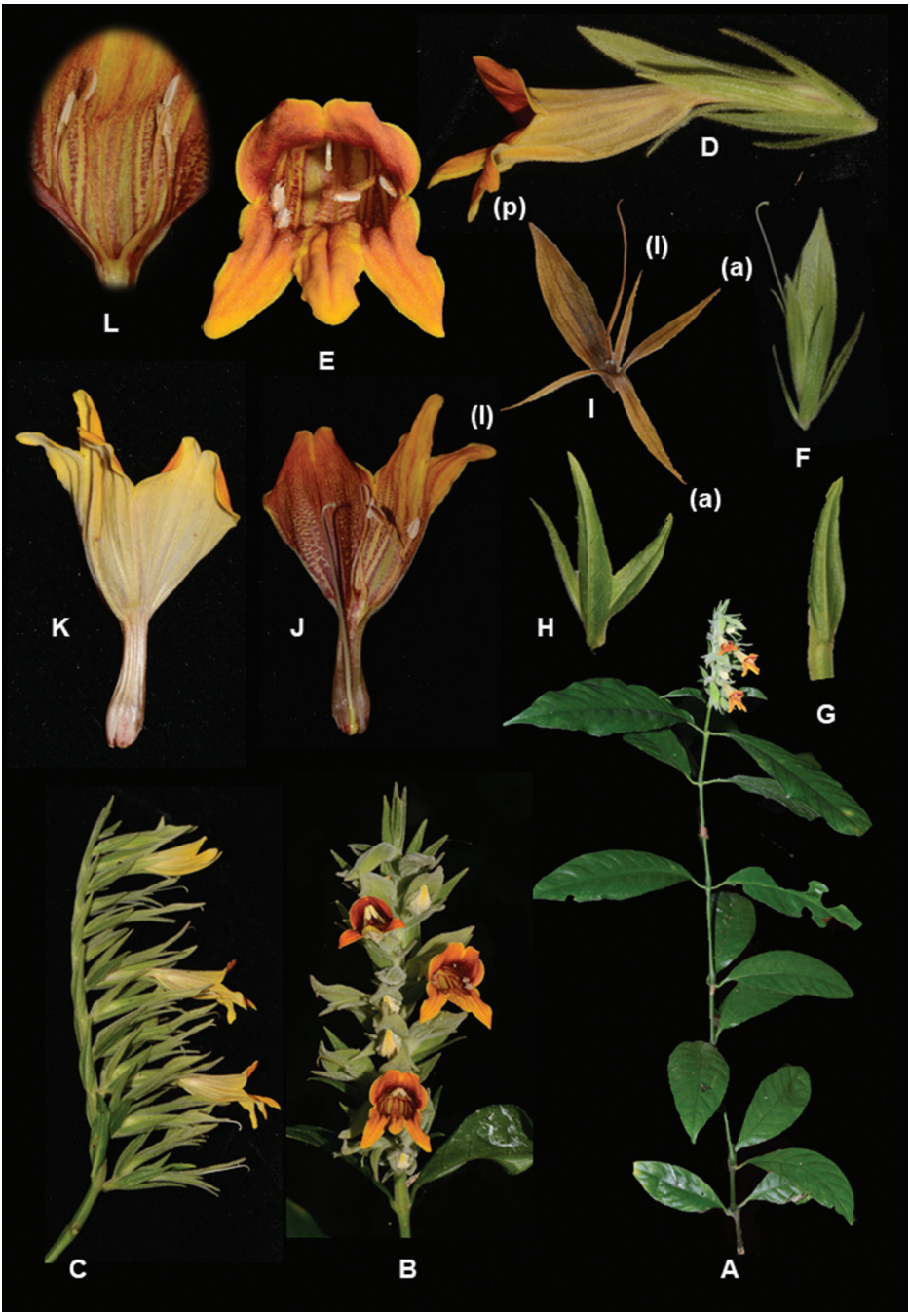


Figure 1. *Chroesthes faizaltahiriana* Siti-Munirah **A** habit of the whole plant **B** inflorescence (front view) **C** inflorescence (side view) **D** flower with bracteoles and calyx **E** perianth lobes (flower from front view) showing the anthers **F** calyx and bracteoles **G** bract **H** bracteoles **I** calyx (a) anterior, (l) lateral, (p) posterior lobes (photo of a dry specimen) **J** corolla (inner view) **K** corolla (outer view) **L** stamens. (All photos by Siti-Munirah MY).

Figure 2. *Chroesthes faizaltahiriana* Siti-Munirah with scale **A** inflorescence **B** one floral unit with calyx and bracteole **C** corolla lobes (All photos by Siti-Munirah MY).

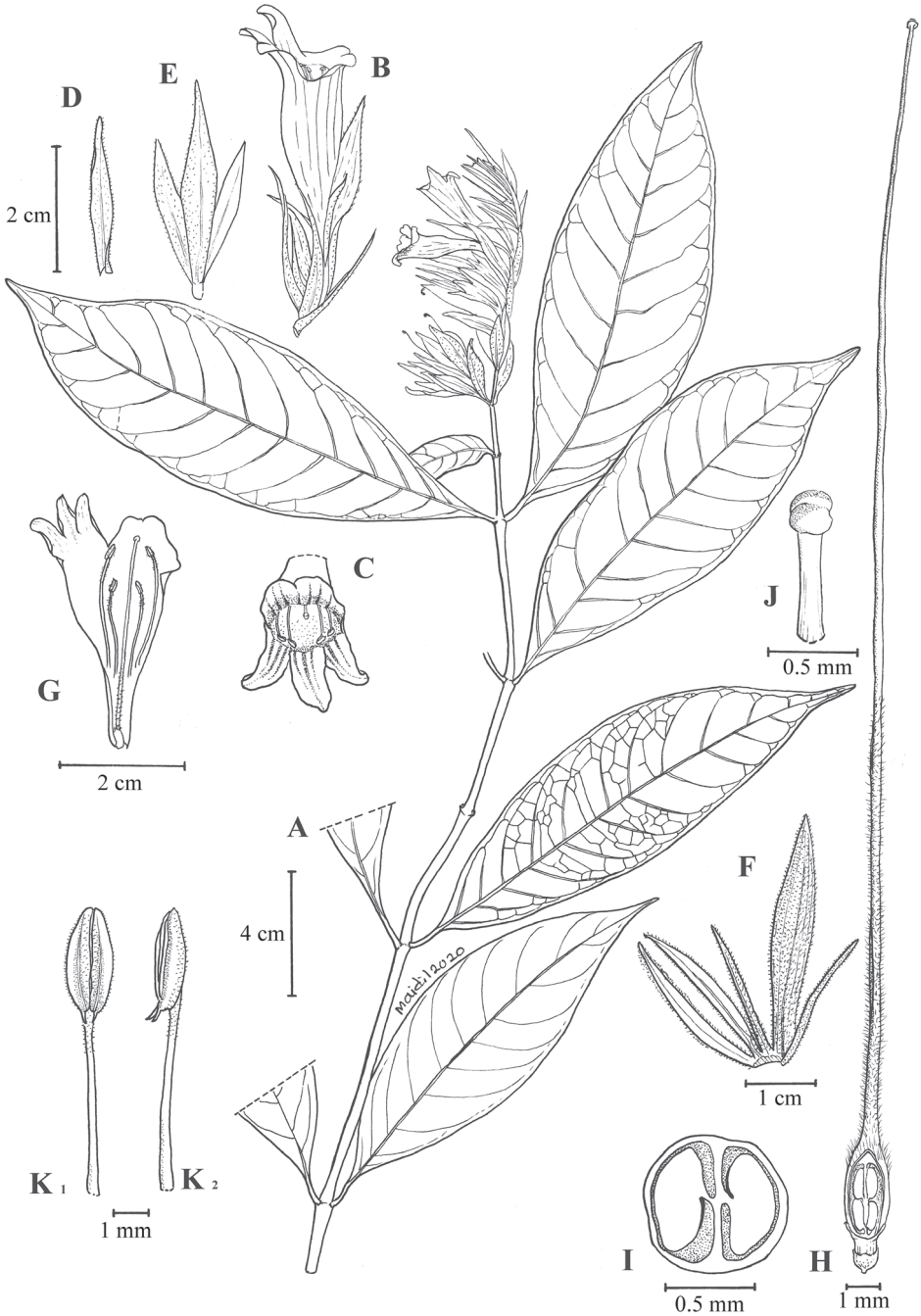


Figure 3. *Chroesthes faizaltahiriana* Siti-Munirah **A** habit **B** a flower with bracteoles and calyx front side view **C** perianth lobes (flower from front view) **D** bract **E** bracteole **F** calyx **G** corolla (inner view) showing the stamens and pistil **H** pistil **I** cross-section of the ovary **J** stigma **K1** anthers front view **K2** anthers side view. All from *FRI 91215*, drawn by Mohamad Aidil Noordin).

Figure 4. Habitat of *Chroesthes faizaltahiriana* Siti-Munirah **A** plant in its original habitat **B** new logging road beside the population **C** plant found on the bank of an old logging road (white arrow). (All photos by Siti-Munirah MY).

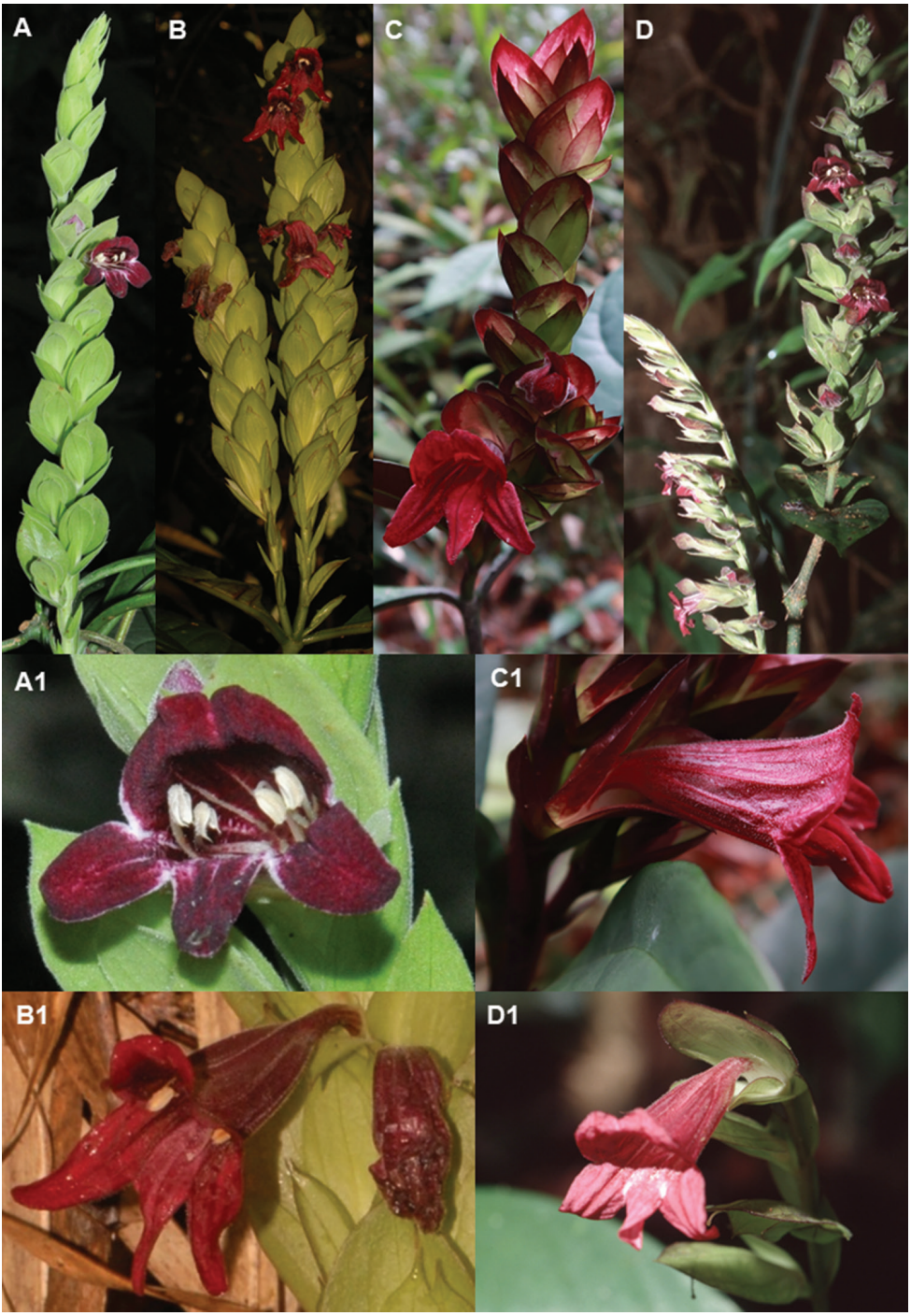


Figure 5. Inflorescence of *Chroesthes longifolia* from four localities **A, A1** Pasoh FR (Negeri Sembilan) **B, B1** Tembat FR (Terengganu) **C, C1** Nerus FR (Terengganu) **D, D1** Gn. Aais FR (Pahang). (Photos: **A, A1** Yao TL **B, B1** Siti-Munirah MY **C, C1** Imin K **D, D1** Sam YY).

And., J. Linn. Soc., Bot. 9: 524. 1867. *Type*: Myanmar, Pegu, Thaungyin, *Brandis s.n.* (holotype CAL).

Description. Shrubs 0.5–3 m tall, anisophyllous. ***Stems*** terete, slender, rarely branched, glabrous. ***Leaves*** petiole 1–2.5 cm; leaf blade elliptic to oblanceolate to lanceolate, 10–16 × 3–7 cm, both surfaces glabrous, secondary veins 6–9 on each side of mid-vein, base cuneate, margin entire or sub-sinuate, apex acuminate. ***Inflorescence*** thyrses 3–7 cm; cymes sessile, 1–3-flowered; peduncles ca.2 cm; bracts elliptic to broadly lanceolate, 3–9 × 1–3 mm, apex acute, glandular-pubescent; bracteoles narrowly elliptic to broadly lanceolate, 4–9 × 0.7–1.2 mm; pedicel 1–5 mm long. ***Calyx*** 1–1.6 cm, outside glandular-pubescent; posterior lobe lanceolate, ovate or subelliptic; anterior lobes connate to two-thirds of their length. lateral lobes linear-lanceolate. ***Corolla*** white with pink or purple spots, ca. 2.5 cm, outside pubescent; tube basal portion ca. 9 mm, throat ca. 1.5 cm; upper lip two-lobed; lower lip three-lobed. Stamens 4, included in throat; filaments 1–1.2 cm, glabrous; anther thecae 2.1–2.3 mm, pubescent at the apex and along sides, basal spur pointed; ovary apex pubescent; style ca. 2.5 cm. Capsule subellipsoid to obovoid, 1.2–1.6 cm, glabrous or only at apex pubescent, four-seeded. Seeds subcircular in outline.

Distribution. Myanmar, N Thailand, N Laos, N Vietnam, SW China (Yunnan), Malaysia. In Peninsular Malaysia, recorded from one specimen collected from the trail to Gunung Padang, Ulu Brang, Terengganu in 2010 (FRI 66129) (Map 1).

Ecology. In Peninsular Malaysia, found in a lowland dipterocarp forest at 473 m a.s.l., under a canopy near a small river. (Trail to Gunung Padang).

Conservation status. Critically Endangered B2 ab(ii). Following the IUCN Red List Categories and Criteria (IUCN 2019), this species is assessed as critically endangered at a national level in Peninsular Malaysia because it is currently known from only one specimen in one locality. It is certainly a very rare species. The forest area is lowland dipterocarp forest which was previously logged in the past and it is not a Totally Protected Area. Globally, its conservation status possibly lists it as least concern (LC), by its wide range distribution.

Specimen examined. Terengganu: Hulu Terengganu, Ulu Brang, Ummul-Nazrah et al. FRI 66129 (KEP).

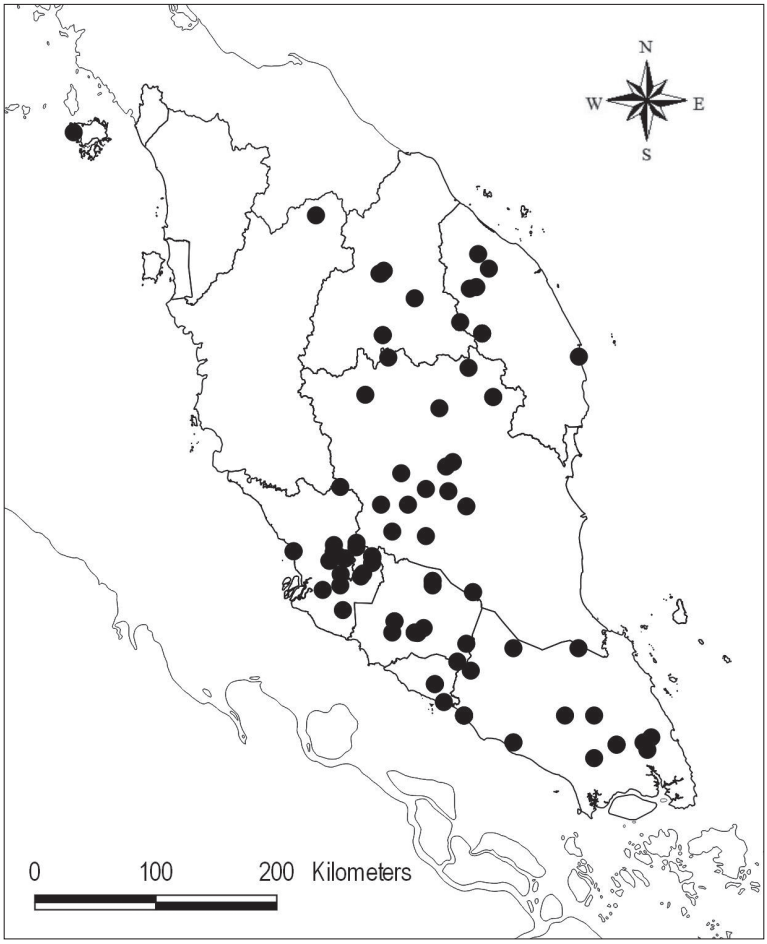
Chroesthes longifolia (Wight) B. Hansen

Figs 7, 8

Chroesthes longifolia (Wight) B. Hansen Nordic J. Bot. 3: 210. 1983. *Basionym*: *Lepidagathis longifolia* Wight, Icones Plantarum Indiae Orientalis 4 (4): 8–9, Pl: t.1564. 1850; Ridley, The Flora of the Malay Peninsula 2: 587. 1923. *Type*: Malaysia, Malacca, *Griffith s.n.* (lectotype K).

Description. Shrub 1–2.5 m high. ***Stem*** branches terete, glabrous. ***Leaves*** with petiole 0.5–2 cm long; lamina lanceolate, 16–24 × 3.4–8 cm, glabrous, lateral nerves up to

Figure 6. *Chroesthes lanceolata* (T. Anderson) B. Hansen collected from Gunung Padang, Terengganu. (FRI 66129).



Map 2. Distribution of *Chroesthes longifolia* (●) in Peninsular Malaysia.

14 pairs, base attenuate, margin entire, apex acuminate. ***Inflorescence*** terminal raceme branching, up to 25 cm long; flowers secund, peduncles up to ca. 2.5 cm; one bract at each node being sterile, the other bract subtending one flower; bracts lanceolate, 15–20 × 4.5–6 mm, base obtuse, apex acuminate, glandular-pubescent, nerves conspicuous; bracteoles lanceolate, 10 × 3 mm, as bracts; pedicel very short. ***Calyx*** 1.6–1.8 cm long, posterior and anterior lobes elliptic-lanceolate, acuminate, conspicuously nerved, one posterior lobe, elliptic-lanceolate, 18 × 8–9 mm, 3–5 nerved/conspicuously nerved, apex acuminate; two anterior lobes, elliptic-lanceolate, 16 × 5–6 mm, 1-nerved/conspicuously nerved, apex acute; two lateral lobes, linear-lanceolate, 10–12 × 0.5 mm, one-nerved; all glandular-pubescent on both sides; lateral segments linear, one-nerved, glandular-pubescent on both sides; calyx greenish to purplish on the upper part or at the apex. ***Corolla*** bilabiate, dark purple-maroon (or claret), 2–3 cm long; outer surface dark purple, dark maroon, claret, sometimes turning white, glandular-pubescent outside; inner surface, dark purple, dark maroon, claret, sometimes whitish on nerves and

base, glabrous; narrow part of tube 0.6 cm long, inflated part 1 cm; upper lip shortly emarginate, ca. 3–4 mm long, lower lip deeply trifid, ca. 5 mm long. Stamens 4, inserted at the base of the inflated part of the corolla; longer pair filament 1 cm, shorter pair filament 0.8 cm, filaments 0.8–1 cm long, glabrous, sometimes with glandular trichomes; anthers thecae ca. 1 mm long, glandular-pubescent along the back, bicalcarate at the base. Pistil whitish-green, stigma capitate; ovary glabrous; style ca. 1.4 cm long, pubescent. Capsule ca. 1.5 cm long, glabrous.

Distribution. Malaysia. Endemic to Peninsular Malaysia: Kedah, Perak, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Melaka and Johor (Map 2).

Ecology. In primary lowland forest up to upper hill dipterocarp forest at 60 to 700 m a.s.l. Sometimes also found in logged and disturbed forests.

Conservation status. Least concern (LC). This is a widespread species throughout Peninsular Malaysia. However, many old collections are from places that have already changed its habitat. Development of these areas has led to population declines. However, its occurrence still has a wide range of distribution and many are still in totally protected areas.

Specimens examined. Peninsular Malaysia. Johor: Batu Pahat: 6 November 1892, *Lake HW* s.n. (SING); *Ibid.*, November 1900, *Ridley HN* 11127 (SING); Johor Bahru: Sedenak, August 1908, *Ridley HN* 13496 (SING); Kluang: 16 November 1922, *Holttum RE* SFN9268 (SING); Kluang FR, 28 August 1950, *Sinclair J* SFN38950 (SING); Kota Tinggi: Gn. Panti, 23 June 1963, *Burkill HM* HMB 3178 (SING); Mawai, 9 September 1934, *Corner EJH* s.n. (SING); Mawai Rd., 24 January 1961, *Sinclair J* 10563 (SING); Sg. Linggiu, 26 July 1991, *Tay EP* 91-0052 (SING); Sg. Linggiu, 26 July 1991, *Tay EP* 91-52 (KEP); Muar: Bkt. Keyara, 1902, *Fox W* 11283 (SING); Gn. Ledang, June 1892, *Ridley HN* s.n. (SING); *Ibid.*, Muar, April 1901, *Curtis C* s.n. (SING); Segamat: Sg. Juasseh, 28 June 1970, *Samsuri A* SA304 (SING); **Kedah:** Langkawi, Gn. Machinchang FR, 17 March 1969, *Chan YC* FRI11209 (SING); **Kelantan:** Gua Musang, 14 July 1935, *Henderson MR* SFN29657 (KEP,SING); Gua Musang: Relai FR, 1 September 1992, *Hamid* H2 (KEP); Kuala Krai: Gn. Stong, 5 March 1924, *Mhd Nur* SFN12180 (SING); Stong FR, 31 March 2009, *Rosdi M* FRI66253 (KEP SING); Stong Tengah FR, 6 February 2007, *Chew MY* FRI53481 (KEP); Kuala Lumpur, February 1890, *Curtis C* 2362 (SING); **Melaka:** Jasin: Air Panas, 5 September 1886, *Watchman under Derry* 667 (SING); Air Panas, November 1893, *Goodenough JS* 1690 (SING); Chabau, 22 September 1885, *Alvins MV* 2256 (SING); Merlimau, June 1889, *Derry R* 221 (SING); **Negeri Sembilan:** Jelebu: Pasoh FR, 3 June 1987, *LaFrankie JV* LJV2276 (KEP); *Ibid.*, 4 July 1982 *Kiew R* RK1186 (KEP); *Ibid.*, 30 August 2010, *Yao TL* FRI65469 (KEP); Kuala Pilah: Angsi FR, Gn. Angsi, 10 December 1930, *Syed A* FMS23764 (KEP, SING); Senaling, 24 June 1930, *Corner EJH* s.n. (SING); Senaling Inas,18 November 1929, *Symington CF* FMS 21356 (KEP, SING); Senaling Inas FR, 28 November 1922, *Holttum RE* 9788 (SING); Rembau: Perhentian Tinggi, December 1898, *Ridley HN* s.n. (SING); **Pahang:** Bentong: Clough FR, Cpt. 37, 16 July 1958, *Kochummen KM* KEP78709 (KEP); Bentung, Karak FR, 14 July 1924, Best, GA, SFN13899 (SING); Jerantut: Gn. Aais FR, 5 July 2004, *Sam YY* FRI49062 (KEP); *Ibid.*, 6 July 2004, *Chua LSL*

Figure 7. *Chroesthes longifolia* (Wight) B. Hansen. The drawing from the original protologue. Source: Icon. Pl. Ind. Orient. 4 (1850) t. 1564.

FRI46691 (KEP); Kota Gelanggi, August 1891, *Ridley HN* 2174 (SING); P. Tawar, July 1891, *Ridley HN* s.n. (SING); Taman Negara Gn. Tahan, 31 July 1996, *Kiew R* RK3978 (KEP); Taman Negara, Sg. Riul, 12 July 1970, *Everett B* FRI14436 (KEP);

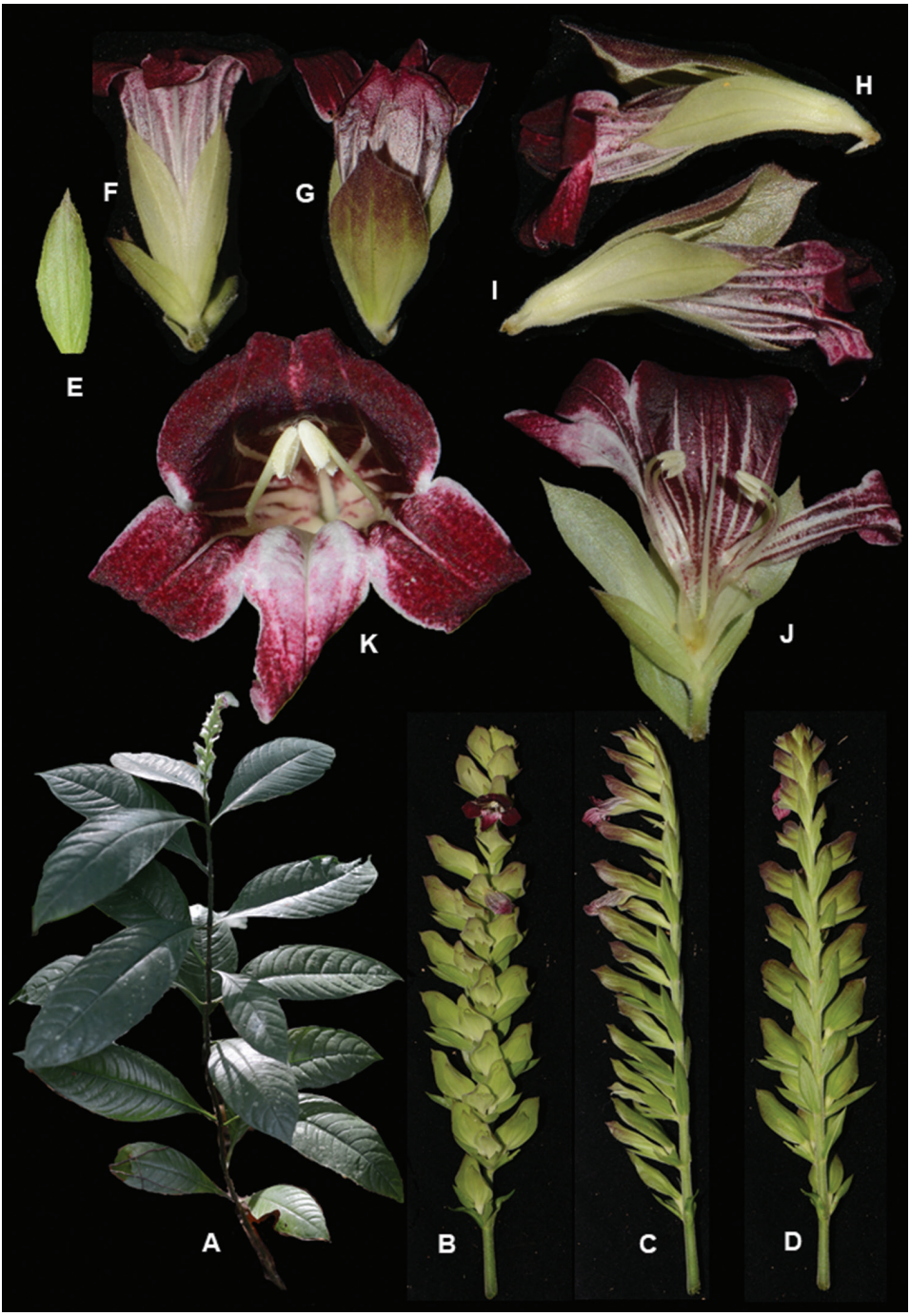


Figure 8. *Chroesthes longifolia* (Wight) B. Hansen, collected from Taman Negara, Pahang (Kuala Keniam) **A** habit **B–D** inflorescence **B** front view **C** side view **D** back view **E** bract **F–I** flower with bracteoles and calyx **F** from the top view **G** lower view **H–I** side view **J** corolla (inner view) showing the stamens and pistil **K** corolla lips (flower from front view) showing anthers (All photos by Siti-Munirah MY).

Taman Negara Ulu Sat Tg. Petir, 12 July 1970, *Whitmore TC* FRI15267 (KEP); Taman Negara Kuala Keniam, 7 September 2020, *Siti Munirah MY* FRI94881 (KEP); Lipis: Chegar Perah,14 October 1927, *Henderson MR* SFN19364 (SING); Maran: Jengka FR, 10 April 2001, *Sam YY* FRI 46518 (KEP); Temerloh: Gn. Benom, 14 June 1968, *Teo LE* 2714 (SING); Temerluh: Fort Iskandar, 2 March 1950, *Woods MC* KL1720 (SING); *Ibid*., 2 March 1959, *Woods MC* KL1720 (KEP); Gn. Senyum, 30 July 1928, *Henderson MR* s.n. (SING); Kemasul FR, 16 September 2006, *Hamid* FMS10598 (KEP); Krau WR, 10 November 1999, *Damahuri S* FRI45314 (KEP); Krau WR 12 July 2007, *Mohd Hairul MA* FRI58921 (KEP); **Perak:** Hulu Perak: Belum FR, 30 December 1993, *Davison GWH* UPM 6077 (KEP); **Selangor:** Gombak: Bkt. Lagong FR, 5 May 1976, *Chan YC* FRI23956 (KEP, SING); *Ibid*., 11 November 1959, *Kochummen KM* KEP94047 (KEP, SING); Kanching FR, 17 September 1925, *Strugnell EJ* FMS10508 (KEP); Kepong, 27 May 1970, *Teo LE* 2862 (SING); Ulu Gombak, 4 July 1918, *Sanger-Davies AE* FMS2376 (KEP, SING); Hulu Langat: Bkt. Batu Balai, 1 August 1959, *Gadoh U* KL1630 (KEP); Bkt. Enggang, 3 April 1930, *Symington CF* FMS 24134 (SING); Bkt. Tangkol, 1 October 1959, *Gadoh U* KL1810 (KEP); Bkt. Tangkol, 25 July 1959 *Gadoh U* KL1621 (KEP); Ulu Langat, 7 June 1979, *Rajoo* SA237 (KEP); Hulu Selangor: Gading Forest Reserve, 19 July 1969, *Chan YC* FRI11235 (KEP); Klang, Klang Water Catchment Forest, 12 March 1922, *Burkill IH* SFN6849 (SING); Petaling: Air Hitam Forest Reserve, 12 September 1985, *Kiew R* RK2065 (KEP); Petaling Rd., 21 June 1889, *Ridley HN* s.n. (SING); Sungai Buloh Forest Reserve, 7 October 1926, *Strugnell EJ* 12495 (KEP); Sungai Buloh Forest Reserve, 13 August 1923, *Hamid* FMS 8888 (KEP); Labu River, *Ridley HN* s.n. (SING); Sungai Buloh, 12 December 1923, *Mhd Nur* SFN11886 (SING); Sungai Buloh, 23 November 1956, *Burkill HM* HMB (SING); **Terengganu:** Dungun: Bukit Bauk, 25 January 1994, *Kiew R* RK3793 (KEP); Hulu Terengganu: Sg. Petuang, 26 March 1974, *Ng FSP* FRI22026 (KEP); Tasik Kenyir, 3 August 2007, *Kamarul Hisham M* FRI52138 (KEP, SING); Tasik Kenyir, 3 August 2007 *Julius A* FRI56144 (KEP); Tembat Forest Rerserve, 6 April 2009, *Rosdi M* FRI66281 (KEP); Tembat Forest Reserve, 3 April 2009, *Siti Munirah MY* FRI67889 (KEP); Setiu: Ulu Setiu Forest Reserve, 7 March 2002, *Saw LG* FRI44390 (KEP); Nerus Forest Reserve, Gunung Sarut, 24 June 2019, *Imin K* FRI 94046 (KEP).

Notes. Ridley (1923) mentioned *Chroesthes macrantha* as present in Peninsular Malaysia based on Wray 3385 from Perak and that it is similar to *C. longifolia*. Hansen (1983) suggested that *C. macrantha* was probably a form of *C. longifolia*, but he had not observed Wray 3385, so was not able to clarify its status. Efforts to search for this specimen have also been unsuccessful. To date, the status of this taxon is uncertain. Besides, this name also appears not to have been validly published.

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Achnanthidium tinea sp. nov. – a new monoraphid diatom (Bacillariophyceae) species, described on the basis of molecular and morphological approaches

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Abstract

A new monoraphid diatom species *Achnanthidium tinea* Tseplik, Kulikovskiy, Kociolek & Maltsev, **sp. nov.** is described from Indonesia. The species is described on the basis of molecular and morphological analyses. According to molecular data the new species belongs to the clade that includes strains of *Achnanthidium minutissimum*, *Achnanthidium saprophilum* and *Achnanthidium digitatum*. Morphologically, the new species differs quite significantly from other species of the same genus because of linear-elliptic valves with almost parallel sides and strongly radiate striae and a butterfly-shaped fascia on the raphe valve. The morphology and phylogeny of the new species are discussed, and thoughts on the current state of the taxonomy of the genus *Achnanthidium* are expressed. Our work shows the importance of using molecular data in diatom systematics and also demonstrates the need to investigate rarely studied regions of our planet.

Keywords

Achnanthidium, Bacillariophyceae, Indonesia, molecular investigations, new species

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Introduction

The genus *Achnanthidium* Kützing was first described by Kützing (1844) and for a long time it was considered a subgenus of *Achnanthes* Bory s.l. (Cleve 1895). Its status as a separate genus was restored by Round et al. (1990) and afterwards Round and Bukhtiyarova (1996) proposed a new diagnosis which significantly narrowed the genus boundaries. The improved diagnosis included such features as small linear-lanceolate to elliptic-lanceolate valves, radiate uniseriate striae, external distal raphe ends that are straight or curved to one side and sternum that widens in the center of the valve. Currently, two morphological groups are distinguished within the genus: the *Achnanthidium minutissimum* (Kützing) Czarnecki species complex has straight external distal raphe ends, while the *Achnanthidium pyrenaicum* (Hustedt) Kobayashi species complex has external distal raphe ends that are distinctly curved in one direction (Kobayashi 1997). A third group, previously recognized for *A. exiguum* (Grunow) Czarnecki and its relatives (Karthick et al. 2017), has been established as a separate genus, *Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek (Kulikovskiy et al. 2020c).

Recent studies include descriptions of many new species belonging to this genus (Rimet et al. 2010; Kulikovskiy et al. 2011; Novais et al. 2011; Krahn et al. 2018; Yu et al. 2019, etc.), as well as studies of type materials of known species using light and scanning electron microscopy (Hlúbiková et al. 2011; Van de Vijver et al. 2011). The studies of type materials primarily concern large species complexes and their main aim is to define separate species more clearly.

Taxonomy within the genus *Achnanthidium* is a rather complicated issue. Species boundaries are often not clear enough due to the fact that morphological features alone may not be sufficient to unequivocally identify species, and because values of quantitative features often overlap in similar species, further complicating their separation (Kulikovskiy et al. 2016a; 2020b; Jahn et al. 2017; Tseplik et al. 2020). These problems require extensive molecular research, both while describing new species and while studying species already known to science. At present, the *Achnanthidium* genus includes about two hundred species (Kociolek et al. 2020b). Molecular data is available for very few taxa, and correct identification of the representatives of this genus based only on morphological features is often difficult (Kulikovskiy et al. 2014, 2016a; Andreeva et al. 2016; Maltsev and Kulikovskiy 2017; Maltsev et al. 2018, 2019).

Achnanthidium species are widely distributed in various freshwater habitats around the world and can be important indicators of environmental conditions (Ponader and Potapova 2007). However, many regions remain poorly studied and the probability of finding new species is quite high, like in Lake Baikal (Kulikovskiy et al. 2011, 2012, 2013, 2015, 2016b, c, 2020b) or Southeast Asia (Kulikovskiy et al. 2018; Liu et al. 2018; Glushchenko et al. 2016, 2017, 2018, 2019, 2020; Kezlya et al. 2020). Hustedt (1937a–c, 1938a, b; 1939; 1942) first documented freshwater diatoms from Indonesia, and of the nearly 800 taxa reported, 315 of them (ca. over 40%) were new to science. Still, Indonesia is a country not only with a high level of

endemism in many groups of living organisms, but also taxa still to be discovered or reinterpreted (Hamsher et al. 2014; Kapustin et al. 2017, 2019, 2020; Kociolek et al. 2018; Kulikovskiy et al. 2019b, 2020a; Rybak et al. 2019). Of the 39 monoraphid diatoms reported in the genera *Cocconeis* Ehrenberg and *Achnanthes* Bory by Hustedt (1937a–c, 1938a, b; 1939) alone, 10 (26%) were described as new. There have been no modern taxonomic studies of *Achnanthidium* in Indonesia. The purpose of the present report is to provide light and scanning electron microscopic observations, as well as DNA sequence data, in support of the description of a new *Achnanthidium* species from Sulawesi, Indonesia.

Materials and methods

Sample collection

The sample used in the present report was collected from Indonesia by I.I. Ivanov on 22.09.2010, and designated I227 from the Sulawesi Island, Temple Lake, periphyton, scraping from macrophytes, t=26.5 °C, pH=8.7, conductivity=277 µS cm^{-1}, 04°06.923'N, 119°58.613'E.

Culturing

Monoclonal strains were established by micropipetting single cells under an inverted microscope. Non-axenic unialgal cultures were maintained in WS liquid medium (Andersen 2005) for one month. The strain investigated here was designated Ind296.

Preparation of slides and microscopic observation

The sample and the monoclonal culture were treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 hours. Afterwards, the samples were boiled in concentrated hydrogen peroxide (≈37%) to dissolve organic matter. After decanting and refilling up to 100 ml with deionized water, the suspension was spread on to coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax (refraction index =1.73). Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective (×100, n.a. 1.4, differential interference contrast) and Axiocam Erc 5s camera (Zeiss). Valve ultrastructure was examined using a JSM-6510LV scanning electron microscope (IBIW, Institute for Biology of Inland Waters RAS, Borok, Russia).

For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm Au in an Eiko IB 3. Sample and slides are deposited in the collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia. The type slide was designated 04133.

All images acquired from the slides were processed using Adobe Photoshop CC (19.0). Length and breadth of the valves were measured on the LM images, and striae and areolae density was measured on the SEM images. The numbers given in brackets in the description are means with standard deviations.

Molecular investigations

Total DNA of monoclonal cultures was extracted using InstaGene Matrix according to the manufacturer's protocol. A fragment of 18S rDNA (382 bp, including V4 domain) was amplified using primers D512for and D978rev following Zimmermann et al. (2011). Amplification of the 18S rDNA fragment was carried out using the premade mix ScreenMix (Evrogen, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for 18S rDNA fragment were: an initial denaturation of 5 min at 95 °C, followed by 35 cycles at 94 °C for denaturation (30 s), 52 °C for annealing (30 s) and 72 °C for extension (50 s), and a final extension of 10 min at 72 °C.

The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5%), colored with SYBR Safe (Life Technologies, United States). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affimetrix, USA) according to the manufacturer's protocol. 18S rDNA fragment was decoded from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, USA), followed by electrophoresis using a Genetic Analyzer 3500 sequencer (Applied Biosystems).

Editing and assembling of the consensus sequences were carried out by comparing the direct and reverse chromatograms using the Ridom TraceEdit program (ver. 1.1.0) and Mega7 (Kumar et al. 2016). Newly determined sequence and DNA fragments from 151 other diatoms, which were downloaded from GenBank (taxa and Accession Numbers are given in the Suppl. material 1), were included in the alignments. Three centric diatom species were chosen as the outgroups. The nucleotide sequences of the 18S rDNA gene were aligned separately using the Mafft v7 software and the E-INS-i model (Katoh and Toh 2010). The resulting alignment had lengths of 404 characters.

The dataset was analyzed using the Bayesian inference (BI) method implemented in Beast ver. 1.10.1. (Drummond and Rambaut 2007) to construct phylogeny. For each of the alignment partitions, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 (Darriba et al. 2012). This BIC-based model selection procedure selected TIM1+I+G model, shape parameter α = 0.4210 and a proportion of invariable sites (pinvar) = 0.3400. We used the GTR model of nucleotide substitution instead of TIM1, given that it was the best matching model available for the Bayesian inference method. A Yule process tree prior was used as a speciation model. The analysis ran for 15 million generations with chain sampling every 1000 generations. The parameters-estimated convergence, effective sample size (ESS) and burn-in period were checked using the software Tracer ver. 1.7.1. (Drummond and Rambaut 2007). The initial 25% of the trees were removed, the rest retained to reconstruct a final phylogeny. The phylogenetic tree and posterior probabilities of its

branching were obtained on the basis of the remaining trees, having stable estimates of the parameter models of nucleotide substitutions and likelihood. Maximum Likelihood (ML) analysis was performed using the program RAxML (Stamatakis et al. 2008). The nonparametric bootstrap analysis with 1000 replicates was used. The statistical support values were visualized in FigTree ver. 1.4.4 and Adobe Photoshop CC (19.0).

Results

***Achnanthidium tinea* Tseplik, Kulikovskiy, Kociolek & Maltsev, sp. nov.**
Figs 1–3

Holotype. Slide no 04133 in collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia, represented here by Fig. 1E.

Reference strain. Sample Ind296, isolated in sample I227.

Type locality. Indonesia. Sulawesi Island, Temple Lake, periphyton, 04°06.923'N, 119°58.613'E, 5 m. elev., *leg*. I.I. Ivanov, *22.09.2010*.

Description. ***LM*** (Fig. 1A–R). Frustules rectangular in girdle view, raphe valve very slightly concave. Valves linear-elliptic with gradually narrowing ends. Length 14.7–17.5 µm (16.2 ± 0.9; n=17), breadth 4.0–5.0 µm (4.5 ± 0.3; n=17). The raphe valve possesses a straight filiform raphe, which lies in a narrow linear axial area. The central area is represented by a symmetrical butterfly-shaped fascia that reaches the valve margins on both sides. Striae on raphe valve strongly radiate, curved. The rapheless valve possesses a narrow lanceolate axial area. Central area absent, on some valves somewhat shorter striae in the center are present. Striae parallel in the center on the valve, slightly radiate near the valve ends.

SEM, external view (Figs 2A–C, 3A). Central raphe ends are straight and drop-shaped (Fig. 2A, white arrows). Distal raphe ends curve strongly to one side of the valve (Fig. 2A, white arrowheads). Striae on the raphe valve 30–35 in 10 µm (32.5 ± 2.5 in 10 µm; n=4). Areolae elliptical or rounded in shape, approximately 40 in 10 µm. Striae on the rapheless valve 30–33 in 10 µm (31 ± 1.2 in 10 µm; n=4). Areolae small, also rounded or elliptical, approximately 50 in 10 µm. Shorter striae in the center are clearly visible in SEM; this often occurs only on one side of the valve (Fig. 3A, white arrow).

SEM, internal view (Figs 2D, 3B–D). Central raphe ends are simple and straight (Fig. 2D, white arrows). Distal raphe ends terminate in helictoglossae (Fig. 2D, white arrowheads). Shorter striae in the center are clearly visible in SEM, this often occurs only on one side of the valve (Fig. 3B, C, white arrows).

Etymology. Epithet refers to the butterfly-like shape of the fascia on the raphe valve of the new species; *tinea* meaning moth in Latin.

Distribution. As yet known only from type locality.

Molecular data (Fig. 4)

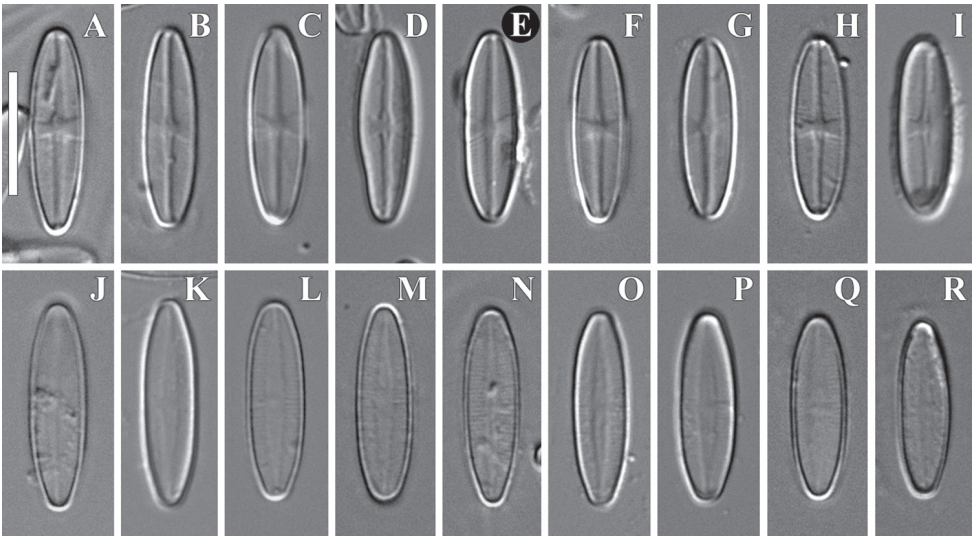

Figure 1. A–R *Achnanthidium tinea* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. LM, DIC, size diminution series. Slide no 04133. Holotype (**E**). Scale bar: 10 µm.

Our new species belongs to the large clade with monoraphid diatoms and sister clade with gomphocymbelloid diatoms. Strain *A. tinea* sp. nov. combined (BI 100; ML 100) with two strains of *A. minutissimum* AW2 and Ashort2 and *A. saprophilum* D06-036. 15 other strains of *A. minutissimum* combined to form a sister branch together with three strains of *A. digitatum* and *A. gladius* Tseplik et al. Other monoraphid taxa from genera *Pauliella*, *Psammothidium*, *Planothidium*, *Cocconeis*, *Lemnicola* and *Gogorevia* spp. formed sister clades to the branch containing these *Achnanthidium* taxa in the molecular tree.

Discussion

In terms of the data from both morphology and molecular sequence data, the new species *A. tinea* sp. nov. belongs to the genus *Achnanthidium*. Morphological features present in *A. tinea* and characteristic for this genus include: linear-elliptical valve shape, sternum that widens near the center of the valve and external distal raphe endings that are curved to one side. The last feature allows us to attribute the new species to the *A. pyrenaicum* species complex.

We compared *A. tinea* sp. nov. with other representatives of the genus *Achnanthidium*. *A. tinea* sp. nov. possesses a rather unusual combination of features for the genus: linear-elliptic valves with parallel sides and narrowed ends and a pronounced butterfly-shaped fascia on the raphe valve. After carrying out the morphological comparison, we identified several species most similar in morphology to *A. tinea* sp. nov.

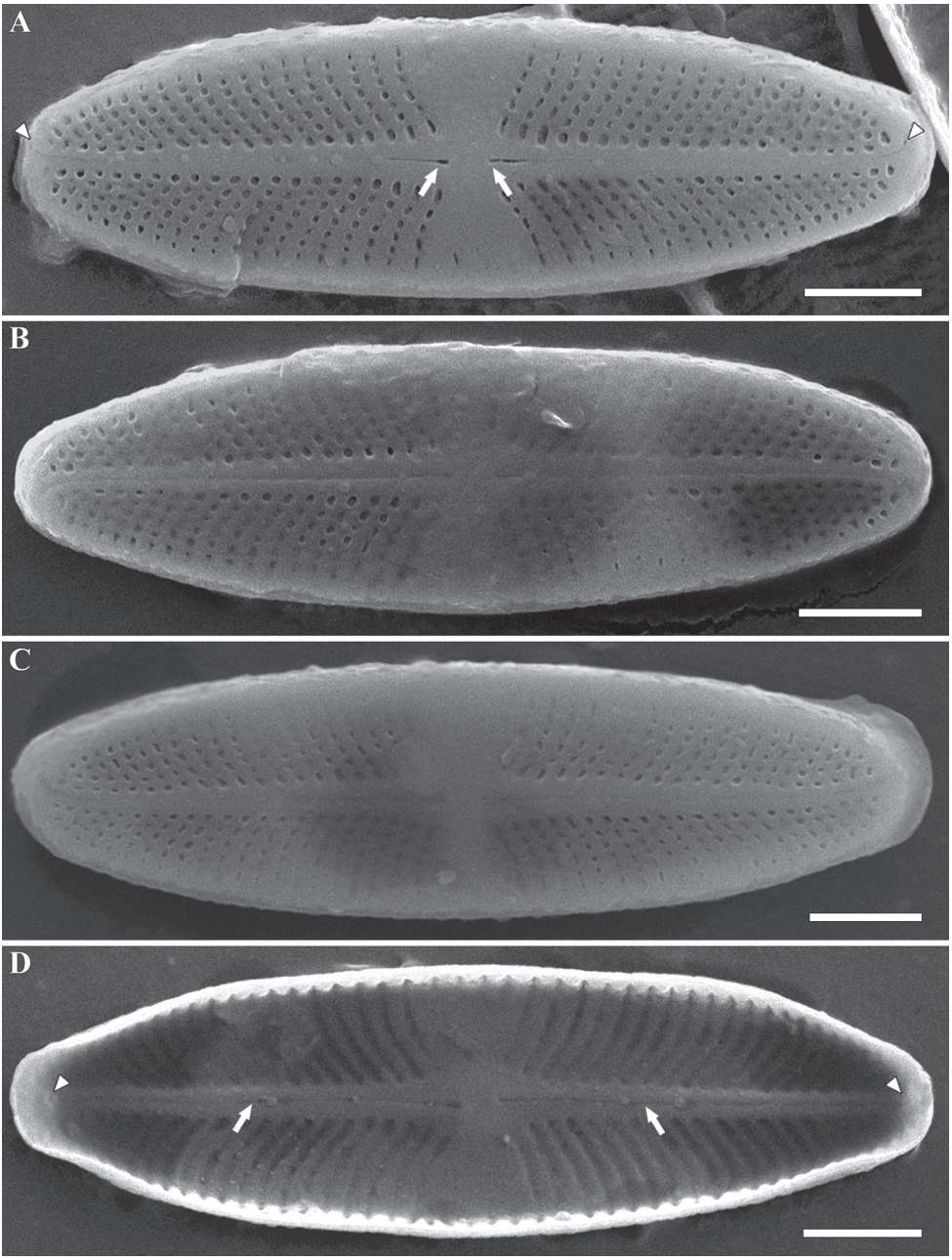

Figure 2. A–D *Achnanthidium tinea* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. SEM. Sample no 04133. Raphe valves **A–C** external views **D** internal view **A** white arrows shows the central raphe ends. White arrowheads shows the distal raphe ends **D** white arrows shows the central raphe ends. White arrowheads shows the helictoglossae. Scale bars: 2 µm.

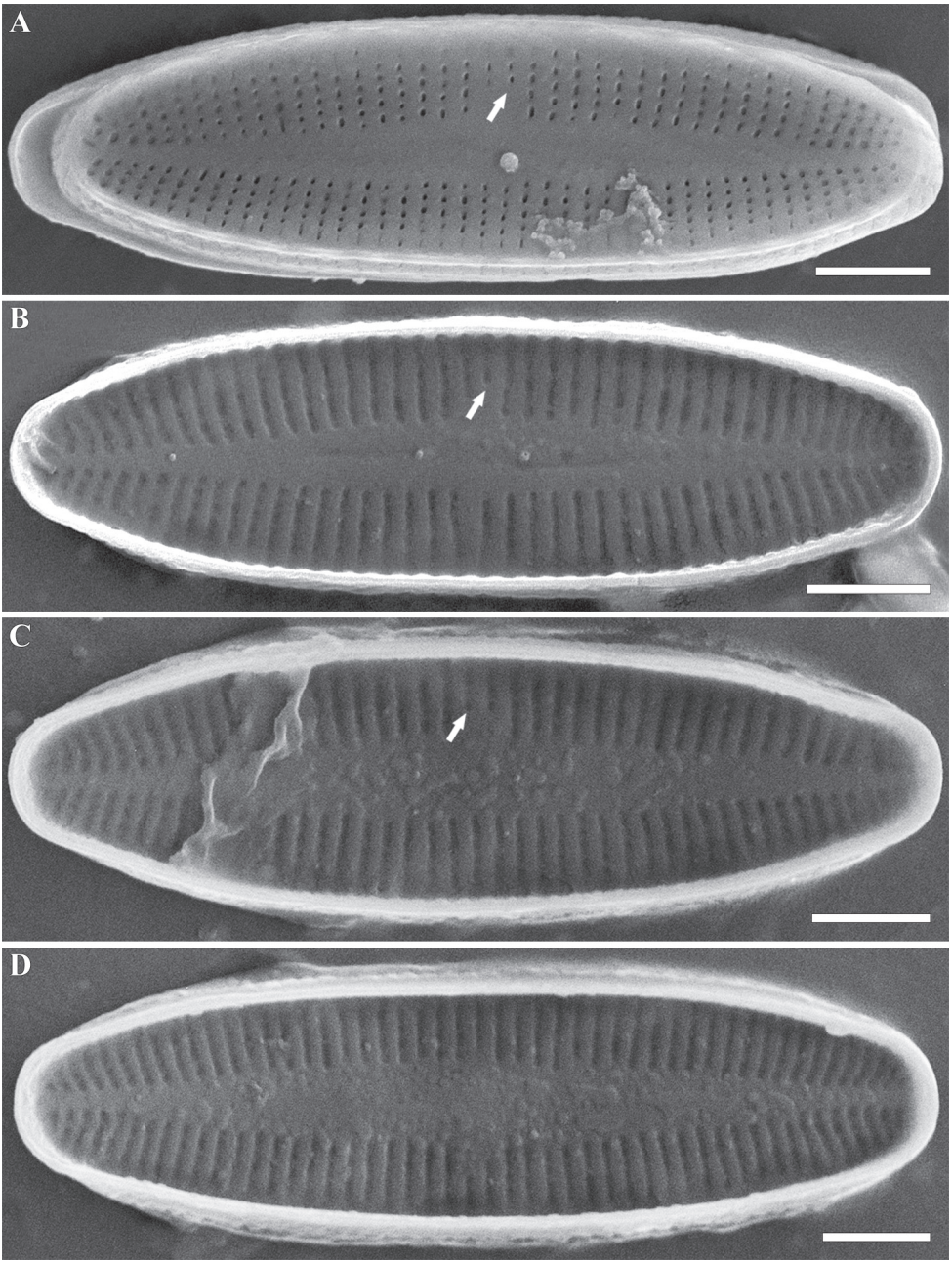

Figure 3. A–D *Achnanthidium tinea* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. SEM. Sample no 04133. Rapheless valves. **A** external view **B–D** internal views **A–C** white arrows shows the short striae. Scale bars: 2 μm.

Figure 4. Bayesian tree of *Achnanthidium tinea* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. (indicated in bold) constructed from a concatenated alignment of 152 partial 18S rDNA sequences of 404 characters. Values above the horizontal lines are bootstrap support from RAxML analyses (<50 are not shown); values below the horizontal lines and to the right of the slash mark are Bayesian posterior probabilities (<90 are not shown). All sequences have strain numbers (if available). Species of centric diatoms were used as an outgroup. * is 100% statistical support.

In terms of valve shape, the species most similar to the new species is *Achnanthidium deflexum* (Reimer) Kingston (Potapova and Ponader 2004), but it can be quite easily distinguished from *A. tinea* sp. nov. by the absence of the central area and by parallel and more widely spaced striae on the raphe valve (20–22 in 10 µm in *A. deflexum*, 30–35 in 10 µm in *A. tinea* sp. nov.). Another species similar to *A. tinea* sp. nov. in terms of valve shape is *Achnanthidium dolomiticum* Cantonati & Lange-Bertalot (Cantonati and Lange-Bertalot 2006). It differs from the new species by having more widely rounded valve ends and the central area represented by a narrow rectangular fascia. Under the scanning electron microscope, it is also possible to observe straight external distal raphe ends in *A. dolomiticum*, while in *A. tinea* sp. nov. they are curved. *Achnanthidium delmontii* Pérès, Le Cohu & Barthès (Pérès et al. 2012) also resembles *A. tinea* in terms of valve shape and, like *A. tinea* sp. nov., belongs to the *A. pyrenaicum* species complex. But *A. delmontii* has a narrower rectangular fascia and more widely-spaced, weakly radiate striae on both valves (raphe valves: 30–35 in 10 µm in *A. tinea* sp. nov., 20–26 in 10 µm in *A. delmontii*; rapheless valves: 30–33 in 10 µm in *A. tinea* sp. nov., 18–22 in 10 µm in *A. delmontii*). Two other species that somewhat resemble *A. tinea* sp. nov. were studied by Morales et al. (2011), namely *Achnanthidium cadimae* Morales, Fernández & Ector and *Achnanthidium peruvianum* Morales & Ector. *A. cadimae* can be differentiated from our new species by its narrowly elliptic valves that are smaller than *A. tinea* sp. nov. (10–13 µm versus 14.7–17.5 µm), its asymmetrical fascia, and the axial area on its rapheless valve that is very narrow and almost linear versus a somewhat broader lanceolate one in *A. tinea* sp. nov. *A. peruvianum* also has a smaller fascia and a narrower axial area on its rapheless valve than *A. tinea* sp. nov., and its valve ends are more broadly rounded. A final species that is similar in valve shape is *Achnanthes tropica* Hustedt, illustrated with line drawings by Hustedt (1937b, Plate XIII, figs 28–32) and described from Java (Hustedt 1937b, p. 200). This species also has fine striae (reported as "zart" by Hustedt and described as 26–30/10 µm), but coarser than in *Achnanthidium tinea*. While no SEM work has yet been done on Hustedt's species, the light microscope images of this taxon published by Simonsen (1987, plate 326, figs 20–28) suggest this species might be better placed in the genus *Nupela* Vyverman & Compére.

In general, due to the above-mentioned unusual combination of features possessed by the new species, its similarity with other representatives of the genus is mostly quite superficial, and *A. tinea* sp. nov. is easily distinguishable from other species even in light microscopy.

On the phylogenetic tree, the strain of *A. tinea* sp. nov. forms a separate branch within a clade that includes other species of *Achnanthidium* and other monoraphid diatoms (e.g. *Gogorevia*, *Psammothidium*, *Planothidium*, *Pauliella*, *Cocconeis*, *Lemnicola*) and the Cymbellales. The group was referred to as the Monoplacatae by Mereschkowsky (1902) and has been recovered in previous phylogenetic analyses (e.g. Thomas et al. 2016). Within this large group, *A. tinea* belongs to a large clade comprised of strains of several *Achnanthidium* species, including *A. minutissimum*, *Achnanthidium digitatum* Pinseel, Vanormelingen, Hamilton & Van de Vijver, *Achnanthidium saprophilum* (Kobayashi & Mayama) Round & Bukhtiyarova and *Achnanthidium gladius* Tseplik,

Kulikovskiy, Glushchenko & Genkal. As discussed above, morphologically, none of these species is similar to *A. tinea* sp. nov. The clade including this species is sister to another clade that comprises strains of other monoraphid genera, namely *Pauliella* Round & Basson, *Psammothidium* Bukhtiyarova & Round and *Cocconeis* Ehrenberg.

Our understanding of the phylogenetic relationships of the monoraphid diatoms continues to yield fascinating new insights at the levels of genus and species (e.g. Round and Basson 1997; Moser et al. 1998; Witkowski et al. 2000; Krammer and Lange-Bertalot 2004; Riaux-Gobin et al. 2012), and a richer understanding of the evolution of the monoraphid condition (Kociolek et al. 2019). The description of new species within genera such as *Achnanthidium* is quite an important area of research for the taxonomy of this genus, but also shows how the genus might be understood for ecological analyses (Potapova and Hamilton 2007). An integrated molecular and morphological approach to species-level identification and understanding phylogenetic relationships of those taxa will provide a more complete picture of the taxonomy of the genus, allow for the construction of a natural classification, and facilitate further research.

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Supplementary material 1

Taxa and DNA sequence data used in phylogenetic analysis

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Data type: species data

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Phylogeny and historical biogeography analysis support Caucasian and Mediterranean centres of origin of key holoparasitic Orobancheae (Orobanchaceae) lineages

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Abstract

The extensive diversity of the tribe Orobancheae, the most species-rich lineage of holoparasitic Orobanchaceae, is concentrated in the Caucasus and Mediterranean regions of the Old World. This extant diversity has inspired hypotheses that these regions are also centres of origin of its key lineages, however the ability to test hypotheses has been limited by a lack of sampling and phylogenetic information about the species, especially in the Caucasus region. First, we assessed the phylogenetic relationships of several poorly known, problematic, or newly described species and host-races of four genera of Orobancheae occurring in the Caucasus region–*Cistanche*, *Phelypaea*, *Phelipanche* and *Orobanche*–using nuclear ribosomal (ITS) and plastid (*trnL–trnF*) sequence data. Then we applied a probablistic dispersal-extinction-cladogenesis model of historical biogeography across a more inclusive clade of holoparasites, to explicitly test hypotheses of Orobancheae diversification and historical biogeography shifts. In sum, we sampled 548 sequences (including 196 newly generated) from 13 genera, 140 species, and 175 taxa across 44 countries.

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We find that the Western Asia (particularly the Caucasus) and the Mediterranean are the centre of origin for large clades of holoparasitic Orobancheae within the last 6 million years. In the Caucasus, the centres of diversity are composed both of long-branch taxa and shallow, recently diversified clades, while Orobancheae diversity in the Mediterranean appears to represent mainly recent diversification.

Keywords

Biodiversity hotspot, chronogram, *Cistanche*, divergence time, historical biogeography, *Orobanche*, *Phelipanche*, *Phelypaea*

Introduction

The tribe Orobancheae is the oldest and most species-rich of the three lineages of holoparasites comprising the cosmopolitan family Orobanchaceae, with a crown age dating to the mid-Miocene (McNeal et al. 2013; Schneider and Moore 2017). In its current circumscription the Orobanchaceae includes the holoparasites that have always comprised Orobanchaceae s. str., and hemiparasites traditionally included in Scrophulariaceae (Olmstead et al. 2001; McNeal et al. 2013). In sum, this is the largest parasitic plant family with 102 genera and over 2,100 species (Nickrent 2020) which together with its variety of trophic modes makes it a valuable model for studying the evolution and physiology of parasitism (Westwood et al. 2010).

The Mediterranean Basin and Caucasus region of western Asia are centres of extant diversity for the two most diverse genera in the Orobancheae, *Orobanche* L. and *Phelipanche* Pomel (ca. 150 and 60 described species, respectively) (Piwowarczyk et al. 2019), and are more generally recognised as one of the world's hotspots of biodiversity (Mittermeier et al. 2005). Recent taxonomic and field studies in the Caucasus have helped clarify the nomenclature, taxonomy, and distribution of taxa from four genera (*Orobanche* L., *Phelipanche* Pomel, *Phelypaea* L. (= *Diphelypaea* Nicolson) and *Cistanche* Hoffmannsegg & Link), and revealed many endemic and host-specific species in this region that had previously been overlooked (e.g., Piwowarczyk 2015; Piwowarczyk et al. 2015, 2017a, b, c, d, 2018a, b, c, 2019, 2020a, 2021). Other researchers have refined the understanding of these four genera in the Mediterranean Basin, as well as the monotypic *Boulardia* F.W. Schultz. (e.g., Foley 2001; Carlón et al. 2003, 2005, 2008; Domina and Arrigoni 2007; Jeanmonod and Habashi 2007; Pujadas Salvà 2009; Domina et al. 2011, 2013; Frajman et al. 2013). Together, the Mediterranean and the Caucasus have been hypothesised as refugia for both plant and animal lineages during the Pleistocene ice ages (Taberlet et al. 1998; Hewitt 1999; Lumibao et al. 2017), and some authors even propose the Caucasus together with the Middle East and Central Asian high mountains as the main area of origin of Old World broomrapes (*Orobanche* and *Phelipanche*, Rätzel and Uhlich 2004).

While regions of high extant diversity for any lineage may be the result of *in situ* diversification, this is not necessarily the case. Thus, hypotheses of historical biogeography must be explicitly tested. Schneider and Moore (2017) used a statistical

phylogenetic framework to infer the divergence times and historical biogeography of the Orobancheae to the extent possible given the limitations of a depauperate fossil record and the increased rates of molecular evolution that are characteristic of parasitic plants (Bromham et al. 2013). While an important first step, their study focused on New World taxa and therefore lacked the taxonomic sampling or granularity of geographical data to evaluate biogeographical patterns within the Old World.

The aims of this study were two-fold. First, we sought to assess previously unknown phylogenetic relationships of Caucasian Orobancheae using nuclear ribosomal (ITS region) and plastid (*trnL–trnF*) DNA sequences. Second, we sought to evaluate the historical biogeography of Old World Orobancheae using a probabilistic dispersal-extinction-cladogenesis (DEC) model. In particular, we wanted to evaluate the hypothesis of Western Asia (especially the Caucasus) and the Mediterranean as potential refugia and/or centres of origin for major species-rich clade in the Orobancheae.

Materials and methods

Taxonomic sampling and data collection

For the initial phylogenetic analysis, we studied Caucasian species of *Cistanche*, *Phelypaea*, *Phelipanche* and *Orobanche*, mainly collected from Georgia, Armenia, Azerbaijan and Russia between 2014 and 2019. Specimens of some species were collected in other countries or taken from herbaria (B, ERCB, HMMNH, IRKU, KTC, LE, MW, herb. Ó. Sánchez Pedraja), or sequences were downloaded from GenBank. In total, 13 genera, 175 taxa representing 140 species (548 sequences, including 196 as new), from 44 countries, were analysed (see Suppl. material 1: Table S1). For the majority of samples, hosts were precisely identified. We assessed infraspecific variation by sampling more than one individual, often from different localities and host species. Voucher information, as well as geographic origin or GenBank accession numbers are listed in Suppl. material 1: Table S1. Newly collected plant specimens were deposited in KTC, ERCB (herbarium codes according to Index Herbariorum, Thiers 2017). Systematic division was adopted according to Beck (1930) and Teryokhin et al. (1993), the scheme followed explicitly or implicitly by most researchers, and some recent taxonomic changes made by Piwowarczyk et al. (2017a, 2018d, 2019) (Fig. 1).

Material used for DNA extraction was freshly collected and silica gel-dried or was obtained from herbarium vouchers. For phylogenetic studies we used two types of sequences: nuclear ITS region (internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, later referred to as ITS) and plastid *trnL–trnF* sequence (RNA-Leu (*trnL*) intron, the partial *trnL* gene, and the intergenic spacer between the *trnL* 3' exon and tRNA-Phe (*trnF*) gene region's plastid DNA). These two regions are commonly used for species-level phylogenetic inference, including in the Orobancheae (ITS: Schneeweiss et al. 2004; Carlón et al. 2005, 2008; Park et al. 2008; Schneider et al. 2016; Fu et al. 2017; Piwowarczyk et al. 2018d; *trnL–trnF*: Schneider et al. 2016;

Piwowarczyk et al. 2018d). DNA extraction and sequence amplification procedures follow the methods of Piwowarczyk et al. (2018d).

Phylogenetic inference

Sequences were aligned with MAFFT v7.407 (Katoh and Standley 2013), manually corrected and trimmed. The final number of sequences and length of alignments were: for ITS 229 sequences of 671 positions, in the case of *trnL–trnF* 153 sequences, 1,337 positions long. Separate ITS and *trnL–trnF* trees were inferred instead of concatenating them into a single analysis for two main reasons: first, although preliminary trees inferred from each sequence were generally congruent, certain species did show conflicting placements (described below), perhaps due to differences in plastid versus nuclear inheritance. Second, the ITS tree is much richer in samples, because of greater availability in GenBank. Information about sequences (newly obtained and downloaded from GenBank) used in phylogenetic analysis is presented in Suppl. material 1: Table S1.

For both sequence alignments, Maximum Likelihood (Figs 1, 2) and Bayesian (Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2) phylogenetic trees were generated with *Lindenbergia sinaica* (Decne.) Benth. used as outgroup. Maximum Likelihood (ML) trees were calculated with IQ-TREE multicore version 1.6.12 (Nguyen et al. 2015) software, with ultrafast bootstrap approximation (2,000 bootstrap replicates). Substitution models were auto-determined by IQ-TREE using the Bayesian Information Criterion (BIC) (SYM+I+G4 for ITS and TVM+F+R3 for *trnL–trnF*). Bayesian phylogenetic trees were generated using MrBayes v. 3.2.6 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) with the following main settings: ngen = 10,000,000, samplefreq = 500, nchains = 4, checkfreq = 100,000, diagnfreq = 5,000, stopval = 0.01, stoprule = yes, relburnin = yes, burninfrac = 0.25 and, lset applyto = (all) nst = 6 rates = invgamma (for ITS) or lset applyto = (all) nst = 6 rates = gamma (for *trnL–trnF*). Substitution models according to BIC (SYM+I+G for ITS and GTR+G for *trnL–trnF*) were determined by IQ-TREE software. The trees were visualised by iTOL tool (Letunic and Bork 2016).

Historical biogeography

To infer a chronogram for historical biogeography analysis of the tribe Orobancheae we used the ITS, PhyA, and PhyB Orobancheae alignments of Schneider and Moore (2017), improved in six ways:

1. Taxonomic coverage for *Orobanche* and *Phelipanche* was expanded based on this study.
2. Taxonomic coverage for *Cistanche* was expanded by using sequence data submitted to GenBank by Ataei (2017). Recent phylogenetic evidence support many more lineages in this genus than previously recognized (Ataei 2017; Ataei et al. 2020).

Names for some of these lineages have been proposed but not yet validly published (Ataei 2017).

3. Sequences for *Gleadovia* Gamble & Prain and *Phacellanthus* Siebold & Zucc. – first published by Fu et al. (2017) and available on GenBank – were added, resulting in complete taxonomic coverage at the genus level, except for the monotypic Mexican genus *Eremitilla* Yatsk. & J.L.Contr.
4. The *trnL–trnF* plastid locus was added for most taxa based on newly generated data or pre-existing sequences (Suppl. material 1: Table S1). Although nrDNA and cpDNA partitions support conflicting relationships for a few taxa, the key nodes associated with major biogeographic transitions and discussed herein are supported by both analyses.
5. A 637 bp region of the PhyA gene was excluded from analysis because it was poorly alignable. This region appears only in our sequences for *Boschniakia himalaica* Hook. f. & Thomson ex Hook. f. and *Aphyllon ludovicianum* (Nutt.) A.Gray but not for any other species.
6. Samples for *Aphyllon californicum* (Cham. & Schltdl.) A.Gray subspecies *feudgei*, *grande*, *grayanum*, and *jepsonii* were replaced with different samples for which both ITS and *trnL–trnF* sequences were available.

Sequences matrices for each gene were aligned separately using Geneious 9.1.8 (Biomatters, Auckland, New Zealand; Kearse et al. 2012), then concatenated into a single supermatrix comprised of a 1986bp ITS + *trnL–trnF* backbone plus 3375 bp of phytochrome sequence from a subset of 20 taxa. In this case, we decided that the better branchlength estimates broadly across the tree by using multiple genes generally outweighed errors introduced for particular tips that may have conflicting ITS and *trnL–trnF* topologies. This supermatrix was used to infer a chronogram by implementing an uncorrelated lognormal relaxed clock model and a GTR+Γ substitution model in the software Revbayes v. 1.0.11 (Höhna et al. 2014). Our starting tree was generated using default parameters in RAxML-HPC v8, run on XSEDE through the CIPRES portal (Stamatakis 2014), rooted based on the results of previous comparable molecular phylogenetic studies (McNeal et al. 2013; Fu et al. 2017; Schneider and Moore 2017), and made ultrametric with a root age set to 25 (Ma) using the rate-smoothing function chronos in the R package 'ape' v. 5.3 (Paradis and Schliep 2019). The same divergence time calibrations and other analysis parameters were used as in Schneider and Moore (2017), except we used a new starting tree and the Markov Chain Montecarlo (MCMC) analysis was run for 4,000 iterations as a pre-burnin to tune the proposal parameters then sampled every 100 iterations for 50000 iterations with the first 15% of samples discarded as burn-in.

Each iteration consisted of 472 moves randomly scheduled from 394 possible moves. Stationarity was assessed using Tracer v.1.7.1 (Rambaut et al. 2018) and the effective sampling size of each important parameter exceeded 200: likelihood, prior, each GTR parameter and the shape parameter for the gamma distribution for each partition, speciation and extinction rates, root time, and clade ages of *Orobanche* s.l. and *Cistanche*.

For biogeographical analysis, the global range of Orobancheae was divided into six non-overlapping regions based on physical geography and natural phytogeographic divisions (Fig. 3): (1) Europe/Mediterranean, including Central, North, Eastern and Southern Europe with Mediterranean Basin (Iberian, Italian and Balkan peninsulas with northern Africa – north from Sahara Desert, and western and southern parts of the peninsula of Turkey); (2) Western Asia, which includes Anatolia in Turkey, the Arabian Peninsula, Iran, the Levant, Mesopotamia, the Sinai Peninsula, and Caucasus (with Transcaucasia); (3) Central Asia, including the area from the Caspian Sea to western China, and from Afghanistan, through Turkmenistan, Tajikistan, Uzbekistan, Kyrgyzstan, and Kazakhstan to the south to Russia (with Ciscaucasia) in the north; (4) East Asia, from central China eastward (Hong Kong, Macao, Mongolia, the Korean peninsula, Japan, and Taiwan) and including Australia for *Orobanche cernua* var. *australiana* (F. Muell.) Beck, the only taxon apparently native to that continent in our study; (5) Africa, south of the Mediterranean Basin (Saharan and sub-Saharan); and (6) the New World. In general, a taxon was not considered to inhabit a region if < 5% of its known range fell within the respective region boundary. To determine the range for individual species, we used a variety of peer-reviewed sources (e.g., Novopokrovskij and Tzvelev 1958; Wu and Raven 1998; Pusch and Günther 2009; Cullen 2010; Tzvelev 2015; Ataei 2017; Freeman et al. 2019; Piwowarczyk et al. 2019) and continually updated databases (Domina and Raab-Straube 2010; Sánchez Pedraja et al. 2016), supported by our knowledge acquired during field and herbaria research. Ataei (2017) was used to determine the distribution of undescribed *Cistanche* taxa who we follow along with Ataei et al. (2020) because they have the most comprehensive set of genetic data. However, some taxonomic and distributional ranges conflict with other recent treatments (Moreno Moral et al. 2018), highlighting the need for continued evaluation in this genus.

Ancestral geographical ranges were inferred by applying a dispersal-extinction-cladogenesis (DEC) model of historical biogeography to the maximum clade credibility (MCC) tree from the Bayesian analysis. The DEC model, also implemented in RevBayes, allows for sympatric speciation, allopatric speciation and anagenetic range expansion and contraction (Ree and Smith 2008). Two independent MCMC replicates were run for 1,000 iterations as a pre-burn-in to tune the proposal settings, then sampled every 5 iterations for 10000 iterations. Each iteration consisted of 11 moves randomly scheduled from 3 possible moves. Stationarity was also assessed using Tracer.

Results

Phylogenetic relationships

The most important results of our phylogenetic analyses clarified the position of many previously unsampled Caucasian species (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). We also showed the phylogenetic relations of the newly described species, i.e., *Phelipanche zangezuri* Piwow. et al., *P. hajastanica* Piwow. et al., and *P. sevanensis* Piwow. et al., *O. javakhetica* Piwow. et al., *O. arpica* Piwow. et al. and *O. zajaciorum* Piwow.

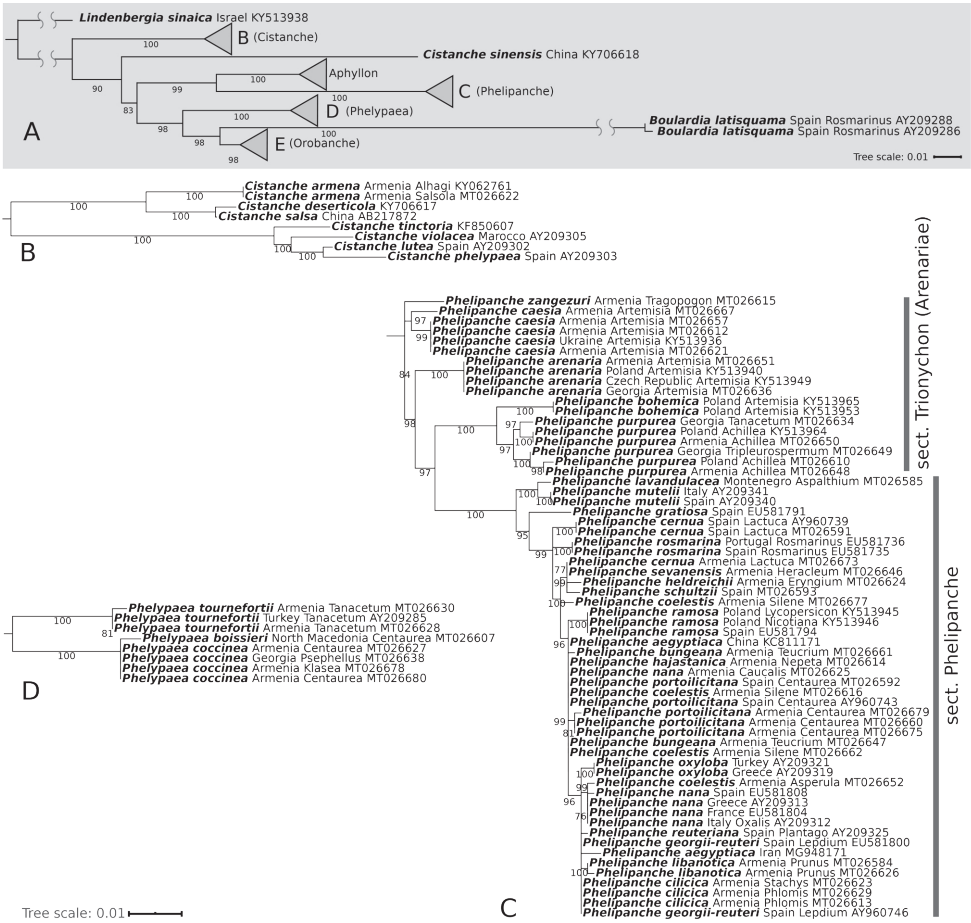

Figure 1. Rooted Maximum Likelihood phylogenetic tree constructed using ITS sequences. Numbers near branches show ultrafast bootstrap values (values ≥ 75 are shown). The bar represents the amount of genetic change (nucleotide substitutions per site) **A** summary of backbone (generic) relationships, branches connecting the outgroup *Lindenbergia* and *Boulardia* are shortened to fit the figure **B–E** relationships of taxa within the genera *Cistanche*, *Phelipanche*, *Phelypaea*, and *Orobanche* respectively. Species names, the country of origin, host species (if available) and GenBank number are included on the phylogeny tip labels.

Consistent with previous studies, the studied genera were each strongly supported as monophyletic (Bootstrap (BS) ≥ 90, Posterior Probability (PP) = 1.0).

Cistanche

ITS (*trnL–trnF* data was not available) trees show that *Cistanche armena* (K. Koch) M.V. Agab. (samples from two different hosts, *Alhagi* Gagnebin and *Salsola* L.) is closely related to *C. deserticola* Ma and *C. salsa* (C.A. Mey.) Beck (BS = 100, PP = 1.00), and with the later one it has sometimes been confused (Fig. 1, Suppl. material 2: Fig. S1).

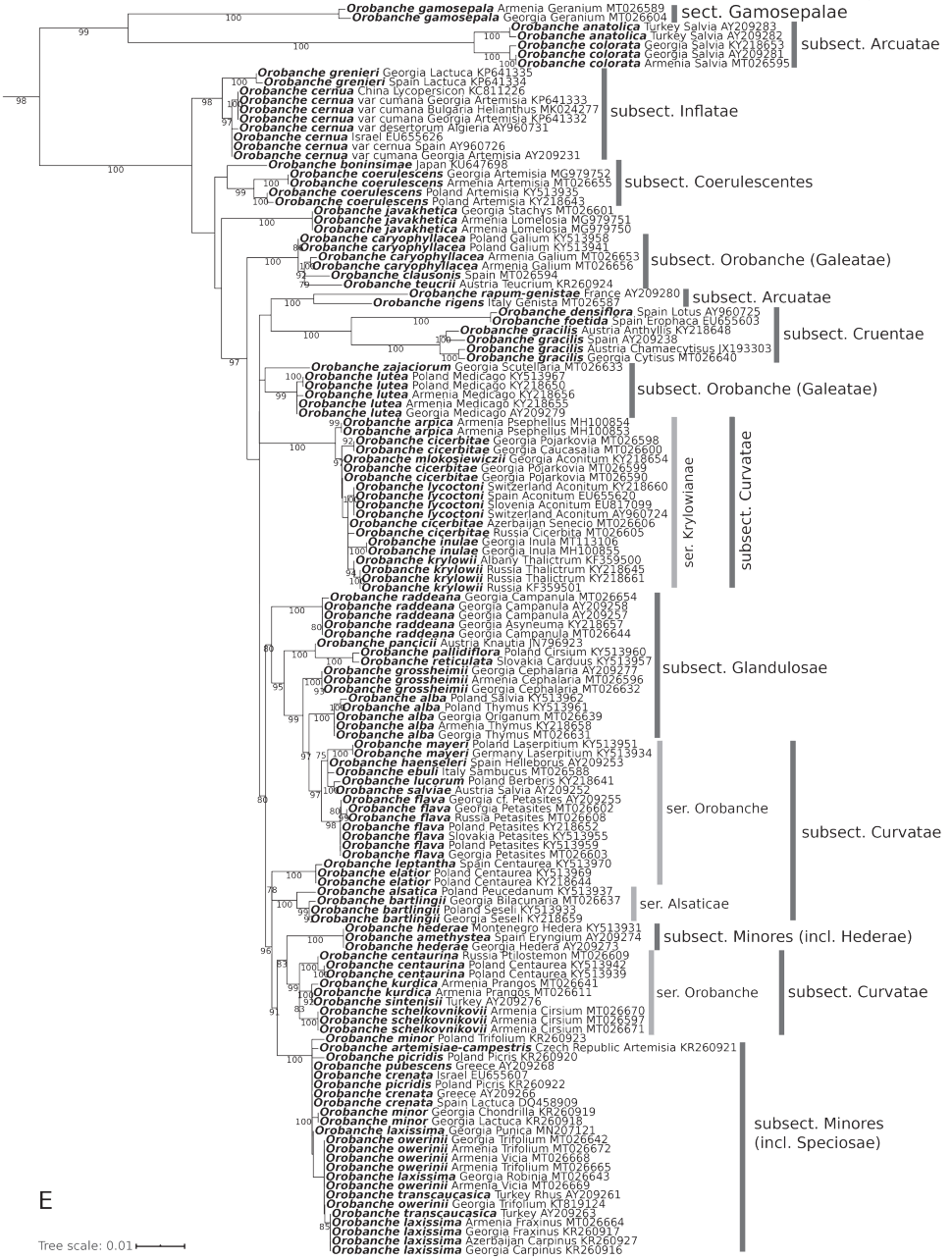

Figure 1. Continued.

Phelypaea

The three species from genus *Phelypaea*, *P. tournefortii* Desf. and *P. coccinea* (M. Bieb.) Poir. are clearly separated (BS = 100, PP = 1.00), however *P. boissieri* (Reut.) Stapf, first

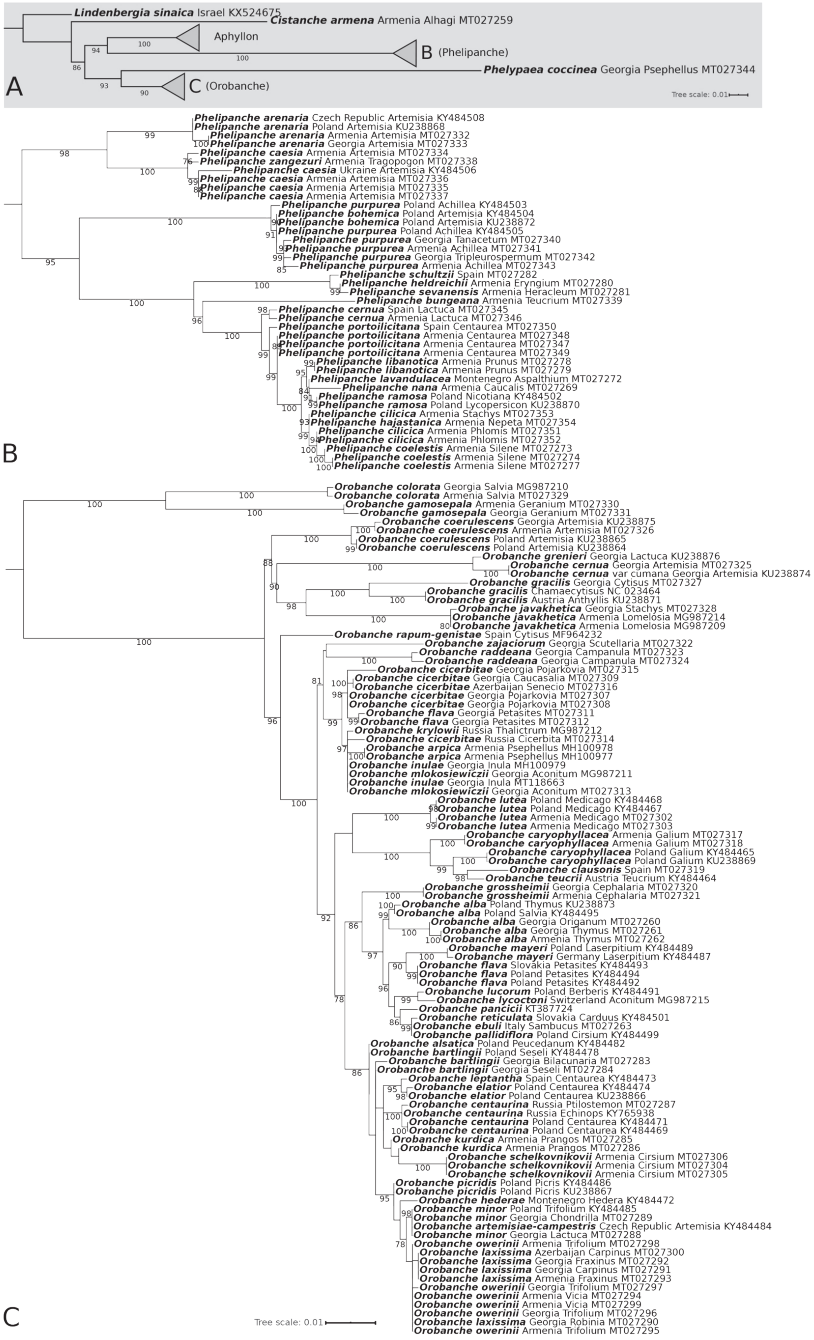

Figure 2. Rooted Maximum Likelihood phylogenetic tree constructed using plastid *trnL–trnF* spacer sequences. As an outgroup, *Lindenbergia sinaica* was used. Numbers near branches show ultrafast bootstrap values (values ≥ 75 are shown). The bar represents the amount of genetic change (nucleotide substitutions per site) **A** summary of backbone (generic) relationships **B** *Phelipanche* clade **C** *Orobanche* clade. Species names, the country of origin, host species (if available) and GenBank number are included on the phylogeny tip labels.

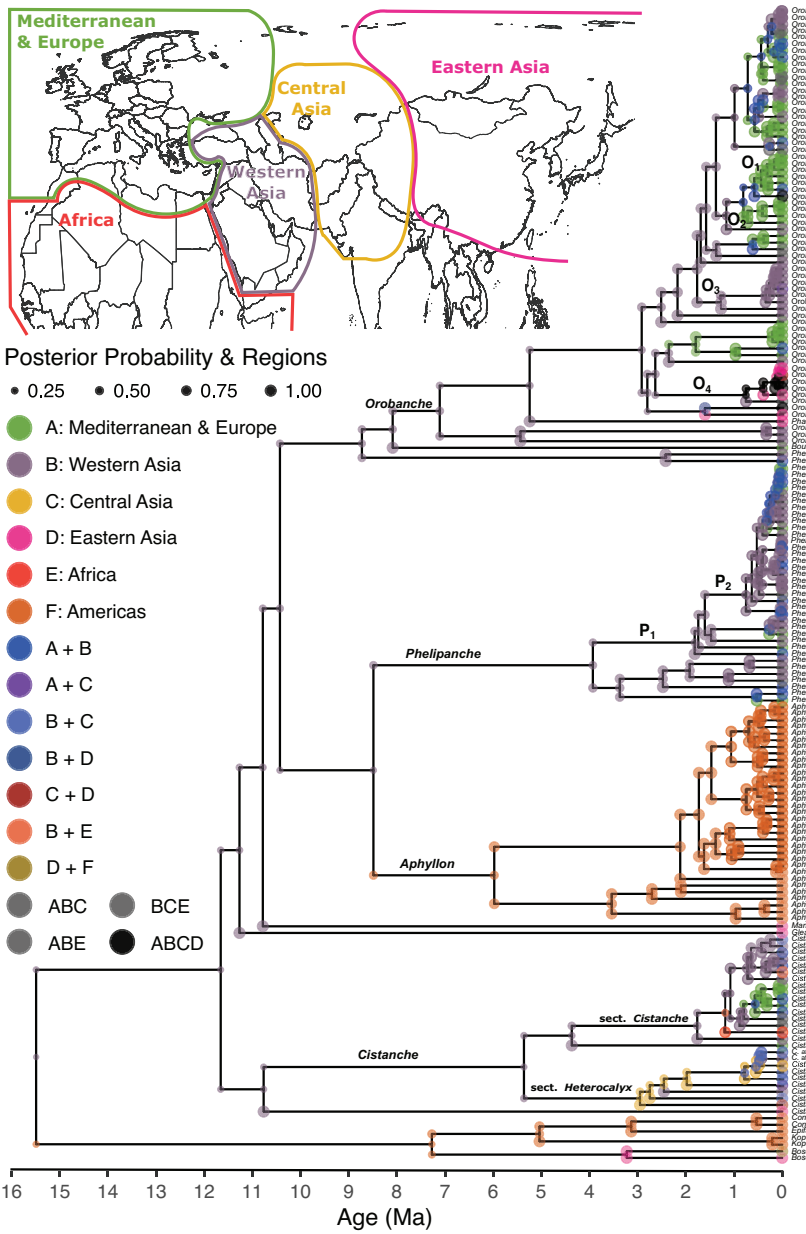

Figure 3. Historical biogeography of tribe Orobancheae, reconstructed using a dispersal-extinction-cladognesis model implemented in RevBayes (maximum likelihood topology, maximum clade credibility branch lengths). Coloured circles at tips represent the current biogeographical range of each sampled taxon. Circles on each node represent the reconstructed ancestral area of the most recent common ancestor of the two daughter lineages, while circles on either side of the node show the reconstructed areas immediately following cladogenesis. Circle size is proportional to posterior probability. Each colour represents a different biogeographical region or combination of regions as indicated by the map and legend to the left of the chronogram. Tip labels for *Cistanche* follow nomenclature of Ataei et al. (2020). Asterisks indicate names proposed by Ataei (2017) but not yet validly published.

sequenced for this study, seems to be very similar to *P. coccinea*. Amplification of *trnL–trnF* in *Phelypaea* samples was successful only in the case of *P. coccinea*, so the above analysis was based only on ITS (Fig. 1, Suppl. material 2: Fig. S1).

Phelipanche

Based on ITS data *P. zangezuri* is separated from the clade of *P. caesia* (Rchb.) Soják (BS = 97, PP = 0.90) and the clade containing remain *Phelipanche* species (BS = 98, PP = 0.85). By contrast, *trnL–trnF* trees do not indicate separation of *P. zangezuri* and *P. caesia*. Rather, samples of *P. arenaria* form a sister clade to these two species, and together form a well-supported lineage (BS = 98, PP = 1.00) separated from the rest of *Phelipanche* (BS = 95, PP = 0.96) (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). *P. sevanensis* is closely related to the group of *P. schultzii* (Mutel) Pomel and *P. heldreichii* (Reut.) Soják on all trees, and to *P. cernua* Pomel. on the ITS trees, (BS = 99, PP = 0.99) (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). However, *P. hajastanica* is found in the group of slightly differentiable species on the ITS tree (Fig. 1), while on the *trnF-trnL* tree it is close to *P. cilicica* (Beck) Soják (BS = 99, PP = 0.92) (Fig. 2).

Our results showed the relationship of samples from different parts of the range of disjunctive species, such as *P. portoilicitana* (A. Pujadas & M.B. Crespo) Carlón et al. and *P. cernua*. Whereas *trnL–trnF* sequences of *P. cernua* places samples from Armenia and Spain are grouped in the same clade (BS = 98, PP = 0.94), on the ITS tree, the European samples are separated from Caucasian sample which is in the same clade as *P. sevanensis*, *P. schultzii* and *P. heldreichii* (BS = 99, PP = 0.99). Also, *P. portoilicitana*, both on ITS and *trnL–trnF* trees, show differences between samples from Armenia and Spain (Figs 1, 2, Suppl. material 3: Fig. S2).

Orobanche

Orobanche gamosepala Reut. is genetically distinct (BS = 100, PP = 1.00) from *O. anatolica* Boiss. & Reut. ex Reut./*O. colorata* K. Koch and together these species are grouped in sister clade to the rest of *Orobanche* species (ITS: BS = 99, PP = 1.00, *trnL–trnF*: BS = 100, PP = 1.00) (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2).

ITS sequence data indicates that *O. cicerbitae* (Uhlich & Rätzel) Tzvelev is not closely related to *O. flava* Mart. ex F.W. Schultz, however on the *trnL–trnF* trees *O. cicerbitae* from Georgia and Azerbaijan forms a common clade with *O. flava* from Georgia (BS = 98, PP = 0.97), whereas Central European samples of *O. flava* are distant (Fig. 1, Suppl. material 2: Fig. S1).

ITS sequences (Fig. 1, Suppl. material 2: Fig. S1) of high mountain *Orobanche* species, such as *O. krylowii* Beck, *O. cicerbitae*, *O. arpica*, *O. mlokosiewiczii* Piwow. et al., *O. inulae* Novopokr. & Abramov and *O. lycoctoni* Rhiner showed that they are closely related, and form a separated clade (ITS: BS = 100, PP = 1.00, *trnL–trnF*: BS = 99, PP = 1.00) included in *O.* ser. *Krylowianae* Piwow. et al. Probably these

species diverged relatively recently and can be an example of recent rapid radiation. Another interesting phenomenon is the placement of the *trnL–trnF* sequence (Fig. 2, Suppl. material 3: Fig. S2) of *O. lycoctoni* on phylogenetic trees near *O. lucorum* A. Braun ex F.W. Schultz, (BS = 99, PP = 1.00), a species distantly related to the sect. *Krylowianae* species.

The phylogenetic position of Caucasian endemic species with unclear affinity has also been presented, in particular those previously classified in inappropriate subsections, such as *O. schelkownikovii* Tzvel., *O. grossheimii* Novopokr., *O. raddeana* Beck, and *O. laxissima* Rätzel & Uhlich (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2, and discussion below).

Little within-species variation is shown among the samples from different host species taken from the following species: *O. laxissima*, *O. alba* Stephan ex Willd., *O. bartlingii* Griseb., *O. caryophyllacea* Sm., *O. cicerbitae*, *O. gracilis* Sm., *O. centaurina* Bertol., *O. minor* Sm., *O. owerinii* (Beck) Beck, *O. raddeana*, *O. schelkovnikovii*, *P. cilicica*, *P. coelestis* (Reut.) Soják, *P. purpurea* (Jacq.) Soják and *P. coccinea* (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2).

Historical biogeography

We find negligible support (PP < 0.4) for any single hypothesis ancestral range of lineages older than 6 million years. However, most diversification in the Orobancheae has happened relatively recently (Tables 1, 2, Fig. 3). We focus below on *Cistanche*, *Phelipanche* and *Orobanche* because these are the three most diverse lineages in the

Table 1. Divergence times with credible intervals (95% highest probability density (HPD)) and inferred historical biogeography of selected clades. Biogeographical regions defined in Methods and Figure 3.

Clade	Crown Age (Ma)		Biogeography	
	Mean	95% HPD	Region	Posterior Prob.
Cistanche sect. *Heterocalyx*	3.0	2.0–4.2	Central Asia	0.28 (0.42 for clade excluding *C. deserticola*)
			Western Asia	0.14 (0.27)
Phelypaea	2.4	1.4–3.7	Western Asia	0.46
			Western Asia + Med/Europe	0.11
Phelipanche	3.9	2.8–5.5	Western Asia	0.41
			Europe/Mediterranean	0.09
			Both	0.11
Phelipanche clade P_1	1.8	1.1–2.4	Western Asia	0.38
			Western Asia + Med/Europe	0.32
Phelipanche clade P_2	0.753	0.52–1.1	Western Asia	0.58
			Western Asia + Europe/Med	0.34
Orobanche clade O_1	0.44	0.26–0.67	Europe/Mediterranean	0.93
Orobanche clade O_2	0.72	0.46–1.0	Europe/Mediterranean	0.99
Orobanche clade O_1+O_2	0.81	0.52–1.1	Western Asia + Europe/Med	0.50
			Europe/Mediterranean	0.24
Orobanche clade O_3	1.27	0.75–1.8	Western Asia	0.93
Orobanche clade O_4	0.75	0.40–1.1	Europe/Mediterranean + Western Asia	n/a
			+ Central Asia	0.12
			+ East Asia	0.10
			+ Both	0.27

Table 2. Divergence times of species or clades endemic or nearly endemic to the Caucasus region.

Species or clade, or paraphyletic group[a]	Taxa	Divergence time[a] (Ma)	95% HPD
Clade	*Phelypaea coccinea* + *P. tournefortii*	2.4	1.4–3.7
Clade	*Orobanche anatolica* + *O. colorata* + *O. gamosepala*	5.4	3.5–7.6
Paraphyletic	*Orobanche arpica*, *O. cicerbitae* on *Caucasalia*, *O. cicerbitae*, *O. inulae*, *O. mlokosiewiczii*, *O. cicerbitae* on *Pojarkovia* (+ widespread *O. krylowii*)	1.3	0.75–1.8
Species	*Orobanche zajaciorum*	1.7	1.2–2.3
Species	*Orobanche raddeana*	2.2	1.7–3.3
Species	*Orobanche grossheimii*	1.2	0.74–1.5
Clade	*Orobanche laxissima* + *O. owerinii* + *O. transcaucasica*	0.08	0.02–0.15
Species	*Orobanche javakhetica*	2.4	1.6–3.2
Species	*Orobanche kurdica*	0.08	0.0002–0.23
Species	*Orobanche schelkovnikovii*	0.50	0.30– 0.69
Species	*Phelipanche bungeana*	1.48	0.93–2.0
Clade	*Phelipanche coelestis* + *Phelipanche* "on *Astrodaucus*"	0.15	0.05–0.26
Species	*Phelipanche hajastanica*	0.28	0.16–0.40
Clade	*Phelipanche heldreichii* + *P. sevanensis*	0.19	0.08–0.32
Species	*Phelipanche* "on *Artemisia*"	1.1	0.52–1.8
Species	*Phelipanche* "on *Genista*"	0.20	0.10–0.31
Species	*Phelipanche pulchella*	0.41	0.21–0.61
Species	*Phelipanche zangezuri*	0.68	0.33–1.1

[a]Crown age indicated for clades of <1 species and paraphyletic groups; stem age indicated for single species.

Old World and we found relatively high support for some biogeographical patterns. *Phelypaea* probably originated in West Asia (PP = 0.46) or was more widespread in West Asia and Caucasus, Europe and the Mediterranean (PP = 0.11).

Cistanche

The phylogeny of *Cistanche* appears to be structured by geography, with clades of species endemic to particular areas. For example, we find weak support for a Central Asian ancestor of *Cistanche* sect. *Heterocalyx* sensu Ataei, non Beck (composed of *C. salsa*, *C. bamianica* Ataei ined. (Ataei 2017), *C. bilobata* Ataei ined. (Ataei 2017), *C. deserticola*, *C. ambigua* (Bunge) Beck (= *C. trivalvis* (Trautv.) Korsh.), *C. tomentosa* Ataei ined. (Ataei 2017), *C. ridgewayana* Aitch. & Hemsl. and *C. persica* Ataei ined. (Ataei 2017)) (PP = 0.28; 0.42 for the subclade excluding the more widespread *C. deserticola*). Several extant species such as *C. persica*, *C. tomentosa*, and *C. salsa* extend further west into the Europe/Mediterranean region; we inferred that these are the result of recent range expansions (Fig. 3).

We found support that the clade of species *C. algeriensis* Ataei ined. (Ataei 2017), *C. almeriensis* Ataei ined. (Ataei 2017), *C. phelypaea* (L.) Cout. and *C. violacea* (Desf.) Hoffmanns. & Link in *Cistanche* sect. *Cistanche* sensu Ataei (= *Cistanche* sect. *Eucistanche* Beck, p.p. max.) originated from an ancestor that was either widespread throughout the European/Mediterranean region and Western Asia (PP = 0.47), or just restricted to Europe/the Mediterranean (PP = 0.44). We also inferred a Western Asian origin for the clade of species *C. chabaharensis* Ataei ined. (Ataei 2017), *C. tubulosa*

(Schenk) Hook. f., *C. senegalensis* (Reut.) Beck, *C. laxiflora* Aitch. & Hemsl., and *C. flava* (C.A. Mey.) Korsh. (PP = 0.95), although extant species are found throughout Western and Central Asia today.

Phelipanche

We found moderate support for a Western Asia origin of *Phelipanche* (PP = 0.41) approximately 2.8–5.5 million years ago, with alternative biogeographical hypotheses much more weakly supported (Table 1). Within the genus we found strong support for two general observations. First, important subclades of *Phelipanche* also likely originated in Western Asia or were more widespread into Europe or the Mediterranean as well. These include the large subclades designated P_1 and P_2 in Figure 3, the crown ancestors of which were most probably limited to Western Asia (PP = 0.38 and 0.58, respectively), but may have had a larger range extending into Europe/the Mediterranean Basin as well (PP = 0.32 and 0.34). Crown ancestors of clades nested within P2 were inferred to be limited Western Asia with even higher probability (PP > 0.7) with dispersal out of this region by some extant species (e.g., *P. gratiosa* (Webb) Carlón et al. (Canary Islands, endemic) to the Mediterranean & Europe, as well as the subclade *P. libanotica* (Schweinf. ex Boiss.) Soják + *P. reuteriana* (Rchb. fil.) Carlón et al. + *P. oxyloba* (Reut.) Soják + *P. georgii-reuteri* Carlón et al. + *P. cilicica* + *P. aegyptiaca* (Pers.) Pomel), and independent range expansions into Europe/the Mediterranean Basin from an ancestor limited to Western Asia in the sister species *P. lavandulacea* (Rchb.) Pomel and *P. mutelii* (F.W. Schultz) Pomel (PP ≥ 0.75).

Similarly, we find it most probable that the most widespread and often weedy species of *Phelipanche* had direct stem ancestors limited in range to Western Asia. These include *Phelipanche arenaria* (Borkh.) Pomel (PP = 0.71), *P. caesia* (PP = 0.5), *P. ramosa* (L.) Pomel (PP = 0.90), and *P. aegyptiaca* (PP = 0.40, with the next most probable origin as Europe/the Mediterranean, PP = 0.22).

Orobanche

Similar to *Phelipanche* we infer a Western Asian origin for ancestral *Orobanche* (PP = 0.43; 0.39 for *Orobanche* + *Boulardia*). Four key subclades are diagnosable by their biogeographic affinities. The first and second subclades are closely related and comprise predominantly Europe/Mediterranean species that have diversified *in situ* (O_1 + O_2 in Table 1, Fig. 3; ancestral range Europe/Mediterranean, PP = 0.93 and 0.99). The larger clade, also including the widespread *O. alba* and the Caucasian endemic *O. grossheimii*, likely originated in Western Asia (PP = 0.58; Fig. 3, Suppl. material 4: data S1 and Suppl. material 5: data S2) or Western Asia + Europe/the Mediterranean (PP = 0.24). A Western Asian origin is even more probable for the several more inclusive clades of *Orobanche* moving toward the root (PP = 0.66 – 0.84). The third key clade, O_3, is composed exclusively of Caucasian endemics, except for the more widespread species *O. krylowii* (indicated as O_3 in Fig. 3) and also originated in Western Asia (PP = 0.93, Tables 1, 2). Finally, the fourth clade (O_4) consists of several widespread species including *O. cernua* L., *O. amoena* C.A. Mey., *O. cumana* Wallr., *O. pycnostachya*

Hance, and *O. grenieri* F.W. Schultz and was also inferred to have a widespread common ancestor, though the exact geography is uncertain (Table 1). The top three most probable biogeographic states for the common ancestor encompass the regions Europe/Mediterranean plus Western Asia as well as either Central Asia, Eastern Asia, or both, but together these hypotheses only represent half of the posterior density (Table 1).

Discussion

Phylogenetic relationships

Cistanche

C. armena was described by Koch (1843) as *Phelypaea armena*, synonymised with *P. salsa* C.A. Mey by Boissier (1879) and transferred to *C. salsa* by Beck, where it has remained in synonym by subsequent authors (Ataei 2017; Ataei et al. 2020). However, recent morphological study has indeed shown that *C. armena* differs clearly from *C. salsa* (Piwowarczyk et al. 2019). The occurrence of this *Cistanche* species in the Caucasus requires further field and molecular studies, however at this point *C. armena* is known only from Armenia, and *C. salsa* and *C. fissa* (C.A. Mey.) Beck probably are absent from Armenia or Georgia (Piwowarczyk et al. 2019).

Phelypaea

This genus includes three holoparasite species (*P. coccinea*, *P. boissieri*, and *P. tournefortii*) that parasitize Asteraceae hosts. *Phelypaea coccinea*, a parasite of *Psephellus* Cass. and *Centaurea* L., rarely *Klasea* Cass., occurs in the Caucasus and Crimea, while *P. tournefortii*, a parasite of *Tanacetum* L., occurs in the Caucasus and Turkey (Piwowarczyk et al. 2019). However, *P. boissieri* shows a different distribution; it occurs in the Balkans (Albania, Greece, North Macedonia), and Western Asia (Turkey, Iraq and Iran), parasitises *Centaurea* (similarly *P. coccinea*), and occasionally *Cousinia* Cass. in Iraq (Piwowarczyk et al. 2019). The molecular (Fig. 1) and morphological features that separate *P. coccinea* and *P. boissieri* – i.e., corolla-tube short and cup-shaped; corolla-lobes broadly obovate-orbicular to orbicular, overlapping; anthers hairy (Piwowarczyk et al. 2019) – are not fully differentiating. Thus, further research into variability and the inclusion of more samples for genetic analysis are required.

Phelipanche

The phylogenetic relations of the newly described species, i.e., *P. zangezuri* (Piwowarczyk et al. 2018a), *P. hajastanica* (Piwowarczyk et al. 2017c), and *P. sevanensis* (Piwowarczyk et al. 2017b) are presented (Figs 1, 2).

Phylogenetic analysis of two species previously known mainly from the Mediterranean area and later found in the Caucasus, i.e., *P. portoilicitana* and *P. cernua*

(Piwowarczyk et al. 2019), showed some differences between samples collected from these different parts of the range. This may indicate the ongoing process of speciation despite similarity in host association (Figs 1, 2).

ITS (Fig. 1) poorly differentiates some species aggregates in *Phelipanche* sect. *Phelipanche*, while it does well in the sect. *Trionychon* (Wallr.) Piwow. & Ó. Sánchez (Piwowarczyk et al. 2018d, = sect. *Arenariae* Teryokh.).

Orobanche

The recently described *O. flava* subsp. *cicerbitae* Uhlich & Rätzel [≡ *O. cicerbitae* (Uhlich et Rätzel) Tzvelev] parasitising *Cicerbita* Wallr. and *Senecio propinquus* Schischk. is distantly related to *O. flava*, at least as far as ITS (Fig. 1, Suppl. material 2: Fig. S1) and morphological (Piwowarczyk et al. 2017a) analyses have shown. On the ITS tree *O. cicerbitae* belong to clade *O.* subsect. *Curvatae* (Beck) Piwow. et al., particularly with species of the *O.* series *Krylowianae* clade (Piwowarczyk et al. 2017a). However, trees based on *trnL–trnF* sequences show displacement of samples of Caucasian *O. flava* close to *O. cicerbitae* (Fig. 2, Suppl. material 3: Fig. S2). A similar phenomenon is the placement of *trnL–trnF* sequence (Fig. 2, Suppl. material 3: Fig. S2) of *O. lycoctoni* near *O. lucorum*, a species relatively distant to *Krylowianae* species. This phenomenon may be explained by hybridisation and requires further research. In this case *O. cicerbitae* might be a species formed by the crossing of *O. flava* (or related species) as a female parent and one of the species belonging to ser. *Krylowianae*, but this supposition requires further study. In the Caucasus, both species often occupy the same habitats (humid tall herb vegetation) and grow with their hosts (*Caucasalia* B. Nord, *Senecio* L., *Pojarkovia* Askerova vs. *Petasites* Mill.) next to each other, thus facilitating gene flow.

We confirm that the newly described *O. javakhetica* (Piwowarczyk et al. 2018b) is distinct from the other studied species. ITS sequence does not resolve its position within other *Orobanche* species. However, trees based on *trnL–trnF* sequences (Fig. 2, Suppl. material 3: Fig. S2) show a common clade with *O. gracilis* (*O.* subsect. *Cruentae* Teryokhin) (BS = 98, PP = 1.00). Morphologically, there seems to be some similarity to the *O.* subsect. *Orobanche* (subsect. *Galeatae* sensu Teryokhin) and *O.* subsect. *Curvatae* (particularly with species of the *O.* ser. *Krylowianae*) (Piwowarczyk et al. 2018b). Finding other new species related to *O. javakhetica* will be helpful in confirming the phylogenetic relationships of this species.

Orobanche schelkovnikovii was incorrectly included in the *O.* trib./Grex *Galeatae* sensu Beck by Novopokrovskij and Tzvelev (1958). This erroneous determination of herbarium specimens collected by Schelkovnikov as *O. caryophyllacea* by Grossheim is surely the reason for its inclusion in this group. The corolla is not helmet-shaped at the apex, which clearly indicates that it should be in *O.* subsect. *Curvatae* (Piwowarczyk et al. 2019). The ITS trees indicate that *O. schelkovnikovii* belong to *O.* subsect. *Curvatae* and formed a clade with *O. centaurina* (syn. *O. kochii* F.W. Schultz, Zázvorka et al. 2019), *O. sintenisii* Beck and *O. kurdica* Boiss. & Hausskn. (syn. *O. rosea* Tzvel., Piwowarczyk et al. 2019) (Fig. 1) (BS = 99, PP = 0.98). *O. kurdica* is also morphologically most similar to *O. centaurina*, but clearly distinct based on some features and host

affinity (Piwowarczyk et al. 2019). However, the sample named as *O. sintenisii* from Turkey (AY209276) was probably identified incorrectly. Based on host and locality it most likely belongs to *O. kurdica* (Fig. 1, Suppl. material 2: Fig. S1, Suppl. material 1: Table S1).

According to Novopokrovskij and Tzvelev (1958) *O. grossheimii* belongs to the group subsect. *Curvatae*, but the presence of dark coloured reddish or violet glandular hairs and morphology of the flowers may indicate that it belongs to the *O*. subsect. *Glandulosae* (Beck) Teryokhin (*O*. subsect. *Glandulosae* Novopokr., Piwowarczyk et al. 2019), which seems to be confirmed in our phylogenetic study (Fig. 1, Suppl. material 2: Fig. S1).

The newly described species *O. zajaciorum* (Piwowarczyk 2015) is clearly separated from other species based on ITS and *trnL–trnF* data, but its precise relationship to other *Orobanche* remains to be unclear. On the ITS tree it formed a separated clade with *O. lutea* Baumg. (subsect. *Orobanche* ≡ *O*. subsect. *Galeatae* sensu Teryokhin), however with low support (BS < 75) (Fig. 1). Morphologically, *O. zajaciorum* is a close relative to *O.* subsect. *Orobanche* (*O*. subsect. *Galeatae* sensu Teryokhin) – especially due to the helmet-shaped upper lip and the relatively narrowly tubular flower, however the species of *O.* Grex *Galeatae* sensu Beck (1930) are – with the exception of *O. clausonis* Pomel – much taller and have larger flowers, usually broad bidentate calyx segments, and different hosts (Piwowarczyk 2015).

Orobanche rapum-genistae Thuill., *O. rigens* Loisel. vs *O. colorata*/*O. anatolica* placed by Beck (1930) in Grex *Arcuatae* (*O.* subsect. *Arcuatae* Teryokhin) in our phylogenetic trees show significant discrepancy (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2).

Orobanche gamosepala is genetically very distinct, yet nested within *Orobanche*, forming a clade with *O. anatolica*/*O. colorata* (*O.* subsect. *Arcuatae*) (ITS: BS = 99, PP = 1.00, *trnL–trnF*: BS = 100, PP = 1.00) that is sister to the clade containing all other *Orobanche* species (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). This species was described previously as the monotypic genus *Necranthus* Gilli from northeastern Turkey based on a calyx anatomy similar to *Boschniakia* C.A. Meyer ex Bong. and *Xylanche* Beck (Gilli 1968). Beck (1930) placed this species in trib./Grex *Galeatae*, and later Teryokhin et al. (1993) included this species in its own section *O*. sect. *Gamosepalae* Teryokh. according to its distinct calyx anatomy and seed micromorphology. In a more recent micromorphological study of Caucasian Orobanchaceae seeds, the position of *O. gamosepala* on the dendrogram is closer to *O. colorata* than to the remaining *Orobanche*, which is in accordance with the above results, and based on the shape of the seed cells, to *Cistanche* (Piwowarczyk et al. 2020b). *O. gamosepala* together with *O. colorata* and *O. anatolica* is one of the oldest lineages of Orobancheae (Table 2, see below).

According to some authors (e.g., Novopokrovskij and Tzvelev 1958; Domina and Raab-Straube 2010) *O. colorata* only grows in the countries of the Caucasus area (with ± glabrescente inflorescence), and is replaced in Turkey, Iran and Iraq by the *O. anatolica* s. str. (with ± lanate inflorescence). However, taxonomic and chorological restrictions corresponding to each taxon are not entirely clear (Piwowarczyk et al. 2019). In the Caucasus and Turkey (*O. anatolica* var. *glabrescens* Post and *O. anatolica*

var. *leucopogon* (Boiss. & Hausskn. ex Boiss.) Beck) both individuals with glabrescente and lanate inflorescences are often found, and lanate indumenum is especially present when the plants are young, but not only. In the ITS trees (Fig. 1, Suppl. material 2: Fig. S1) *O. anatolica* (from Turkey) and *O. colorata* (Caucasus) formed a common clade but the difference between them remains unclear (BS = 100, PP = 1.00).

Orobanche raddeana is a Caucasian endemic parasitising on Campanulaceae (*Campanula* L., *Asyneuma* Griseb. & Schenk). The ITS tree may suggest that it is related to species from the subsect. *Glandulosae* (Fig. 1, Suppl. material 2: Fig. S1), which is also supported by morphological features, while based on *trnL–trnF* sequences (Fig. 2, Suppl. material 3: Fig. S2) *O. raddeana* forms a common clade with species mostly from the *O.* subsect. *Curvatae* ser. *Krylowianae*. It is worth noting that *O. raddeana*, described later as *O. alba* var. *raddeana* (Beck) Beck, is relatively distant to *O. alba*. Recently, the name *O. raddeana* was changed as a new species, *O. campanulae* Rätzel et al. (Rätzel et al. 2018), but in our opinion – despite the correction of their studies on this taxon – the traditional interpretation of the name of *O. raddeana* is valid and must be retained in order to avoid disadvantageous nomenclatural changes entailed by the strict application of the rules (Turland et al. 2018: Art. 14). For this reason, the authors of the new species (*O. campanulae*) should propose the conservation of the name *O. raddeana* according to ICN, even though other names, e.g., *O. glabrata* C.A. Mey could have priority.

Within the *O.* subsect. *Inflatae* Beck, *O. grenieri* (parasitic on mainly *Lactuca* L.) is clearly distinguished morphologically and phylogenetically from related species (*O. cernua* and *O. cumana*), as has already been shown (Piwowarczyk et al. 2015). The taxonomic relationships of the polymorphous species *O. cernua* and *O. cumana* are not entirely clear. Some researchers recognised *O. cumana* as a separate species, and others as varieties or subspecies of *O. cernua*. *O. cumana* parasitises cultivated plants, mainly *Helianthus* L. and Solanaceae (*Lycopersicon* Mill. L., *Nicotiana* L.). The problem with identifications arises when *O. cumana* parasitises wild species, i.e., *Artemisia* L. (sometimes *Xanthium* L.), like the closely allied typical *O. cernua*, because morphological differences between these two species cannot always be easily seen.

Species from subsect. *Minores* Teryokhin and subsect. *Speciosae* Teryokhin are highly polymorphic, especially regarding colour, inflorescence length and variability of flower, as well as range of hosts. In our research we used Caucasian samples of *O. laxissima* (a parasite of various tree species, i.e., *Fraxinus* L., *Carpinus* L., *Punica* L., *Robinia* L.), *O. owerinii* (a parasite of herbaceous hosts, i.e., *Trifolium* L., *Vicia* L.), and *O. minor* (samples from *Chondrilla* L. and *Lactuca* hosts) (Figs 1, 2). Tzvelev considered that *O. owerinii* is a polymorphic species, represented by several races, which can be treated as distinct species. *O. owerinii* is probably closely related to *O. crenata* Forssk. and replaces it in the upper montane zone of Asia Minor and Caucasian territories (Novopokrovskij and Tzvelev 1958). However, the typical *O. crenata* has not been confirmed in the Caucasus. *O. laxissima* seems to be very similar morphologically to *O. transcaucasica* Tzvel., apparently also a parasite on shrubs and trees and described in a hornbeam-oak forest from the Shemackha region in Azerbaijan by

Tzvelev (1957). Rätzel and Uhlich (2004) mistakenly assigned *O. laxissima* to the *O.* subsect. *Galeatae* sensu Teryokhin (*O.* trib. *Galeatae* sensu Beck). Morphologically (Piwowarczyk et al. 2019) and molecularly (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2), the species clearly belongs to the *O.* subsect. *Speciosae* (*O.* trib./Grex *Speciosae* sensu Beck). *O. laxissima* can be confused by an inexperienced researcher with *O. owerinii*, especially in the herbarium materials without details about the host. Our results support this affinity (Figs 1, 2, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). Moreover, *O. laxissima*, *O. owerinii* seem to be phylogenetically similar, especially on ITS trees, with several other species. The recent diversification of these lineages (Tables 1, 2, Fig. 3) could explain why some of these species have not entirely diverged.

Molecular studies do not indicate the validity of dividing species into subsect. *Speciosae* because the species included here are both very morphologically and genetically similar to the subsect. *Minores*. Similar conclusions can be used to merit the inclusion of *Vitellinae* Teryokhin, *Hederae* Teryokhin, and *Camptolepides* Teryokhin in separate subsections when they are clearly similar to species from the subsect. *Minores* and *Inflatae* (respectively). The results presented here suggest that the currently distinguished systematic division of *Orobanche*-based morphology is frequently inconsistent with the phylogenetic studies and thus needs revision, regarding both phenotypic traits and molecular analyses, for example, the heterogeneous subsect. *Curvatae* is clearly resolved as polyphyletic (Fig. 1). Furthermore, our knowledge of some poorly understood species in sections in the *Phelipanche* genus requires further taxonomic, field (especially in Western and Central Asia), and molecular research.

Biogeography

We found strong support for Western Asia as the centre of origin for large subclades of *Phelipanche*, *Orobanche*, and *Cistanche* (Table 1, Fig. 3), followed by both diversification *in situ* as well as dispersal out of this region over the last 1–2 million years (Table 2, Fig. 3). This supports previous hypotheses that Western Asia, especially the Caucasus region and nearby high mountains in the Middle East and Western Asia, as important centres of origin for Eurasian Orobancheae (Rätzel and Uhlich 2004), despite our uncertainty in the biogeography of the ultimate common ancestor of *Orobanche* + *Phelipanche* + *Aphyllon*. About 30 species of holoparasitic Orobancheae are known as endemic (15 confirmed species) to the Caucasus or have most of their range there. High-mountain genetic lineages with subalpine habitats are especially unique, such as species from the ser. *Krylowianae* (*O. inulae*, *O. mlokosiewiczii*, *O. cicerbitae*, and *O. arpica*), as well as *O. gamosepala*, *O. grossheimii*, *O. raddeana*, *O. javakhetica*, *O. schelkovnikovii*, and *O. zajaciorum*. The extant diversity in this region is a combination of clades of recently diverged (i.e., neoendemic) species such as those in ser. *Krylowianae*, as well as species on relatively long branches without close relatives (e.g., *O. raddeana*, *O. javakhetica* and *Phelipanche bungeana* (Beck) Soják) or species-poor clades subtended by long branches (e.g., *Phelypaea*, or *O. anatolica* + *O. colorata*; Table 2). Thus, the Caucasus region may

be considered as one of mixed endemism for parasitic Orobancheae (sensu Mishler et al. 2014). However, we do not have the temporal resolution to determine if these long-branch parasite species have always been range-limited, or have gone through expansions and subsequent contraction due to climatic or other ecological shifts.

The broader floristic and geological history of the Caucasus and high mountain region does provide some clues to the processes that its status as a centre of extant diversity, a centre of origin for large portions of this diversity, and potentially a region of mixed endemism for holoparasitic Orobancheae. The Caucasus has an unusually high proportion of endemic and relict species for a continental, non-tropical region (Tarkhnishvili 2014). Approximately 25% of vascular plant species found in the Caucasus are endemic, as well as unique vegetation types such as Colchic and Hyrcanian forests with relict tree species (Kikvidze and Oshawa 2001). The Caucasian oreoxerophytic flora has a historical connection with the Mediterranean and Asia Minor due to Pleistocene migration from Asia Minor eastwards. Following the retreat of the glaciers, xerophytic flora from the Irano-Turanian region and mountains of Central Asia also migrated to the Caucasus, with simultaneous degradation of the mesothermophilous forest vegetation (Nakhutsrishvili and Abdaladze 2005). The southern part of the Caucasus in Armenia is also located in the Irano-Anatolian biodiversity hotspot (as well as northeastern Iran and Iraq, and central and eastern Turkey). This is the only global biodiversity hotspot entirely inside Southwest Asia (Noroozi et al. 2018), with over 40% endemic plant species (Mittermeier et al. 2005). Longstanding explanations for the unique flora in this region highlight the role the Caucasus and high mountains have played as a refugium for many elements of the pre-glacial Tertiaty flora during cooling of the Pliocene and Pleistocene, and aridification during the Upper Pleistocene and Holocene (Kuznetsov 1909; Fedorov 1952; Kharadze 1960). Along with aridification, another important contributor to the flora was the Pleistocene migration of plants from Asia Minor and post-glacial xerophytic migrants from the mountains of central Asia (Nakhutsrishvili and Abdaladze 2005; Zernov 2006). Characteristics such as a dissected, heterogeneous topography, a large altitudinal range, and a relatively mild climate subsequently helped preserve these floristic elements (Kikvidze and Oshawa 2001). Consistent with this pattern, some of the oldest lineages of Orobancheae that include extant Caucasus endemics were probably also found in Western Asia at their time of divergence during the Pliocene and Pleistocene (Tables 1, 2, Fig. 3). These refugia may also explain the disjunct ranges of many *Orobanche* and *Phelipanche* species present both in Western Asia (especially the Caucasus) and further west in Europe, such as *O. grenieri*, *P. cernua*, *P. portoilicitana* and the Carpathian mountain species *O. flava* (Piwowarczyk et al. 2019).

However, we also found a number of very recent diversification events in Orobancheae, pointing to recent *in situ* speciation as a complementary mechanism that explains the high levels of endemism in this region (Table 2, Fig. 3). For example, the diversification of the *Orobanche laxissima* + *O. owerinii* + *O. transcaucasica* clade was exceptionally recent (most likely in the last 150,000 years, but this may exceed the precision of our analysis). Many species of Orobanchaceae are associated with

calcareous habitats that probably favour speciation and are abundant throughout the Caucasus (Kikvidze and Oshawa 2001). Many of the same geographical and ecological factors that have made western Asia a refugium for *Orobanche* and *Phelipanche* species also likely contributed to subsequent diversification, such as topographic and habitat heterogeneity, and a diverse flora of potential host species, many of which are also endemic or of limited range. In the postglacial, continental climate of western Asia, suitable xerophytic habitat has replaced forest in many areas. The expansion of steppe, subalpine, subalpine or steppe communities – and more importantly the potential host plants occupying them – may explain the diversification of Orobancheae in this region.

By contrast, the biodiversity of *Orobanche* and *Cistanche* that evolved in Europe and especially in the Mediterranean Basin appears to have done so more recently than that in West Asia, although we cannot confidently infer ancestral states of lineages greater than 5 million years (Table 1, Fig. 3). Iberia, Italy, and the Balkans are three well-studied refugial regions of Mediterranean Europe where thermophilic species persisted through glacial periods, (Bennett et al. 1991; Comes and Kadereit 1998, 2003; Taberlet et al. 1998; Hewitt 1999; Habel et al. 2014) however our analysis is not fine-grained enough to distinguish among them. Collectively, the Mediterranean region has been recognised as another of the world hotspots of biodiversity, with more than 25,000 known vascular plant species, mostly endemic. The Mediterranean flora consists of low species-genus ratios, with many primarily long-lived taxa restricted to island or mountain habitats, probably as paleoendemics of likely Tertiary origin (Blondel and Aronson 1999). This unique plant diversity and endemism are the result of several key factors: paleogeological and climatic history, biogeography, and ecogeographical heterogeneity, with evidence that adaptive radiation has taken place relatively recently (Blondel and Aronson 1999; Comes 2004). Several radiations within *Orobanche* and *Cistanche* have taken place over the last million years (Table 1, Fig. 3), but the clade of *O. foetida* Poir., *O. densiflora* Salzm. ex Bertol., *O. sanguinea* C. Presl, *O. austrohispanica* M.J.Y. Foley, and the more widespread *O. gracilis* likely began diversifying earlier, about 2–3 million years ago. Like the origin of many other Mediterranean flora elements, the ancestors of each of these clades came from western Asia, then moved into the Mediterranean and diversified (Fig. 3; Blondel and Aronson 1999). An alternative hypothesis, more strongly supported for *Phelipanche* than *Orobanche*, is that a widespread ancestor growing across Europe and western Asia may have given rise to both Western Asian and European/Mediterranean clades (the second most probable ancestral states of many *Phelipanche* and *Orobanche* common ancestors, Suppl. material 4: data S1 and Suppl. material 5: data S2).

We conclude with a cautionary note that we were not able to exhaustively sample the Orobancheae, in particular certain species of *Orobanche*, such as *O.* sect. *Kotschyinae* Teryokhin from the Middle East and western and central Asia. The addition of certain other lineages, such as species in *O.* subsect. *Coerulescentes* Teryokhin would likely strengthen the importance of diversification in East Asia. Finally, our results within *Cistanche* are sensitive to changing taxonomic concepts.

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The DNA sequence data generated and analysed during this work are available in the GenBank repository [https://www.ncbi.nlm.nih.gov/genbank/]. Alignments for the biogeography data generated or analysed during this study are available in Suppl. material 4.

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Supplementary material 1

Table S1

Authors: Renata Piwowarczyk, Adam C. Schneider, Grzegorz Góralski, Dagmara Kwolek, Magdalena Denysenko-Bennett, Anna Burda, Karolina Ruraż, Andrzej J. Joachimiak, Óscar Sánchez Pedraja

Data type: List of taxa and sequences analysed

Explanation note: List of taxa and sequences analysed (* sequences obtained from GenBank).

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Link: https://doi.org/10.3897/phytokeys.174.62524.suppl1

Supplementary material 2

Figure S1

Authors: Renata Piwowarczyk, Adam C. Schneider, Grzegorz Góralski, Dagmara Kwolek, Magdalena Denysenko-Bennett, Anna Burda, Karolina Ruraż, Andrzej J. Joachimiak, Óscar Sánchez Pedraja

Data type: phylogenetic tree

Explanation note: Rooted Bayesian phylogenetic consensus tree inferred from ITS sequences (outgroup: *Lindenbergia sinaica*). Numbers near branches show Bayesian posterior probabilities ≥ 0.75. The bar represents the amount of genetic change (nucleotide substitutions per site). Species names, the country of origin, host species (if available) and GenBank accession number are included on the phylogeny tip labels.

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Link: https://doi.org/10.3897/phytokeys.174.62524.suppl2

Supplementary material 3

Figure S2

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Data type: phylogenetic tree

Explanation note: Rooted Bayesian phylogenetic consensus tree constructed using plastid *trnL–trnF* spacer sequences (outgroup: *Lindenbergia sinaica*). Numbers near branches show Bayesian posterior probabilities ≥ 0.75. The bar represents the amount of genetic change (nucleotide substitutions per site). Species names, the country of origin, host species (if available) and GenBank accession number are included on the phylogeny tip labels.

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Link: https://doi.org/10.3897/phytokeys.174.62524.suppl3

Supplementary material 4

Data S1

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Data type: DNA sequence alignment (fasta format)

Explanation note: Sequence matrix used for biogeography analysis.

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Link: https://doi.org/10.3897/phytokeys.174.62524.suppl4

Supplementary material 5

Data S2

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Data type: tree file

Explanation note: Historical biogeography reconstruction (maximum clade credibility tree).

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