PhytoKeys 135: 1–10 (2019)
doi: 10.3897/phytokeys.135.38531
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Ottelia fengshanensis, a new bisexual species of *Ottelia* (Hydrocharitaceae) from southwestern China

Zhi-Zhong Li[1,2], Shuang Wu[3], Chun-Yu Zou[4], Yan Liu[4], Guang-Wan Hu[1,5], Samuli Lehtonen[6], Qing-Feng Wang[1,5], Jin-Ming Chen[1]

1 *CAS Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, CN-430074, China* **2** *University of Chinese Academy of Sciences, Beijing, CN-100049, China* **3** *Guangxi Association for Science and Technology, CN-530022, China* **4** *Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, CN-541006, China* **5** *Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan, CN-430074, China* **6** *Herbarium, Biodiversity Unit, University of Turku FI-20014 Turku, Finland*

Corresponding author: *Qing-Feng Wang* (qfwang@wbgcas.cn); *Jin-Ming Chen* (jmchen@wbgcas.cn)

Academic editor: *Peter Boyce* | Received 25 July 2019 | Accepted 10 September 2019 | Published 30 October 2019

Citation: Li Z-Z, Wu S, Zou C-Y, Liu Y, Hu G-W, Lehtonen S, Wang Q-F, Chen J-M (2019) *Ottelia fengshanensis*, a new bisexual species of *Ottelia* (Hydrocharitaceae) from southwestern China. PhytoKeys 135: 1–10. https://doi.org/10.3897/phytokeys.135.38531

Abstract

Ottelia fengshanensis, a new species (Hydrocharitaceae) from southwest China is here described and illustrated. Comparing its morphological features to putative close relatives *O. guanyangensis*, it has 3–4 flowers (vs. 2–5) each spathe, hexagonal-cylindric fruit, white styles (vs. yellow), green leaves (vs. dark green) and fruit tiny winged (vs. winged obviously). Molecular phylogenetic investigation of four DNA sequences (ITS, *rbc*L, *trn*K5' intron and *trn*S-*trn*G) and the Poisson Tree Processes model for species delimitation (PTP) analysis, further resolves *O. fengshanensis* as a new species that is close to *O. guanyangensis* with distinct support.

Keywords

karst, bisexual flowers, molecular phylogeny

Introduction

Ottelia Persoon (1805:1) has about 22 species and is widely distributed in the tropical, subtropical and temperate regions. In comparison with other genera within the family Hydrocharitaceae, *Ottelia* is morphologically complex and variable, e.g. the leaf

type of the genus is exceptionally erratic even within an individual depending on the developmental stage, as well as within the varieties or populations (Li et al. 2018). The flower sexuality varies within species and flowers can be either bisexual or unisexual. Southwestern China possesses complex terrain and various ecosystems and is a center of diversity for *Ottelia* species (Chen et al. 2017, Zhai et al. 2018). To date, six species and three varieties of *O. acuminata* Dandy (1934: 132) have been recorded from the area with narrowly endemic distribution in karst rivers or lakes. Among these, just three species, *O. alismoides* Persoon (1805: 273), *O. balansae* Dandy (1934: 137) and *O. guanyangensis* Z.Z. Li, Q.F. Wang & S. Wu (2018: 294) are bisexual and can only be found in specific karst regions, except for the widespread species *O. alismoides* (Cook et al. 1984, Cook and Urmi-Konig 1984, Li 1981).

In 2017–2018, we found and reported a new bisexual species *O. guanyangensis* in Guilin City, China (Li et al. 2018). We deemed that there are some previously undetected potentially new *Ottelia* species in Guangxi province's karst steams (Fig. 1). We made further aquatic plant investigations in Guangxi province, China, in 2018. From the Fengshan County, we found once again a species with bisexual flowers which generally appeared to be like *O. balansae*. Based on investigations of herbarium specimens in GXMG, HIB, IBSC, KUN and PE, and literature review, only three bisexual species of *Ottelia* are known from China. These are *O. guanyangensis*, a species described in 2018 (Li et al. 2018), *O. balansae*, and *O. alismoides*, the latter two recorded from "Flora of China". Compared to the recorded three bisexual species, it was interesting that the population from Fengshan county had some unique flowers (e.g. white styles and over three flowers each spathe) and leaf traits (e.g. triplinerved with obvious cross-

Figure 1. Distribution record of *Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang (red triangle) from Fengshan county, Guangxi province, China.

veins). We transplanted several individuals to the greenhouse at Wuhan Botanical Garden, Chinese Academy of Sciences, to observe the growth. Here we formally describe and discuss this taxon as a new species based on careful morphological observations and molecular phylogeny.

Material and methods

Morphological study

The morphological characteristics of the new species were collected during fieldwork in July 2018. We randomly selected 10 individuals, took pictures of each part and measured the characteristics of flowers, leaves and fruits (Fig. 2). The pollen grains of new species were gold-coated, and photographed using a Hitachi S-800 SEM system at Wuhan Botanical Garden, CAS. Simultaneously, we collected voucher specimens and several fresh leaves were dried using silica gel for DNA extraction. For further detailed morphological analysis, we transplanted five living individuals to a greenhouse at Wuhan Botanical Garden. We also observed the characteristics of flowers, leaves and fruits of these two bisexual species in our greenhouse for further comparative analysis (Table 1).

Table 1. The voucher information and GenBank accession numbers for the sequences of internal transcribed spacer (ITS) and three cp regions (*trn*S-*trn*G, *rbc*L and *trn*K5' intron) in the present study.

Taxon	Individual code	Locality	Voucher no.	Acceession No.			
				ITS	*rbc*L	*trn*K5' intron	*trn*S-*trn*G
O. acuminata var. *jingxiensis*	01	Jingxi, Guangxi	HIB-Otte010	MG751780	MH257624	MH257642	MH257660
	12	Debao, Guangxi	HIB-Otte009	MG751781	MH257628	MH257646	MH257664
	19	Du'an, Guangxi	HIB-Otte012	MG751782	MH257630	MH257648	MH257666
O. acuminata var. *crispa*	10_1, 10_2	Luguhu, Yunan	HIB-Otte011	MG751784/ MG751785	MH257626/ MH257627	MH257644/ MH257645	MH257662/ MH257663
O. acuminata var. *acuminata*	9	Heqing, Yunan	HIB-Otte003	MG751786	MH257625	MH257643	MH257661
	15	Jianchuan, Yunan	HIB-Otte006	MG751787	MH257637	MH257655	MH257673
	30	Caohai, Guizhou	HIB-Otte014	MG751788	MH257633	MH257651	MH257669
O. acuminata var. *lunanensis*	16	Shilin, Yunnan	HIB-Otte008	MG751789	MH257629	MH257647	MH257665
O. acuminata var. *songmingensis*	21_1, 21_2	Songming, Yunnan	HIB-Otte007	MG751790/ MG751791	MH257631/ MH257632	MH257649/ MH257650	MH257667/ MH257668
O. balansae	29	Huaxi,Guizhou	HIB-Otte005	MG751792	MH257634	MH257652	MH257670
O. emersa	41	Guigang, Guangxi	HIB-Otte004	MG751794	MH257638	MH257656	MH257674
O. cordata	40	Haikou, Hainan	HIB-Otte001	MG751795	MH257639	MH257657	MH257675
O. alismoides	42	Changping, Fujian	HIB-Otte002	MG751796	MH257640	MH257658	MH257676
O. guanyangensis	32	Guanyang, Guangxi	HIB-Otte015	MG751797	MH257635	MH257653	MH257671
	34	Guanyang, Guangxi	HIB-Otte016	MG751798	MH257636	MH257654	MH257672
O. fengshanensis	35	Fengshan, Guangxi	HIB-lzz51	MK531550	MK531552	MK531553	MK531551
B. japonica		Wuyishan, Fujian	HIB-Bly001	MG751799	MH257641	MH257659	MH257677

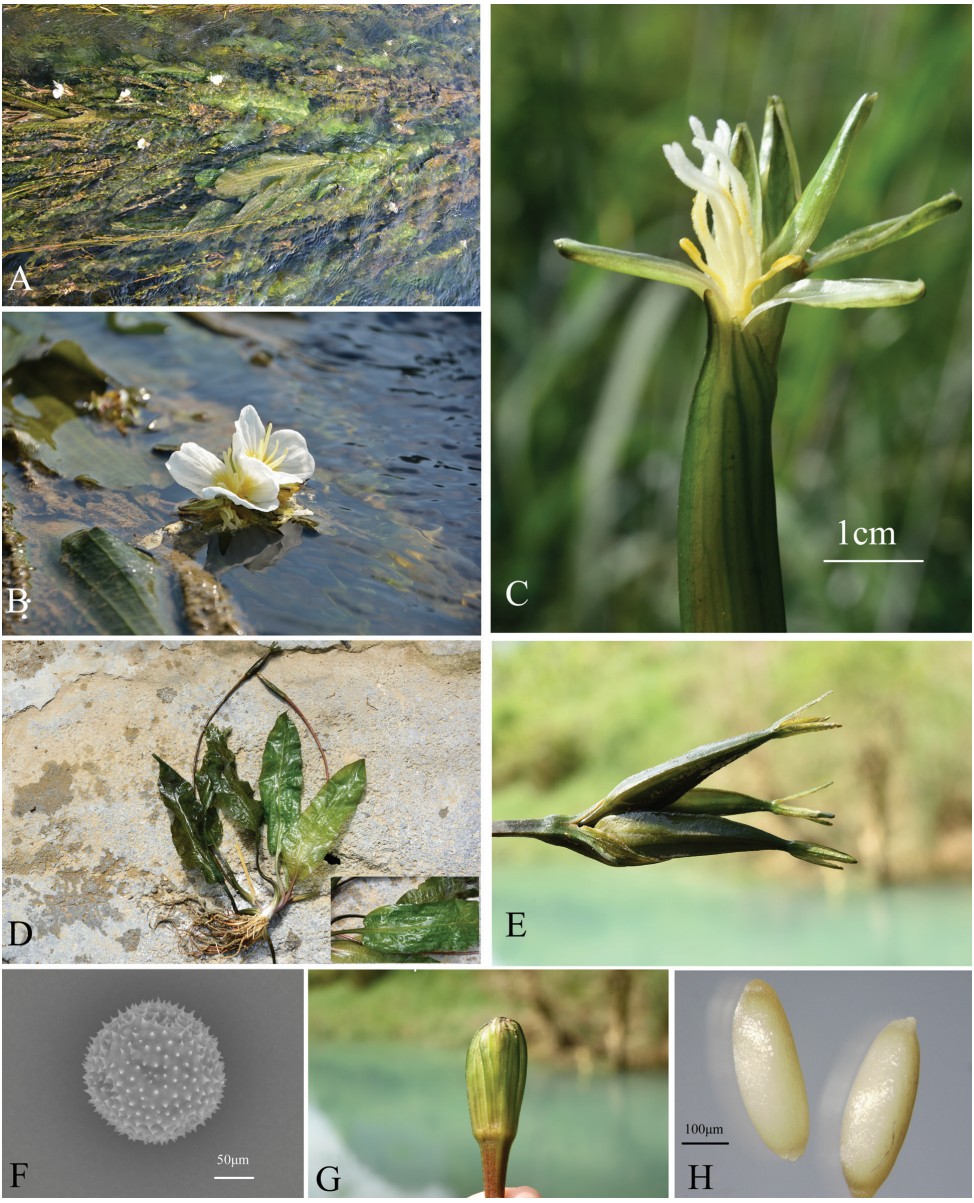

Figure 2. *Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang. **A** Habitat **B** flowering plant **C** bisexual flower with red-green sepals **D** individual and leaf: triplinerved with conspicuous cross veins **E** fruit: Hexagonal-cylindric with tiny wings **F** the character of pollens by SEM **G** spathe **H** seeds.

Phylogenetic analysis

Total genomic DNA of one sample, collected from Fengshan county, Hechi city, Guangxi province, was extracted following Li et al. (2018). One nuclear DNA region (ITS) and three chloroplast DNA regions (*trn*S-*trn*G, *rbc*L and *trn*K5' intron) were sequenced; the primers and PCR protocols followed Li et al. (2018). The same sequence

regions from other species were downloaded from the NCBI (Table 2). The sequence alignments were made using MAFFT with default settings (Kuraku et al. 2013). The best nucleotide substitution model was detected using jModeltest 2.1.4 (Darriba et al. 2012) with the Akaike Information Criterion (AIC). The Maximum Likelihood (ML) analysis was made using IQtree with 5000 bootstrap replicates (Nguyen et al. 2003). The Bayesian Inference (BI) was analyzed by MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2015), with 20,000,000 generations and four chains run with sampling after every 2000 generations. The first 25% of generations were discarded and a majority rule consensus tree (> 50%) was computed from the remaining trees. In order to test molecular support for species delimitation in *Ottelia*, the Poisson Tree Processes model for species delimitation (PTP) was applied to the tree with the following parameters: 500,000 generations; thinning: 100; burnin: 0.1 and seed: 123 (Zhang et al. 2013).

Results and discussion

The comparison among three bisexual species, *O. fengshanensis*, *O. guanyangensis* and *O. balansae*, is presented in Table 2. The new species had unique features, including the number of flowers, white styles, trinerved venation with distinct cross veins and longer leaf shape.

Morphological characters distinguish *O. fengshanensis* from the three bisexual species. For *O. alismoides*, there is only one flower in each spathe and it is easy to distinguish from the new species. However, *O. guanyangensis* and *O. balansae*, which are

Table 2. Morphological characters comparison among *Ottelia fengshanensis*, *Ottelia guanyangensis* and *Ottelia balansae*.

Characters	*Ottelia fengshanensis*	*Ottelia guanyangensis*	*Ottelia balansae*
Flowers	bisexual	bisexual	bisexual
Sepals	1.0–1.5 cm, red green	1.0–1.5 cm long, red brown	2.0–2.5 cm long, green
Stamens	3;filaments 3.0–5.0 mm long	3; filaments 5.0–7.0 mm	3; filaments 4.0–5.0 mm
Ovary	5–10 cm long, hexagonal-cylindric to cylinder	4–5 cm long, hexagonal-cylindric	3.5–5.0 cm long, triangularcylindric
Styles	3, bifid to base,white	3, bifid nearly to base,yellow	3.5–5.0 cm long, yellow
Spathe	3-4 (3) flowered	2–5 flowered	3–11 flowered
Leaf shape	Linear or oblong, 30–70 × 8–14 cm,base rounded, apex acute or obtuse;petiole 8.0–10.0 cm long	linear, 15–50 × 2.5–4.0 cm, base rounded, apex acute, petiole 8.0–13.0 cm long	oblong or ovate, 20–40 × 6.0–8.0 cm, base truncate, rounded, or cordate, apex acute or rounded, petiole ca. 20 cm long
Texture	green, opaque, thick ca. 0.8 mm	dark green, opaque, thick ca. 1.2 mm	green, translucent, thick ca. 0.5 mm
Venation	trinerved with obvious cross veins, distance 4.0–6.0 cm to base, longitudinal veins 9	trinerved with obvious cross veins, distance 4.0–6.0 cm to base, longitudinal veins 9	basal veins, longitudinal veins 7
Fruit	hexagonal-cylindric, winged unobviously	hexagonal-cylindric, winged	narrowly elliptic, unwinged
Seed	fusiform, ca. 1.0 mm long	fusiform, ca. 1.5 mm long	cylindric to fusiform, ca. 3.0 mm long
Pollen	spheroidal, inaperturate, ca. 40 × 40 μm	spheroidal, inaperturate, ca. 35 × 45 μm	spheroidal, inaperturate, ca. 49 × 53 μm
Flowering time	April to November	April to October	June to November

distributed in Guangxi province and Guizhou province respectively, are closest to the new species. The critical diagnostic characters of *O. fengshanensis* include having white styles, longer leaf shape and number of flowers in each spathe. Moreover, these three species are also isolated geographically, *O. fengshanensis* was only found in Fengshan county, but *O. guanyangensis* was found in Guilin city. *O. balansae* was only recorded in Guizhou province based on a recent survey. Karst terrain will play an important role in species divergence in this lineage.

Four sequence regions (ITS, *trn*S-*trn*G, *rbc*L and *trn*K5' intron) were aligned and concatenated into a 3623 bp sequence. 605 variable nucleotides were detected. Two clades were displayed with high support (BS= 70, PP= 0.7). PTP analysis further recognized four species with *O. fengshanensis* having the highest support (0.678). Based on phylogenetic analyses, *O. fengshanensis* was resolved as sister to *O. guanyangensis* with high support (BS= 100, PP= 1.0) and only distantly related to *O. balansae*, which clusters together with *O. acuminata* (Fig. 4), and based on PTP analysis, *O. balansae* was not supported as a species, but was more likely to be treated as a bisexual variety of *O. acuminata*. In combination, the morphological and molecular phylogenetic analyses support that *O. fengshanensis* is a distinct species closely related to *O. guanyangensis*, a species also distributed in Guangxi province.

Ottelia possesses complex floral traits and may have bisexual and unisexual flowers. Based on the previous studies (He 1991, Chen et al. 2012) bisexual flowers have evolved multiple times in *Ottelia*. Here we report a new bisexual species *O. fengshanensis* and verify that bisexual flower indeed has multiple origins in *Ottelia*. *Ottelia fengshanensis* probably has a common ancestor with the unisexual *O. acuminata* var. *songmingensis*. Besides, we also suggest that *O. balansae* should be treated as a variety of *O. acuminata*. This point has also been put forward by Yu Ito et al. (2019). It will also help us have a better understanding of the diversity and evolution of sex evolution in *Ottelia*.

Description of the new species

***Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang, sp. nov.**
urn:lsid:ipni.org:names:77202741-1
Fig. 3

Description. The new species is similar to *Ottelia guanyangensis* in having bisexual flowers, three stamens, but differs through having (3)-4 flowers in each spathe (vs. 2–5), white styles (vs. yellow), green leaves (vs. dark green) and by fruits which are tiny winged (vs. obviously winged).

Type. CHINA. Guangxi, Hechi City, Fengshan County, elev. 507 m, 24°34'20"N, 107°10'17"E, 11 September 2018, *Z. Z. Li & S. Wu-Otte51* (holotype HIB-lzz51!).

Annual or perennial herb. Rhizome, short. Leaves entirely submerged, dark green and opaque, linear or oblong, 30–70 × 8–14 cm, base rounded, apex acute or obtuse; longitudinal veins 9; midrib conspicuous, stretched to the apex, becoming trinerved with obvious cross-veins at a distance of 5–7 cm from the base; petiole smooth, green, 8.0–10.0 cm long,

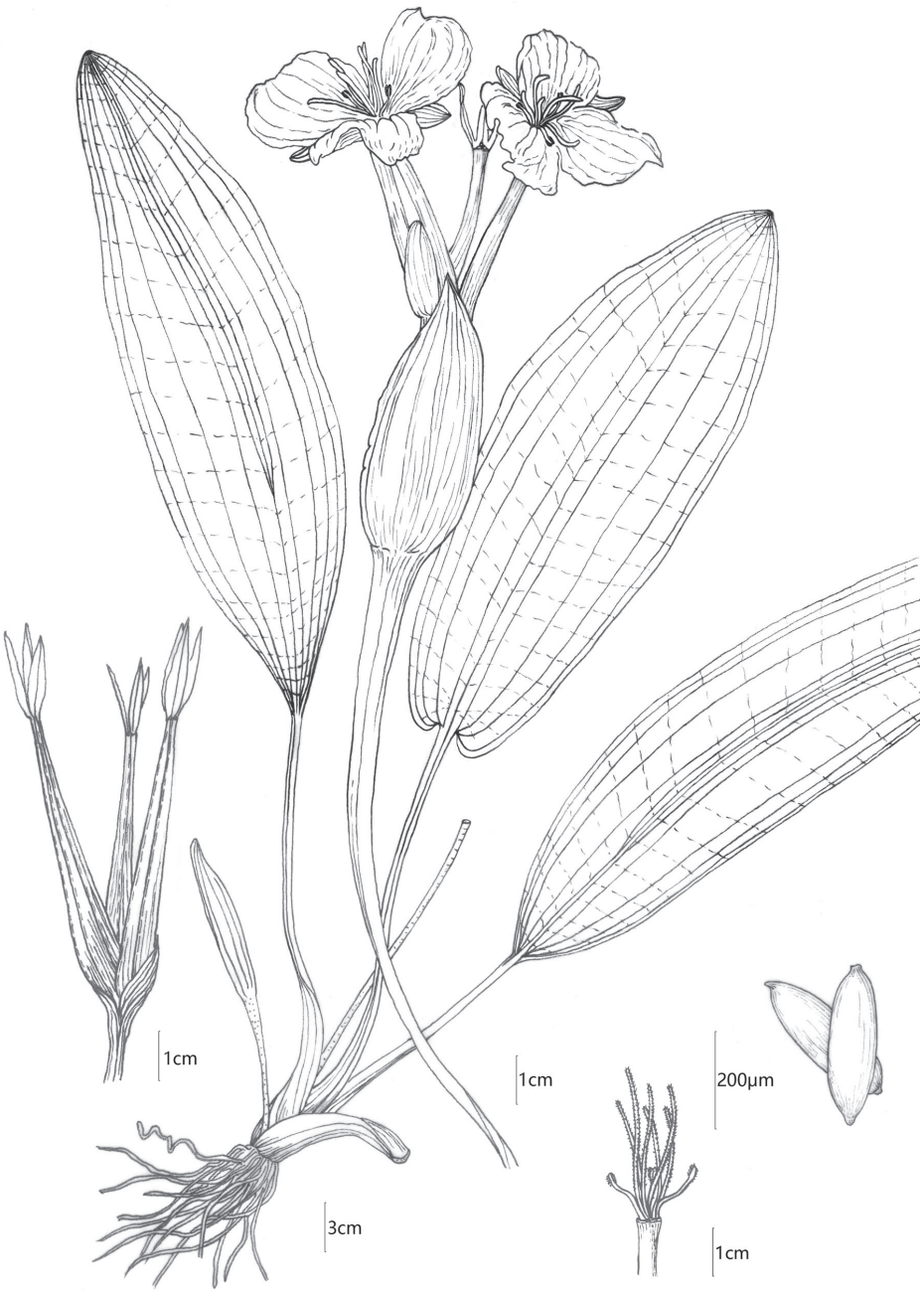

Figure 3. Illustration of *Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang. Drawn by Shuai-Jie Li.

the base expanded into a sheath. Spathe oblate, ca 3. 0 × ca. 3.5 cm, warty along edges or smooth, longitudinally ribbed and winged on the lateral margins, containing 3–4 (3) flowers; flowers bisexual; sepals reddish green, 1.0–1.5 × ca. 0.5 cm, with longitudinal ribs; petals white with yellow base, obovate, ca. 2.0 × ca. 2.0–2.5 cm, with longitudinal pleats;

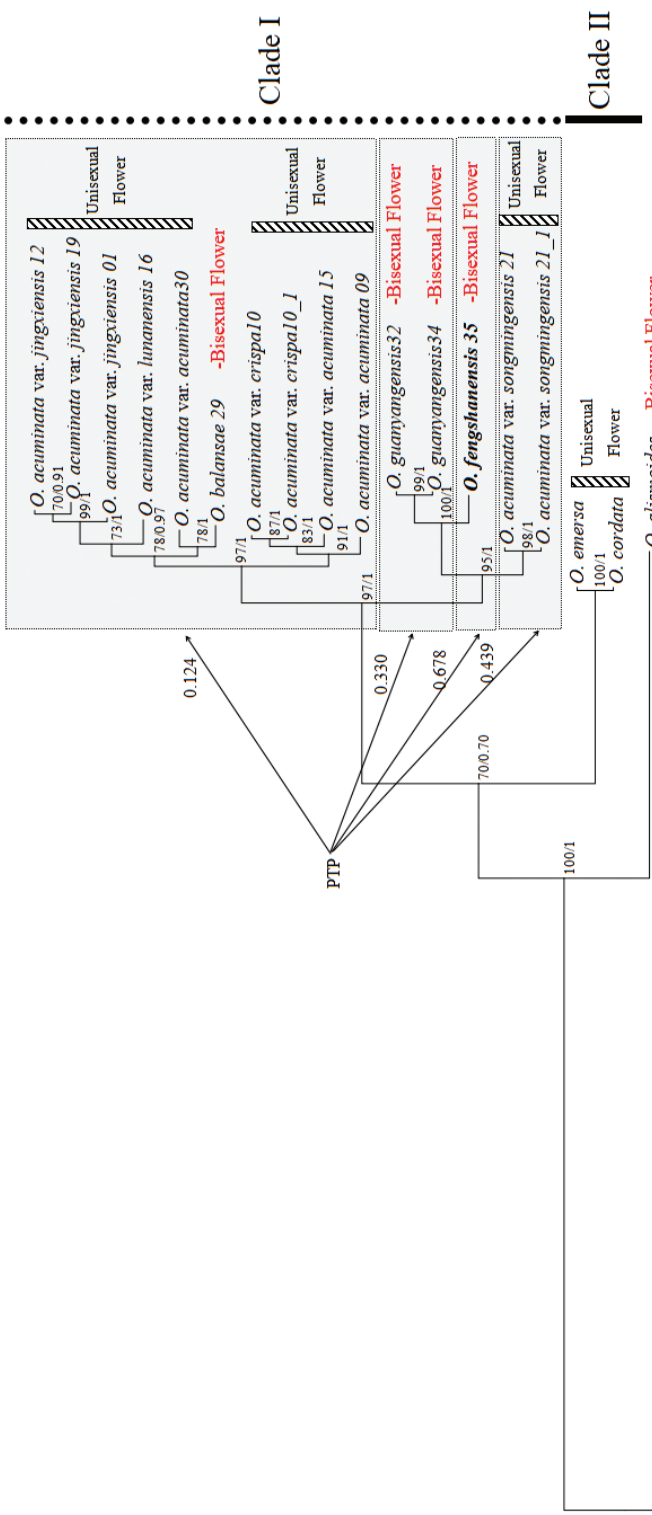

Figure 4. Phylogenetic tree and PTP analysis of *O. fengshanensis* sp. nov. and *Blyxa japonica* as an outgroup. Posterior probabilities (PP > 0.70) and bootstrap values (BS > 70) based on Bayesian Inference and maximum likelihood (ML) analysis are shown above the branches.

stamens 3, opposite to sepals, anthers elliptic, connective obscure, filaments 3.0–5.0 mm long; glands 3, 0.5–1.0 × 0.5–1.5 mm, opposite to petals, pale yellow. Ovary hexagonal-cylindric to the cylinder, 5–10 cm long, with 3 carpels; styles 3, white, slender and hairy, 1.2–1.5 cm long, stigma bifid, divided to base; stigmas 6, liner and hairy, ca. 8 mm long. Fruit a hexagonal-cylindric capsule, with 6 inconspicuous wings, dark green, with persistent calyx, 4.0–9.0 cm × ca. 6.5 mm, always longer than spathe. Seeds numerous, fusiform, ca. 1.0 mm long, both ends hairy. Pollen, subglobose, ca. 40μm in diam, with spiny granules.

Distribution and habitat. *Ottelia fengshanensis* is known from a single population in Fengshan County, Guangxi Province, China. The species inhabits a karst river less than 1.5 m in depth. Due to the complex underground river system in the karst region, it is probable that the species occurs in nearby areas as well.

Conservation status. Only one population of new species was found at Fengshan County, Guangxi Province, China. Although it might be distributed in adjacent karst rivers. Until now, approximately 50–100 individuals were found in a single population. However, there is not enough information on population size and dynamics. According to the IUCN Red List Categories and Criteria (IUCN 2017), we suggested that the species be evaluated as Data Deficient (DD).

Phenology. The new species was found in flower from April to November.

Etymology. The epithet is derived from the name of Fengshan County, which is the only known locality of occurrence.

Other specimens examined (paratypes). CHINA. Guangxi, Hechi City, Fengshan County, elev. 507 m, 11 September 2018, *Z. Z. Li & S. Wu Otte 056* (HIB!)

Acknowledgments

This study was supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB31000000), Wuhan Botanical Garden (CAS) (No. Y655261W03), and the Special Funds for the Young Scholars of Taxonomy of the Chinese Academy of Sciences (ZSBR-013).

References

Chen LY, Chen JM, Gituru WR, Wang QF (2012) Generic phylogeny, historical biogeography and character evolution of the cosmopolitan aquatic plant family Hydrocharitaceae. BMC Evolutionary Biology 12(1): e30. https://doi.org/10.1186/1471-2148-12-30

Chen JM, Du ZY, Long ZC, Gichira AW, Wang QF (2017) Molecular divergence among varieties of *Ottelia acuminata* (Hydrocharitaceae) in the Yunnan-Guizhou Plateau. Aquatic Botany 140: 62–68. https://doi.org/10.1016/j.aquabot.2017.03.001

Cook CDK, Urmi-Konig K (1984) A revision of the genus *Ottelia* (Hydrocharitaceae). 2. The species of Eurasia, Australasia and America. Aquatic Botany 20(1–2): 131–177. https://doi.org/10.1016/0304-3770(84)90033-0

Cook CDK, Symoens JJ, Urmi-Konig K (1984) A revision of the genus *Ottelia* (Hydrocharitaceae). 1. Generic considerations. Aquatic Botany 18(3): 263–274. https://doi.org/10.1016/0304-3770(84)90068-8

Dandy JE (1934) Notes on Hydrocharitaceae. 1. The delimitation and subdivision of *Ottelia*. Journal of Botany (London) 72: 132–137.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

He JB (1991) Systematic Botanical and Biosystematic Studies on Ottelia in China. Wuhan University Press, Wuhan, 5–20.

Ito Y, Tanaka N, Barfod AS, Bogner J, Li J, Yano O, Gale SW (2019) Molecular phylogenetic species delimitation in the aquatic genus *Ottelia* (Hydrocharitaceae) reveals cryptic diversity within a widespread species. Journal of Plant Research 132(3): 335–344. https://doi.org/10.1007/s10265-019-01109-7

IUCN (2017) Guidelines for using the IUCN Red List categories and criteria. Version 13. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) leaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Research 41(W1): 22–28. https://doi.org/10.1093/nar/gkt389

Li H (1981) Classification, distribution and phylogeny of the genus *Ottelia*. Zhiwu Fenlei Xuebao 19: 29–42.

Li ZZ, Liao K, Zou CY, Liu Y, Hu GW, Wang QF, Chen JM (2018) *Ottelia guanyangensis* (Hydrocharitaceae), a new species from southwestern China. Phytotaxa 361(3): 294–300. https://doi.org/10.11646/phytotaxa.361.3.5

Nguyen LT, Schmidt HA, Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Persoon CH (1805) Synopsis Plantarum: seu Enchiridium botanicum, complectens enumerationem systematicam specierum hucusque cognitarum. vol. 1, 1. Parisiis Lutetiorum, C.F. Cramerum, 273 pp. https://doi.org/10.5962/bhl.title.638

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Zhai SH, Shen YG, Yang XH (2018) Population Genetics of the Endangered and Wild Edible Plant *Ottelia acuminata* in Southwestern China Using Novel SSR Markers. Biochemical Genetics: 56(3): 235–254. https://doi.org/10.1007/s10528-018-9840-2

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics (Oxford, England) 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

PhytoKeys 135: 11–20 (2019)
doi: 10.3897/phytokeys.135.30069
http://phytokeys.pensoft.net

DATA PAPER

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

The FLORIVON flora survey in the Netherlands between 1902 and 1950

Laurens B. Sparrius[1], Joop van Heeswijk[1], Gerard M. Dirkse[2], Michiel J.J.M. Verhofstad[1]

1 *FLORON Plant Conservation Netherlands, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands* **2** *Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands*

Corresponding author: *Laurens B. Sparrius* (sparrius@floron.nl)

Academic editor: *Ricarda Riina* | Received 10 June 2019 | Accepted 27 September 2019 | Published 30 October 2019

Citation: Sparrius LB, van Heeswijk J, Dirkse GM, Verhofstad MJJM (2019) The FLORIVON flora survey in the Netherlands between 1902 and 1950. PhytoKeys 135: 11–20. https://doi.org/10.3897/phytokeys.135.30069

Abstract

In 1902, the nationwide citizen science project, known as FLORIVON, for mapping the flora of the Netherlands was launched, resulting in the publication of a complete flora atlas in 1980. Until 2004, the atlas dataset of the fieldwork between 1902 and 1950 had only been partly digitised and observations were aggregated and anonymised. Between 2001 and 2018, the dataset has been entirely digitised from the original field forms, including notes on non-native taxa. This paper presents key characteristics and figures of the dataset and provides an overview of the historical survey project, the digitisation process and subsequent validation of the data. The dataset is currently curated in the National Database Flora and Fauna and published in GBIF.

Keywords

Biodiversity, citizen science, digitization, mapping, taxonomy, vascular plants

A brief history of flora mapping in the Netherlands

In March 1902, the National Herbarium of the Netherlands (L) and the Dutch Botanical Society started a citizen science project – nowadays referred to as FLORIVON – to map the flora of the entire country of the Netherlands, led by J.W.C. Goethart and W.J. Jongmans (Goethart 1902). During the project, observations were noted by checking taxon names on a special recording form. A field survey was carried out for each map grid cell of 1.3 × 1.01 km.

Starting from the autumn of 1902 until 1907, small numbers of distribution maps were published on an irregular basis to show participants the progress of the work. From 1908 to 1923, only a few participants continued their work, mainly during the so-called *Unio* summer meetings of the Dutch Botanical Society (Smit and Verschoof 1980). In 1924, a new group of botanists, led by J.L. van Soest and J.G. Sloff, continued the mapping project (Verschoof 1978). Another group, led by W.C. de Leeuw, focused on mapping the changes in the flora after the construction of the Afsluitdijk, a dam that caused the Zuiderzee to transform from a salt water body into the a freshwater lake (Westhoff 1964).

In 1930, the IVON foundation (Institute for Vegetation Research in the Netherlands) was founded by J.W.C. Goethart and aimed to unite all botanists working on plant surveys. Between 1930 and 1939, many grid cells were surveyed and preliminary maps were compiled and published in several journals (e.g. Sloff 1935). During and after World War II, the survey project slowed down. Although the project never formally ended, 1950 could be considered as the final year of the field surveys.

It was only in 1980 that the data were compiled into an atlas of the flora of the Netherlands with maps on 5 × 5 km spatial resolution. The atlas was produced by J. Mennema and co-workers at the National Herbarium in Leiden (Mennema et al. 1980).

In 1988, FLORON was founded as a spin-off from the National Herbarium to continue the vascular plant surveys by volunteers and build a database by digitising distribution data of vascular plants. At first, the Atlas of the Flora of the Netherlands (published in 1980) was digitised to have quick access to historical distribution maps. Between 2001 and 2018, all original field forms, opportunistic observations on handwritten notes, letters, vegetation relevées and literature data were digitised by Joop van Heeswijk and compiled into the FLORIVON dataset which is described in this paper.

Methods

Sampling protocol

The basis of the survey scheme was a map of grid cells 1.3 × 1.01 km covering the Netherlands. Grid cells were assigned to participants by the project organisation. Each grid cell was then surveyed for several hours to one day aiming to make a complete list of all wild vascular plants occurring in the area. Survey data were recorded on field forms with abbreviations of scientific taxon names printed on them (Fig. 1). Nomenclature followed the second edition of the Prodromus Florae Batavae (Vuyck 1901). Additionally, miscellaneous observations, vegetation relevées and literature records (from 1832 until 1953) were submitted on special forms or in handwritten or typewritten letters. Most observations include the grid cell code, taxon name, date and up to 9 names of co-observers (Table 1). In total, 56,103 forms were digitised, of which 47,060 were field survey forms, 8,279 written notes and 764 vegetation relevées. The average number of taxa per form was 47. Most of the field forms contained higher numbers of taxa, while written notes usually reported only 1–5 taxa (Table 2).

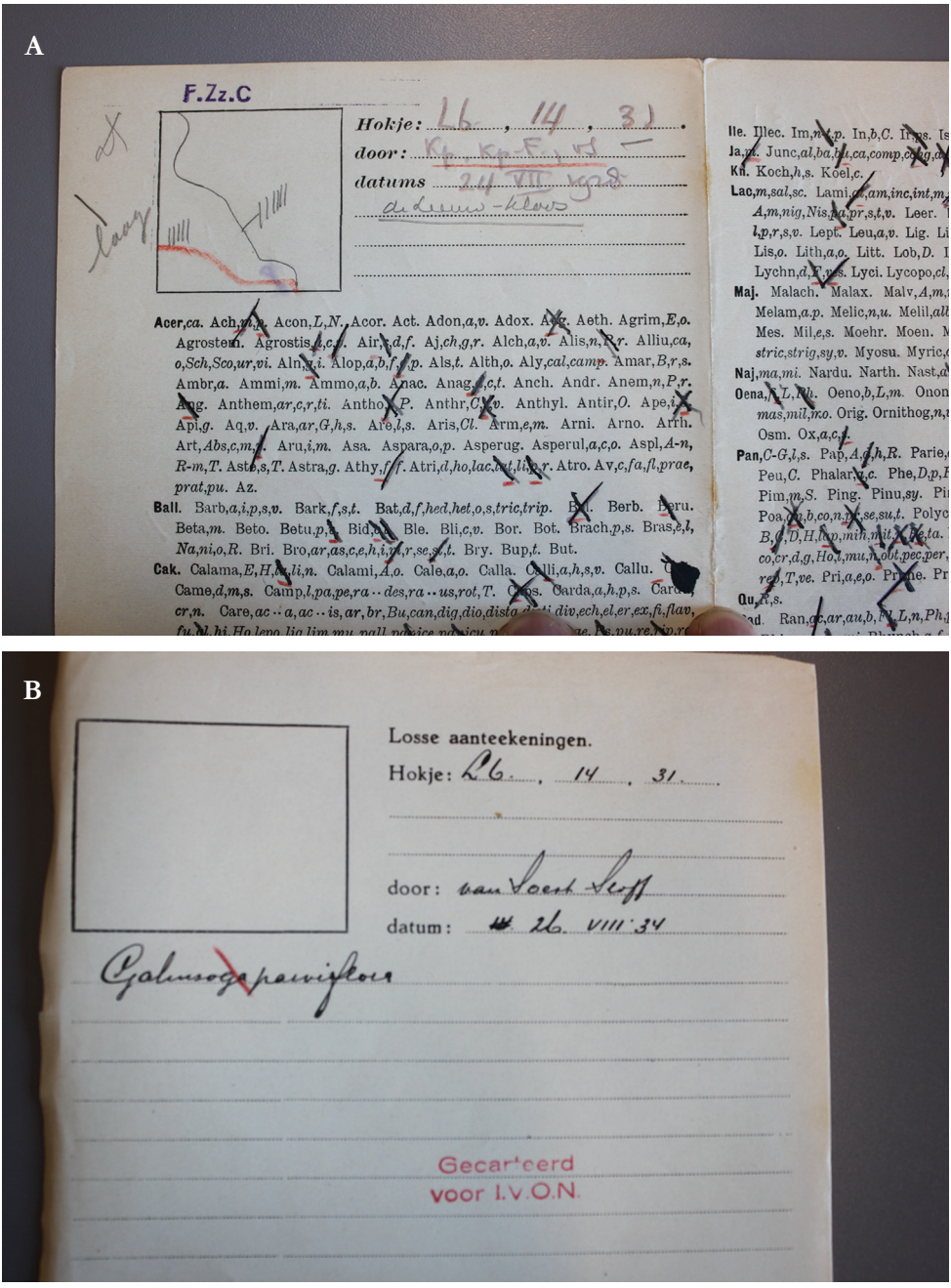

Figure 1. Samples of the FLORIVON survey forms: **A** field form showing a square with a drawing of the surveyed area, space for writing down grid square code ('hokje'), location name, observer name(s) and date, followed by two pages of taxon abbreviations that surveyors had to cross out after observation **B** written note with header data containing the grid cell code (e.g. L6.12.31), observer's name and the survey date. Stamps confirm that the data have been included in printed atlas volumes.

Table 1. Top four observers per decade in the main survey period of FLORIVON with the number of surveys performed in brackets. In total, 572 people were mentioned as observers in the dataset.

1900–1909	1910–1919	1920–1929	1930–1939	1940–1949
L. Vuyck (2369)	L. Vuyck (1477)	A. Koopmans (867)	J. Sloff (6722)	J.L. van Soest (768)
J.W.C. Goethart (1293)	A.W. Kloos (577)	D. Koopmans-Forstmann (634)	J.L. van Soest (3448)	J. Sloff (715)
W.J. Jongmans (1123)	J. Sloff (407)	J.L. van Soest (254)	T. Weevers (2187)	G. Sissingh (417)
M.J. Blijdenstein (998)	D. Lako (284)	A.W. Kloos (189)	Joh. Jansen (1756)	V. Westhoff (364)

Table 2. Number of taxa recorded per form in the FLORIVON dataset.

Number of taxa mentioned on a survey form	Number of survey forms
1–5	11089
6–11	7495
12–25	8212
25–50	8516
50–00	13310
100–378	8080

Data processing and quality control

Survey forms were digitised using Turboveg (Hennekens and Schaminée 2001), a computer programme usually used for handling phytosociological relevées, with customised species dictionaries matching the taxonomy and nomenclature of the field survey forms. All additional written information on the forms, including additional taxon names of, for example, non-native taxa, additional survey dates and remarks were temporarily included in the Turboveg header record and extracted afterwards.

Taxon names were mapped to current names using a translation table between the Prodromus Flora Batavae (Vuyck 1901) and a more recent checklist of vascular plants in the Netherlands (Groen et al. 1999). The original taxon name or its abbreviation is kept in the database. Grid cells codes were translated to geographical coordinates. Observer names were mapped to existing observer identifiers in the National Database Flora and Fauna.

Records without an observation date were assigned to the entire survey period of 1902–1950. Records without a valid taxon name or missing grid cell codes were omitted from the final dataset. Records with locations entirely outside the country or in the sea were also omitted. A total of 5,530 records were cleaned. The number of digitised observations after this first data cleaning step was 2,638,919.

Validation of the digitised observations was performed with an automated procedure which involved trying to find a match for each observation in a dataset, based on printed volumes of the Atlas of the Flora of the Netherlands (Mennema et al. 1980) and other digitised literature and collection records in the National Database Flora and Fauna, which had been validated in the past.

In the FLORIVON dataset, 142,838 observations did not match validated data sources and were considered for a manual review. Of the remaining unmatched observations, 110,889 records of common taxa were validated, i.e. taxa occurring in 30% or more of the 5 × 5 km grid squares in the Netherlands. A total of 2,415 records of less common taxa were validated if they were present in neighbouring grid cells. Further unmatched records, rare taxa, were validated by Gerard Dirkse by plotting them on a map for visual in-

terpretation (17,427 observations). These observations were validated if they matched the geographical pattern of all other valid observations of the taxon. Herbarium specimens and publications mentioning an observation were also taken into account during validation. In the validation process, 12,107 out of 142,838 records were deleted (ca. 3%).

The validated dataset was added to the NDFF Verspreidingsatlas (http://www.verspreidingsatlas.nl), which is the platform FLORON uses to curate datasets. Simultaneously, the dataset was published through the GBIF Integrated Publishing Toolkit (IPT).

Personnel

Joop van Heeswijk performed the digitisation between 2001 and 2018 as voluntary work. Laurens Sparrius performed the validation of the dataset. Gerard Dirkse assisted with the validation of non-native and doubtful taxa. Naturalis Biodiversity Center (Leiden) is hosting the physical archive with field forms and notes.

Dataset

GBIF Dataset description

The dataset is curated on the NDFF Verspreidingsatlas data platform and will be updated on GBIF annually if any changes are made. Included Darwin Core terms are: occurrenceID, type, language, licenserightsHolder, accessRights, references, datasetName, basisOfRecord, eventDate, decimalLatitude, decimalLongitude, geodeticDatum, coordinateUncertaintyInMeters, scientificName, kingdom, taxonRank, scientificNameAuthorship.

Excluded information: Complete observer biographies, source type (field list, publication, specimen, vegetation relevée), location names and remarks were not included in the published dataset, but can be found in the source (curation) database, which can be accessed with the link below. This information was excluded due to privacy reasons or because it was deemed irrelevant.

Object name: FLORIVON
Format name: Darwin Core Archive format
Format version: 1.0
Character encoding: UTF-8
Language: English
Licence: http://creativecommons.org/licenses/by-nc/4.0/legalcode
First publication date: 2019/09/01
Distribution: http://www.verspreidingsatlas.nl:8080/ipt
DOI: https://doi.org/10.15468/ke2ody
Curation website: https://www.verspreidingsatlas.nl/waarnemingen
Number of records: 2,626,773

Taxonomic coverage

The dataset only includes taxa of vascular plants (Kingdom Plantae: clade Tracheophyta). Most of the taxa are native to the Netherlands. Occasionally, non-native taxa were recorded. Nomenclature follows the last edition of the Flora of the Netherlands (van der Meijden 2005). Non-native taxa not listed in this Flora follow The Plant List (The Plant List 2013).

The dataset contains distribution data of 2502 taxa at species or intraspecific level divided over 138 plant families. The plant families with the most observations in the dataset belong to the Asteraceae and Poaceae (Table 3).

Some taxa in FLORIVON are currently accepted as lumped taxa, which makes it impossible to compare taxon distributions for certain taxa (Table 4).

Temporal coverage

The dataset contains observations and literature data from 1832 to 1953. Most of the data were collected between 1902 and 1950 as part of the FLORIVON citizen science project (Fig 2).

Table 3. Top-25 of 137 plant families in the FLORIVON dataset.

Plant family	Number and percentage of observations
Asteraceae	326856 (12.4%)
Poaceae	292948 (11.1%)
Fabaceae	142223 (5.4%)
Rosaceae	124470 (4.7%)
Caryophyllaceae	108542 (4.1%)
Lamiaceae	108048 (4.1%)
Apiaceae	103661 (3.9%)
Plantaginaceae	100695 (3.8%)
Brassicaceae	91438 (3.4%)
Polygonaceae	75203 (2.8%)
Cyperaceae	71567 (2.7%)
Ranunculaceae	63497 (2.4%)
Juncaceae	50209 (1.9%)
Primulaceae	42433 (1.6%)
Amaranthaceae	33551 (1.2%)
Boraginaceae	33050 (1.2%)
Rubiaceae	32086 (1.2%)
Salicaceae	30801 (1.1%)
Ericaceae	29673 (1.1%)
Caprifoliaceae	28348 (1%)
Betulaceae	26600 (1%)
Violaceae	24663 (0.9%)
Onagraceae	23652 (0.9%)
Urticaceae	22418 (0.8%)
Orchidaceae	21735 (0.8%)

Table 4. Taxa in the Prodromus Florae Batavae and FLORIVON that are now considered lumped taxa.

Scientific names of combined taxa	Number of observations
Myosotis laxa subsp. *cespitosa* / *scorpioides*	14792
Festuca rubra / *arenaria*	14738
Agrostis stolonifera / *gigantea*	8930
Betula pendula / *pubescens*	7961
Juncus bufonius / *ambiguus*	6991
Ranunculus aquatilis / *peltatus*	5881
Arenaria leptoclados / *serpyllifolia*	5660
Polypodium vulgare / *interjectum*	5283
Dryopteris carthusiana / *dilatata*	4700
Bolboschoenus maritimus / *laticarpus*	3979
Thymus pulegioides / *serpyllum*	3326
Atriplex prostrata / *longipes*	2781
Nasturtium microphyllum / *officinale*	2279
Aphanes arvensis / *australis*	1955
Potamogeton pusillus / *berchtoldii*	1649
Agrostis canina / *vinealis*	1496
Scrophularia auriculata / *umbrosa*	1375
Salicornia europaea / *procumbens*	1333
Veronica anagallis-aquatica / *catenata*	1319
Viola reichenbachiana / *riviniana*	1296
Malva neglecta / *pusilla*	1263
Elytrigia atherica / *maritima*	785
Festuca brevipila / *lemanii*	428
Ranunculus aquatilis / *baudotii*	418
Glyceria notata / *declinata*	226
Aster lanceolatus / *ontarionis*	188
Galeopsis ladanum / *angustifolia*	121
Trifolium campestre / *dubium*	103
Cerastium pumilum / *glutinosum*	35

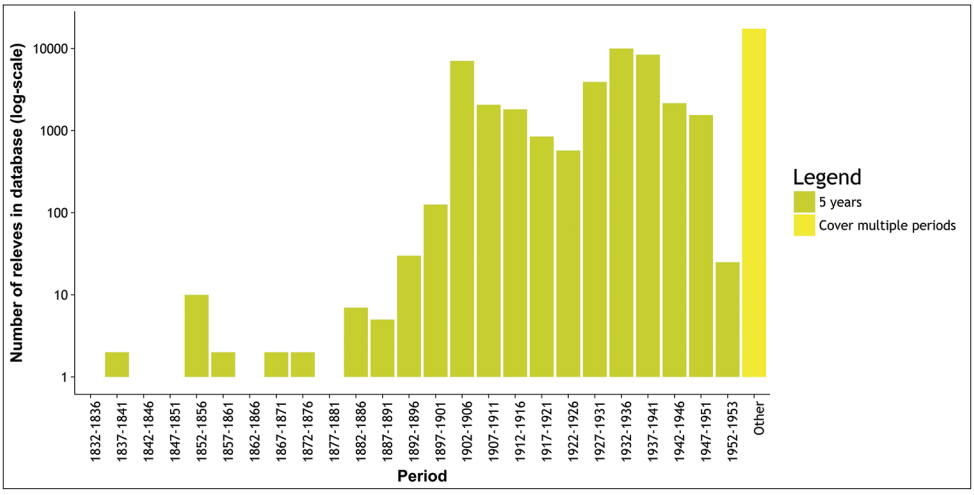

Figure 2. Number of surveys per 5 year period during the course of FLORIVON project.

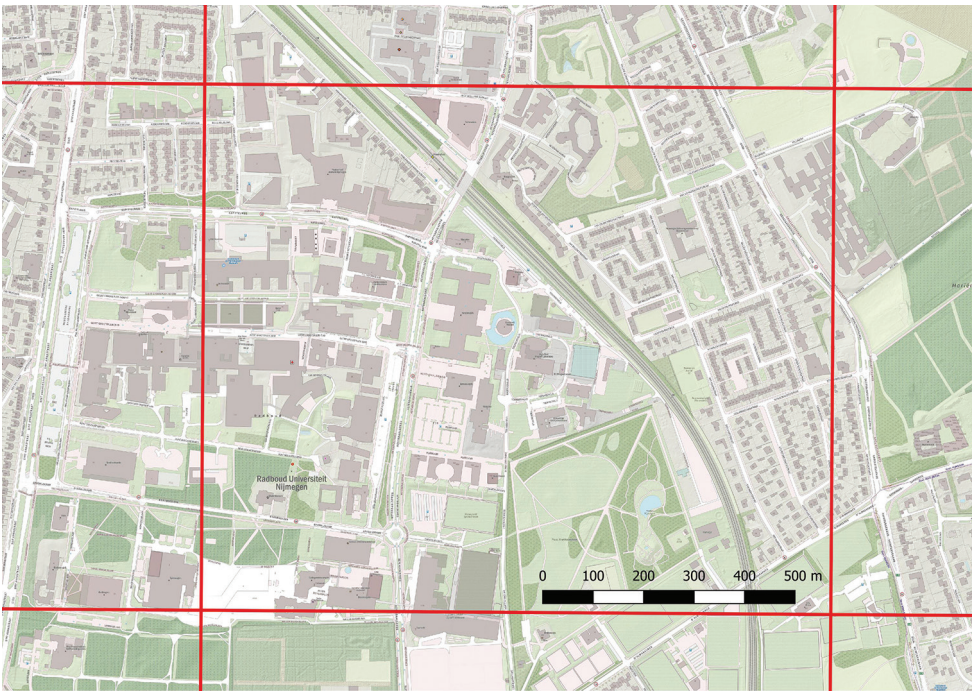

Figure 3. Example of a FLORIVON grid cell of 1.3 × 1.01 km, the smallest spatial unit in which data were collected. Map: OpenStreetMap.

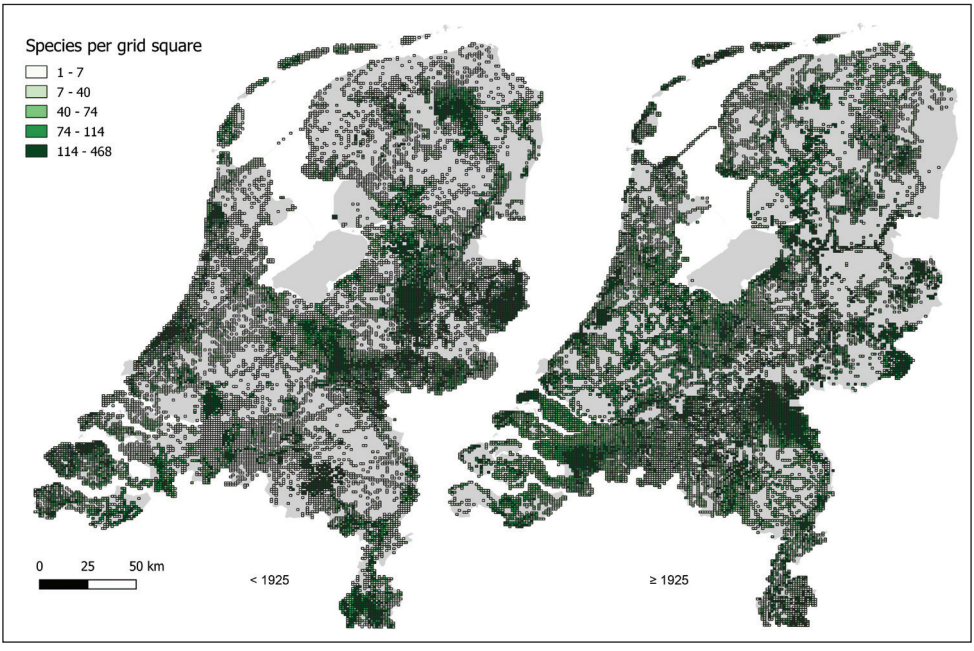

Figure 4. Maps of the Netherlands showing the number of taxa recorded per grid cell before and after 1925 in the context of the FLORIVON project.

Spatial coverage

The dataset covers the entire country of the Netherlands as it was in the period 1902–1950. At that time, the southern part of the province of Flevoland did not yet exist (Hoeksema 2007). Additionally, minor changes were made to the border with Germany and Belgium after World War II (Wijchgel 2008).

Survey data were collected in small grid cells of 1.3 × 1.01 km (*kwartierhok*) (Fig. 3), 16 of which can be combined into a larger grid cell of 5.0 × 4.167 km (*uurhok*), which is used on some forms. The grid system was created in 1902 by the botanical community itself because, until 1920, a km grid was lacking on the topographical military maps. These grid cells differ from the currently used grid, in which the smallest grid cells are 1 × 1 km and follow the Dutch National Coordinate Reference System (ESPG: 28992).

The periods before and after 1925 show different patterns of survey intensity, which should be taken into account when using the data for further analysis (Fig. 4).

Acknowledgements

The authors thank Berry van den Hoorn and Wout Holverda for providing office space and assistance at Naturalis Biodiversity Center. Eddy Weeda for his opinion on *Thymus* and *Scrophularia* taxonomy. Baudewijn Odé, Jan van Groenendael, Kees Groen, Wil Tamis and Ruud van der Meijden (†) for starting the project. Funding was obtained from the Prins Bernhard Cultuurfonds for the years 2001–2004 and Radboud University for the year 2011. The Dutch node of the Global Biodiversity Information Facility (NLBIF) funded the last five years (2014–2018) of the digitisation project.

References

Goethart JWC (1902) Plantenverbreidingskaartjes voor Nederland. De Levende Natuur 7: 17–18.

Groen K, van Heeswijk J, van der Meijden R, van Moorsel R (1999) Kwalitatieve verbetering van de historische floradatabank FLORIVON: algemene soorten en indicatorsoorten van vochtige en droge milieus. Leiden.

Hennekens SM, Schaminée JHJ (2001) TURBOVEG, a comprehensive data base management system for vegetation data. Journal of Vegetation Science 12(4): 589–591. https://doi.org/10.2307/3237010

Hoeksema RJ (2007) Three stages in the history of land reclamation in the Netherlands. Irrigation and Drainage 56(S1): S113–S126. https://doi.org/10.1002/ird.340

Mennema J, Quené-Boterenbrood AJ, Plate CL (1980) Atlas van de Nederlandse flora 1. Amsterdam, 1–226. https://doi.org/10.1007/978-94-009-9191-0

Sloff JG (1935) Plant-maps for the Netherlands 1:3.000.000. Blumea 2: 1–23.

Smit P, Verschoof BJM (1980) De geschiedenis van het plantengeografisch onderzoek in Nederland, in het bijzonder van het IVON. In: Mennema J, Quené-Boterenbrood AJ, Plate CL (Eds) Atlas van de Nederlandse Flora 1. Amsterdam, 18–25.

The Plant List (2013) The Plant List version 1.1. https://www.theplantlist.org

van der Meijden R (2005) Heukels' flora van Nederland (23th edn).

Verschoof BJM (1978) Professor Dr. Ir. J.L. van Soest tachtig jaar. Gorteria (Leiden) 9: 118–120.

Vuyck L (1901) Prodromus Florae Batavae (2nd edn). F.E. MacDonald, Nijmegen.

Westhoff V (1964) In memoriam W.C. de Leeuw. Natuur en Landschap 18: 257–258.

Wijchgel M (2008) Wanneer grenzen worden gecorrigeerd. Selfkant en Elten onder Nederlands bestuur 1949–1963. Universiteit van Amsterdam, Amsterdam.

PhytoKeys 135: 21–34 (2019)
doi: 10.3897/phytokeys.135.38050
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Two new species of *Chlorospatha* section *Orientales* (Araceae) from western Andes in Colombia

Allison Muñoz-Castillo[1,2], Leonardo Guevara-Ibarra[1,2], Laura Clavijo[2,3], Alejandro Zuluaga[1,2]

1 *Departamento de Biología, Facultad de Ciencias, Universidad del Valle, Calle 13 # 100-00, Cali, Colombia* **2** *Grupo de Investigación Ecología y Diversidad Vegetal, Universidad del Valle, Cali, Colombia* **3** *Jardín Botánico Joaquín Antonio Uribe de Medellín, calle 73 # 51D-14, Medellín, Colombia*

Corresponding author: *Alejandro Zuluaga* (zuluaga.alejandro@correounivalle.edu.co)

Academic editor: *Peter Boyce* | Received 7 July 2019 | Accepted 6 September 2019 | Published 6 November 2019

Citation: Muñoz-Castillo A, Guevara-Ibarra L, Clavijo L, Zuluaga A (2019) Two new species of *Chlorospatha* section *Orientales* Araceae) from western Andes in Colombia. PhytoKeys 135: 21–34. https://doi.org/10.3897/phytokeys.135.38050

Abstract

Two new species of *Chlorospatha* (section *Orientales*) from the western slope of the Cordillera Occidental in the departments of Valle del Cauca and Choco (Colombia) are described here. The new species represent the first records of section *Orientales* for Colombia, which was previously known only from the eastern Andes in Ecuador. The two new species are similar to *C. longipoda*, *C. hannoniae* and *C. boosii*. *Chlorospatha minima* **sp. nov**. is differentiated by its small overall size (less than 30 cm tall), blade strongly inequilateral with smooth adaxial surface, and spadix less than 2.2 cm long. *Chlorospatha silverstonei* **sp. nov**. is differentiated by its large overall size (30–60 cm tall), 1–3 leaves per plant, and quilted adaxial blade surface.

Keywords

Araceae, Chlorospatha, new species, taxonomy, tropical Andes, section Orientales

Introduction

The tribe Caladieae (Araceae) comprises 11 genera and 326 species restricted to Tropical America (Grayum 1986, Cusimano et al. 2011, Boyce and Croat 2011) and 8 species (genus *Hapaline* Schott) in Tropical Asia. The center of diversity of the tribe is Colombia with about 35% of the species, most of them endemic (Grayum 1986, Croat and Hannon 2015, Croat et al. 2017). The genus *Chlorospatha* Engl., with 68 species (Croat and Hannon 2015), is the second largest in the tribe after *Xanthosoma* Schott with 201 species (Boyce and Croat 2011). Morphological characters distin-

guishing these two genera and others in the tribe have been conflictive (Mayo and Bogner 1988), and the phylogenetic relationships within Caladieae need to be further studied. Cusimano and collaborators (2011) included in their phylogeny only one species of *Chlorospatha* and recovered it as sister of *Xanthosoma*; however, Gonçalves (personal communication) found some species of *Chlorospatha* nested within *Xanthosoma*, using molecular data.

Until 1981, and for almost 100 years, the genus *Chlorospatha* was monotypic with *C. kolbii* Engl. as the only species described. Madison (1981) combined the genus *Caladiopsis* Engl. with *Chlorospatha*, transferred two species from *Caladium* and *Xanthosoma* into *Chlorospatha*, and published three new species in the genus (Croat and Hannon 2015). For the next 20 years, only six new species were described until Croat and Hannon (2004) published a revision of *Chlorospatha* of Antioquia, Colombia, and in 2015, a comprehensive taxonomic treatment that included the description of 39 new species, 19 from Colombia and 12 from Ecuador, one new subspecies, and four unnamed taxa. For a complete history of the taxonomy of the genus *Chlorospatha* see Croat and Hannon (2015).

Most species of *Chlorospatha* have narrow distribution ranges; furthermore, the level of endemism in *Chlorospatha* is the highest among the genera of Araceae in Colombia, with 43 (63%) species endemic to the country (vs. 23 [33%] endemic to Ecuador). Despite the large number of species in Colombia, fewer collections were available compared with Ecuador, highlighting the need for more botanical exploration in the country and the potential for the discovery of several new species (Croat and Hannon 2015). Croat and Hannon (2015) listed 370 collections of *Chlorospatha* from herbaria across the world, but they probably studied more since in the manuscript they mentioned that there are 226 collections from Ecuador, 55 from Central America, and 122 from Colombia. The collection and study, in the past three years, of ca. 70 new collections of *Chlorospatha* from Colombia resulted in the discovery of at least four new species; here we name and describe two new species of *Chlorospatha* from the western slopes of the Colombian Andes. The two new species belong to section *Orientales* which presents a style not expanded into a mantle, sessile stigma, and was previously, known only from the eastern slopes of the Ecuadorian Andes.

Materials and methods

We assembled a database with all collections from Croat and Hannon (2015), the TROPICOS database, and the Colombian herbaria COL, CUVC and COAH. Additionally, we reviewed all the collections missing in the most recent revision of the genus (Croat and Hannon 2015) and, between 2012 and 2018, carried out eight expeditions to Serranía de los Paraguas and six expeditions to the Anchicaya river basin, where we collected the two new species. We follow Croat and Hannon (2015) for terminology and its use in the descriptions. All measurements were made from dried herbarium material unless otherwise mentioned.

Results

Our database comprised, in total, 572 collections of *Chlorospatha*, representing 70 species (including the two described here), with 214 collections from Colombia, seven of them belonging to the two new species. The number of collections per species was very low, with 22 species known only from the type collection, 13 only from two, 27 from less than 10, and merely eight species known from more than 10 collections.

Taxonomic description

***Chlorospatha minima* Zuluaga & Muñoz-Castillo, sp. nov.**
urn:lsid:ipni.org:names:77202843-1
Figs 1–4

Type. COLOMBIA. Valle del Cauca: municipio Dagua, corregimiento El Queremal, old road Cali-Buenaventura, 6 km from El Queremal, 3°33'45.6"N, 76°45'27.1"W, 1050–1100 m, 20 May 2017, A. Zuluaga, L. Guevara, M. Llano & A. Muñoz 1645 (**holotype**: CUVC!; **isotypes**: COL!, MO!)

Diagnosis. *Chlorospatha minima* can be distinguished from other species in section *Orientales* by its overall small size (less than 30 cm tall), smooth adaxial leaf surface, 1–2 inflorescences per axil, and spadix 20.4–22.8 mm long. Additionally, it differs from *C. silverstonei* sp. nov., the other species of this section in the western slopes of the Andes, by having three collective veins (vs. two in C. *silverstonei* sp. nov.), and the primary lateral (secondary) and minor veins glabrous on the abaxial surface (vs. scale-like indument).

Terrestrial herb, 10–25(–30) cm tall; stem subterranean, decumbent, with cataphylls quickly deciduous; internodes 6.6–7.4 × 5.4–7.8 mm, drying matte, dark brown; cataphylls brownish green, 5.2–6.2 cm long, acuminate at apex, drying matte, reddish brown. Leaves 2 to 5, erect-spreading; petioles 8.3–28.2 cm long, fleshy, glabrous, semiglossy, green with faint darker transverse markings, drying matte, dark brown, sheathed 5.2–9.4(–12.0) cm or (1/5–)1/3–1/2 of its total length, rarely more than 1/2; sheath decurrent onto the petiole apex; free portion of the petiole 0.6–3.7 mm diam. midway; blades broadly triangular-ovate, inequilateral, 5.8–14.2(–16.3) × 2.6–10.1 cm, 1.5 to 2.2 times longer than wide, weakly hastate at base, acuminate at apex, usually slightly broader across anterior lobe than at base, not constricted at petiole insertion, glabrous, conspicuously discolor, distance tip to tip across posterior lobes 2.2–9.2 cm wide; both surfaces smooth, glossy, drying semiglossy; abaxial surface with several layers of cells forming a reticulum, 0.3 to 0.4 mm diam.; anterior lobe 5.1–11.9 × 2.6–10.1 cm, 1 to 2 times longer than wide, 2.2 to 5.7 times longer than posterior lobe, wider near petiole insertion, rarely asymmetrical; posterior lobes directed toward base, 0.9–4.6 (–5.2) × 0.8–4.4 cm, 0.7 to 1.4 times longer than wide, narrowly rounded to obtuse at apex, slightly broader at petiole insertion, ± symmetrical, sinus

parabolic to spathulate; midrib and major venation usually darker than the surface, round-raised and drying ± flattened abaxially; primary lateral veins 3, rarely 4, per side, arising at 30°–60°, rarely 70°, straight to weakly curving towards the margin; secondary veins abaxially sunken, drying visible and darker than the surface, the primary lateral and minor veins glabrous on the abaxial surface; 3 collective veins that originate from first, second and third basiscopic veins, respectively, ± parallel to margin; basal veins coalescent into a prominent posterior rib, 1–2(–3) acroscopic, 2–3(–4) basiscopic veins; minor veins slightly visible abaxially. Inflorescences erect, 1 to 2 per axil; cataphylls of inflorescence not visible outside the sheath; peduncle held within the sheath, 34.0–51.5(–76.0) × 0.4–1.2 mm, drying dark brown to black; spathe erect (all measurements for the spathe and spadix made from spirit material), 27.0–29.8 mm long, apiculate at apex, 6.6–7.0 mm (1.1 to 1.3 times) longer than spadix; spathe tube green or pale green on outer surface, rarely maroon-tinged, 10.0–12.9 × 4.2–4.3 mm, drying dark brown to black on outer surface; spathe blade maroon-tinged, with green veins on outer surface, green on inner surface, drying dark brown or black, ca. 16.9 mm long, erect after anthesis, then marcescent; spadix erect, 20.4–22.8 mm long, sessile, adnate basally to the spathe for 2.3–3.1 mm (1/5 to 1/3 of the length of pistillate portion); pistillate portion light green, 8.3–8.6 × 2.0 mm; pistils ca. 1.3 mm diam.; stigma light green, sessile, ca. 0.4 mm diam.; fertile staminate portion white, 11.2–12.9 × 2.8–3.0 mm, cylindrical, rounded at apex, drying whitish brown; synandria ca. 1.3 mm diam., coherent; sterile portion white, 2.2–2.5 × 2.7 mm, wider at apex, drying whitish brown; sterile flowers with straight borders, 1.2 mm diam. (viewed from above). Infructescence (measurements made from spirit material) erect or pendent, brown, ca. 25.5 × 8.0 mm, drying mate, dark brown on outer surface; berries green, 2.3–3.9 mm diam.; seeds white, (6–)20 to 24 per berry, 1.2–1.5 × 0.7–0.9 mm, ovoid to ellipsoid, longitudinally striate, minutely white-strophiolate.

Etimology. The epithet *minima* refers to the small size of this species, less than 30 cm tall, the smallest in the genus.

Distribution and ecology. *Chlorospatha minima* is endemic to the western slopes of the Colombian Andes in the department of Valle del Cauca. It has been found only in one locality on the old road Cali-Buenaventura at 1000 m, inhabiting humid forest and growing close to a waterfall (Fig. 1).

Conservation status. Despite more than four years of extensive field work, *Chlorospatha minima* is known only from one population located outside the Farallones de Cali National Natural Park, in an area frequently visited by tourists. Because the estimated extent of occurrence is less than 100 km^2, the only population known has less than 100 individuals, and the quality of habitat is declining, *C. minima* could be assessed as Critically Endangered, according to the IUCN criteria (IUCN 2012, 2017).

Discussion. *Chlorospatha minima* belongs to section *Orientales*, characterized by having a stylar region lacking a mantle (Fig. 2B). However, all species in section *Orientales*, as recognized by Croat and Hannon (2015), are endemic to the eastern slopes of the Ecuadorian Andes, whereas *C. minima* and *C. silverstonei* sp. nov. (also described

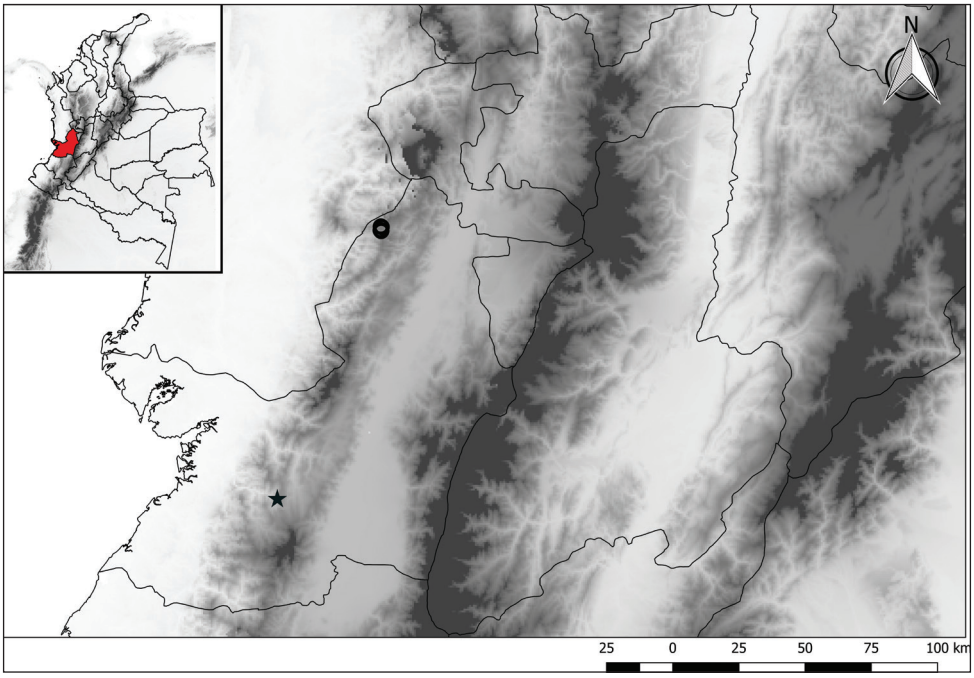

Figure 1. Distribution map of *Chlorospatha minima* (star) and *C. silverstonei* (circle).

here) are endemic to the western slopes of the Colombian Andes. *Chlorospatha minima* is similar to *C. silverstonei* (see discussion under this species), *C. longipoda* (K.Krause) Madison, *C. hannoniae* Croat, and *C. boosii* Croat & L.P.Hannon, but it differs from these four species in having an overall smaller size, less than 30 cm tall (vs. 30–60 cm). Additionally, *C. minima* is the only species with smooth adaxial leaf surface (vs. quilted or bullate) (Fig. 3), cataphylls of inflorescence not visible outside the petiole sheath, and 1 to 2 inflorescences per axil (vs. 1 to 7) (Table 1).

Chlorospatha minima differs from *C. longipoda* in having leaves broadly triangular-ovate vs. narrowly ovate or ovate-elliptic, base of blade slightly hastate vs. subcordate to subsagittate, and (6)20–24 seeds per berry (vs. 7–8). It differs from *C. hannoniae* in having leaves weakly hastate at base vs. sagittate or subsagittate, apex of spathe apiculate vs. cuspidate, and erect spadix vs. slightly curving forward. Finally, *C. minima* differs from *C. boosii* in having 2–5 leaves that are held erect (vs. 8 to 12 leaves) (Table 1). Also, in both species described here, we observed several layers of apparently dead cells on the abaxial surface forming a reticulum visible on dried specimens (Fig. 2F–G, 2M–N). This is not mentioned on the description of other species of section *Orientales*; therefore this could be a potential diagnostic character.

Specimens examined. COLOMBIA. **Valle del Cauca**: municipio Dagua, corregimiento El Queremal, 3°33'45.6"N, 76°45'27.1"W, 1159 m, 17 Mar 2018, Zuluaga et al. 2328 (CUVC!).

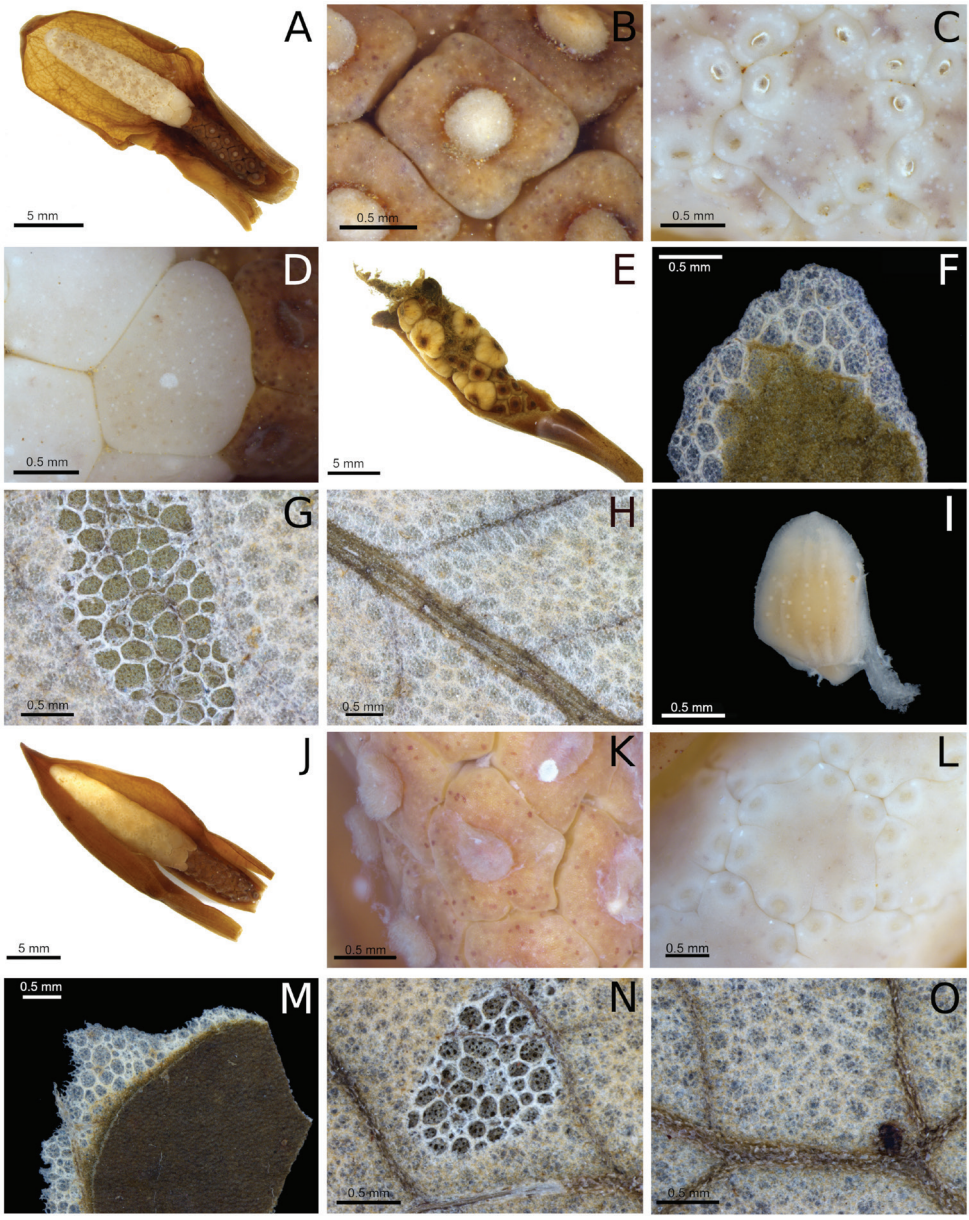

Figure 2. Inflorescence and leaf characters of *Chlorospatha minima* and *C. silverstonei*. **A–I** *C. minima* **J–O** *C. silverstonei* **A** inflorescence **B** female flowers **C** male flowers **D** sterile flowers **E** infructescence **F** adaxial blade surface, note the reticulum of cells in the peeling of abaxial surface **G** abaxial blade surface **H** primary lateral and minor veins glabrous on abaxial surface **I** seed **J** inflorescence **K** female flowers **L** male flowers **M** adaxial blade surface, note the layers of cells **N** abaxial blade surface **O** primary lateral and minor veins with scale-like indumentum on abaxial surface. (Photographs by Juan Felipe Ortega and the imaging laboratory at the Biology graduate program at Universidad del Valle).

Table 1. Morphological comparison of *Chlorospatha minima*, *C. silverstonei*, *C. longipoda*, *C. hannoniae* and *C. boosii*.

	C. minima	*C. silverstonei*	*C. longipoda*	*C. hannoniae*	*C. boosii*
Plant size	10–25 (–30 cm)	30–60 cm	40 cm	50 cm	30–50 cm
Bulbils	Absent	absent	absent	Present	present
Cataphylls	quickly deciduous	persisting ± intact	Remnants of old cataphylls persisting ± intact to semi-intact.	ultimately deciduous	quickly deciduous
Number of leaves	2–5	1–3	3–5	8–14	8–12
Leaf shape	broadly triangular-ovate	broadly ovate to rounded	narrowly ovate or ovate-elliptic	ovate-cordate, occasionally broadly subtriangular	ovate (occasionally subsagittate in juvenile plants)
Base of blade	inaequilateral, slightly hastate	cordate, rarely slightly hastate	subcordate to subsagittate	sagittate or subsagittate	cordate-subcordate
Apex of blade	acuminate	acuminate to cuspidate, almost always mucronate at apex	weakly to moderately acuminate to bluntly acute or apiculate at apex	weakly acuminate to apiculate at apex	weakly acuminate or apiculate at apex
Blade size	5.8–14.2(–16.3) × 2.6–10.1 cm	9.3–27.7 × 4.9–17.7(–21.2) cm	(10.0–)15.5–21.5 × (3.5–)5.0–13.0 cm	16.0–20.5 × 10.0–15.5 cm	(16.0–)19.0–27.5 × (7.0–)13.0–19.5 cm
Adaxial leaf surface	Smooth	quilted	quilted	broadly quilted and sub-bullate	broadly quilted
Abaxial leaf surface	broadly reticulate, with dead cells layers	reticulate, with dead cells layers	reticulate, narrowly colliculate along all venation	reticulate, narrowly minutely colliculate along all venation	reticulate, narrowly minutely colliculate along all venation
Diameter of reticulum on abaxial surface of the leaf	0.3–0.4 mm diam.	0.2–0.3 mm diam.	present, not seen	present, not seen	present, not seen
Secondary and minor venation on lower surface	sunken	prominulous	convex or moderately to narrowly round-raised	prominulous	slightly raised
Indument of veins in lower surface	Absent	present	unknown	unknown	unknown
Number of acroscopic veins	1–2(–3)	1–3(–4)	2–3	unknown	2–3
Number of basiscopic veins	2–3(–4)	3–4(–5)	2–3	unknown	3 to 4
Number of primary lateral veins	3(–4) pairs	2–4(–6) pairs	4–6 pairs	(3–)4 pairs	3–4 pairs
Angle of primary lateral veins	30°–60°(70°)	30°–70°(90°)	17°–45°	45°–65°	25°–55°
Number of collective veins	3	2	2–3(–4)	2(–3)	3
Inflorescences per axil	1 to 2	1 to 4	1 to 6	3 to 5	4 to 7
Cataphylls of inflorescence	not visible outside petiole sheath	visible outside petiole sheath	visible outside petiole sheath	visible outside petiole sheath	visible outside petiole sheath
Portion of the spathe exceeding the spadix	6.6–7 mm	4.3–5.0(–25.7) mm	10.0–35.0 mm	7.0–10.0 mm	20.0–40.0(60.0) mm
Spadix position	Erect	erect	erect, occasionally curving forward at anthesis	slightly curving forward	erect
Spadix length	20.4–22.8 mm	25.0–37.3 mm	(33.0–)43.0–53.0 mm	38.0–50.0 mm	56.0–69.0 mm
Length of spadix adnate to spathe	2.3–3.1 mm	3.3–5.1 mm	6.0–8.0 mm	2.0–3.0 mm	3.0–7.0 mm
Proportion of adnate portion	1/3	1/3	1/2	1/4 or less	1/4 to ca. 1/2
Pistillate portion length	8.3–8.6 × 2.0 mm	8.7–16.1 × 2.5–2.9 mm	(7.0–)10.0–18.0 × 2.0–3.5 mm	7.0–12.0 × 2.0–4.0 mm	10.0–15.0 × 3.0–3.5 mm
Staminate portion length	11.2–12.9 × 2.8–3.0 mm	13.6–19.6 × 4.1–4.4 mm	20.0–30.0 × 2.0–3.0 mm	25.0–35.0 × 3.0–3.5 mm	34.0–45.0 × 3.5–4.0 mm
Sterile portion length	2.2–2.5 × 2.7 mm	3.1–3.9 × 3.7–4.3 mm	4.0–8.0 × ca. 2.0 mm	7.0–12.0 × 2.0–3.0 mm	5.0–9.0 × 2.5–3.0 mm
Border of sterile flowers	Straight	irregular	irregular	irregular	irregular
Color of spathe in fruit	Brown	black	entirely green or occasionally purple-tinged	unknown	unknown
Seeds per berry	(6–)20 to 24	6 to 20	7 to 8	unknown	unknown

Chlorospatha silverstonei **Zuluaga & Muñoz-Castillo, sp. nov.**
urn:lsid:ipni.org:names:77202844-1
Figs 1–3, 5

Type. COLOMBIA. Valle del Cauca: municipio El Cairo, Reserva Natural de la Sociedad Civil "Cerro El Inglés", camino al límite departamental entre Valle y Chocó. 4°44'13.3"N, 76°18'7.7"W, 2120–2230 m, 8 Oct 2017, A. Zuluaga & M.E. Cardona 1946 (**holotype**: CUVC!; **isotypes**: COL!, MO!)

Diagnosis. *Chlorospatha silverstonei* can be distinguished from the other species in section *Orientales* by having 1–3 leaves per plant, an overall larger size (30–60 cm tall) and a small spadix (25–37.3 mm long). Additionally, it differs from *C. minima* sp. nov., the other species in this section from the western slopes of the Colombian Andes, in having two collective veins (vs. three in *C. minima* sp. nov.), the primary lateral and minor veins with scale like indument on the abaxial surface (vs. glabrous).

Terrestrial herb, 30–60 cm tall; stem decumbent, with remnants of cataphylls persisting ± intact; internodes 5.2–20.2 × 6.8–12.5 mm, drying matte, dark brown; cataphylls brownish green, (3.1–)4.4–8.5 cm long, acuminate at apex, drying faintly glossy, reddish brown. Leaves 1 to 3, erect-spreading; petioles 15.2–46.8(–52.4) cm long, free portion of the petiole 1.72–7.18 mm diam. midway, fleshy, glabrous, semiglossy, irregularly dark purple-mottled with longitudinal dark purple lines, drying dark brown to black, sheathed 2.7–11.5(–15.0) cm, less than 2/5 of its total length; sheath decurrent on to the petiole apex; blades broadly ovate to rounded, glabrous, conspicuously bicolor, 9.3–27.7 × 4.9–17.7(–21.2) cm, 1.2 to 2.3 times longer than wide, cordate at base, rarely slightly hastate, acuminate to cuspidate at apex, almost always mucronate at apex, usually slightly broader across anterior lobe than at base, distance tip to tip across posterior lobes 3.1–13.9(–20.5) cm, not constricted at petiole insertion; adaxial surface quilted, glossy, drying brownish green; abaxial surface reticulate, glossy, drying green to yellow-green, with several layers of cells forming a reticulum, 0.2 to 0.3 mm diam.; anterior lobe 7.2–17.4(–18.5) × 4.9–17.7(–21.2) cm, 0.8 to 1.7(–2.0) times longer than wide, 1.5 to 3.6(–4.0) times longer than posterior lobes, broader near petiole insertion, ± symmetrical; posterior lobes directed toward base, 2.1–9.6 × 1.7–8.3 cm, 0.68 to 1.45 times longer than wide, rounded to obtuse at apex, weakly broader at base, slightly inequilateral, the inner side narrower, sinus spathulate to ovate; midrib and major venation narrowly sunken adaxially, round-raised, drying ± flattened and usually darker than surface abaxially; primary lateral veins 2 to 4 per side, rarely 5–6, arising at 30°–70°, rarely 90°, straight to weakly curved towards the margin; secondary and minor veins darker than the surface, prominulous, forming a conspicuous reticulum abaxially, more visible when dried, the primary lateral and minor veins with scale-like indumentum abaxially, only visible in dried material under the microscope; collective veins 2, the outermost arising from the first and second basiscopic veins, ± parallel to margin, the innermost arising from the third basiscopic vein; basal veins coalescent into a prominent posterior rib, 1–3(–4) acroscopic veins, 3–4(–5) basiscopic veins. Inflorescences erect (all measurements made from spirit material), 1 to 4 per axil; cataphylls of the inflorescence visible outside the petiole sheath,

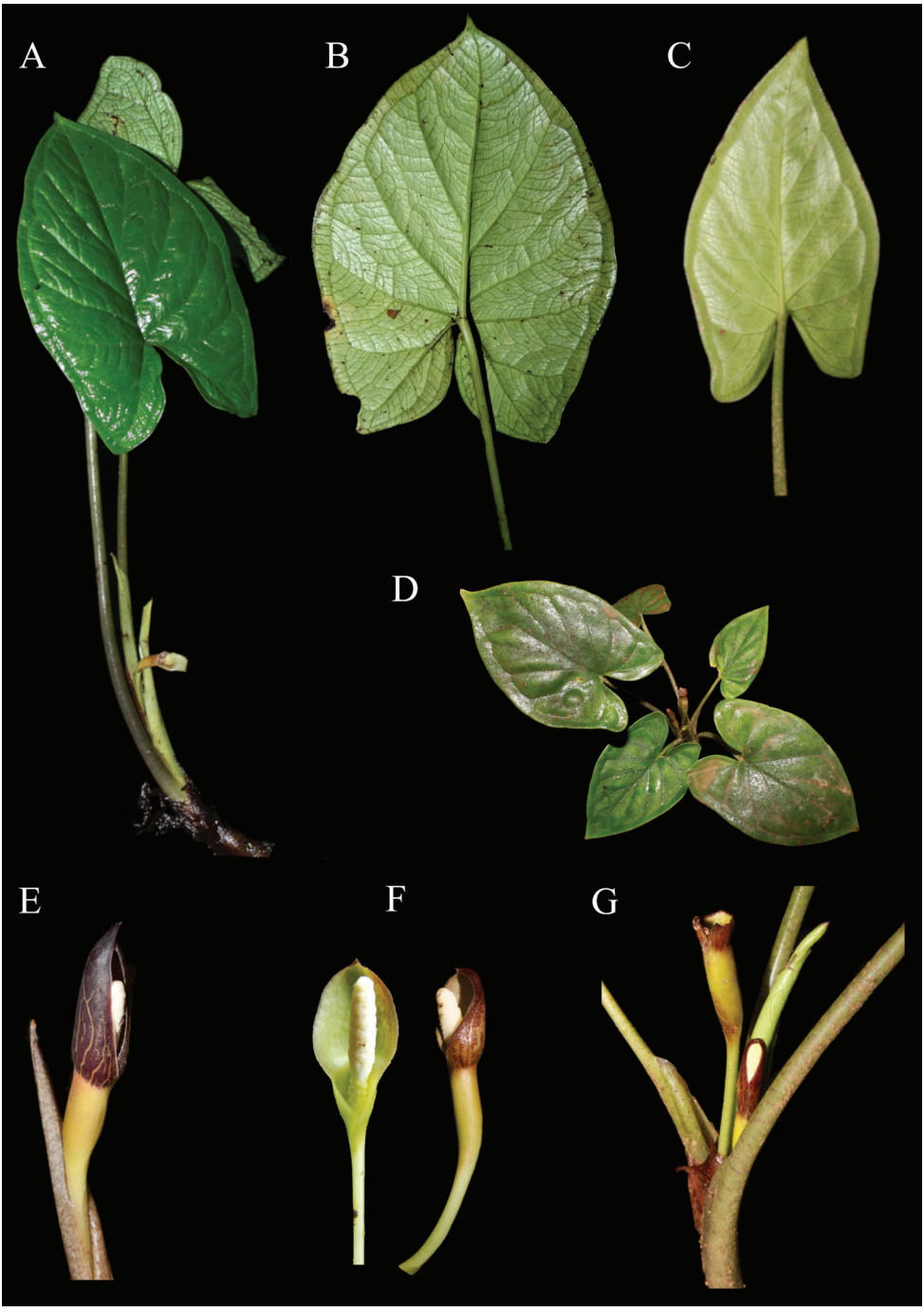

Figure 3. Main morphological characters of *Chlorospatha silverstonei* and *C. minima*. **A, B, E** *C. silverstonei* **C, D, F, G** *C. minima* **A** adult plant **B** abaxial blade surface **C** abaxial blade surface **D** adult plant **E** cataphyll and inflorescence in post anthesis, note spathe acuminate and longer than the spadix **F** Inflorescence in anthesis (right) and post-anthesis (left) **G** shoot, showing young infructescence with deciduous spathe blade and young inflorescence.

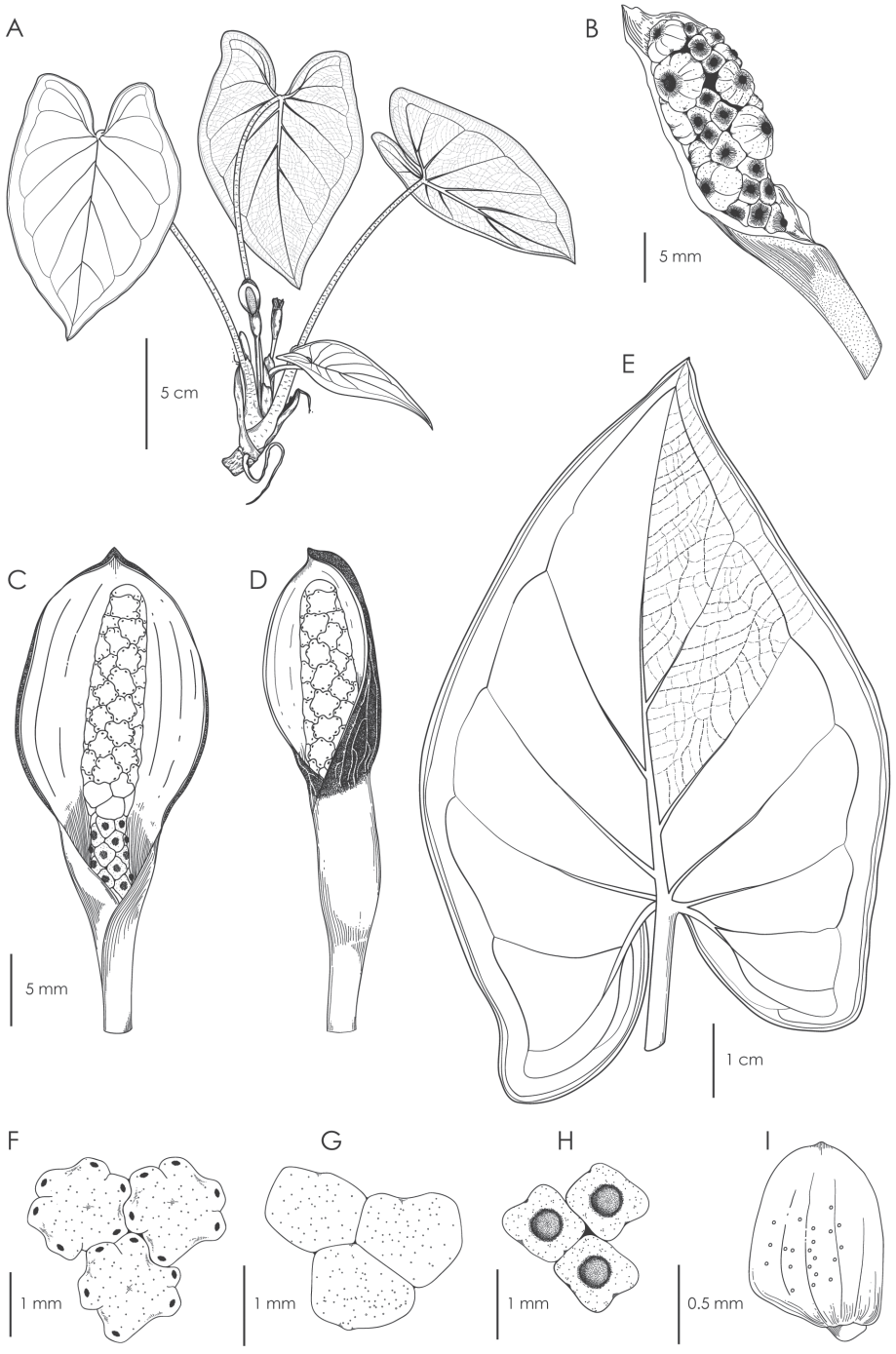

Figure 4. Illustration of *Chlorospatha minima* **A** adult plant with inflorescence **B** infructescence **C** inflorescence at anthesis **D** inflorescence on post-anthesis **E** abaxial surface of leaf blade; note reticulate venation and collective veins **F** upper view of male flowers **G** upper view of sterile flowers **H** upper view of female flowers **I** seed. (Drawn by Eileen Muñoz from the holotype A. Zuluaga et al. 1645).

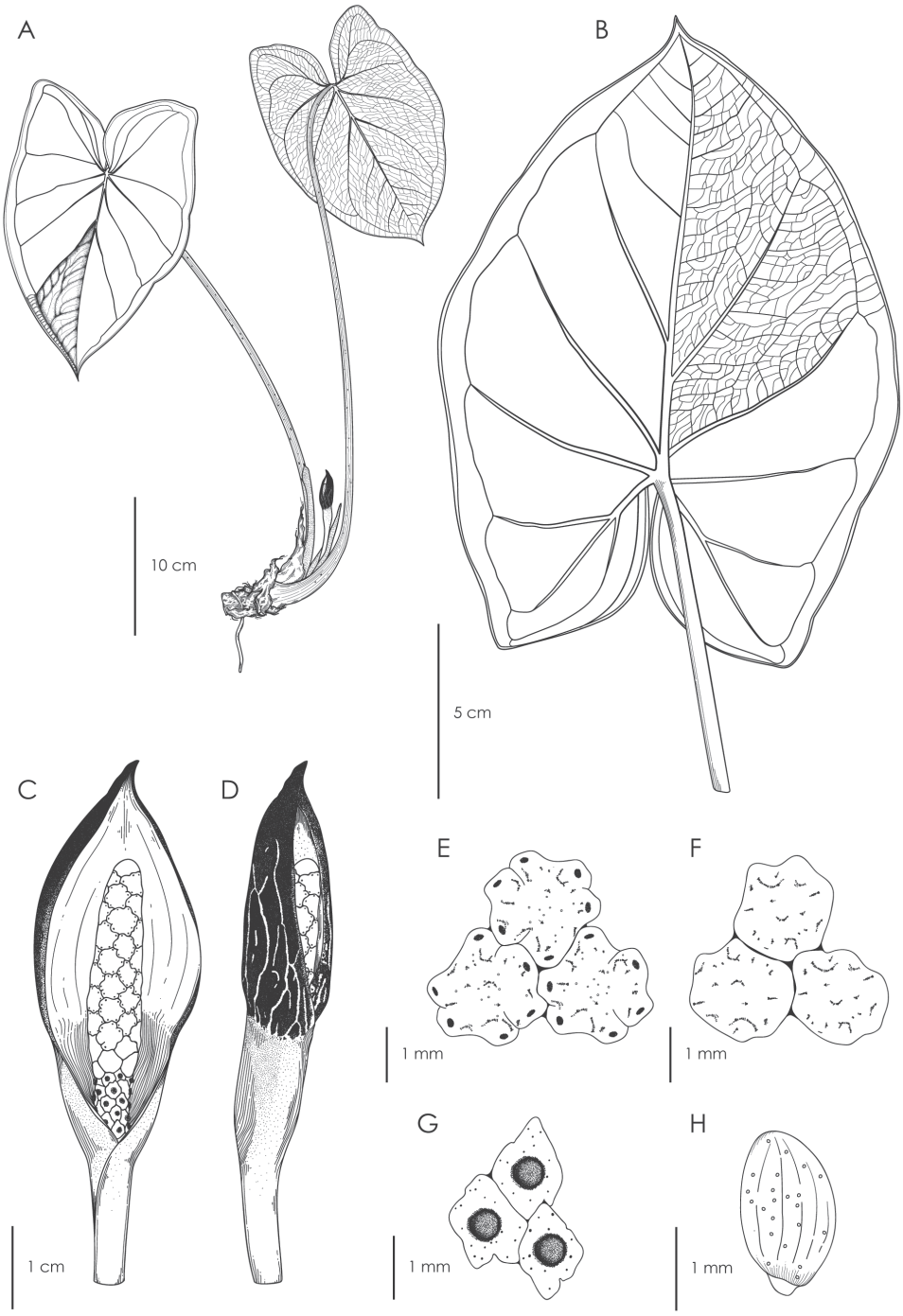

Figure 5. Illustration of *Chlorospatha silverstonei* **A** adult plant with inflorescence **B** abaxial surface of leaf blade; note reticulate venation and collective veins **C** Inflorescence in female anthesis **D** Inflorescence in post-anthesis **E** upper view of male flowers **F** upper view of sterile flowers **G** upper view of female flowers **H** seed. (Drawn by Eileen Muñoz from the holotype A. Zuluaga et al. 1946).

irregularly dark purple-mottled, drying dark brown to black; peduncle 42.8–53.1 (–112.7) × 0.8–1.3 mm, held within the sheath, drying dark brown to black; spathe erect, 32.2–62.9 mm long, acuminate at apex, 4.3–5.0(–25.7) mm longer than spadix (1.2 to 1.7 times longer than spadix); spathe tube pale green on outer surface, rarely maroon-tinged, 8.7–23.3 × 5.0–6.3 mm, drying dark brown to black on outer surface; spathe blade maroon-tinged, with green veins on outer surface, green on inner surface, 18.7–39.7 mm long, drying dark brown or black, erect after anthesis, marcescent in fruit; spadix erect, 25.0–37.3 mm long, sessile, adnate basally to the spathe for 3.3–5.1 mm (ca. 1/3 of the length of the pistillate portion); pistillate portion light green, 8.7–16.1 × 2.5–2.9 mm; pistils coherent, ca. 1.0 mm diam.; stigma light green, sessile, ca. 0.3 mm diam.; fertile staminate portion white, 13.6–19.6 × 4.1–4.4 mm, slightly conical, rounded at apex, drying whitish brown; synandria ca. 1.1 mm diam., coherent; sterile portion white, 3.1–3.9 × 3.7–4.3 mm, wider at apex, drying whitish brown; sterile flowers with irregular borders, ca. 1.4 mm (viewed from above). Infructescence erect or pendent, brown, 18.5–32.6 × 4.8–10.3 mm, drying dark brown on outer surface; berries drying pale tan; seeds white, 6 to 20 per berry, 1.5–2.0 × 0.9–1.2 mm, ovoid to ellipsoid, longitudinally striate, minutely strophiolate, drying brown.

Etimology. *Chlorospatha silverstonei* is named in honor of Dr. Phillip Silverstone-Sopkin (1939–2018), an American botanist who lived and worked in Colombia for 39 years. He was a faculty member at Universidad del Valle until 2014 and an *ad Honorem* professor since 2015. Additionally, he was the director of the herbarium Luis Sigifredo Espinal Tascon at the same University for 17 years. Dr. Silverstone-Sopkin collected more than 13000 botanical specimens, especially from the department of Valle del Cauca, and carried out several explorations in the region where this species was found.

Distribution and ecology. *Chlorospatha silverstonei* is endemic to the western slopes of the Colombian Andes, along the border between the departments of Valle del Cauca and Chocó. It grows in cloud forests between 1900 and 2300 m. It has been collected in two natural reserves, "Cerro El Inglés" and "Alto Galapagos" (Fig. 1), where it has been found widespread in the dark understory, with high humidity and, sometimes, flooded ground. This species has been recorded flowering in October and January. Information about pollination is still lacking but we observed individuals of a species of Brachonidae (Hymenoptera) visiting the inflorescence during female anthesis.

Conservation status. *Chlorospatha silverstonei* has been found in two localities along the Serranía de los Paraguas mountain range, with an estimated extent of occurrence larger than 38000 km^2. In these two localities there are several populations of this species with abundant individuals; therefore, *C. silverstonei* is preliminary categorized as Least Concern (LC), according to the IUCN criteria (IUCN 2012, 2017).

Discussion. *Chlorospatha silverstonei* is similar to *C. minima*, *C. longipoda*, *C. hannoniae*, and *C. boosii*, but it differs from these four species in having fewer leaves (1–3 vs. 2–14) (Table 1). *Chlorospatha silverstonei* differs from *C. minima* in having 1–3 leaves (vs. 2–5 in *C. minima*), longer petioles, 15.2–46.8(–52.4) cm long (vs. 8.3–28.2 cm), that are irregularly dark purple-mottled with longitudinal dark purple lines (vs. green

with darker transverse markings), blade broadly ovate to rounded (vs. broadly triangular-ovate), two collective veins (vs. three), fertile staminate portion slightly conical (vs. cylindrical), infructescence 18.5–32.6 × 4.8–10.3 mm (vs. 25.5 × 8.0 mm) and seeds 1.5–2.0 × 0.9–1.2 mm (vs. 1.2–1.5 × 0.7–0.9 mm). Finally, *C. silverstonei* differs from *C. hannoniae* and *C. boosii* in having cataphylls persisting ± intact (vs. cataphylls ultimately deciduous or quickly deciduous) and the absence of bulbils.

Specimens examined. COLOMBIA. Chocó: municipio Sipí, Reserva Natural Cerro El Inglés, debajo del sitio Santicos, 4°45'22.0"N, 76°18'12.9"W, 2000 m, 17 Oct 2016, A. Zuluaga et al. 1321 (CUVC!). **Valle del Cauca:** municipio El Cairo, Reserva Natural Cerro El Inglés, camino desde la divisoria de aguas hasta la cabaña de investigadores, 4°44'23.9"N, 76°18'15.0"W, 2100–2200 m, 22 Jan 2016, A. Zuluaga et al. 946 (CUVC!); camino a Los Santicos, 4°45'15.5"N, 76°18'02.3"W, 2250 m, 17 Oct 2016, A. Zuluaga et al. 1305 (CUVC!); reserva natural Alto Galapagos, near to the border Chocó-Valle del Cauca, 2018, A. Zuluaga et al. (CUVC!).

Acknowledgements

We thank the non-governmental organization Serraniagua for allowing us to conduct fieldwork in the Reserves Cerro El Inglés and Alto Galapagos in Serranía de los Paraguas mountain range. We also thank Juan Felipe Ortega and the imaging laboratory at the Biology graduate program at Universidad del Valle for providing some of the images. Finally, we thank Monica Carlsen and Peter Boyce for their meticulous reviews and for their suggestions to improve this manuscript.

References

Boyce PC, Croat TB (2011 onwards) The Überlist of Araceae, Totals for Published and Estimated Number of Species in Aroid Genera. http://www.aroid.org/genera/180211uberlist.pdf [accessed 07.2019]

Croat TB, Hannon LP (2004) *Chlorospatha* of Antioquia (Colombia). Aroideana 27: 2–37.

Croat TB, Hannon LP (2015) A Revision of the genus *Chlorospatha* (Araceae). Annals of the Missouri Botanical Garden 101(1): 1–259. https://doi.org/10.3417/2005079

Croat TB, Delannay X, Hannon LP, Ortiz OO (2017) A revision of *Xanthosoma* (Araceae). Aroideana 40(2): 4–581.

Cusimano N, Bogner J, Mayo SJ, Boyce PC, Wong SY, Hesse M, Hetterscheid WL, Keating RC, French JC (2011) Relationships within the Araceae: Comparison of morphological patterns with molecular phylogenies. American Journal of Botany 98(4): 654–668. https://doi.org/10.3732/ajb.1000158

Grayum MH (1986) New taxa of *Caladium*, *Chlorospatha*, and *Xanthosoma* (Araceae: Colocasioideae) from southern Central America and northwestern Colombia. Annals of the Missouri Botanical Garden 73(2): 462–474. https://doi.org/10.2307/2399124

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK, iv + 32 pp.

IUCN (2017) Guidelines for Using the IUCN Red List Categories and Criteria. Version 13. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Madison M (1981) Notes on *Caladium* (Araceae) and its allies. Selbyana 5(3–4): 342–374.

Mayo SJ, Bogner J (1988) A new species of *Caladium* (Araceae) with notes on generic delimitation in the Colocasioideae-Caladieae. Willdenowia (18): 231–242.

PhytoKeys 135: 35–38 (2019)
doi: 10.3897/phytokeys.135.46629
http://phytokeys.pensoft.net

CORRESPONDENCE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

The first report of *Nervilia lilacea* Jum. & H. Perrier (Orchidaceae, Epidendroideae) from Kenya and the Northern Hemisphere

Jing Tian[1,2], Vivian Kathambi[1,2,3], Peris Kamau[4], Geoffrey Mwachala[4], Itambo Malombe[4], Guang-Wan Hu[1,2]

1 *CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, Hubei, China* **2** *Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan 430074, Hubei, China* **3** *University of Chinese Academy of Sciences, Beijing 100049, China* **4** *East African Herbarium, National Museums of Kenya, P.O. Box 451660-0100, Nairobi, Kenya*

Corresponding author: *Guang-Wan Hu* (guangwanhu@wbgcas.cn)

Academic editor: *M. Simo-Droissart* | Received 16 September 2019 | Accepted 30 October 2019 | Published 11 November 2019

Citation: Tian J, Kathambi V, Kamau P, Mwachala G, Malombe I, Hu G-W (2019) The first report of *Nervilia lilacea* Jum. & H. Perrier (Orchidaceae, Epidendroideae) from Kenya and the Northern Hemisphere. PhytoKeys 135: 35–38. https://doi.org/10.3897/phytokeys.135.46629

Abstract

Nervilia lilacea is recorded from Kenya as well as the Northern Hemisphere for the first time. A plate of ink drawing and a distribution map are provided based on the new collection.

Keywords

Flora of Kenya, illustration of *Nervilia lilacea*, new record, Orchidaceae, taxonomy

Introduction

Nervilia Commerson ex Gaudichaud-Beaupré in Freycinet (1829: 421) comprises ca. 80 species, distributed from tropical, subtropical and warm temperate regions of Africa, Asia, Australia, and the Southwest Pacific islands (Govaerts et al. 2019). There are 15 species of *Nervilia* recorded in Africa (Govaerts et al. 2019). Among these spe-

cies, five of them have been recorded in Kenya (Petersson 1990, 1991; Stewart and Campbell 1996; Olszewski 2004; Nusbaumer et al. 2011; Govaerts et al. 2019). In April 2018, during a field survey in Nandi Forest, *N. lilacea* Jumelle & Perrier (1912: 197) was collected from Kenya (the northern side of the equator) for the first time. We report it here with a plate of ink drawing and a distribution map based on the new collection.

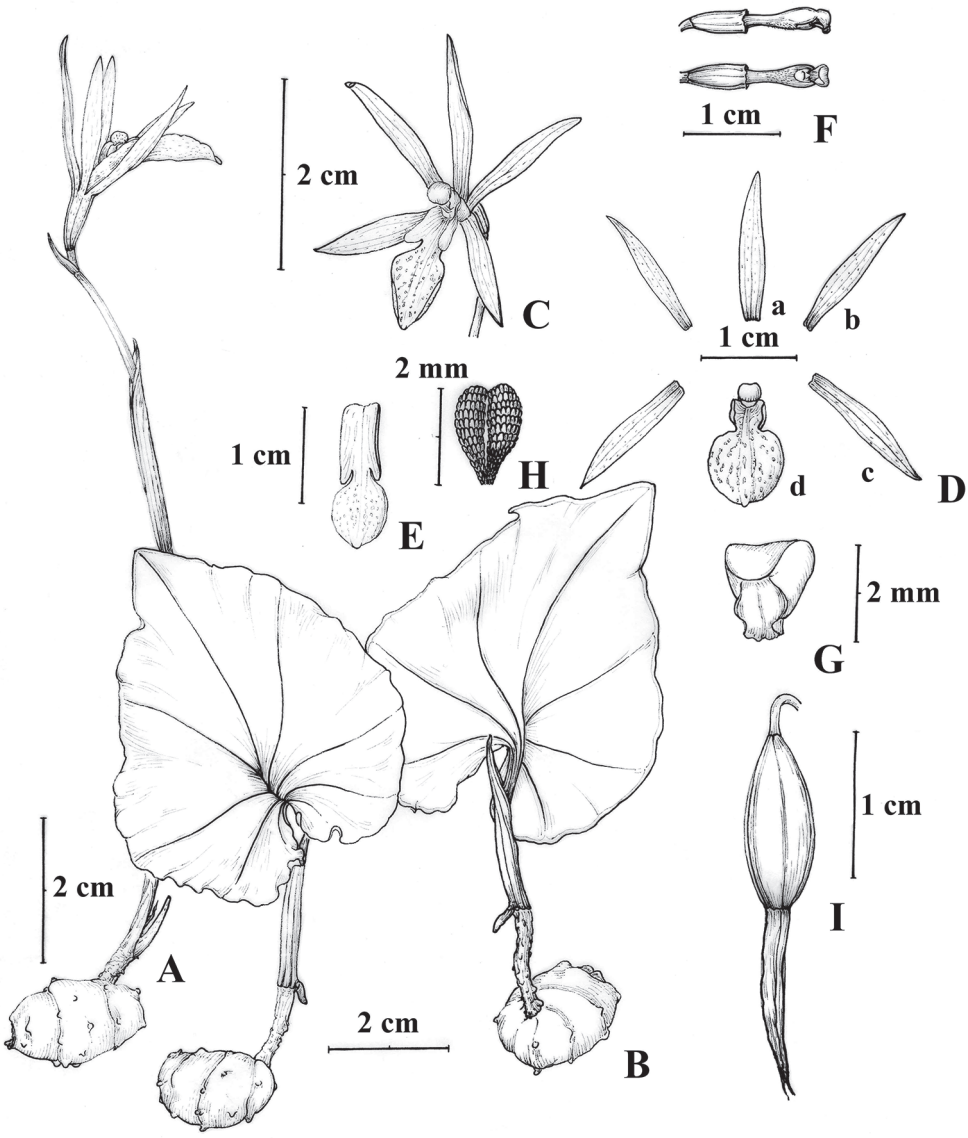

Figure 1. *Nervilia lilacea* **A** plant with flower **B** plant with leaf (adaxial and abaxial leaf) **C** flower, frontal view **D** floral pieces dissected (**a** dorsal sepal **b** lateral petal **c** lateral sepal **d** lip) **E** lip **F** column, lateral and ventral view **G** anther **H** pollinia **I** capsule (Jing Tian drew it from *FOKP-1530* specimen).

New record

***Nervilia lilacea* Jum. & H.Perrier, Ann. Fac. Sci. Marseille 21(2): 197, 1912**
Fig. 1

= *Nervilia gassneri* Börge Pett. in Nord. J. Bot. 9: 492. 1990. Type. Malawi: Southern Prov., Zomba Distr., Zomba Plateau, 1530 m, 15 July 1984. *Petersson and Gassner 359* (holotype: UPS, image seen!; isotype: BP, K, image seen!, LISC, LMU, MAL, NHT, SRGH).

Type. Madagascar: Centre, massif de Manonarivo, bois humides, 1000 m, fl., *Perrier de la Bâthie 1873* (holotype: P [P00094725], image seen!).

Specimens examined. Kenya. Nandi North District, Spetonok, 0°22'32"N, 35°00'29"E, elevation 2000 m, 22 April 2018, *FOKP-1530* (EA, HIB).

Distribution. Madagascar, Malawi, South Africa, Tanzania, Zambia, Zimbabwe, Kenya (new record). (Fig. 2)

Habitat and phenology. Tropical rain forest floor margins at elevation 200–2000 m a.s.l.. Flowering from March to April was observed in Kenya.

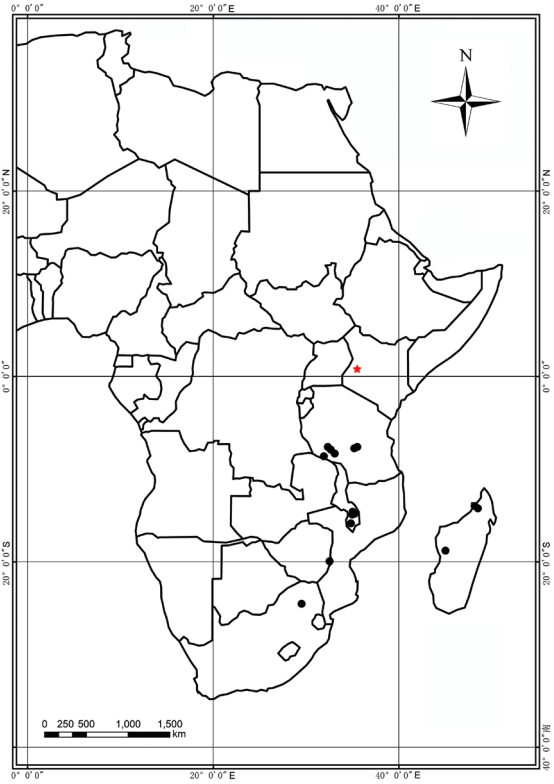

Figure 2. Distribution map of *Nervilia lilacea*, with the new collection *FOKP-1530* shown by a red star [other points re-drawn after Petersson (1990, 1991) and specimen records]

Acknowledgments

Thanks to the Kenya Forest Service for the permission of field investigation. This work was supported by a grant from Sino-Africa Joint Research Center, CAS (SAJC201614).

References

Freycinet LCD (1829) Voyage autour du monde, entrepris par ordre du roi. Chez Pillet Aîné, Paris, 522 pp.

Govaerts R, Dransfield J, Zona S, Hodel DR, Henderson A (2019) World checklist of Orchidaceae. Facilitated by the Royal Botanic Gardens, Kew. http://wcsp.science.kew.org [Retrieved 29 October 2019]

Jumelle H, Perrier H (1912) Les *Nervilia* et les *Bulbophylum* du Nord-Ouest de Madagascar. Annales de la Faculte des Sciences de Marseille 21: 187–216.

Nusbaumer L, Cribb P, Gautier L (2011) *Nervilia gassneri* Börge Pett. from Africa is conspecific with the Malagasy *N. liacea* Jum. & H.Perrier. Candollea 66(1): 127–130. https://doi.org/10.15553/c2011v661a14

Olszewski TS (2004) A provisional checklist of the continental African Orchidaceae. 5. Vanilloideae. Polish Botanical Journal 49(2): 123–134.

Petersson B (1990) Studies in the genus *Nervilia* (Orchidaceae) in Africa. Nordic Journal of Botany 9(5): 487–497. https://doi.org/10.1111/j.1756-1051.1990.tb00539.x

Petersson B (1991) The Genus *Nervilia* (Orchidaceae) in Africa and the Arabian Peninsula. Orchid Monographs (Vol. 5). Rijksherbarium/Hortus Botanicus, Leiden, 90 pp.

Stewart J, Campbell B (1996) Orchids of Kenya. Timber Press Inc, Portland Oregon, 146–147.

PhytoKeys 135: 39–47 (2019)
doi: 10.3897/phytokeys.135.39036
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

A new species of *Chrysosplenium* (Saxifragaceae) from Northeastern China

Yong-In Kim[1], Jae-Seo Shin[1], Sangwoo Lee[3], Jia-Hui Chen[2], Sangho Choi[3], Jin Hee Park[4], Young-Dong Kim[1]

1 *Department of Life Sciences, Multidisciplinary Genome Institute, Hallym University, Chuncheon 24252, South Korea* **2** *CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China* **3** *International Biological Material Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, South Korea* **4** *Freshwater Bioresources Research Division, Nakdonggang National Institute of Biological Resources, Sangju 37242, South Korea*

Corresponding author: *Young-Dong Kim* (ydkim@hallym.ac.kr)

Academic editor: *Y. Mutafchiev* | Received 16 August 2019 | Accepted 8 November 2019 | Published 25 November 2019

Citation: Kim Y-I, Shin J-S, Lee S, Chen J-H, Choi S, Park JH, Kim Y-D (2019) A new species of *Chrysosplenium* (Saxifragaceae) from Northeastern China. PhytoKeys 135: 39–47. https://doi.org/10.3897/phytokeys.135.39036

Abstract

This study describes and illustrates *Chrysosplenium macrospermum* Y.I.Kim & Y.D.Kim, a new plant species from Changbaishan Mt. (Baekdusan Mt.) in northeastern China. The species is most similar to *Chrysosplenium valdepilosum* in the series *Pilosa* but is readily distinguishable by short arching sterile branches, multiple (up to 3) flowering stems, and smooth surfaced seeds (without tubercles), which are ca. 30–50% larger than those of other members in the series.

Keywords

Saxifragales, seed morphology, sterile branch, taxonomy

Introduction

Chrysosplenium L. (Saxifragaceae) is a genus of small succulent and fragile herbs characterized by tetramerous flowers with petaloid sepals (Bensel and Palser 1975, Soltis 2007, Soltis et al. 2001). It is composed of approximately 70 species, mainly distributed in temperate regions of the Northern Hemisphere, except for two disjunctive species in Chile (Hara 1957, Spongberg 1972, Pan 1986, Ye and Zhang 1994, Wakabayashi and

Takahashi 1999, Han and Kang 2012, Bhaumik 2014, Kim and Kim 2015, Liu et al. 2016, Kim et al. 2018, Wakabayashi et al. 2018). They usually exhibit dramatic morphological changes in the shapes of sterile branches during flowering and fruiting periods, and variations in size depending on the environmental conditions (e.g., humidity, light). For these reasons, correct identification and species delimitation have been the most challenging taxonomic tasks in relation to this genus. More detailed and comprehensive morphological studies encompassing various developmental periods have led to the discovery of five new *Chrysosplenium* species over the past five years (Bhaumik 2014, Kim and Kim 2015, Liu et al. 2016, Kim et al. 2018, Wakabayashi et al. 2018).

Recently, molecular phylogenetic approaches have provided valuable assistance in the effort to detect cryptic lineages in many plant groups, including *Chrysosplenium*. During an ongoing phylogenetic study of *Chrysosplenium* series *Pilosa* Maxim., we came across a new taxon that was collected near Tianchi Crater Lake in Changbaishan, Jilin, in China. Additional fieldwork was conducted in July 2017 to collect flowering individuals and seeds for more detailed morphological examinations. After a comprehensive examination of herbarium specimens (at HHU, TI, KB, KH, KWNU, KUS, IUI, KYO, and PE and at the Global Plants website of JSTOR) and literature related to *Chrysosplenium* (Franchet and Savatier 1878, Nakai 1914, Kitagawa 1934, Ohwi 1934, Hara 1957, Pan 1986, Pan and Ohba 2001, Han and Kang 2012, Kim and Kim 2015, Kim et al. 2018), we recognized that the taxon is a new species and belongs to the series *Pilosa*. Here, the new species is described and illustrated.

Materials and methods

Photographs of the plant habit and macro-morphological characters were taken in the field. Morphological observations and measurements of the new species were conducted based on living and dried specimens and preserved materials. All morphological characters were observed and photographed with a Zeiss Stemi SV 11 Apo stereoscopic microscope and a Zeiss AxioCam MRc 5 microscope camera. Seed coat characters were examined by a Hitachi S-3400N scanning electronic microscope.

Taxonomic treatment

Chrysosplenium macrospermum **Y.I.Kim & Y.D.Kim, sp. nov.**
urn:lsid:ipni.org:names:77203161-1
Figs 1, 2, 3A1, A2

Diagnosis. *Chrysosplenium macrospermum* is most similar to *Chrysosplenium valdepilosum* (Ohwi) S.H. Kang & J.W. Han, 2011 (see Han et al. 2011), but the former is readily distinguishable by short arching sterile branches, multiple (up to 3) flowering stems, and smooth surfaced seeds (without tubercles), which are ca. 30–50% larger than those of other members in the series *Pilosa* (Figure 3).

Type. China. Jilin: near Tianchi (Cheon-Ji in Korean) Crater Lake to Changbaishan Mt. (Beakdusan Mt. in Korean), Antu County, Changchun, 42°01'44.80"N, 128°03'59.22"E, elev. 2,610 m, 26 Jul. 2017, *KYI-2017001* (holotype HHU; isotypes HHU, KB, KRIB).

Description. Perennial herbs. Small (up to 7 cm), hermaphroditic. Roots thick fibrous. Flowering stem(s) 1–3, erect, 2–7 cm long, sometimes branched, tetragonal in the cross-section, sparsely pilose along the edges, light green to green, with 2(3) sterile branches arising from the base; sterile branches 1–1.5 cm long, stout, arch-shaped, sparsely pilose. Leaves simple, estipulate, petiolate. Basal leaves (1) or 2, opposite, petiole 3–15 mm long, blade up to ca. 1 × 1 cm, flabelliform. Cauline leaves of flowering stem(s) 1–4, opposite or rarely alternate, attached at 1/2 or below of the stem; petiole 1–10 mm long, entirely ciliate; blade 2–10 × 3–11 mm, flabelliform, apex subtruncate to rounded, base attenuate, margins obscurely undulate to crenate or distinctly obtusely dentate (3–7 teeth), translucent white or brown ciliate, both surfaces glabrous. Leaves of sterile branches, opposite, 4–8 pairs; petiole 4–15 mm long, entirely ciliate; blade to 1.5 × 1.5 cm, suborbicular or widely ovate to ovate, apex rounded, base cuneate to narrowly cuneate, margins crenate with 3–10 flat obscure teeth, translucent white or brown ciliate, upper surface sparsely pilose near the margin, green to pale green, lower surface sparsely pilose along the veins, greenish grey. Inflorescence 5- to 30-flowered cyme, surrounded by leaf-like bracts; pedicel 1–3 mm long, sparsely pilose. Bracteal leaves yellow during flowering, turning to greenish yellow after anthesis; petiole 1–3 mm long, entirely ciliate; blade 2–9 × 2–10 mm, flabellate, obdeltoid, spatulate, apex obtuse to subtruncate, base narrowly cuneate to cuneate, margins obscurely undulate to crenate or distinctly obtusely dentate, 2–7 teeth, sparsely translucent white or brown ciliate, both surfaces glabrous, greenish-grey. Flowers tetramerous, actinomorphic; sepals 4 (2 pairs), free, petaloid, 1 pair overlapping the other in bud, erect, yellow, 2–4 × 2–3 mm, widely obovate to widely subelliptic, glabrous, 3-veined, apex obtuse to truncate, slightly recurved to outside, persistent; petals absent; stamens 8, biseriate, ca. 2 mm long, shorter than sepal; filaments narrow conical, ca. 1.5 mm long; anthers yellow, 2-locular, ca. 0.5 mm long, longitudinally dehiscent; pistil 2-carpellate, semi-inferior, ovary 1-locular, ovules at 2 parietal placentae, styles 2, free, ca. 1 mm long, stigma round. Fruit a capsule, light green, glabrous, ca. 6 mm long, 2-lobed (horn shaped), lobes slightly unequal, dehiscent along the adaxial suture; seeds numerous, light brown, ellipsoid, with a raphe on one side, thick-walled, 935–1021 × 511–566 μm, seed surface covered with minute deciduous papillae, without tubercles.

Etymology. The specific epithet of the new species refers to the distinctly larger size of the seeds compared with those of other members in the series *Pilosa*.

Vernacular name. Cheon Ji Gwaeng I Nun (Korean pronunciation); 천지괭이눈 (Korean name), Tiān Chí Jīn Yāo (Chinese pronunciation); 天池金腰 (Chinese name)

Distribution. *Chrysosplenium macrospermum* is only known from Changbaishan Mt. in Jilin Province of China, at an elevation of ca. 2,600 m. To date, only a few subpopulations with approximately 5,000 individuals have been discovered near Tianchi Crater Lake. In the absence of additional data, we presently score it as Data Deficient (DD) according to the IUCN Red List criteria (IUCN 2001).

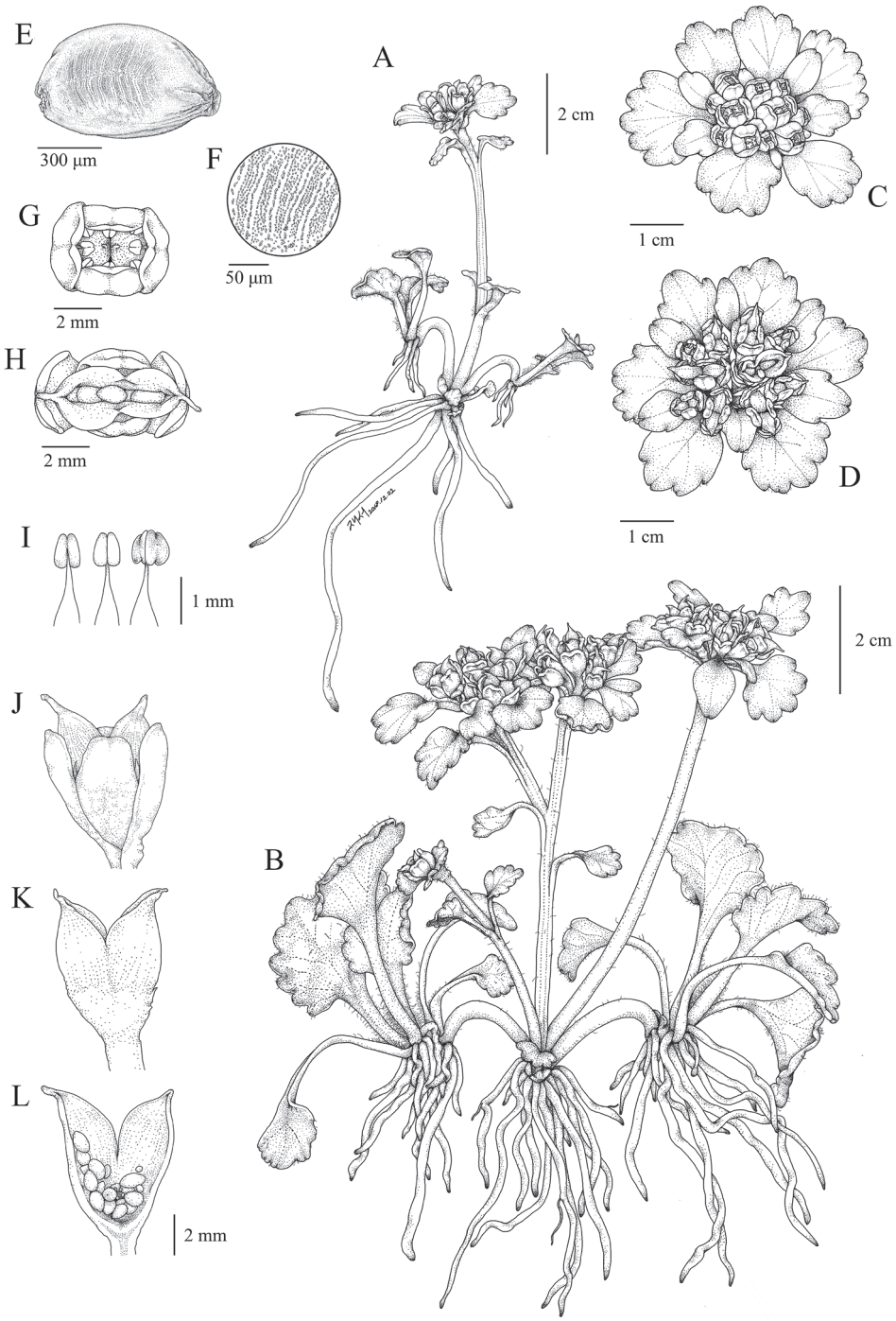

Figure 1. *Chrysosplenium macrospermum* Y.I.Kim & Y.D.Kim, sp. nov. **A** flowering individual **B** fruiting individual **C** inflorescence and bracteal leaves **D** infructescence and bracteal leaves **E** seed **F** seed coat, enlarged **G** flower (top view) **H** capsule, after dehiscence (top view) **I** stamen at various stages **J** capsule with persistent sepals (side view) **K** capsule, sepals removed **L** capsule, longitudinal section.

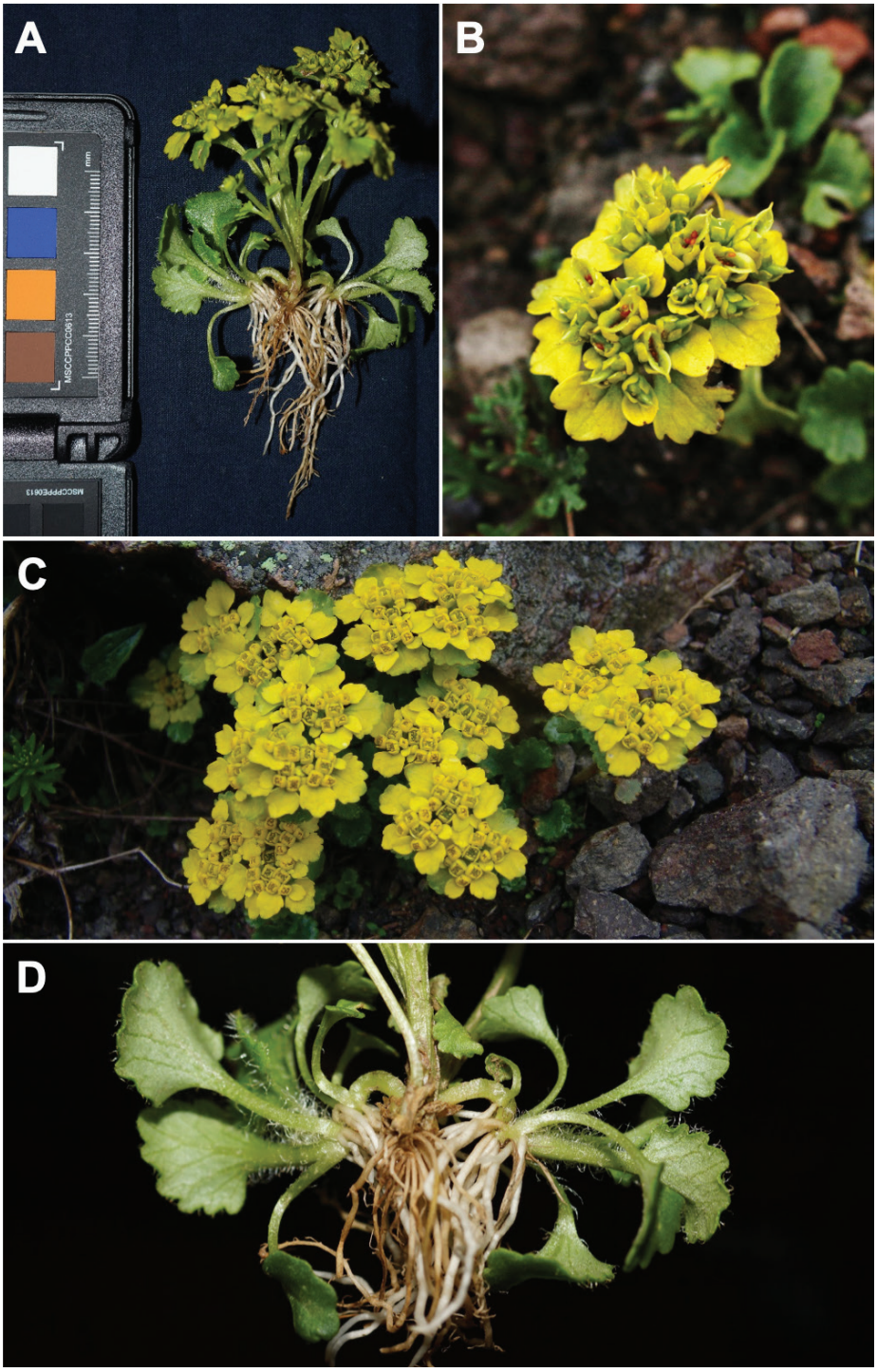

Figure 2. *Chrysosplenium macrospermum* Y.I.Kim & Y.D.Kim, sp. nov. **A** fruiting individual **B** infructescence, bracteal leaves and seeds in capsules **C** plant habit during flowering **D** fruiting individual showing short arch-shaped sterile branches and thick fibrous roots.

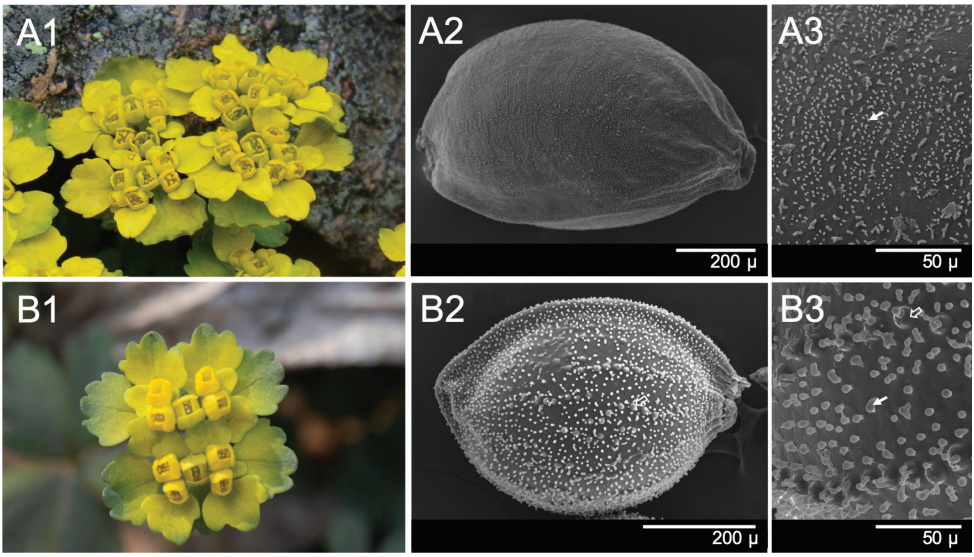

Figure 3. *Chrysosplenium* spp. inflorescence and seeds. **A** *C macrospermum* Y.I.Kim & Y.D.Kim, sp. nov., inflorescence with bracteal leaves (**A1**), seed surface, scanning electron micrograph, 140× (**A2**) and 600× (**A3**) **B** *C. valdepilosum*, inflorescence with bracteal leaves (**B1**), seed, scanning electron micrograph, 350× (**B2**) and 600× (**B3**). White solid arrows indicate deciduous papilla (**A3, B3**) and blanked arrows indicate tubercle (**B2, B3**).

Ecology. *Chrysosplenium macrospermum* occurs in alpine tundra, where it grows in humid and semi-shaded areas near the Tianchi volcanic crater along with *Papaver radicatum* var. *pseudoradicatum* (Kitag.) Kitag., *Bistorta ochotensis* Kom., *Micranthes laciniata* (Nakai & Takeda) S. Akiyama & H. Ohba, *Sedum rosea* (L.) Scop., and *Pedicularis verticillata* L. The flowering period of this species is from late May to early July, and the fruiting period is from July to August.

Additional specimens examined (paratype). China. Jilin: near Tianchi (Cheon-Ji in Korean) Crater Lake to Changbaishan Mt., Antu County, Changchun, 25 Apr. 2014, *D.K. Lee-2014001* (HHU), *D.K. Lee-2014002* (HHU), *D.K. Lee-2014003* (HHU), 42°01'44.80"N, 128°03'59.22"E, elev. 2610 m, 26 Jul. 2017, *KYI-2017002* (HHU), *KYI-2017003* (HHU), *KYI-2017004* (HHU), *KYI-2017005* (HHU), *KYI-2017006* (KB).

Notes. The new taxon and *C. valdepilosum* exhibit a high degree of morphological similarity upon flowering (Fig. 3) but can be distinguished by several characters, including the size of the seed, the excrescence of the seeds, the developmental form of the sterile branch, and the hair type on the leaves of the sterile branch (Table 1). *Chrysosplenium macrospermum* occurs only in the vicinity of Tianchi Lake (elev. 2190 to 2610 m). It is the only species of the series Pilosa that grows in the vast Changbaishan Mt. region. The geographical distributions of other members of series Pilosa, including *C. valdepilosum* (endemic to Korea), do not overlap with that of *C. macrospermum*.

Table 1. Comparison of the key features of *Chrysosplenium macrospermum* and *C. valdepilosum*.

Character	*C. macrospermum*	*C. valdepilosum*
Root	thick, stout	filiform, rather soft
Sterile branch	arch-shaped	straight
upper surface of leaf	sparsely pilose near the margin	pilose
Flowering stem	1–3	1
hair type	sparsely pilose	pilose
color	Green	green and purple (lower part of stem)
branched	often branched	not branched
Seed		
size	length/width range 935–1021/511–566 μm	length/width range 578–758/409–589 μm
surface	smooth (without tubercles)	with tubercles

Key to species of *Chrysosplenium* series *Pilosa* modified from Kim et al. (2018)

1 Sepals white. Anthers dark red **2**
– Sepals yellow or greenish. Anthers yellow **3**
2 Stamens longer than or equal to sepals. Ovary superior. Seeds with tubercles... ..... ***C. album***
– Stamens shorter than sepals. Ovary subsuperior. Seeds smooth.... ***C. hebetatum***
3 Sterile branches often hypogeous, filiform, with bulbil at top..... ..... ***C. maximowiczii***
– Sterile branches epigeous without bulbil **4**
4 Seeds without tubercles **5**
– Seeds with tubercles 7
5 Sterile branches arch-shaped. Flowering stem(s) 1–3, sometimes branched. Seeds 935–1021 × 511–566 μm ..... ***C. macrospermum***
– Sterile branches straight (not arch-shaped). Flowering stem 1, not branched. Seeds 528–785 × 369–704 μm **6**
6 Leaves of sterile branches congested at distal end, with white variegated veins on upper surface ..... ***C. flaviflorum***
– Leaves of sterile branches distantly arranged, with silvery dotted upper surface ..... ***C. epigealum***
7 Seed tubercles arranged on inconspicuous longitudinal ridges **8**
– Seed tubercles arranged on prominent longitudinal ridges **10**
8 Leaves of sterile branches densely ciliate ..... ***C. villosum***
– Leaves of sterile branches rarely ciliate **9**
9 Sterile branches branched (at least two times), ca. 30 cm long after fruiting. Leaves of sterile branches with silvery dots, upper surface glabrous. Bracteal leaves yellowish-green ..... ***C. ramosissimum***
– Sterile branches unbranched, less than 15 cm long after fruiting. Leaves of sterile branches without silvery dots, upper surface pilose. Bracteal leaves bright yellow ..... ***C. valdepilosum***

10 Basal leaves persistent **11**
– Basal leaves withered before flowering **13**
11 Sepals yellow. Stamens shorter than sepals ..... ***C. sphaerospermum***
– Sepals light green. Stamens equal to or longer than sepals **12**
12 Stamens equal to or slightly longer than sepals. Ovary 1/2 or 1/3 inferior ..... ***C. rhabdospermum***
– Stamens longer than sepals. Ovary 1/4 inferior or nearly superior ..... ***C. pseudopilosum***
13 Leaves of sterile branches distantly arranged after fruiting. Bracteal leaves golden yellow, yellowish-green or green at flowering **14**
– Leaves of sterile branches congested at distal end after fruiting. Bracteal leaves green **15**
14 Leaves of sterile branches pilose. Bracteal leaves golden yellow at flowering ..... ***C. aureobracteatum***
– Leaves of sterile branches glabrous. Bracteal leaves yellowish-green to green at flowering ..... ***C. pilosum***
15 Seeds ca. 720 × 640 μm, with ca. 18 ridges, densely papillate ..... ***C. barbatum***
– Seeds ca. 640 × 510 μm, with ca. 16 ridges, sparsely papillate ..... ***C. fulvum***

Acknowledgements

This work was supported by a grant from the National Research Foundation of Korea (2015R1D1A1A01057163) and by the KRIBB initiative programme.

References

Bensel C, Palser B (1975) Floral anatomy in the Saxifragaceae sensu lato. IV. Bauerioideae and Conclusions. American Journal of Botany 62(7): 688–694. https://doi.org/10.1002/j.1537-2197.1975.tb14101.x

Bhaumik M (2014) *Chrysosplenium arunachalense* (Saxifragaceae), a new species from India. Kew Bulletin 69(1): 9491. https://doi.org/10.1007/s12225-014-9491-3

Franchet AR, Savatier PAL (1878) Enumeratio plantarum: in Japonia sponte crescentium hucusque rite cognitarum, adjectis descriptionibus specierum pro regione novarum, quibus accedit determinatio herbarum in libris japonicis So mokou zoussetz xylographice delineatarum Volumen Secundum, 358 pp.

Han JW, Kang SH (2012) *Chrysosplenium epigealum* JW Han et SH Kang: A new species of *Chrysosplenium* (Saxifragaceae) from Korea. Korean Journal of Plant Resources 25(3): 346–348. https://doi.org/10.7732/kjpr.2012.25.3.346

Han JW, Yang SG, Kim HJ, Jang CG, Park JM, Kang SH (2011) Phylogenetic study of Korean *Chrysosplenium* based on nrDNA ITS sequences. Korean Journal of Plant Resources 24(4): 358–369. https://doi.org/10.7732/kjpr.2011.24.4.358

Hara H (1957) Synopsis of genus *Chrysosplenium* L. Journal of the Faculty of Science, University of Tokyo, Section III. Botany 7: 1–90.

IUCN (2001) IUCN Red List Categories and Criteria, Version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland and Cambridge, 17 pp.

Kim YI, Kim YD (2015) *Chrysosplenium aureobracteatum* (Saxifragaceae), a new species from South Korea. Novon: A Journal for Botanical Nomenclature 23: 432–436. https://doi.org/10.3417/2013018

Kim YI, Cho SH, Lee JH, Kang DH, Park JH, Kim YD (2018) *Chrysosplenium ramosissimum* Y.I. Kim & Y.D. Kim (Saxifragaceae), a new species from Korea. PhytoKeys 111: 1–10. https://doi.org/10.3897/phytokeys.111.27182

Kitagawa M (1934) Contributio ad cognitionem Florae Manshuricae V. The Botanical Magazine 48: 908. https://doi.org/10.15281/jplantres1887.48.907

Liu H, Luo JL, Liu YQ, Lan DQ, Qin R, Yu XL (2016) A new species of *Chrysosplenium* (Saxifragaceae) from Zhangjiajie, Hunan, central China. Phytotaxa 277(3): 287–292. https://doi.org/10.11646/phytotaxa.277.3.7

Nakai T (1914) Plantae novae Coreanae et Japonicae II. Repertorium Specierum Novarum Regni Vegetabilis 13(17–21): 267–278. https://doi.org/10.1002/fedr.19140131703

Ohwi J (1934) Plantae Novae Japonicae. Repertorium Specierum Novarum Regni Vegetabilis 36: 39–58.

Pan JT (1986) A study on the genus *Chrysosplenium* L. from China. Zhiwu Fenlei Xuebao 24(3): 203–214.

Pan JT, Ohba H (2001) *Chrysosplenium*. In: Wu ZY, Raven PH (Eds) Flora of China, Vol. 8. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 346–358.

Soltis DE (2007) Saxifragaceae. In: Kubitzki, K. (Ed.) Flowering Plants. Eudicots, Springer Berlin Heidelberg, 418–435. https://doi.org/10.1007/978-3-540-32219-1_47

Soltis DE, Nakazawa MT, Xiang QY, Kawano S, Murata J, Wakabayashi M, Jetter CH (2001) Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on matK sequence data. American Journal of Botany 88(5): 883–893. https://doi.org/10.2307/2657040

Spongberg SA (1972) Genera of Saxifragaceae in the southeastern United States. Journal of the Arnold Arboretum 53(4): 409–498.

Wakabayashi M, Takahashi H (1999) A new species of *Chrysosplenium* (Saxifragaceae) from central Honshu, Japan. Acta Phytotaxonomica et Geobotanica 50: 1–12.

Wakabayashi M, Takahashi H, Tomita S (2018) *Chrysosplenium suzukaense* (Saxifragaceae), a New Species from Yoro and Suzuka Mts., Central Honshu, Japan. Acta Phytotaxonomica et Geobotanica 69(1): 41–51.

Ye H, Zhang G (1994) A new species of *Chrysosplenium* from Guangxi. Acta Botanica Austro Sinica 9: 57–59.

PhytoKeys 135: 49–58 (2019)
doi: 10.3897/phytokeys.135.38756
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Camellia debaoensis (Theaceae), a new species of yellow camellia from limestone karsts in southwestern China

Renchuan Hu[1], Sujuan Wei[2], Yongqing Liufu[3], Yunkai Nong[1], Wei Fang[4]

1 *Guangxi Institute of Chinese Medicine and Pharmaceutical Science, Nanning, Guangxi 530022, China* **2** *Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and the Chinese Academy of Sciences, Guilin, Guangxi 541006, China* **3** *Guangxi Museum of Natural History, Nanning, Guangxi 530012, China* **4** *Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*

Corresponding author: *Yongqing Liufu* (lfyongqing@foxmail.com)

Academic editor: *C. Morden* | Received 8 August 2019 | Accepted 1 October 2019 | Published 28 November 2019

Citation: Hu R, Wei S, Liufu Y, Nong Y, Fang W (2019) *Camellia debaoensis* (Theaceae), a new species of yellow camellia from limestone karsts in southwestern China. PhytoKeys 135: 49–58. https://doi.org/10.3897/phytokeys.135.38756

Abstract

Camellia debaoensis R.C.Hu & Y.Q.Liufu, **sp. nov.** is described and illustrated as a new species from southwestern Guangxi, China. It is morphologically similar to *Camellia pubipetala* Y. Wan & S. Z. Huang, *C. mingii* S.X. Yang and *C. tuyenquangensis* D.V. Luong, N.N.H. Le & N. Tran, but it differs from these species in having glabrous young branches, glabrous petiole, glabrous sepals, glabrous petals, glabrous stamens and glabrous ovary, 10 petals, cylindrical ovary and style 3-lobed to 1/6 style length.

Keywords

Camellia, China, limestone flora, taxonomy, Theaceae

Introduction

Guangxi Zhuang Autonomous Region of southern China is an area noted for its karst landscapes (Hou et al. 2010). The limestone region in southwestern Guangxi harbors very high levels of biological diversity and is recognized as one of 20 centers of plant endemism in China (Myers et al. 2000, López-Pujol et al. 2011). Yellow camellia, a subgroup of *Camellia* (Theaceae), are characterized by yellow, waxy and shiny petals (Chang and Ren 1998). Because of their beautiful flowers and useful chemical constituents, yellow camellias have considerable economic value in breeding, as well as traditional Chinese medicine and commercial tea production (He et al. 2016). Before 2007, fewer than

20 yellow camellias from China were recognized, according to the literatures (Ye and Xu 1992; Chang and Ren 1998; Min 2000; Min and Bruce 2007). Most of them are only distributed in southwestern Guangxi, which had been considered as a center of diversity of the yellow camellia. In recent years, many new species of yellow camellia have been reported from northern Vietnam and southern China (e.g. Tran and Nguyet 2005; Orel 2006; Orel and Wilson 2010, 2012; Orel et al. 2012, 2013, 2014a, b; Tran et al. 2012; Tran and Luong 2013; Huang et al. 2014; Orel and Curry 2015; Tran and Le 2015; Luu et al. 2015; Dung et al. 2016; Luong and Le 2016; Luong et al. 2016a, b; Le et al. 2017; Nguyen et al. 2018; Liu et al. 2019), increasing the total to more than 50 species (Tran et al. 2019) and making northern Vietnam another center of yellow camellia diversity. Generally, yellow camellias are rare and highly endemic due to their small population, narrow distribution and excessive gathering. Recently, almost all Chinese yellow camellia species were categorized as Critically Endangered, Endangered, or Vulnerable species in the Threatened Species List of China's Higher Plants (Qin et al. 2017).

During our floristic survey in limestone karsts of Debao County, southwestern Guangxi, in 2015, we collected several specimens from a population of *Camellia* with yellow flowers. In the following three years, this population was documented for flowering and fruiting regularly at the same locality. Morphological comparison between the newly collected specimens and other yellow camellias suggested that the specimens from Debao differed from all the previously described species. Therefore, we here describe this material as a new species.

Materials and methods

Several specimens were collected at the entrance of one of the karst caves of Debao County, Jingde Town, Tuoliang village from 2015 to 2018, and were deposited in the herbaria GXMI, IBK, NHMG, KUN. The morpho-photographs of the plants were taken with a Panasonic LX100 camera. This material was confirmed as a new species based on detailed comparison with all other heretofore known yellow camellias, including specimens deposited at PE, KUN, IBSC, IBK, GXMI, HIB, SYS, GXMG, and description from botanical websites (e.g. http://www.cvh.ac.cn/, https://plants.jstor.org/). Herbarium acronyms follow Thiers (2018). The morphological characters were measured using M & G ARL96004.

Taxonomic treatment

Camellia debaoensis **R.C.Hu & Y.Q.Liufu, sp. nov.**
urn:lsid:ipni.org:names:77203187-1
Figs 1, 2

Diagnosis. Morphologically, the new species is similar to *Camellia pubipetala* Y. Wan & S.Z. Huang, *C. mingii* S.X. Yang and *C. tuyenquangensis* D.V. Luong, N.N.H. Le &

N. Tran, but it differs from these species in having glabrous young branches, glabrous petiole, glabrous sepals, glabrous petals, glabrous stamens and glabrous ovary, 10 petals, cylindrical ovary and 3-lobed to 1/6 style length.

Type. China. Guangxi Zhuang Autonomous Region: Debao County, Jingde Town, Tuoliang village, at the entrance of one of karst caves, rare, 23°29'23.12"N, 106°9'47.27"E, 760 m a.s.l., 13 January 2017 (fl.), *R.C. Hu HRC170113002* (holotype: GXMI!, isotypes: GXMI!, KUN!, NHMG! and IBK!).

Description. Shrubs, 1–3 m tall. Young branches cylindrical, thick, glabrous, yellowish brown or grayish brown, and current year branchlets purplish red. Leaf blade leathery, ovate to long ovate, 6–13 × 3–5 cm, adaxial surface dark green and glabrous, abaxial surface pale green, brown glandular punctuate and veins sparsely spreading villous, veins abaxially elevated and adaxially impressed, secondary veins 5–6 on each side of midvein and connected at the proximal edge, base cuneate to broadly cuneate, apex caudate tip, margin serrulate; petiole 5–12 mm long, glabrous. Flowers subterminal axillary, solitary, 3–4.5 cm diam. Pedicel ca. 4(–6) mm long, thick; bracteoles 4 (or 5), unequal, 1–3 × 2–4 mm, appressed and covering pedicel, oval-triangle, leathery, green and glabrous, margin ciliolate. Sepals 5 (–6), semiorbicular to broadly ovate, 3–5 × 5–8 mm, leathery, glabrous, lightly yellow and occasionally with pink patches, fruiting stage green, margin ciliolate. Petals 10, in three whorls of 3–4 petals, golden yellow, glabrous; outer 3 or 4 petals suborbicular, occasionally with pink patches, 0.7–1.1 × 1 cm; inner orbicular-ovate or oval, 1.2–1.8 × 1.2–2.6 cm, basally connate for 1–3 mm. Stamens numerous, glabrous, ca. 2 cm long; anthers ca. 3 × 1mm; outer filaments connate ca. basal 1/4, ca. 1.6 cm, inner filaments nearly distinct, ca. 1.7 cm. Ovary cylindrical, ca. 2 mm in diam., glabrous, 3-loculed; style 2 cm long, glabrous, base connate, apex 3-lobed to 1/6 style length. Capsule triangle oblate, glabrous, 1.4–1.6 × 1.6–2.8 cm; Seeds brown, hemispherical, pubescent.

Phenology. Flowering from December to February of the next year; fruiting from July to August.

Distribution and habitat. *Camellia debaoensis* grows at the entrance of one of the limestone caves in the karst region of Debao County (Fig. 3), Guangxi, China, accompanied by *Ageratina adenophora* (Sprengel) R. M. King & H. Robinson (Compositae), *Boehmeria penduliflora* Wedd. ex Long (Urticaceae), *Fallopia multiflora* (Thunb.) Harald (Polygonaceae), *Flueggea virosa* (Roxb. ex Willd.) Voigt (Euphorbiaceae), *Pteris vittata* L. (Pteridaceae), *Ficus tikoua* Bur (Moraceae), and *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen et S. M. Almeida ex Sanjappa et Predeep (Fabaceae).

Conservation status. According to currently available data, *Camellia debaoensis* is only found in its type locality and there are only nine adult trees and four saplings, with the distribution restricted to a very limited region (less than 200 m^2). Considering this situation, we consider *Camellia debaoensis* as 'Critically Endangered' (CR) based on the IUCN categories and criteria (IUCN 2017).

Additional specimens examined. China. Guangxi Zhuang Autonomous Region: Debao County, Jingde Town, Tuoliang village, at the entrance of karst cave, rare, ca. 760 m a.s.l., 13 Jan. 2017 (fl.), *R.C. Hu HRC170113001* (GXMI!); the same locality, 21 May 2016 (fr.), *R.C. Hu HRC170521001*(GXMI!); the same locality, 25 Dec. 2015 (fl.), *R.C. Hu & Y.Q. Liufu HRC151225023* (GXMI!).

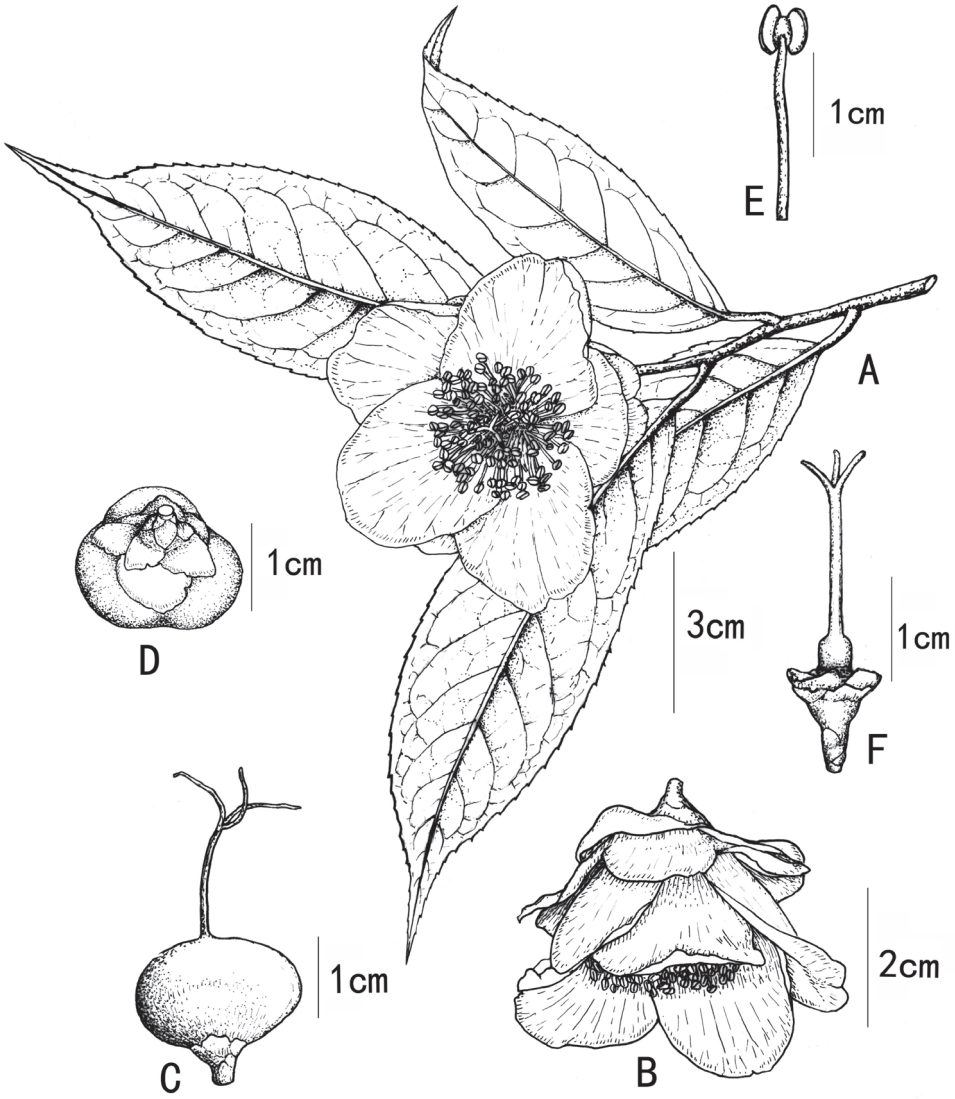

Figure 1. *Camellia debaoensis* R.C.Hu & Y.Q.Liufu, sp. nov. **A** flowering branch **B** lateral view of flower **C** fruit and style **D** fruit, sepals and bracteoles **E** stamen **F** pistil. Drawn by Xincheng Qu.

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

Taxonomic notes. It is noted that there are several classification systems about taxonomic treatments of *Camellia*, represented by Ye and Xu (1992), Chang and Ren (1998), and Min and Bruce (2007). These systems have different taxonomic opinions on sectional taxonomic treatment of yellow camellias. Considering the inclusiveness of the system of Min and Bruce (2007), the new species should be placed in *C.* sect. *Stereocarpus*.

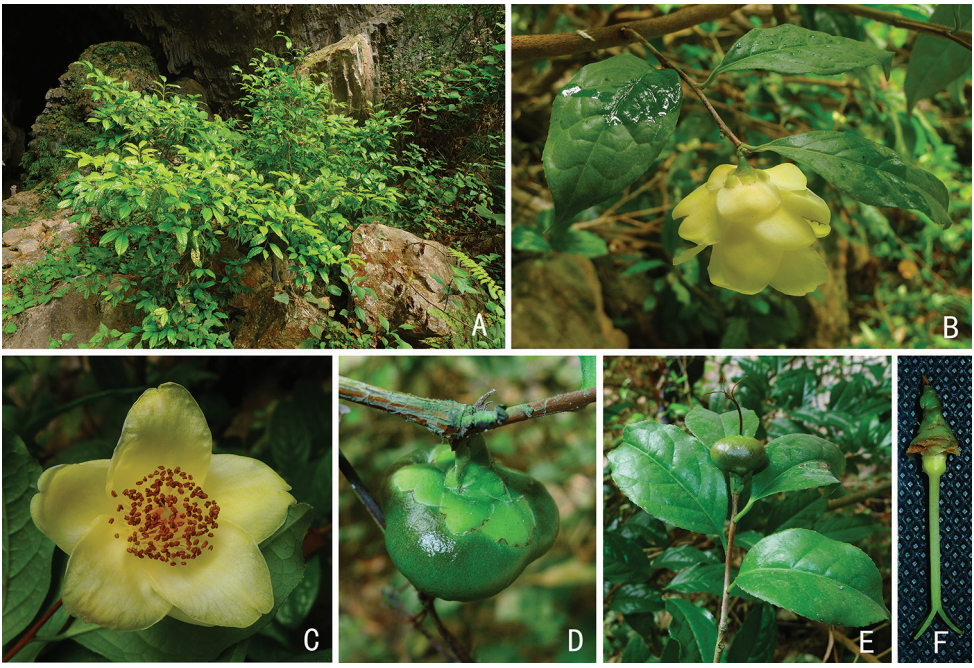

Figure 2. *Camellia debaoensis* R.C.Hu & Y.Q.Liufu, sp. nov. **A** habit **B** flowering branch **C** face view of flower **D** fruit, sepals and bracteoles **E** fruiting branch **F** pistil. Photographed by Renchuan Hu.

Figure 3. Map showing distribution of *Camellia debaoensis* R.C.Hu & Y.Q.Liufu, sp. nov. in southwestern Guangxi, China.

Table 1. Morphological comparison of *C. debaoensis* with other yellow camellias with connate style. Data from Min and Bruce (2007), Orel and Wilson (2010), Orel et al. (2013, 2014a), Luong et al. (2016a, b), Le et al. (2017), Liu et al. (2019).

Taxon	Section	Leaf shape, leaf size	Pedicel length	No. of bracts	No. of petals	Style, extent to which cleft, hairiness	Ovary, carpels, hairiness	Filaments morphology
C. capitata	*Capitatae*	Elliptic to oval, 24.0–27.0 × 10.0–12.0 cm	Sessile	8–10	6	Apex 3-lobed, apically cleft for 1–2 mm, glabrous	3-carpellate, glabrous	Glabrous, outer filaments basally attached to inner petals for ca. 10 mm
C. luteocerata	*Dalatia*	Elliptic to broadly elliptic, 22.0–27.5 × 9.0–11.0 cm	Sessile	6	11–13	Apex 5-lobed, apically cleft to 1/3 style length, glabrous	5-carpellate, glabrous	Glabrous, filaments basally connate for 1/3 of their length, and partially joined to the inner petals
C. bugiamapensis	*Dalatia*	Elliptic, oval to widely elliptic, 18.0–27.5 × 12.0–14.0 (–15.0) cm	Sessile	7	9–11	Apex 5-lobed, apically shortly lobed, white hairs	5–6-carpellate, glabrous	Glabrous, outer filaments basally attach to inner petals for 20–25 mm
C. luteopallida	*Dalatia*	Elliptic to ovate, 16.0–20.0 × 5.0–9.0 cm	Sessile	6–9	12–14	Apex 3-lobed, apically cleft 3–6 mm, dense white hairs	3-carpellate, glabrous	Sparely hairy, filaments basally united to each other to form a 10–13 mm fleshy tube
C. tuyenquangensis	*Chrysantha*	Oblong ovate to narrow elliptic, 14.0–18.0 × 5.0–8.0 cm	10 mm	4–5	12	Apex 3-lobed, apically cleft to 1/2 style length, glabrous	3-carpellate, glabrous	Glabrous, outer filament whole basally connate for 10–14 mm, adnate to petal base
C. thuongiana	*Chrysantha*	Elliptic to oblong elliptic, 9.0–17.0 × 4.0–6.5 cm	8–10 mm	3–4	11–13	Apex 3-lobed, apically cleft to 1/2 style length, glabrous	3-carpellate, pubescent	Glabrous, outer filaments basally connate for 4–5 mm
C. oconoriana	*Chrysantha*	Narrowly elliptic, 30.0–36.5 × 8.0–8.5 cm	30–40 mm	6	6	Apex 3–5-lobed, apically cleft 9–11 mm, finely hairy proximally, glabrous distally	4–5-carpellate, densely tomentose	Glabrous, outer filaments basally connate for ca. 6 mm
C. pubipetala	*Stereocarpus*	Elliptic to ovate, 10.0–17.0 × 5.0–8.0 cm	Subsessile	(4–)6–8	9–13	Apex 3(or 4)-lobed, apically cleft 5–10 mm, tomentose	3(or 4)-loculed, yellowish tomentose	Distinct part pilose, outer filament whorl basally connate for ca. 1/3 of its length
C. mingii	*Stereocarpus*	Elliptic-ovate to narrowly ovate, 10.0–15.0 × 4.0–6.0 cm	3–6 mm	4 or 5	12 or 13	Apex 3-lobed, apically cleft ca.2–3 mm, glabrous or sparsely pubescent	3-carpellate, densely tomentose	Puberulent, outer filaments basally connate for ca. 1/2 of their length
C. debaoensis	*Stereocarpus*	Oval to long oval, 6.0–13.0 × 2.5–5.0 cm	4(–6) mm	4 (or 5)	10	Apex 3-lobed, apically cleft to 1/6 style length, glabrous	3-carpellate, glabrous	Glabrous, outer filaments basally connate for ca. 1/4 of their length

Camellia debaoensis resembles many other yellow-flowering camellia species with connate style, such as *Camellia pubipetala* Y. Wan & S.Z. Huang, *C. mingii* S.X. Yang, *C. tuyenquangensis* D.V. Luong, N.N.H. Le & N. Tran, *C. oconariana* Orel, Curry & Luu, *C. thuongiana* Luong, Anna Le & Lau, *C. luteocerata* Orel, *C. luteopallida* Luong, T.Q.T. Nguyen & Luu, *C. bugiamapensis* Orel, Curry, Luu & Q.D. Nguyen and *C. capitata* Orel, Curry & Luu. These species are placed in various sections within the genus including sections *Stereocarpus* Chang, *Chrysantha* Chang, *Dalatia* Orel and *Capitatae* Orel (Table 1). A key to identifying species of yellow camellia with connate style is provided below. Of these species, the new species is more similar to *C. pubipetala*, *C. mingii*, *C. tuyenquangensis* than to other species by sharing small leaflets, subterminal axillary flowers, 9–13 petals, differentiated bracts, outer filament whorl basally connate, 3-locular ovary, connate style. However, it is well distinguished from these species in having glabrous young branches, glabrous petiole, glabrous sepals, glabrous petals, glabrous stamens and a glabrous ovary (vs. villous in *C. pubipetala* and *C. mingii*), 10 petals (vs. 9–13 in *C. pubipetala*, 12–13 in *C. mingii* and 12 in *C. tuyenquangensis*), cylindrical ovary (vs. ovoid in *C. mingii* and *C. tuyenquangensis*, spherical in *C. pubipetal*) and style 3-lobed to 1/6 style length (vs. 3 (or 4)-cleft to 1/3 style length in *C. pubipetala*, 3-cleft to 1/10 style length in *C. mingii*, 3-cleft to 1/2 style length in *C. tuyenquangensis*). The comparisons between *C. debaoensis* and these species are provided in Table 2. Based on hairs, lamina length, flower size, number of carpels and other characteristics, the new species is also a taxonomic entity distinct from *C. oconariana*, *C. thuongiana*, *C. luteocerata*, *C. luteopallida*, *C. bugiamapensis* and *C. capitata* (Table 1).

Table 2. Morphological comparison of *C. debaoensis* with *C. pubipetala*, *C. mingii* and *C. tuyenquangensis*.

Items	*C. debaoensis*	*C. pubipetala*	*C. mingii*	*C. tuyenquangensis*
Young branches	Glabrous	Gray spreading villous	Densely spreading yellowish villous	Glabrous
Leaf	Ovate to long ovate, 6.0–13 × 2.5–5.0 cm, abaxial veins sparsely spreading villous, secondary veins 5–6	Elliptic-ovate, 10.0–17.0 × 5.0–8.0 cm, abaxial appressed villous but densely spreading villous along midvein, secondary veins 8–10	Elliptic-ovate to narrowly ovate, 10.0–15.0 × 4.0–6.0 cm, abaxial densely spreading villous along veins, secondary veins 7–10	Oblong ovate to narrow elliptic, 14.0–18.0 × 5.0–8.0 cm, abaxial glabrous, secondary veins 9–11
Petiole	5–12 mm long, glabrous	5–10 mm long, villous	5–7 mm long, densely villous	10–15 mm long, glabrous
Pedicel length	4 (–6) mm	3–5 mm	3–6 mm	10 mm
Sepals	Glabrous	Densely puberulent	Inside densely puberulent	Glabrous
Petals	10, glabrous	9–13, outside gray puberulent, inside glabrous	12 or 13, puberulent on both surfaces	12, glabrous
Stamens	2.0 cm long, glabrous	2.5–3.0 cm long, distinct part pilose	3.0 cm long, puberulent	2.5–3.0 cm long, glabrous
Ovary	Cylindrical, glabrous	Spherical, densely tomentose	Ovoid, densely tomentose	Ovoid, glabrous
Style	2 cm long, apex 3-lobed to 1/6 style length, glabrous	Apically 3 (or 4)-lobed to 1/3 style length, tomentose	3.0 cm long, apex 3-cleft to 1/10 style length, glabrous	3 cm long, apex 3-cleft to 1/2 style length, glabrous

Key to identification of species of yellow camellia with connate style

1 Ovary 3 carpellate, style 3-parted; if ovary 3 (or 4) carpellate, style 3 (or 4)-parted, young branches and leaves spreading villous (*C. pubipetala*)..**2**
– Ovary 4–6 carpellate, style (3–) 4–6-parted **8**
2 Petals 9–14 .....**3**
– Petals 6 .....***C. capitata***
3 Young branches, petiole, petals, stamens, ovary and style piliferous.....**4**
– Young branches, petiole, petals, stamens, ovary and style glabrous **5**
4 Petals puberulent on both sides, suborbicular.....***C. mingii***
– Petals adaxial puberulent, inside glabrous, elliptic .....***C. pubipetala***
5 Petals with dense appressed brown hairs; style with dense white appressed hairs.....***C. luteopallida***
– Petals and style glabrous.....**6**
6 Leaf 6–13 × 2.5–5 cm, abaxial veins sparsely spreading villous, secondary veins 5–6; ovary cylindrical; style apex 3-lobed to 1/6 the length of style .... ***C. debaoensis***
– Leaf glabrous; style apex 3-cleft to 1/2 the length of style.....**7**
7 Stamens 2.5–3 cm long; style 30 mm long.....***C. tuyenquangensis***
– Stamens 1.3–1.4 cm long; style 8–9 mm long.....***C. thuongiana***
8 Leaf secondary veins 8–11; pedicel sessile, 1–5 mm long **9**
– Leaf secondary venation 24 pairs; pedicel 30–40 mm long .... ***C. oconoriana***
9 Petals 9–11, with margins sparsely ciliate; style finely tomentose..... ..... ***C. bugiamapensis***
– Petals 11–13, outer 5-petaloid concave; style glabrous ..... ***C. luteocerata***

Acknowledgements

We are grateful to Xu Weibin (Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and the Chinese Academy of Sciences, Guilin) for useful comments on the manuscript, Huang Xiaona, Jiang Changjie and Professor Huang Liangdong (Nanning Golden Camellia Park, Nanning) for species diagnosis, Yang Ping, Wei Guiyuan and Lan Xiangchun (Guangxi Institute of Traditional Medical and Pharmaceutical Sciences, Nanning) for fieldwork assistance, Qu Xincheng for the line drawing. This work was partially supported by the 2017 Chinese Medicine Public Health Service Subsidy Special "National Chinese Medicine Resources Census Project" (GXZYZYPC17-3), the National Natural Science Foundation of China (31760088), the Guangxi Key Laboratory of Traditional Chinese Medicine Quality Standards Independent research topic (GZZZ201605), the Guangxi Science and Technology Project (guike AD16380013).

References

Chang HT, Ren SX (1998) Theaceae. In: Wu CY (Ed.) Flora Reipublicae Popularis Sinicae, vol. 49(3). Science Press, Beijing, 101–112.

Dung LV, Son HT, Ninh T, Nhan PH (2016) *Camellia quangcuongii* (Theaceae), a new species from Vietnam. Shokubutsu Kenkyu Zasshi 91: 226–230.

He DY, Li XY, Wang LL, Zhang P, Li SY, Xu YP (2016) Chemical constituents and pharmacological effects of *Camellia nitidissima.* Zhongguo Shiyan Fangjixue Zazhi 22: 231–234.

Hou MF, López-Pujol J, Qin HN, Wang LS, Liu Y (2010) Distribution pattern and conservation priorities for vascular plants in Southern China: Guangxi Province as a case study. Botanical Studies (Taipei, Taiwan) 51: 377–386.

Huang LD, Liang SY, Ye CX (2014) A new species of *Camellia* from Guangxi, China. Journal Guangdong Landscape Architecture 36: 69–70.

IUCN (2017) Guidelines for using the IUCN red list categories and criteria. Version 13. Prepared by the standards and petitions subcommittee.

Le NHN, Uematsu C, Katayama H, Nguyen LT, Tran N, Luong DV, Hoang ST (2017) *Camellia tuyenquangensis* (Theaceae), a new species from Vietnam. Korean Journal of Plant Taxonomy 47(2): 95–99. https://doi.org/10.11110/kjpt.2017.47.2.95

Liu ZW, Fang W, Liu ED, Zhao M, He YF, Yang SX (2019) *Camellia mingii*, a new species of yellow camellias from Southeast Yunnan, China. Phytotaxa 393(1): 047–056. https://doi.org/10.11646/phytotaxa.393.1.4

López-Pujol J, Zhang FM, Sun HQ, Ying TS, Ge S (2011) Centres of plant endemism in China: Places for survival or for speciation? Journal of Biogeography 38(7): 1267–1280. https://doi.org/10.1111/j.1365-2699.2011.02504.x

Luong VD, Le NHN (2016) *Camellia ninhii* – a new yellow camellia species from Vietnam. International Camellia Journal 48: 117.

Luong VD, Luu HT, Nguyen TQT, Nguyen QD (2016a) *Camellia luteopallida* (Theaceae), a new species from Vietnam. Annales Botanici Fennici 53(1–2): 135–138. https://doi.org/10.5735/085.053.0224

Luong VD, Le A, Nguyen TH, Nguyen TL (2016b) *Camellia thuongiana* – A new yellow camellia species from Vietnam. Dalat University Journal of Science 6(3): 338–344.

Luu HT, Luong VD, Nguyen QD, Nguyen TQT (2015) *Camellia sonthaiensis* (Theaceae), a new species from Vietnam. Annales Botanici Fennici 52(5–6): 289–295. https://doi.org/10.5735/085.052.0502

Min TL (2000) Monograph of the genus *Camellia*. Yunnan Science & Technology Press, Kumming, 78–239.

Min TL, Bruce B (2007) Theaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China. Science Press (Beijing) and Missouri Botanical Garden Press (St. Louis), 366–478.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Nguyen LT, Tran N, Chiyomi U, Hironori K, Luong DV, Hoang ST, Nguyen KD, Nguyen HV, Thai TC (2018) Two new species of *Camellia* (Theaceae) from Vietnam. Korean Journal of Plant Taxonomy 48(2): 115–122. https://doi.org/10.11110/kjpt.2018.48.2.115

Orel G (2006) A new species of *Camellia* section *Piquetia* (Theaceae) from Vietnam. Novon 16(2): 244–247. https://doi.org/10.3417/1055-3177(2006)16[244:ANSOCS]2.0.CO;2

Orel G, Curry AS (2015) In pursuit of hidden camellias: 32 New *Camellia* Species from Vietnam and China. Theaceae Exploration Associates, Sydney, 339 pp.

Orel G, Wilson PG (2010) *Camellia luteocerata* sp. nov. and a new section of *Camellia* (*Dalatia*) from Vietnam. Nordic Journal of Botany 28(3): 281–284. https://doi.org/10.1111/j.1756-1051.2010.00652.x

Orel G, Wilson PG (2012) *Camellia cattienensis*: a new species of *Camellia* (sect. *Archaecamellia*: Theaceae) from Vietnam. Kew Bulletin 66(4): 565–569. https://doi.org/10.1007/s12225-012-9317-0

Orel G, Wilson PG, Curry AS, Luu HT (2012) *Camellia inusitata* (Theaceae), a new species forming a new section (*Bidoupia*) from Vietnam. Edinburgh Journal of Botany 69(2): 347–355. https://doi.org/10.1017/S0960428612000170

Orel G, Wilson PG, Curry AS, Luu HT (2013) *Camellia oconoriana* (Theaceae), a new species from Vietnam. Edinburgh Journal of Botany 70(3): 439–447. https://doi.org/10.1017/S0960428613000103

Orel G, Wilson PG, Curry AS, Truong LH (2014a) Four new species and two new sections of *Camellia* (Theaceae) from Vietnam. Novon 23(3): 307–318. https://doi.org/10.3417/2012076

Orel G, Wilson PG, Luu HT (2014b) *Camellia curryana* and *C. longii* spp. nov. (Theaceae) from Vietnam. Nordic Journal of Botany 32(1): 42–50. https://doi.org/10.1111/j.1756-1051.2013.00399.x

Qin HN, Yang Y, Dong SY, He Q, Jia Y, Zhao L, Yu S, Liu H, Liu B, Yan Y, Xiang J, Xia N, Peng H, Li Z, Zhang Z, He X, Yin L, Lin Y, Liu Q, Hou Y, Liu Y, Liu Q, Cao W, Li J, Chen S, Jin X, Gao T, Chen W, Ma H, Geng Y, Jin X, Chang C, Jiang H, Cai L, Zang C, Wu J, Ye J, Lai Y, Liu B, Lin Q, Xue N (2017) Threatened Species List of China's Higher Plants. Shengwu Duoyangxing 25(7): 696–744. https://doi.org/10.17520/biods.2017144

Thiers B (2018) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium.

Tran N, Le NHN (2015) A new yellow *Camellia* species from North Vietnam. International Camellia Journal 47: 36–39.

Tran N, Luong VD (2013) *Camellia dilinhensis*: A new species from Vietnam. Internal Camellia Journal 45: 87–89.

Tran N, Nguyet HN (2005) *Camellia hamyenensis*: A new yellow species from Vietnam. Internal Camellia Journal 37: 125–127.

Tran N, Hakoda N, Luong VD (2012) A new species of yellow camellias (Sect. *Piquetia*) from Vietnam. Internal Camellia Journal 44: 161–162.

Tran DM, Nguyen TT, Hoang TS, Dang VT, Phung DT, Nguyen VT, Dao TD, Mai TL, Vu TL, Nguyen HT, Nguyen TTP, Tran VD (2019) Golden Camellia: A review. Archives of Current Research International 16(2): 1–8. https://doi.org/10.9734/acri/2019/v16i230085

Ye CX, Xu ZR (1992) A taxonomy *Camellia* Sect. *Chrysantha* Chang. Acta Scientiarum Naturalium Universitatis Sunyatseni 31: 68–77.

PhytoKeys 135: 59–69 (2019)
doi: 10.3897/phytokeys.135.36426
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Silene sunhangii (Caryophyllaceae), a new species from China

Nan Lin[1,2,3,*], Dai-Gui Zhang[4,*], Xian-Han Huang[1,3], Jian-Wen Zhang[1], Jing-Yuan Yang[5], Komiljon Tojibaev[6], Heng-Chang Wang[2], Tao Deng[1]

1 *CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China* **2** *CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, Hubei 430074, China* **3** *University of Chinese Academy of Sciences, Beijing 100049, China* **4** *Key Laboratory of Plant Resources Conservation and Utilization, Jishou University, Jishou, Hunan 416000, China* **5** *Administration of Shennongjia National Park, Shennongjia, Hubei 44241, China* **6** *Central Herbarium of Uzbekistan, Institute of Botany, Academy Sciences of Uzbekistan, Tashkent 100025, Uzbekistan*

Corresponding author: *Heng-Chang Wang* (hcwang@wbgcas.cn); *Tao Deng* (dengtao@mail.kib.ac.cn)

Academic editor: *G.G. del Galdo* | Received 23 May 2019 | Accepted 10 September 2019 | Published 5 December 2019

Citation: Lin N, Zhang D-G, Huang X-H, Zhang J-W, Yang J-Y, Tojibaev K, Wang H-C, Deng T (2019) *Silene sunhangii* (Caryophyllaceae), a new species from China. PhytoKeys 135: 59–69. https://doi.org/10.3897/phytokeys.135.36426

Abstract

Silene sunhangii, a new species of Caryophyllaceae known from only three populations in Hubei and Hunan provinces of central China, is described. Both morphological and molecular data were used to assess the taxonomic status and relationships of this species. Morphologically, *S. sunhangii* is most similar to *S. platyphylla* Franch. from which it differs most readily in having 3-veined elliptical leaves without pubescence, tasseled catacorolla, pale purple to red petals without a linear lobe or narrow tooth and lanceolate, bifid to one third. A phylogenetic analysis based on nuclear ITS region identified the new species as a well-supported, independent lineage. Our new species is nested within a grade that encompasses species representing a polyphyletic *Silene* sect. *Physolychnis* (Benth.) Bocquet. Both the genetic and morphological data support the recognition of *Silene sunhangii* as a distinct species, although there is inconsistency between these two datasets as to the relationships of the new species.

* The authors contributed equally to this study

Keywords
Silene, new species, morphology, phylogeny, China

Introduction

Silene L. (Sileneae, Caryophyllaceae) is the largest genus of Caryophyllaceae Juss., containing over 700 species (Melzheimer 1988; Rautenberg et al. 2010; Oxelman et al. 2011). It is distributed mainly in the Northern Hemisphere, but some species also occur in Africa and South America (Oxelman et al. 2011). Morphologically, *Silene* is characterized by having a synsepalous calyx, 3–5 carpels and a campanulate, clavate or ovate calyx tube. de Candolle (1824) had recognized just eight sections, using several morphological features, including those of habit, inflorescences and stems. Using life form as the primary character, Boissier (1867) recognized 31 sections for the genus, 11 containing annual species and 20 containing perennial species. Previously, the sectional classification within *Silene* was subsequently revised by Chowdhuri (1957) who recognized 44 sections, and it is this scheme that remains in place today. That study was based on a comprehensive sampling of species and a re-assessment of morphological characters. Uncertainties exist as to the number of subgenera that should be recognized for the genus. Rohrbach (1868) recognized two subgenera (subg. *Silene* and subg. *Behenantha* (Otth) Endl., based on seed characters) while Williams (1896) recognized three subgenera (subg. *Gastrosilene* Williams, subg. *Conosilene* Williams and subg. *Eusilene* Williams, based on calyx characters). Recent molecular studies (Oxelman et al. 1997; Petri and Oxelman 2011) support the subdivision of *Silene* into two major clades which correspond to subg. *Silene* and subg. *Behenantha*. Notwithstanding the above, deficiencies still exist within current classifications involving the genus and a comprehensive phylogenetic study is needed, especially as there is a suggestion in the results of both Oxelman et al. (1997) and Petri and Oxelman (2011) that *Silene* may be polyphyletic.

The treatment of *Silene* by Zhou et al. (2001) in the Flora of China recognized 110 species, of which 67 are endemic and geographically restricted within the country. Within China, species of *Silene* are widely distributed and show a large range of morphological variation. Historically, these species have been accommodated in 22 sections that were defined mostly by characters of the stems, petals, calyx and seeds (Zhou et al. 2001).

Field investigations conducted during this study revealed the existence of a distinctive entity of *Silene* in Hubei and Hunan provinces. Morphologically, this entity is most similar to *S. platyphylla* Franch. which occurs in Yunnan, but it differs significantly from that species in the characters of its root, leaves, petals, catacorolla and lobes. These morphological differences are supported by molecular evidence that justify the recognition of the Hubei and Hunan entity as a new species of *Silene* for China. It is therefore described below as *Silene sunhangii*.

Material and methods

Morphology

Natural populations of the new species were collected from three populations in Hubei and Hunan province (Fig. 1, these data were submitted to PANGAEA, accession number 10.1594/PANGAEA.906581). Morphological characters recorded for the new species were based on fresh flowering and fruiting material collected from those populations. *S. platyphylla* were from herbarium material (KUN). A comparison of the new species with similar species is provided in Table 1.

Molecular analyses

Fresh leaves of the new species were dried in silica gel and total genomic DNA was extracted from 10–20 mg dried leaf tissue. Molecular material of *S. platyphylla* was collected from herbarium specimens (Appendix 1). The nuclear ITS locus was used for phylogeny. The PCR protocol used the following conditions: 5 min at 94, followed by 35 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C and then ending with a final extension of 5 min at 72 °C. The ITS primers used were ITS1 and ITS4, as described by White et al. (1990) and Urbatsch et al. (2000). Voucher specimen and GenBank accession information for taxa are listed in Appendix 1. DNA sequences were aligned using MAFFT software and then manually checked (Katoh et al. 2002). A total of 301-taxon data sets, including two newly published

Table 1. Comparison of *Silene sunhangii* with similar species detected by morphology (*S. platyphylla*).

Species	Characters	
	S. sunhangii	***S. platyphylla***
Roots	tuberous	cylindric
Stems	diffuse, 30–80 cm tall, long pubescent	diffuse, 60–100 cm tall, pubescent
Leaves	elliptic, 4–10 × 1–5 cm, glabrous, conspicuously 3-veined	ovate, 6–8 × 3–5 cm, margin ciliate, 3 or 5-veined
Flower diameter	35–40 mm	20 mm
Pedicel length &indumentum	20–30 mm, pubescent	10–30 mm, hairy
Calyx	tubular-clavate, teeth triangular, glabrous	tubular-clavate, teeth triangular-lanceolate with margin ciliate
Petals	pale purple to red, 2.5 cm, catacorolla tasseled, bifid to one third, lobes lanceolate, without a linear lobe or narrow tooth on each side	white or pale red, 2 cm, catacorolla elliptical or linear, bifid to middle, lobes elliptic, with a linear lobe or narrow tooth on each side
Stamens and filaments	stamens and filaments slightly exserted; filaments pubescent	stamens slightly exserted; filaments glabrous
Distribution	China: Western Hubei and north-western Hunan	China: Western Yunnan

sequences, were obtained. Bayesian inference (BI) and Maximum likelihood (ML) analyses were conducted using MrBayes 3.1.2 and RAxML v.6 (Huelsenbeck and Ronquist 2001; Stamatakis 2006), respectively. The best-fitting substitution models GTR for Bayesian inference were selected using ModelTest v.3.8, and branch support was computed with 1, 000 bootstrap replicates (Posada and Crandall 1998). ML analyses were conducted using the GTRGAMMA model with 1, 000 nonparametric bootstrapping replicates.

Results and discussion

Taxonomic treatment

Silene sunhangii **D.G.Zhang, T.Deng & N.Lin, sp. nov.**
urn:lsid:ipni.org:names:77203328-1
Figs 1–3

Type. China. Hubei Province: Shennongjia National Nature Reserve (SNNR) region, Guanmen Mountain, Alt. 1,319 m, 30°08'16.80"N, 110°34'33.59"E, 1 July 2010, Dai-Gui Zhang, et al. 0622 (holotype: KUN!).

Diagnosis. *Silene sunhangii* is morphologically similar to *S. platyphylla*, from which it differs through the root tuberous (not cylindric as *S. platyphylla*), stems 30–80 cm tall (100 cm tall in *S. platyphylla*), leaves elliptic (not obovate in *S. platyphylla*), 3-veined (not 3/5 veined in *S. platyphylla*) and glabrous (not margin ciliate as *S. platyphylla*), flowers 35–40 mm diam. (not 20 mm in *S. platyphylla*), petals purple to red (not white or pale red in *S. platyphylla*), catacorolla tasseled (not elliptic or linear in *S. platyphylla*), lobe limbs divided to 1/3 (more than 1/3 in *S. platyphylla*).

Description. Herbs perennial. Plant with densely ciliate, tuberous roots and dichasial cymose inflorescences containing many flowers. Stems diffuse, 30–80 cm tall, much-branched, pubescent. Leaves elliptic, 4–10 × 1–5 cm, glabrous, conspicuously 3-veined. Pedicel 20–30 mm long, pubescent. Calyx tubular-clavate, ca. 1.5–2 cm long, densely hairy on veins; teeth triangulate, ciliate. Petals pale purple to red, ca. 2.5 cm long; claws exserted beyond calyx; catacorolla tasseled, limbs obovate, bifid to 1/3; lobes lanceolate, without a linear lobe or narrow tooth on each side. Stamens slightly exserted; filaments pubescent. Capsule ovoid, 10–20 mm long. Seeds dark brown, reniform, ca. 1 mm long, with lateral auricular pits (Fig. 2, 3).

Phenology. Flowering occurs from February to April, and fruiting from April to June.

Etymology. The new species is named in honor of Chinese botanist, Prof. Hang Sun, who has made significant contributions to the flora of China.

Distribution, habitat and conservation status. *Silene sunhangii* is presently known from only Hubei and Hunan provinces in central China (Fig. 1). It grows in

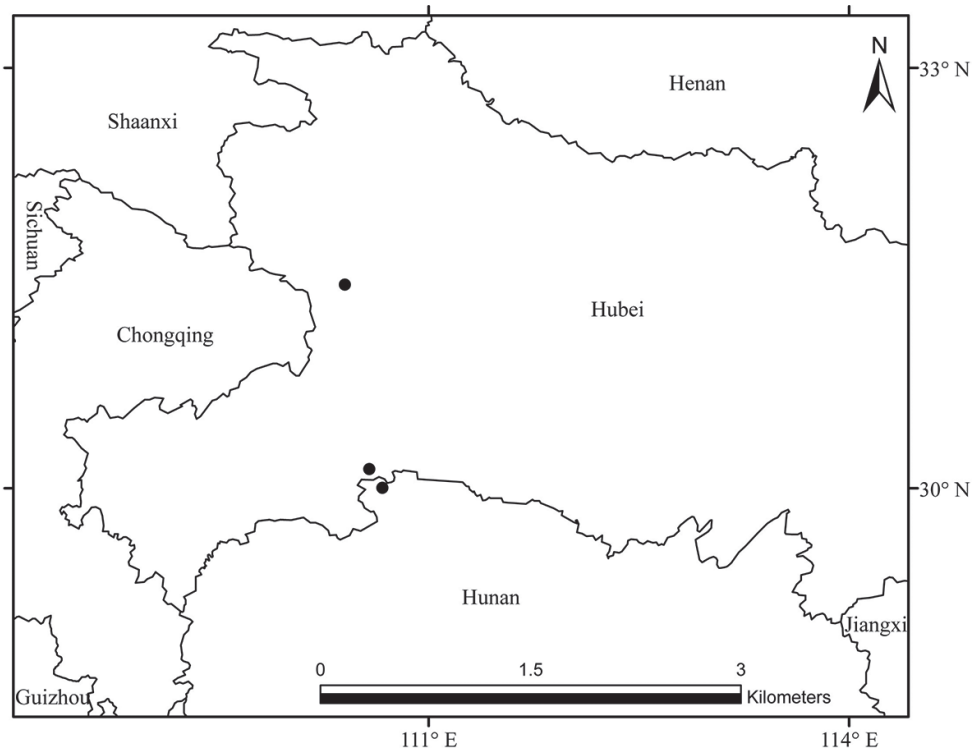

Figure 1. Distribution map of *Silene sunhangii.* The black dots represent locations of *S. sunhangii*.

humid and evergreen or deciduous mixed forest, from 1214–2227 m (Fig. 1). A total of three natural populations have been located, each comprising less than 100 individuals distributed over an area not exceeding 100 m^2. These populations are located within the Shennongjia National Nature Reserve (Hubei province), Houhe Nature Reserve (Hubei Province) and Huping Mountains (Hunan Province), and are therefore well-protected; there are no known threats to these populations. Further field studies are needed to more authoritatively determine the geographic range and frequency of this species. In the meantime, current evidence indicates that *Silene sunhangii* should be assigned the conservation status of "Data Deficient (DD)", following the IUCN Red List Criteria and Categories (IUCN 2017).

Taxonomic notes. *Silene sunhangii* is a perennial with densely ciliate, tuberous roots and dichasial cymose inflorescences containing many flowers. These characters indicate that the new species should be assigned to *Silene* sect. *Cucubaloideae* subsect. *Silene* Chowdhuri. It can be distinguished from all other species of *Silene* that possess lilac to red petals through its root, stem, leaf and corolla characters as described above. Morphologically, *Silene sunhangii* shows greatest similarities with *S. platyphylla*. The diagnosis above enables the two species to be reliably distinguished. *Silene platyphylla* is distributed in western Yunnan.

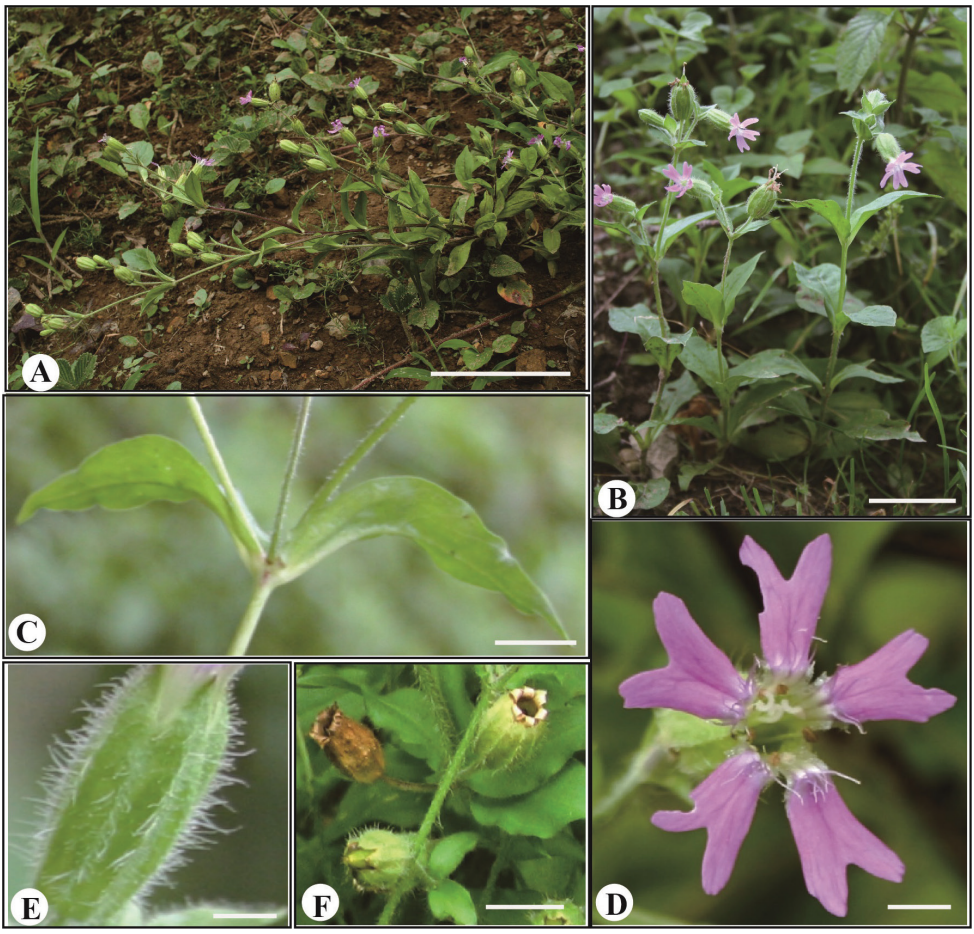

Figure 2. *Silene sunhangii* (from the holotype plant). **A** plant habit **B** plant **C** leaf **D** flower **E** calyx **F** open capsule. Scale bar: 20 cm (**A, B**), 1 cm (**C, D, E, F**).

Molecular phylogenetic analysis

The results of our initial phylogenetic analysis, which included over 300 species, are not shown here but they did confirm the position of the new species within *Silene*. In Fig. 4, we present only those clades (38 species from that original matrix) which are relatively close to the new species. Due to the vagueness of outgroup, we constructed unrooted phylogenetic tree based on 38-taxon of *Silene* (Fig. 4). Clades associated with *Silene platyphylla* are also included because morphological criteria indicate that this species has similarities with *S. sunhangii*.

The aligned matrix consisted of 676 characters from 38 species, of which 165 were variable and 82 were parsimony-informative. Our results based on ITS produced trees with identical topology between BI and ML, and only the tree with bootstrap support values from ML analyses was presented (Fig. 4). According to these results, *Silene sunhangii* is nested within a grade that incorporates a polyphyletic Sect. *Physolychnis*

Figure 3. 1–5 *Silene platyphylla* Franchet (modified from illustration in flora of China), **6–10** *Silene sunhangii*, **1, 6** flowering branch **2, 7** sterile branch **3, 8** petal and stamen **4, 9** pistil **5, 10** root.

(Benth.) Bocquet. *S. sunhangii* is shown to be separated from associated taxa with very high support (BS = 97, PP = 1), and is well-removed from *S. platyphylla*. These results differ from those of the morphological study which placed *S. sunhangii* in sect. *Cucubaloideae* and showed it to be morphologically most similar to S. *platyphylla*. As al-

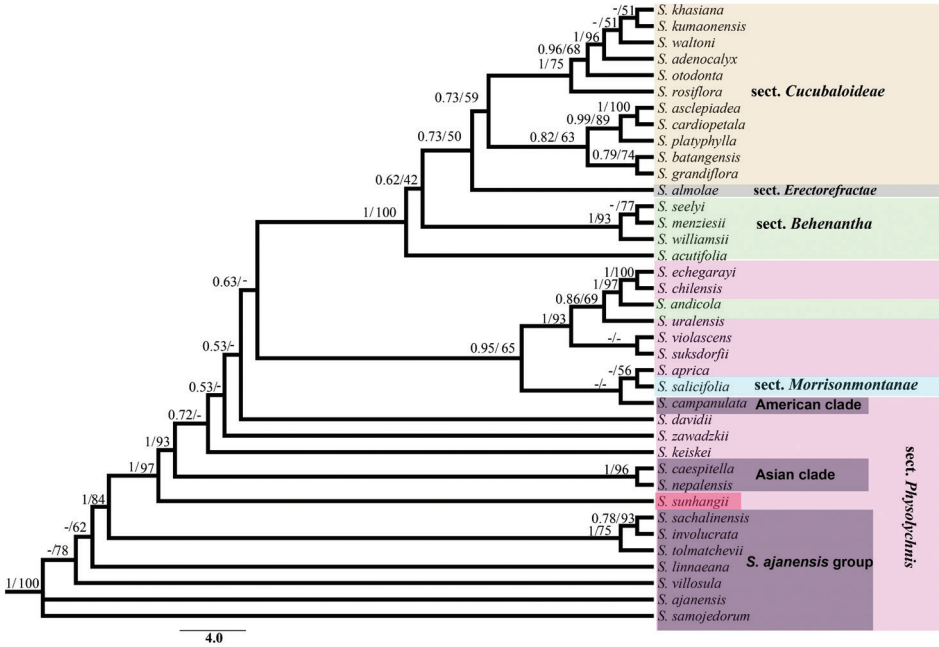

Figure 4. Phylogeny of *Silene* species studied based on ITS region and only bootstrap values >50% are shown. The colored taxa with identical color represent which are from same sect.

ready noted, Sect. *Physolychnis* was resolved as polyphyletic. This section was shown to include the '*S. ajanensis* group', an Asian clade, an American clade, and miscellaneous other species. These results are consistent with those of a previous study by Petri and Oxelman (2011). An unexplainable result was that *S. platyphylla* was well-separated from *S. sunhangii*, and included within a clade containing species of Sect. *Cucubaloideae* Edgew. et Hook. f.. These genetic results do clearly support the morphological data in recognizing *Silene sunhangii* as a distinct species. However, relationships of the new species do require further investigation.

Acknowledgements

We are grateful to the herbarium curators who helped us in our study.

Special thanks to Ling Wang for her excellent illustrations of *S. sunhangii*. The authors are grateful to B.R. Maslin for revising manuscript. Thanks are also due to the reviewers for their useful suggestions.

This study was supported by Conservation Technology Research and Demonstration of Rare and Endangered Plants in Shenlongjia National Park (2018ACA132), Hubei Key Laboratory of Shenlongjia Snub-nosed Monkey Conservation Fund (2018SNJ0009), the Key Projects of the Joint Fund of the National Natural Science Foundation of China (U1802232), the Second Tibetan Plateau Scientific Expedition

and Research (STEP) program (2019QZKK0502), the Major Program of the National Natural Science Foundation of China (31590823), the National Key R & D Program of China (2017YFC0505200), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA20050203), the National Natural Science Foundation of China (31700165), Youth Innovation Promotion Association CAS (2019382), Young Academic and Technical Leader Raising Foundation of Yunnan Province (2019HB039).

References

Boissier E (1867) Flora Orientalis (Vol. 1). Georg H., Basel, Geneva, 567–656.

Chowdhuri PK (1957) Studies in the Genus *Silene*, 22. Notes from the Royal Bot.,Garden Edinburgh 22: 221–278.

de Candolle AP (1824) Prodromus systematis naturalis regni vegetabilis, sive,enumeratio contracta ordinum generum specierumque plantarum huc usquecognitarium, juxta methodi naturalis, normas digesta. Treuttel et Wurtz, Paris, 745 pp. https://doi.org/10.5962/bhl.title.286

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics (Oxford, England) 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

IUCN (2017) Guidelines for using the IUCN Red List Categories and Criteria, Version 13. Prepared by the Standards and Petitions Subcommittee, 108 pp. http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Katoh K, Kei KM, Kuma KI, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14): 3059–3066. https://doi.org/10.1093/nar/gkf436

Melzheimer V (1988) Caryophyllaceae: *Silene* L. In: Rechinger KH (Ed.) Flora Iranica,163, Akad. Druck- und Verlagsanstalt., Graz, Austria, 341–508.

Oxelman B, Lidén M, Berglund D (1997) Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). Plant Systematics and Evolution 206: 393–410. https://doi.org/10.1007/BF00987959

Oxelman B, Rautenberg A, Thollesson M, Larsson A, Frajman B, Eggens F (2011) Sileneae taxonomy and systematics. http://www.sileneae.info

Petri A, Oxelman B (2011) Phylogenetic relationships within *Silene* (Caryophyllaceae) section *Physolychnis*. Taxon 60(4): 953–968. https://doi.org/10.1002/tax.604002

Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics (Oxford, England) 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rautenberg A, Hathaway L, Oxelman B, Prentice HC (2010) Geographic and phylogenetic patterns in *Silene* section *Melandrium* (Caryophyllaceae) as inferred from chloroplast and nuclear DNA sequences. Molecular Phylogenetics and Evolution 57: 978–991. https://doi.org/10.1016/j.ympev.2010.08.003

Rohrbach P (1868) Monographie Der Gattung *Silene*. W. Engelmann, Leipzig, Germany. https://doi.org/10.5962/bhl.title.15462

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics (Oxford, England) 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Urbatsch LE, Baldwin BG, Donoghue MJ (2000) Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chlorplast DNA restriction site data. Systematic Botany 25: 539–565.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Williams FN (1896) A revision of the genus *Silene* L. Botanical Journal of the Linnean Society 32(220–227): 1–196. https://doi.org/10.1111/j.1095-8339.1896.tb00695.x

Zhou LH, Wu ZY, Magnus L, Oxelman B (2001) *Silene* Linnaeus. Flora of China. Science Press, Beijing, 66–100. https://doi.org/10.2478/dna-2013-0004

Appendix 1

Vouchers information and GenBank accession of species used in our study.

Species	GenBank accessions	Herbarium voucher specimens
Silene ajanensis Vorosch.	KX757376	Anja Rautenberg 68 UPS
Silene samojedorum (Sambuk) Oxelman	JX274522	–
Silene villosula (Trautv.) V.V.Petrovsky & Elven	KX757382	Afonina et al. 1983. Vii. 23 S
Silene linnaeana Vorosch.	KX757383	H. Wilh. Arnell S
Silene involucrata (Cham. & Schltdl.) Bocquet	KX757387	Greve Alsosreas Tribsch O
Silene sachalinensis F. Schmidt	KX757394	Popov 1949.Vii.8 LE
Silene tolmatchevii Bocquet	KX757396	M.Karavaev 1945.Vii.6 LE
Silene caespitella F.N. Williams	KX757337	KGB 113 GB
Silene andicola Gillies ex Hook. & Arn.	KX757338	–
Silene violascens (Tolm.) V.V.Petrovsky & Elven	KX757343	H. Solstad, R. Elven 04/1353 O
*Silene chilens*is (Naudin) Bocquet	KX757359	B. Frajman, P. Schonswetter 12153
Silene echegarayi (Hieron.) Bocquet	KX757360	B. Frajman, P. Schonswetter 12176
Silene zawadzkii Herbich	KX757363	Cernoch F 47354 M
Silene davidii (Franch.) Oxelman & Lidén	KX757367	Frida Eggens 86 UPS
Silene salicifolia C.L. Tang	KX757372	Tang 1225 KUN
Silene nepalensis Majumdar	JF978562	KIB-D389
Silene keiskei Miq.	DQ908643	–
Silene suksdorfii B.L. Rob.	DQ908670	–
Silene uralensis subsp. *apetala*	JX274519	–
Silene aprica Turcz. (L.) Bocquet	JF978553	A519
Silene campanulata subsp. *glulosa*	DQ908635	clone 2459
Silene adenocalyx F.N. Williams	KX757269	Poelt J. M
Silene khasiana Rohrb.	KX757270	Einarsson et.al 3025 UPS
Silene waltoni F.N. Williams	KX757272	G. S. Miehe 03-048-12 Miehe
Silene kumaonensis F.N.Williams	KX757273	G. S. Miehe 01-109-08 Miehe
Silene rosiflora Kingdon-Ward	KX757277	G. Miehe SonamCo L.Opgenoorth 04-086-01 Miehe
Silene otodonta Franch.	KX757282	G.Miehe, U.Wuendisch 94-141-15 Miehe

Species	GenBank accessions	Herbarium voucher specimens
Silene asclepiadea Franch.	KX757283	Boufford D. E. et al. 35267 M
*Silene cardiopeta*la Franch.	KX757284	Liden 4-17
Silene grandiflora Franch.	KX757286	KGB 275 GB
Silene batangensis H. Limpr.	KX757288	Miehe 07-26-07 Miehe
Silene williamsii (Britton) Hultén	KX757298	C. Brochmann H. H Grundt
Silene acutifolia Link ex Rohrb.	KX757318	Bengt Oxelman 2554 GB
Silene almolae J.Gay	KX757424	Merxmueller H. & Lippert W. 25372 M
Silene menziesii Hook.	DQ908651	–
Silene seelyi C.V. Morton & J.W. Thomps.	DQ908666	–
Silene sunhangsii	–	KUN060722
Silene platyphylla Franch.	–	KUN0514438

PhytoKeys 135: 71–90 (2019)
doi: 10.3897/phytokeys.135.36714
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Taxonomic studies on the *Chara* section *Hartmania* in Poland based on morphological and molecular data

Jacek Urbaniak[1], Paweł Kwiatkowski[2]

1 *Department of Botany and Plant Ecology, Wrocław University of Environmental and Life Sciences, Poland*
2 *Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Poland*

Corresponding author: *Jacek Urbaniak* (jacek.urbaniak@upwr.edu.pl)

Academic editor: *Regine Jahn* | Received 3 June 2019 | Accepted 19 October 2019 | Published 5 December 2019

Citation: Urbaniak J, Kwiatkowski P (2019) Taxonomic studies on the *Chara* section *Hartmania* in Poland based on morphological and molecular data. PhytoKeys 135: 71–90. https://doi.org/10.3897/phytokeys.135.36714

Abstract

Charophytes are aquatic green macroalgae, which inhabit fresh and brackish water ecosystems. In this study, four species belonging to the genus *Chara* were examined to determine their taxonomic status. Morphological characteristics of the plant bodies as well as plastid *psa*B barcoding genes were applied to test the relations among *Chara* species. Plants were initially classified using morphological features into four species: *C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis*, and twelve quantitative characters were used in a principal component analysis and discriminant analysis to determine groupings among the species and to determine the morphological features that best separated the groups. In the component analysis and discriminant analysis, results showed that only *C. polyacantha* and partly *C. baltica* formed separate groups. The other species *C. hispida* and *C. rudis* were only partially distinguishable. All species from one molecular group, and no differentiation in the *psa*B variability between them has been found.

Keywords

Chara, morphological characters, species identification, taxonomy, Poland

Introduction

Macroscopic algae from the genus *Chara* L. can be commonly found in various water bodies, such as shallow lakes, artificial ponds, slowly running waters or drainage canals. The taxonomy of the genus *Chara*, as well as the other representatives of the Characeae family is not easy, mostly due to the overlapping of morphological features of individual specimens belonging to different species (Sakayama et al. 2002; Nylander et al.

2004; Sakayama et al. 2009; Urbaniak 2010, 2011a, 2011b; Urbaniak and Combik 2013; Schubert 2014). The variability among specimens is probably also due to genetic and ecological (environmental, site-specific) conditions (e.g. water quality, light availability) and resulting phenotypic plasticity or developmental differences (Meiers et al. 1999; Mannschreck 2003).

Taxonomic studies based on charophyte morphology started at the end of the 19th century, and during this initial phase, many people tried to characterize the degree of morphological variation in charophytes and find traits to circumscribe distinct species. Traditionally, in the genus *Chara*, a narrow species concept has been used resulting in about 45 European species (Braun and Nordstedt 1882; Corillion 1957; Krause 1997). Because of overlapping morphological variations in many traits, some workers have used a wider species concept and interpreted the genus to be subdivided into fewer and more polymorphic species (Wood and Imahori 1965) thus discriminating only 18 species (worldwide) including a number of varieties and forms. These differences between understanding and interpreting the species result from a lack of objective methods to determine which characters actually serve to delimit species within the genus. This problematic classification is typical not only for the genus *Chara* (Mannschreck 2003; O'Reilly et al. 2007; Urbaniak and Combik 2013) but also for the genus *Nitella* C.Agardh (Sakayama et al. 2002). Because certain intermediate forms exist between *C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis*, authors treat them in different ways: they either consider them to be separate species (Krause 1997; Urbaniak and Gąbka 2014) or as varieties or forms of *C. hispida* (Wood 1962; Wood and Imahori 1965) (Table 2).

Unfortunately, previous studies of oospore morphology, oospore wall ornamentation (scanning electron microscopy, SEM) and molecular fingerprinting data did not give satisfactory results in delimitating *Chara* species from the section *Hartmania* (Urbaniak and Combik 2013). This could indicate, that *i*) the choice of the method used was not the best solution or *ii*) it showed a very close phylogenetic relationship among *C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis* and all these taxa should be treated as varieties or forms of *C. hispida* according to the monomorphic species concept (Wood and Imahori 1965). SEM studies of the oospore wall ornamentation and dimensions have also been used for species delimitation in the genus *Chara* to suggest that both methods can be helpful in taxonomic decisions regarding species (John et al. 1990; Urbaniak 2011a, 2011b; Urbaniak and Blazencic 2012). Sakayama et al. (2002) showed that the combination of different types of data (SEM, oospore morphology and molecular data) can be more informative than when considered separately and can be used for taxonomic distinction, especially in closely related species of the genus *Nitella* Agardh.

In addition, the DNA barcoding method has been proposed as an alternative method for identifying taxonomic relationships in species of the Characeae family. This method can be used successfully to facilitate biodiversity and taxonomic studies of various plants (Kress et al. 2005). Sakayama et al. (2002) applied different barcoding genes of *mat*K, *rbc*L or *psa*B genes to test whether the distribution of haplotypes among individuals is consistent with species boundaries as they are currently understood. The choice of *rbc*L+*mat*K as a barcode was probably based on the good recovery of the *rbc*L region and high dis-

criminatory power of the *mat*K region, which is one of the most rapidly evolving coding sections of the plastid genome (Hollingsworth et al. 2011). However, Hollingsworth et al. (2009) as well as Shaw et al. (2007) point out that the plastid barcode gene *psa*B can be used that serves good delimitation. The use of *psa*B gene has been tested previously with good results by Sakayama et al. (2005) in a taxonomic re-examination of the genus *Nitella*.

The presented study focuses on four of the most problematic freshwater species (two diplostichous aulacanthous species, *C. hispida* and *C. rudis*, one diplostichous thylacanthous species: *C. polyacantha* and *C. baltica* as representatives of brackish water species (a diplostichous thylacanthous species, in transition to slightly isostichous). All of them belong to the section *Hartmania*. We applied the plastid *psa*B gene as well as morphological observation to test whether the distribution of haplotypes among species is in agreement with the species delimitation.

Methods

Collection of plants and PCR analysis

The plants were collected manually or using a hook directly from the field. We collected mature specimens and determined according to the Krause (1997), Urbaniak and Gąbka (2014), Becker et al. (2016) and van de Weyer (2016) determination keys.

We have used the charophyte names following Krause (1997) and Urbaniak and Gąbka (2014): *C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis*. In case of *C. rudis* a name on species rank that has priority was established recently: *Chara subspinosa* Rupr. An earlier name for the widely used name for *C. polyacantha* is *C. aculeolata* Kütz. (Becker et al. 2016), however, in this case its taxonomic position is not clear. The synonymy is presented in Table 2. In the case of molecular analysis, after collection, the material was placed in glass jars and transported to the laboratory and cultured in laboratory conditions (at room temperature, with light from a north-facing window) in jars filled with tap water. To reduce the influence of contaminating DNA from epiphytes, large filamentous green algae were removed from young plant shoots before DNA extraction by dissection under a stereomicroscope. Only newly grown tissue was used for molecular analysis.

Morphological observations

In the case of morphological observations, after collection, plants were dried and analysed in a laboratory using a stereomicroscope SMZ 800 (Nikon, Tokyo, Japan). The morphological characteristics of the investigated species were described (Table 3) with some examples of studied species with important discriminatory analysis shown in Figs 1–7. The characters used for performing the principal component analysis (PCA) and discriminant analysis (DCA) are shown in Fig. 8, coded and analysed using PCA and DCA discriminatory techniques using Statistica 12.1 software (StatSoft 2010).

Table 1. Specimens studied with GenBank accession numbers and collection sequences used in study.

Species	*psa*B GenBank accession number/locality	Geographical coordinates
C. baltica Bruz.	KX791851/ Puck, Poland	54°42'14.09"N, 18°27'40.70"E
	KX791852/ Swarzewo, Poland	54°45'25.19"N, 18°24'33.91"E
	KX791853/ Rewa, Poland	54°38'11.17"N, 18°30'37.50"E
C. hispida L.	KX791854/ Lake Czarne, Poland	54°00'57.75"N, 22°59'40.22"E
	KX791855/ Lake Mikaszewo, Poland	53°53'15.96"N, 23°21'22.84"E
	KX791856/ Lake Białe, Poland	53°52'03.95"N, 23°02'16.17"E
	KX791857/ Lake Czajcze, Poland	54°06'56.00"N, 22°28'19.44"E
	KX791858/ Lake Wielkie, Poland	53°20'43.74"N, 22°55'58.36"E
	KX791859/ Lake Wigry, Poland	54°00'34.76"N, 23°02'16.89"E
	KX791860/ Lake Mamry, Poland	54°06'24.87"N, 21°46'13.13"E
	KX791861/ Lake Pobłądzie, Poland	54°18'24.30"N, 22°45'25.25"E
	KX791862/ Lake Muliste, Poland	53°54'11.23"N, 23°16'15.58"E
	KX791863/ Lake Staw, Poland	54°01'14.44"N, 22°59'26.91"E
	KX791864/ Lake Jeziorak, Poland	53°43'06.51"N, 19°36'02.98"E
	KX791865/ Lake Śniardwy, Poland	53°47'25.95"N, 21°44'14.71"E
C. polyacantha A. Br.	216/KX791866/ Lake Jasne, Poland	54°07'56.82"N, 22°58'41.09"E
	217/KX791867/ Lake Bilskie, Poland	54°05'03.47"N, 23°05'31.62"E
	218/KX791868/ Lake Wigry, Poland	54°01'48.15"N, 28°08'30.31"E
	219/KX791869/ Lake Kockie, Poland	53°59'40.25"N, 20°51'25.79"E
C. rudis Leonh.	220/KX791870/ Lake Staw Wielki, Poland	53°57'01.46"N, 23°08'42.92"E
	221/KX791871/ Lake Oleckie, Poland	54°03'23.86"N, 22°30'20.63"E
	222/KX791872/ Lake Małe, Poland	54°03'24.09"N, 22°42'09.85"E
	223/KX791873/ Lake Korzęckie, Poland	54°13'29.08"N, 22°34'05.47"E
C. vulgaris L.	DQ229107/Poland	–
N. axiliformis	AB191785/Japan	–
N. pseudoflabellata	AB191766/Japan	–

The morphological features (quantitative characters) used in the analysis for species in *Chara* section *Hartmania* are described in Table 4. No fewer than 30 specimens have been measured in this instance, except for the population *C. polyacantha* (Lake Wigry, Bilskie, Kockie), where only about 23 specimens have been analysed.

Molecular analysis

In addition to the morphological observations, a molecular technique, sequencing of the plastid *psa*B gene, has been conducted. Total genomic DNA was isolated from fresh tissue using liquid nitrogen and a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Cells were disrupted using the Mixer Mill MM400 (Retsch, Haan, Germany). The quality and quantity of the DNA was determined on 1% TBE–agarose gel. The PCR amplification and sequencing of the *psa*B gene was accomplished using the primers described by Sakayama (2008). Analyses were performed in a GeneAmp 9700 Thermal Cycler (Applied Carlsbad, CA, USA). Each 20 µl reaction contained water, 10 mM each of dATP, dCTP, dGTP, and dTTP; 0.5 µM of each primer, 10.0 µl reaction buffer, 0.2 µl DreamTaq DNA Polymerase (Thermo Scientific, Waltham, MA, USA) and 1.0 µL of total genomic DNA. The PCR

Table 2. Classification of selected species from the genus *Chara* (Charophyta).

Krause (1997)	Wood and Imahori (1965)	Becker et al. (2016)
Chara baltica Bruz.	*Chara hispida* var. *baltica* f. *baltica* *Chara hispida* var. *baltica* f. *liljebladi*	*Chara baltica* (Hartman) Bruz.
Chara hispida L.	*Chara hispida* var. *major* f. *major* *Chara hispida* var. *hispida* f. *hispida*	*Chara hispida* L.
Chara polyacantha A. Braun	*Chara hispida* var. *hispida* f. *polyacantha*	*Chara aculeolata* Kütz.
Chara rudis (A. Braun) Leonh.	*Chara hispida* var. *major* f. *rudis*	*Chara subspinosa* Rupr.

Table 3. Comparisons of morphological features of studied species.

Character / Feature	*C. baltica*	*C. hispida*	*C. polyacantha*	*C. rudis*
Plant axis	robust, slender	robust, thick	erect, robust	robust, thick
Plant size	medium size, 6–27 cm high, 1–2 mm in diameter	medium large to large species, up to 18–70 cm high, 4–5 in diameter	medium large to large, 30–75 cm high, up to 5 mm in diameter	medium large to large plants, 23–65 cm high, up to 4–6 mm in diameter
Color	light to dark green	green to greyish green	green to dark green	green to greyish green
Incrustation	unincrusted	moderately to heavily incrusted	moderately incrusted	moderately to heavily incrusted
Internodes	longer or as long as branches	longer than branches	similar length or longer (up to 2 times) than branches	up to 2 times longer than branches
Branchlet	up to 8 branches in a whorl, stout to slender with 5–8 segments	7–10 branches in a whorl, straight and rigid, with 5–9 segments	8–10 branches in a whorl with 6–9 segments	7–10 branches in a whorl, with 6–7 segments
Cortification	diplostichous and slightly thylacanthous	diplostichous, aulacanthous, often isostichous on older internodes	diplostichous and tylacanthous occasionally irregular	diplostichous, strongly heterostichous and aulacanthous
Spine cells	shorter than axis diameter, solitary or in pairs	solitary or in fascicles as long as the axis diameter	in bunches, as long or longer than the axis diameter	sparse in pairs, similar in length as plant axis
Stipulodes	stipulodes in two rows similar in length to spine cells	stipulodes in two rows, uppers are similar to lowers	stipulodes in two rows, as long as axis diameter	stipulodes are in two rows, uppers similar to lowers
Reproduction	monoecious	monoecious	monoecious	monoecious
Oogonia	540–1165 µm long, 515–650 µm wide	415–1200 µm long, 520–770 µm wide	625–1140 µm long, 450–615 µm	890–1210 µm long, 415–750 µm wide
Antheridia	420–630 µmin diameter	490–730 µm in diameter	375–530 µm in diameter	370–480 µm in diameter
Oospores	black, 465–925 µm long and 335–645 µm wide	reddish brown to dark brown, 545–810 µm long, 390–760 µm wide	brown, dark brown or black, 485–900 µm long, 270–585 µm wide	brown to dark brown–almost black, 620–925 µm long, 395–835 µm wide

Table 4. Morphological features used in the analysis of features in four species in *Chara* section *Hartmania*. See Figure 1 for a diagrammatic explanation. Qualitative characters are signed with "[n]" and quantitative with "[cm]".

Feature (see Figure 1)	Abbreviation
Number of branches in second branchlet whorl [n]	IL2
Mean length of branches in second branchlet whorl [cm]	SDL2
Mean number of corticated internodes on branchlets in the second whorl [n]	IOC2
Mean length of stipulodes at the second node [cm]	SDP2
Diameter of the internode above second branchlet whorl [cm]	SN2
Length of spine cells above second branchlet whorl [cm]	DO2
Number of branchlets in third branchlet whorl [n]	IL3
Mean length of branchlets in third branchlet whorl [cm]	SDL3
Mean number of corticated internodes on branchlets in third whorl [n]	IOC3
Mean length of stipulodes at the third node [cm]	SDP3
Diameter of the internode above third branchlet whorl [cm]	SN3
Length of spine cells above third branchlet whorl [cm]	DO3

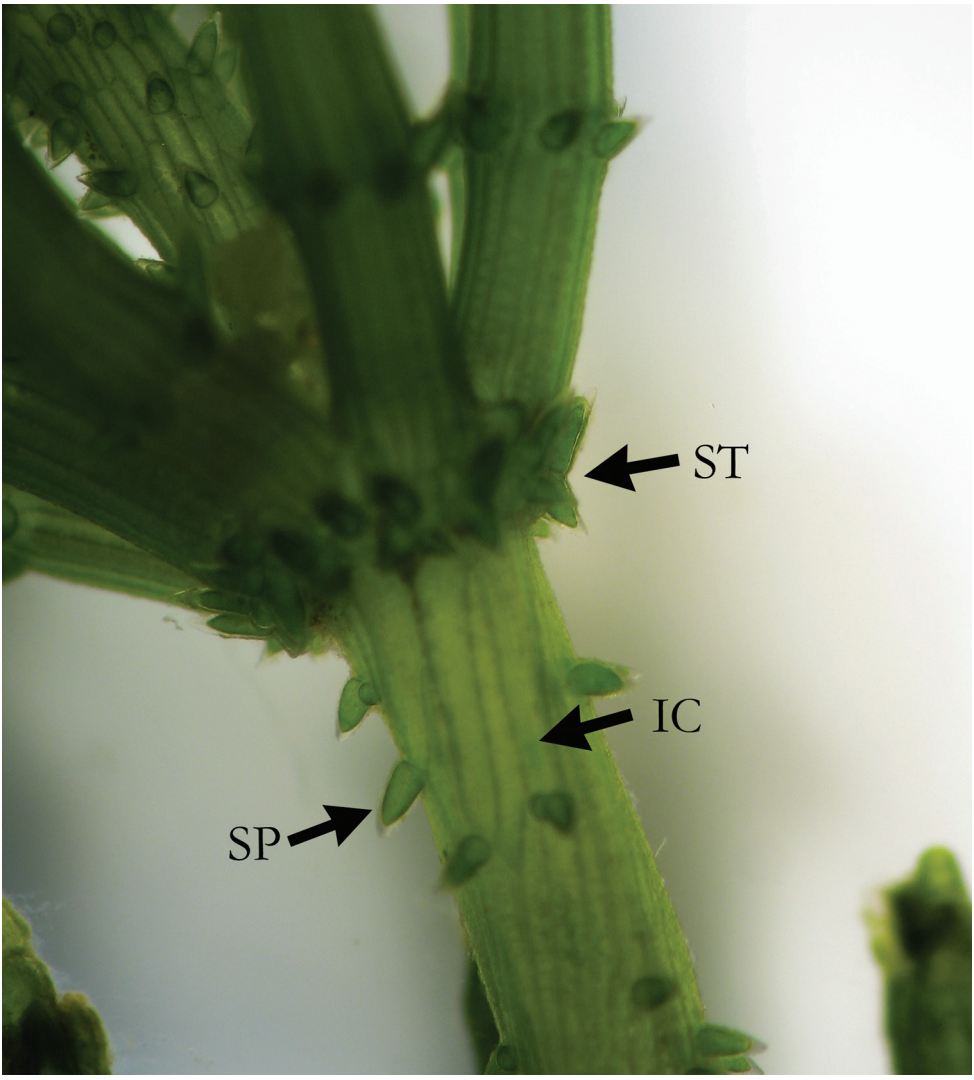

Figure 1. *C. baltica* with short irregular stipulodes (ST), spine cells (SP) shorter than axis diameter, and irregular cortex (IC).

cycle consisted of an initial denaturation at 95 °C for 6 min., followed by 33 cycles at 95 °C for 45 sec., followed by testing the adequate annealing temperature for 45 sec., and elongation 72 °C for 1 min, with a final extension of 10 min at 72 °C. The PCR products were examined for correct length, yield and purity under UV light on 1% agarose gels, stained with SimplySafe. PCR products were purified prior to sequencing reactions, using the Exo–BAP Mix (Eurx, Gdańsk, Poland), and sequenced using the amplification primers. All molecular analyses were performed at the Department of Botany and Plant Ecology, Wrocław University of Environmental and Life Sciences.

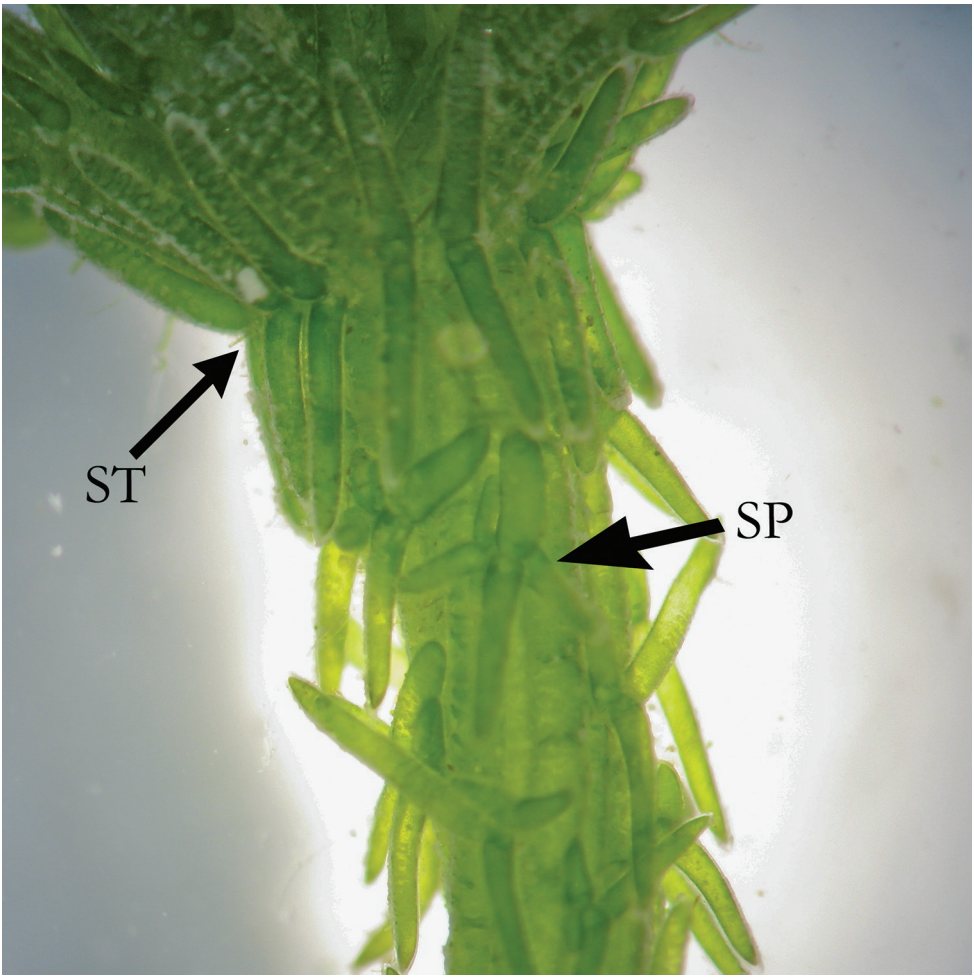

Figure 2. *C. hispida* with stipulodes (ST) and spine cells (SP) in similar length, as long as axis diameter.

Phylogenetic analysis

Prior to the phylogenetic analyses, the *psa*B DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). A tree was constructed using PHYML 3.0 by the maximum likelihood (ML) method (Guindon and Gascuel 2003). Prior to analysis, the KAKUSAN 4 (Tanabe 2011) was used to identify the sequence evolution model that fit the dataset using Akaike's Information Criterion (AIC). The bootstrap proportions (BP) (Felsenstein 1985) used for ML analyses and selected with the GTR + G model selected by KAKUSAN 4 were calculated based on 100 replicates of heuristic searches. The BI analyses were performed using MRBAYES 3.1.2. (Ronquist and Huelsenbeck 2003). The Bayesian inference (BI), were also constructed and com-

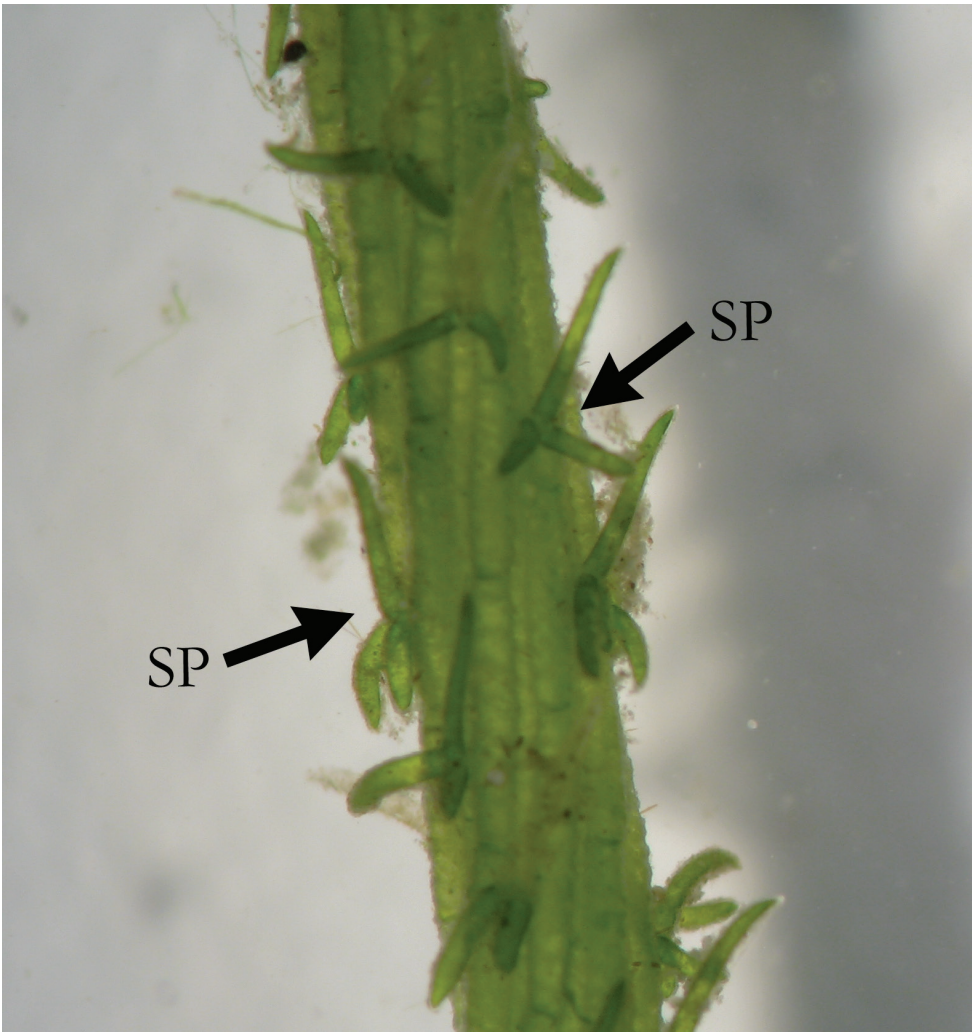

Figure 3. *C. hispida* with spine cells (SP) in bunches.

pared the topologies of the obtained trees to establish and validate the phylogenetic position of the studied species. The substitution models used for each codon position of the *psa*B gene in the BI analyses were GTR + I (1st codon position), GTR + I + G (2nd codon position), and GTR + G (3rd codon position), which were estimated based on AIC and selected by MRMODELTEST 2.3 (Nylander et al. 2004) implemented in PAUP* 4.0b10 (Swofford 2002). The parameters of the substitution models for each codon position were unlinked. The Markov chain Monte Carlo iteration process was stopped at 1,000,000 generations, and the first 25% of generations were discarded as burn-in, whereas the remaining trees were used to calculate a 50% majority–rule tree and to determine the posterior probabilities (PP) of individual branches.

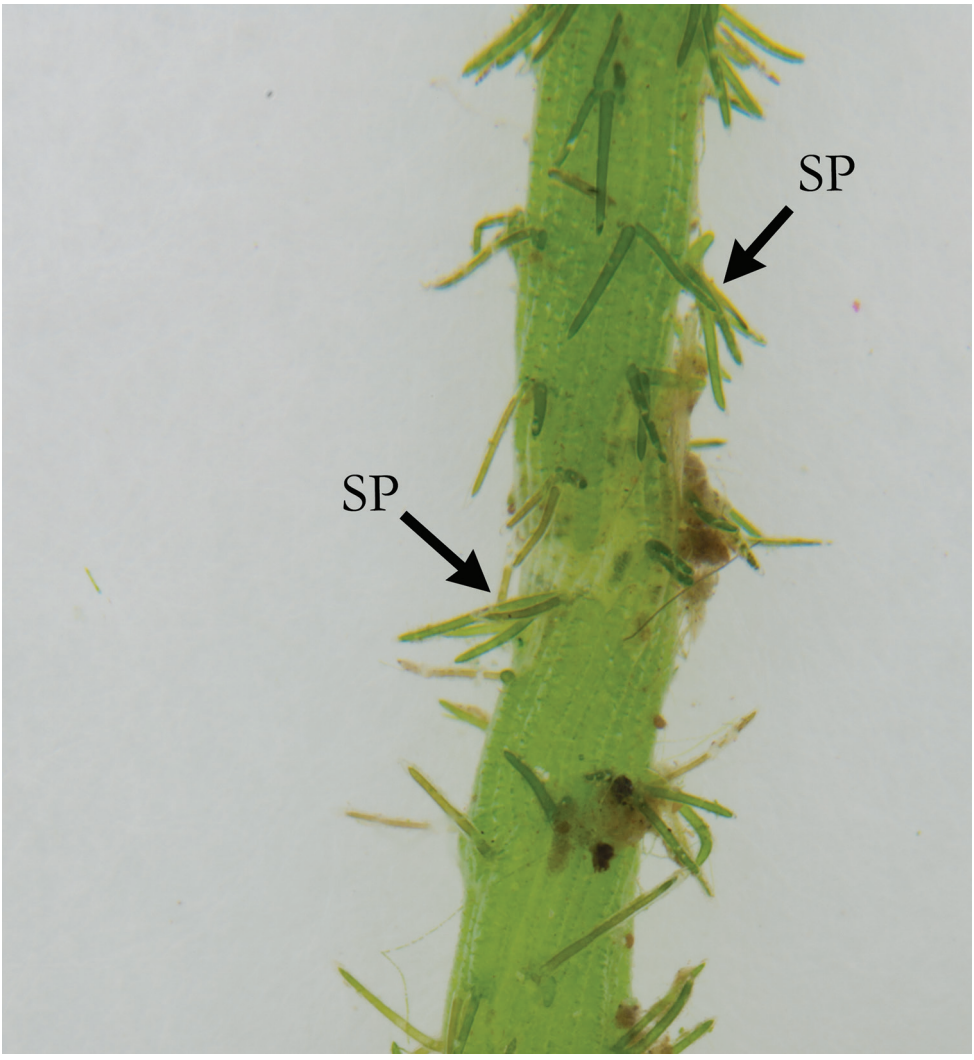

Figure 4. *C. polyacantha* with long spine cells (SP), as long as axis diameter.

Results

The specimens examined in the present study are described in detail in Table 3. In general, all plants were robust and thick, medium to large with plant axis up to 4–6 mm in diameter, except for *C. baltica*, which has a thinner main axis. All plants differed in colour and level of incrustation that determines colour a little. Differences were also noted in the size of internodes and number of branches. All specimens were diplostichous, but sometimes thylacanthous or with occasionally aulacanthous cortex (*C. hispida*, *C. rudis*). The studied plants were monoecious with stipulodes in two rows with spine cells shorter than the axis diameter (*C. baltica*) or with spine cells longer

Figure 5. *C. polyacantha* with extremely long spine cells (SP) and stipulodes (ST) exceeding axis diameter.

or as long as the axis (*C. hispida*, *C. polyacantha*, *C. rudis*). The details of gametangia (oospores, oogonia and antheridia) are in Table 3. All investigated taxa grow in similar water places except *C. baltica*, which is a truly brackish water species and can be found only in the Baltic Sea. The others are cosmopolitan species, found in different aquatic habitats such as lakes, ponds, pools and petland exploitation pools, with a wide ecological range, growing in both mesotrophic and eutrophic water.

The first three components in the PCA explained 25.1%, 21.3% and 17.5% of the total morphological variation. Four groups that correspond to the four species can be distinguished along the first and third axes (Fig. 9). Specimens that key out to *Chara rudis* and *C. polyacantha* were separated from specimens that key out to the other two species along the first axis. Specimens that key out to *Chara polyacantha* and *C. Baltica* were separated from the other specimens along the third axis. Some specimens assigned to species using the conventional key characters were incorrectly grouped in the

Figure 6. *C. rudis* with short, regular stipulodes (ST).

PCA, and this occurred for all species. The first component that separated the specimen groups (PC1, 25.1%), was made up largely of mean branchlet length in the second and third branchlet whorls, the diameter of the internode above the second branchlet whorl and length of spine cells above the third branchlet whorl. This component resulted in positive values for specimens that key out to *C. hispida* and *C. rudis* in contrast to specimens that keyed out to *C. baltica* and *C. polyacantha*, which had negative values. The third component (PC3, 17.5%) was made up of differences in the mean number of branchlets in the second and third branchlet whorls and the diameter of the internode above the third branchlet whorl. Specimens that keyed out to *C. polyacantha* and *C. rudis* had negative values in this component, which allowed them to be distinguished from specimens that keyed out to *C. baltica* and *C. hispida*, which had positive values.

In the discriminant analysis, specimens were assigned to species groups on the basis of the classical taxonomic approach. After analysis, the first three canonical functions accounted for 96% of the total variation (first 46%, second 24% and third 24%). The analysis showed that 11 out of the 14 characters were useful for differentiating the specimens. The

Figure 7. *C. rudis* with opposite spine cells (SP) on the axis.

other characters were not significant. The individuals of *C. polyacantha*, *C. baltica* and *C. rudis* form well-separated groups, and *C. hispida* overlaps *C. rudis* and *C. baltica* (Fig. 10).

Analysis of the *psa*B gene of *Chara* species showed a smaller resolution than on the tree produced with sequences from the *Nitella* genus (Sakayama et al. 2009). Out of the 1,461 analysed base pairs included in the *psa*B sequence analyses, 157 were informative with respect to parsimony. Almost all investigated specimens formed one congruent and unresolved clade that group all of the studied specimens. A phylogenetical tree based on the *psa*B sequences is shown in Fig. 11, and as can be seen, the four studied species belonging to section *Hartmania* (*C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis*) form a coherent group with high bootstrap support in ML and BI analyses. The *psa*B sequences were almost identical with no nucleotide differentiation between species.

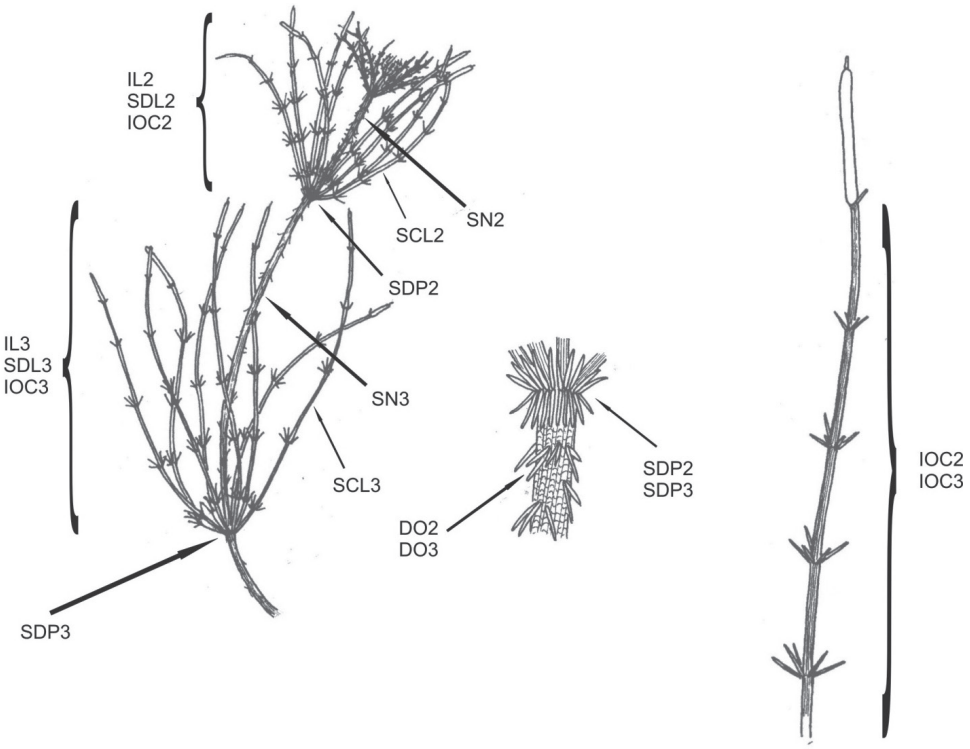

Figure 8. Axis, internode and node complex and branchlet characteristics measured in this study. Abbreviations of the morphological features are given in Table 4. Figure after Bruinsma et al. (1988), modified.

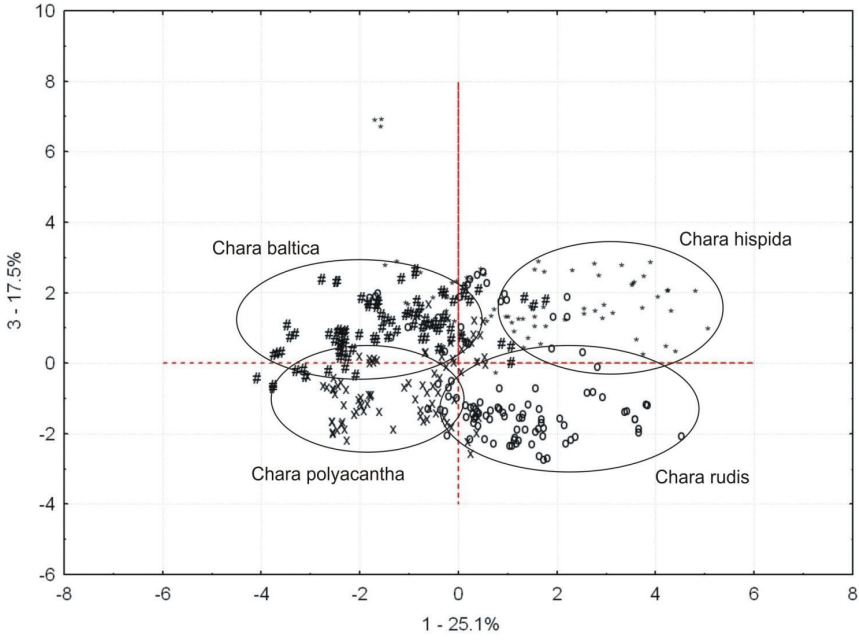

Figure 9. Principal Components Analysis ordination of the species from section *Hartmania*.

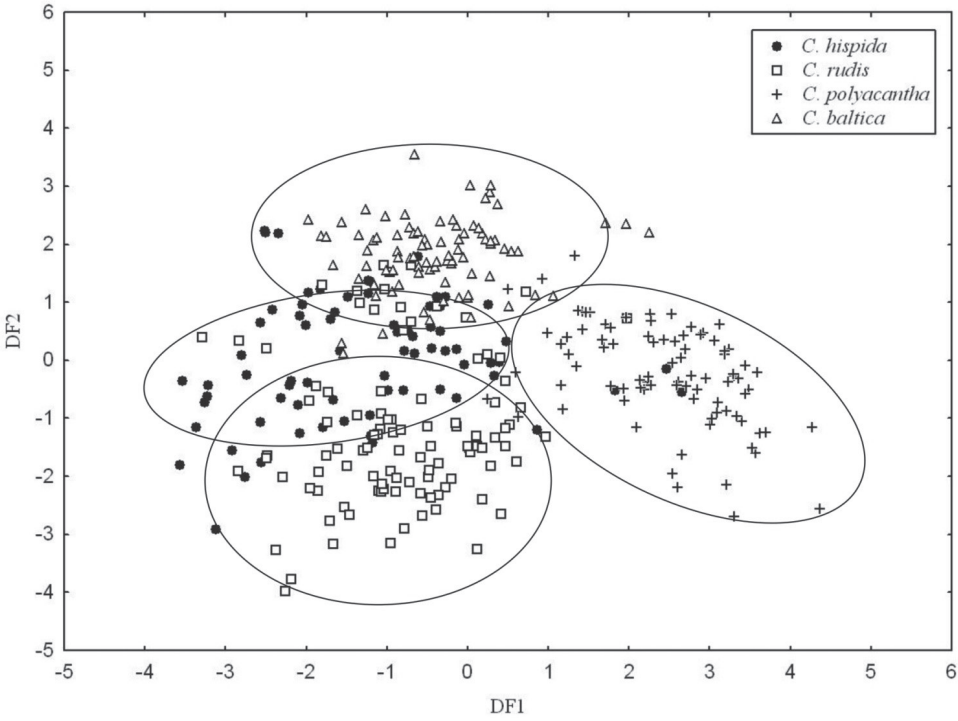

Figure 10. Discriminant analysis of the species from section *Hartmania*.

Discussion

The results of numerous studies indicate that a combination of various morphological data with molecular sequences can be helpful for distinguishing charophyte species, as well as making various taxonomic decisions or explaining the phylogenetic relationship between species (Sakayama et al. 2005; Urbaniak 2010; Urbaniak and Blazencic 2012).

In comparison to other authors, and especially to more recently published data on the morphological features of charophytes (Becker et al. 2016), we have observed several differences in plant characters. The specimens of *C. baltica* presently growing in the Polish part of the Baltic Sea are in general of similar length as presented in Becker et al. (2016) and no plants that reach 90 cm (i.e. *C. baltica* var. *liliebladi*) have been observed. In the case of *C. hispida* and *C. rudis*, oogonia, antheridia and oospores are in general of similar size, except for the length of oogonia measured in Polish *C. hispida* that can be shorter (minimum size 415 μm) than described in Becker et al. (2016). These authors described oogonia of *C. rudis* with a minimum breadth of 600 μm (Becker et al. 2016) whereas the Polish specimens were smaller (minimum breadth 415 μm). Both examples show how big the differences can be in measurements of oospores, oogonia and antheridia in charophytes.

The multivariate analysis of *C. hispida* and *C. rudis* based on vegetative traits gives some additional explanation of the taxonomy of species belonging to section *Hartmania*.

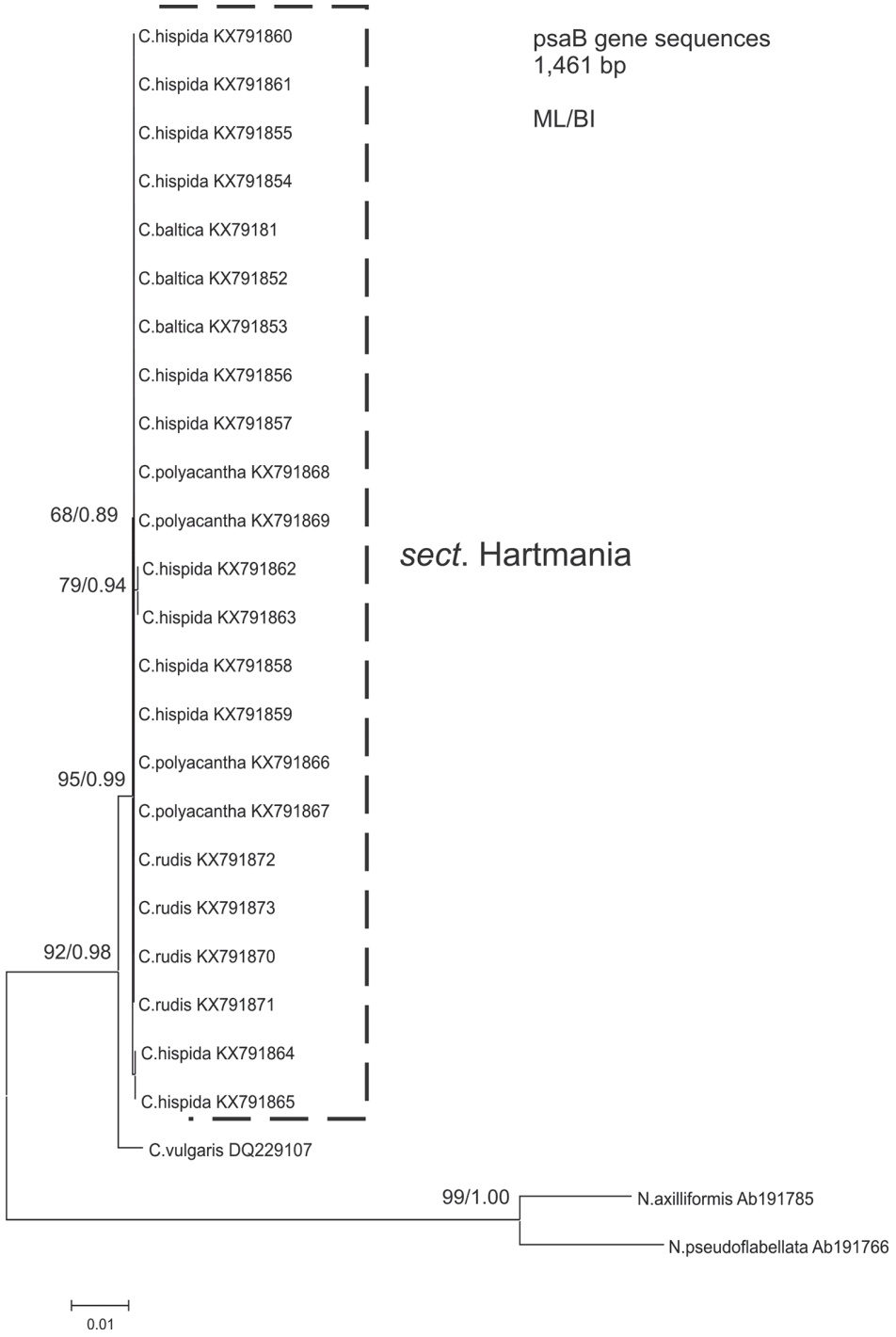

Figure 11. Phylogenetic tree inferred from maximum-likelihood (ML) analysis of *psa*B gene sequence data for the Charophyceae (Characeae) and outgroup taxa, with LM bootstrap support (BP)/bayesian interference (BI) indicated at the nodes.

The result obtained during the DCA seems to be clearer than obtained from the PCA, probably due to the different algorithms used in the analyses that can explain the differences: DCA emphasises characters that distinguish groups while suppressing the variation within groups, whereas PCA tends to accentuate the within-group variation (Řepka 2003). In general, analyses of the morphological characters show that the studied species form more or less separated groups, but all seem to be closely related.

The PCA results were used to demonstrate the differences among the species, and most specimens could be allocated to particular taxa. Both figures support the amalgamation of *C. hispida* and *C. rudis*, and maintenance of the species *C. baltica* and *C. polyacantha* as similar to how they were presented previously (Urbaniak 2010). On the other hand, several specimens were mis-allocated along the first and third axes (Fig. 9). DCA demonstrated close relations among taxa, but material assigned to *C. polyacantha* formed the most separated group. The other three species groups: *C. baltica*, *C. hispida* and *C. rudis* formed closely related groups. Despite that, *C. baltica* can reliably be distinguished by a combination of morphological characters and by their occurrence in different habitats (Krause 1997; Urbaniak and Gąbka 2014). *C. baltica* and *C. polyacantha* differ greatly in their morphology and both taxa are differentiated not only by morphological characters but also by ecological preferences. *C. baltica* is a typically brackish water species, whereas *C. polyacantha*, *C. hispida* and *C. rudis* are typically fresh water species. This contrasts with Wood and Imahori (1965) who treated these taxa as varieties of *C. hispida* (Table 2). However, despite good segregation of the majority of specimens in these two species, there are still a number of specimens that have overlapping characteristics. This is likely to be a result of a close phylogenetic relationship between those species (Boegle et al. 2007), which has also been contradicted by the present results.

On the other hand, it supports the thesis that all these species are morphologically very similar, and that 'transitional forms' commonly exist between them. The so-called 'transitional forms' are probably not real hybrids, but rather forms that visualize possible plasticity that can be noted in the genus *Chara*. In this group of species: *C. hispida*, *C. rudis*, *C. polyacantha* and *C. baltica* 'transitional forms' are those that display features intermediate between species, or the features are not clear enough for determination. In the case of *C. rudis* and *C. hispida*, spine cells are the main distinguishing character, and they are normally in twos or threes in *C. hispida*, but in pairs lying one above the other along the axis in *C. rudis* (Urbaniak and Gąbka 2014), Fig. 7. Both features can be found on the same plant, and this sometimes makes determination difficult or impossible. The transitional forms of *Chara* species do not grow only as morphologically mixed populations. They can occur also in populations where most of the specimens are easily allocated to one species or the other. Quantitative characters, including those of cortication and general appearance of habit, are generally very variable and cannot offer reliable characters for determination. Earlier authors did not always use all the characters identified as important by DCA in this work. This could be quite complicated in the routine determination but may be necessary to reach a proper understanding of the taxonomy of the group (Urbaniak

2010); however, a really deep understanding of the taxonomic relationships among the group depends on both molecular and morphological studies on different populations of species within the section Hartmania.

The analysis of phylogenetic sequence data reveals a strictly close relationship between *C. baltica*, *C. hispida*, *C. polyacantha* and *C. rudis* (Fig. 11). The results based on the *psa*B cpDNA sequences show one clade on the phylogenetic tree, which is not exactly congruent with the morphological analyses, but contradicts the previously found taxonomic relations between species (Urbaniak 2011a, 2011b; Urbaniak and Combik 2013). Our *psa*B phylogeny clearly revealed that the species of section *Hartmania* are monophyletic and the groups of sequences (section *Hartmania*) form a cluster containing all individuals together. The lack of genetic variability in them did not differ at all in the species and showed a lack of discrimination, as similar as in Schneider et al. (2015), who found that one large and unresolved group consisted of species such as *C. intermedia*, *C. hispida*, *C. horrida*, *C. baltica*, *C. polyacantha* and *C. rudis*. Results based on more data analysed showed that many more species that can differ morphologically or genetically are placed in *C. hispida* cluster (Schneider et al. 2016). This, in particular, can contradict that all studied species are very closely related, but on the other hand, the *psaB* seems to be not the best marker for studying phylogenetic and taxonomic relations between species from the genus *Chara*. This, however, is not in accordance with the previous work e.g. on the genera *Nitella* (charophyta). Sakayama et al. (2005) found that *psa*B can concatenate with other genes or morphological analysis of oospore wall ornamentation gave successful discrimination, but in presented results, morphologically different species were not differentiated by molecular analysis. This could rather support the hypothesis on the close phylogenetical and evolutionary relations that exist between species from the section *Hartmania*.

Although morphological and molecular data separately are not ideal tools for species delimitation, together they are important and useful when combined with other types of data (Sakayama 2008). Such studies are being published at an increasing rate and are discovering cryptic species (Bickford et al. 2007). Lack of differentiation based on barcodes or fingerprinting techniques allows for the reinterpretation of some particular taxa in the charophytes, particularly in the genus *Chara*. The obtained results show that close taxonomic relations between studied species are not questionable, however, more adequate data, used molecular markers and performed on a wider spectrum of taxa, are needed for a better understanding of such relations.

Conclusion

We have shown that morphological features allow for differentiation of the investigated *Chara* species. *C. polyacantha* formed separate clusters in both PCA and DCA, and *C. rudis* had intermediate features. Molecular analyses showed that all species definitely comprise one closely related group and no differentiation in the *psa*B variability between them has been found.

References

Becker R, Blindow I, Doege A, Franke T, Gregor T, Hamann U, Jager D, Jorda C, Kabus T, Korsch H, Korte E, Kusber WH, Patzold F, Raabe U, Schubert H, Teppke M, van de Weyer K, Wolff P (2016) Beschreibung der Characeen-Arten Deutschlands. In: Arbeitsgruppe Characeen Deutschlands (Eds) Armleuchteralgen. Die Characeen Deutschlands. Springer Verlag, Berlin Heidelberg, 209–572. https://doi.org/10.1007/978-3-662-47797-7_12

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22(3): 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Boegle MG, Schneider S, Mannschreck B, Melzer A (2007) Differentiation of *Chara* intermedia and *C. baltica* compared to *C. hispida* based on morphology and amplified fragment length polymorphism. Hydrobiologia 586(1): 155–166. https://doi.org/10.1007/s10750-006-0571-8

Braun A, Nordstedt CFO (1882) Fragmente einer Monographie der Characeen. Nach den hinterlassenden Manuscripten A. Braun's. Abhandlungen der Königliche Akademie der Wissenschaften zu Berlin, Physikalish – Mathematische Klasse, 1882(1): 1–221.

Bruinsma J, Krause W, Nat E, Raam J (1988) Determinatie table voor kranswieren in de Benelux. Stichting Jeugdbondsuitgeverij, Utrecht, 101.

Corillion R (1957) Les Charophycées de France etd'Europe Occidentale, Otto Koeltz, Koenigstein, 499.

Felsenstein J (1985) Confidence limits on phylogenies, an approach using bootstrap. Evolution; International Journal of Organic Evolution 38: 16–24. https://doi.org/10.1111/j.1558-5646.1984.tb00255.x

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5): 696–704. https://doi.org/10.1080/10635150390235520

Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, Chase MW, Cowan RS, Erickson DL, Fazekas AJ, Graham SW, James KE, Kim K-J, Kress WJ, Schneider H, van AlphenStahl J, Barrett SCH, van den Berg C, Bogarin D, Burgess KS, Cameron KM, Carine M, Chacon J, Clark A, Clarkson JJ, Conrad F, Devey DS, Ford CS, Hedderson TAJ, Hollingsworth ML, Husband BC, Kelly LJ, Kesanakurti PR, Kim JS, Kim Y-D, Lahaye R, Lee H-L, Long DG, Madrinan S, Maurin O, Meusnier I, Newmaster SG, Park C-W, Percy DM, Petersen G, Richardson JE, Salazar GA, Savolainen V, Seberg O, Wilkinson MJ, Yi D-K, Little DP (2009) A DNA Barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America 106(31): 12794–12797. https://doi.org/10.1073/pnas.0905845106

Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. PLoS One 6(5): e19254. https://doi.org/10.1371/journal.pone.0019254

John DM, Moore JA, Green DR (1990) Preliminary Observations on the Structure and Ornamentation of the Oosporangial Wall in *Chara* (Charales, Chlorophyta). British Phycological Journal 25(1): 1–24. https://doi.org/10.1080/00071619000650011

Krause W (1997) Charales (Charophceae). Gustav Fischer Verlag, Jena, 202 pp.

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America 102(23): 8369–8374. https://doi.org/10.1073/pnas.0503123102

Mannschreck B (2003) Genetische und morphologische Differnzierung ausgewahlter Arten der Gattung Chara. Phd Thesis, Technical University Munich, Shaker Verlag, 166.

Meiers ST, Proctor WV, Chapman RL (1999) Phylogeny and biogeography of *Chara* (Charophyta) inerferred from 18S rDNA sequences. Australian Journal of Botany 47(3): 347–360. https://doi.org/10.1071/BT97089

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. Systematic Biology 53(1): 47–67. https://doi.org/10.1080/10635150490264699

O'Reilly CL, Cowan RS, Hawkins AA (2007) Amplified fragment length polymorphism genetic fingerprinting challenges the taxonomic status of the near – endemic species *Characurta* Nolte ex Kütz. (Characeae). Botanical Journal of the Linnean Society 155(4): 467–476. https://doi.org/10.1111/j.1095-8339.2007.00713.x

Řepka R (2003) The Carex muricata aggregate in the Czech Republic: Multivariate analysis of quantitative morphological characters. Preslia 75: 233–248.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3, Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sakayama H (2008) Taxonomy of *Nitella* (Charales, Charophyceae) based on comparative morphology of oospores and multiple DNA marker phylogeny using cultured material. Phycological Research 56(3): 202–215. https://doi.org/10.1111/j.1440-1835.2008.00502.x

Sakayama H, Nozaki H, Kasaki H, Hara Y (2002) Taxonomic re-examination of *Nitella* (Charales, Charophyceae) from Japan, based on microscopical studies of oospore wall ornamentation and *rbc*L gene sequences. Phycologia 41(4): 397–408. https://doi.org/10.2216/i0031-8884-41-4-397.1

Sakayama H, Miyaji K, Nagumo T, Kato M, Hara Y, Nozaki H (2005) Taxonomic reexamination of 17 species of Nitella subgenus *Tieffallenia* (Charales, Charophyceae) based on internal morphology of the oospore wall and multiple DNA marker sequences. Journal of Phycology 41(1): 195–211. https://doi.org/10.1111/j.1529-8817.2005.04133.x

Sakayama H, Kasai F, Nozaki H, Watanabe MM, Kawachi M, Shigyo M, Krienitz L (2009) Taxonomic reexamination of *Chara* globularis (Charales, Charophyceae) from Japan based on oospore morphology and rbcL gene sequences, and the description of *C. leptospora* sp. nov. Journal of Phycology 45: 917–927. https://doi.org/10.1111/j.1529-8817.2009.00700.x

Schneider SC, Rodrigues A, Boe TF, Ballot A (2015) DNA barcoding the genus *Chara*: Molecular evidence recovers fewer taxa than the classical morphological approach. Journal of Phycology 51(2): 367–380. https://doi.org/10.1111/jpy.12282

Schneider SC, Nowak P, Von Ammon U, Ballot A (2016) Species differentiation in the genus *Chara* (Charophyceae): Considerable phenotypic plasticity occurs within homogenous genetic groups. European Journal of Phycology 51(3): 282–293. https://doi.org/10.1080/09670262.2016.1147085

Schubert H (2014) Phylogeny and taxonomy of charophytes, good news from a battlefield of concepts. Journal of Phycology 50(5): 773–775. https://doi.org/10.1111/jpy.12212

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. American Journal of Botany 94(3): 275–288. https://doi.org/10.3732/ajb.94.3.275

StatSoft (2010) STATISTICA 12. (data analysis software system), version 9.1. Tulsa.

Swofford DL (2002) PAUP* – Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0b10. Sinauer Associates, Sunderland.

Tanabe AS (2011) Kakusan 4 and Aminosan, two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Molecular Ecology Resources 11(5): 914–921. https://doi.org/10.1111/j.1755-0998.2011.03021.x

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W, Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Urbaniak J (2010) Analysis of morphological characters of *Chara baltica*, *C. hispida*, *C. horrida* and *C. rudis* from Europe. Plant Systematics and Evolution 286(3–4): 209–221. https://doi.org/10.1007/s00606-010-0301-6

Urbaniak J (2011a) An SEM and light microscopy study of the oospore wall ornamentation in Polish charophytes (Charales, Charophyceae) – genus *Chara*. Nova Hedwigia 93(1–2): 1–28. https://doi.org/10.1127/0029-5035/2011/0093-0001

Urbaniak J (2011b) An SEM study of the oospore wall ornamentation in Polish charophytes (Charales, Charophyceae) – genus *Lychnothamnus*, *Nitella* and *Nitellopsis*. Nova Hedwigia 93(3–4): 537–549. https://doi.org/10.1127/0029-5035/2011/0093-0537

Urbaniak J, Blazencic J (2012) SEM study of oospore characteristics in endemic and endangered Balkan Charophytes. Cryptogamie. Algologie 33(3): 277–288. https://doi.org/10.7872/crya.v33.iss3.2012.277

Urbaniak J, Combik M (2013) Genetic and morphological data fail to differentiate *Chara* intermedia from *C. baltica*, or *C. polyacantha* and *C. rudis* from *C. hispida*. European Journal of Phycology 48(3): 253–259. https://doi.org/10.1080/09670262.2013.805249

Urbaniak J, Gąbka M (2014) Polish Charophytes. An illustrated guide to identification. Wydawnictwo Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław, 120. https://www.researchgate.net/profile/Jacek_Urbaniak2

van de Weyer K (2016) Bestimmungsschlüssel. In: Arbeitsgruppe Characeen Deutschlands (Eds) Armleuchteralgen. Die Characeen Deutschlands. Springer Verlag, Berlin Heidelberg, 193–208. https://doi.org/10.1007/978-3-662-47797-7_11

Wood RD (1962) New combinations and taxa in the revision of Characeae. Taxon 11: 7–25. https://doi.org/10.2307/1216853

Wood RD, Imahori KA (1965) A revision of the Characeae. First part, Monograph of the Characeae. J. Cramer Verlag, Weinheim, 904 pp.

PhytoKeys 135: 91–104 (2019)
doi: 10.3897/phytokeys.135.37011
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Molecular phylogeny of *Hiptage* (Malpighiaceae) reveals a new species from Southwest China

Ke Tan[1,3], Hai-Lei Zheng[2], Shu-Peng Dong[1], Ming-Xun Ren[1,3]

1 *Key Laboratory of Genetics and Germplasm Innovation of Tropical Special Forest Trees and Ornamental Plants, College of Ecology and Environment, Hainan University, Haikou 570228, China* **2** *Wild Dali Nature Education and Research Center, Dali 671000, Yunnan, China* **3** *Center for Terrestrial Biodiversity of the South China Sea, Hainan University, Haikou 570228, China*

Corresponding author: *Ming-Xun Ren* (renmx@hainanu.edu.cn)

Academic editor: *R.F. Almeida* | Received 10 June 2019 | Accepted 3 November 2019 | Published 5 December 2019

Citation: Tan K, Zheng H-L, Dong S-P, Ren M-X (2019) Molecular phylogeny of *Hiptage* (Malpighiaceae) reveals a new species from Southwest China. PhytoKeys 135: 91–104. https://doi.org/10.3897/phytokeys.135.37011

Abstract

Hiptage is an Asia-endemic genus of Malpighiaceae currently placed in the tetrapteroid clade, representing one of the seven inter-continent dispersions from New to Old World. A molecular phylogeny based on sequences of the internal transcribed spacer (ITS) region was recovered for the first time for the genus. Our results showed that the most recent common ancestor of *Hiptage* probably originated in the South Indo-China Peninsula and diversified in this region. Based on phylogenetic evidence and relevant morphological traits, we propose a new species; *Hiptage incurvatum* is characterised by mericarps with arcuate anterior lateral wings, two large glands on the dorsal sepals, and small glands on the remaining sepals. The new species is from Mt. Cangshan, Dali City (25°35'N, 100°02'E) in North Yunnan, Southwest China and is notable for its occurrence at high altitude, 1400 m (the highest distribution currently known for the genus). The implications of this unusual species for the dispersal and evolution of the genus are discussed.

Keywords

Asia, *Hiptage incurvatum*, Malpighiales, taxonomy, tetrapteroid clade

Introduction

Hiptage Gaertn. is a genus of Malpighiaceae currently comprising ca. 40 species (Anderson et al. 2006; Chen and Funston 2008; Ren 2015; Yang et al. 2018). Its species typically grow as woody lianas in the margins of evergreen and seasonal rainforests, in

river valleys, or on limestone hills of South and Southeast Asia (Sirirugsa 1991; Srivastava 1992; Chen and Funston 2008). The genus is characterised by its many-flowered thyrse bearing mirror-image flowers with nectar secreting sepal glands, and a single mericarp per flower bearing three lateral wings (Anderson et al. 2006; Ren et al. 2013; Zhang et al. 2016). Mirror-image flowers are a sexual polymorphism in which the style is deflected away from the floral axis, resulting in mirror images between the left-styled flower and right-styled flowers, facilitating cross-pollination (Jesson and Barrett 2002; Ren et al. 2013). Currently, the genus is placed in the tetrapteroid clade, one of the ten major lineages recovered for Malpighiaceae by Davis and Anderson (2010). *Hiptage* was represented in that phylogeny by six species and recovered as sister to *Flabellariopsis* R.Wilczek, an African endemic, and together both genera form a poorly supported clade, sister to the Neotropical genus *Carolus* W.R.Anderson (Davis and Anderson 2010). However, the monophyly of *Hiptage* has never been properly tested by the inclusion of its type species (Jacobs 1955; Davis and Anderson 2010; Zhang et al. 2016).

During recent field studies addressing the pollination ecology of *Hiptage* in North Yunnan, two populations of an unusual morphotype of *Hiptage benghalensis* were discovered near Pingpo Town, in Mount Cangshan near Dali City. After molecular and morphological analyses, based on the nuclear internal transcribed spacer (ITS) region and on the comparison of living and herbarium specimens (including type specimens of all currently accepted names in the genus), we concluded that these abovementioned populations represent an undescribed species of *Hiptage*. We present a molecular phylogeny sampling 17 of 39 species of *Hiptage*, including a discussion on the systematics and biogeography of the genus, besides the formal description of the new species and an updated key for the genus in China.

Materials and methods

Molecular analysis

We sampled most species of *Hiptage* occurring in the Philippines, Thailand, Vietnam, Singapore and Southwest China, to explore the phylogenetic relationships of the suspected new species (Table 1). The sequences of nuclear ribosomal Internal Transcribed Spacer (ITS) region of 17 species (with some species with multi accessions) of *Hiptage* were generated and analysed. The ITS sequences of two American-endemic species of *Mascagnia* (i.e., *M. australis* and *M. divaricata*) were obtained from GenBank and used as outgroups. Total genomic DNA was extracted from dried leaf material following a modified CTAB method (Doyle and Doyle 1987). All polymerase chain reactions (PCR) were carried out in 25 μl volumes consisting of 1 μl sample DNA, 12.5 μl 2 × Taq PCR master Mix (Aidlab Biotechnologies Co. Ltd), 1 μl each primer (10 μmol/ml), and a final volume adjusted to 25 μl with double distilled water. The ITS region was amplified with the primers ITS17SE and ITS26SE (Sun et al. 1994). We used an amplification profile with an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 40 seconds at 94 °C, 20 seconds at 69 °C,

1 min at 72 °C, and a final 10 min extension at 72 °C. The PCR products were sequenced from both directions using an ABI3730XL sequencer.

The original chromatograms from both directions of the ITS sequences were evaluated with PhyDE (Müller et al. 2010) for base confirmation and contiguous sequences editing. All sequences were aligned manually in MEGA v.7 (Kumar et al. 2016). Ambiguous positions were excluded from the alignments. The Akaike Information Criterion (AIC), which allows non-nested models to be evaluated, was used as a selection criterion (Kumar et al. 2016), and the GTR + I + G model was used in both ML and BI analyses. Maximum Likelihood (ML) analysis was performed with optimal substitution models suggested by MEGA v.7 to carry out 1000 bootstrap (BS) replicates analyses. Bayesian inference (BI) was performed with MrBayes v.3.1 (Ronquist and Huelsenbeck 2003) with a Markov chain Monte Carlo (MCMC) simulations were run for 10 000 000 generations and sampled every 1000 generations. The first 2500 trees (25% of total trees) were discarded as burn-in. The remaining trees were summarised in a 50% majority-rule consensus tree, and the posterior probabilities (PP). The obtained tree was edited using Figtree v. 1.4.3 (Morariu et al. 2008). Sequences were deposited in GenBank and the alignment and phylogenetic trees in TreeBASE (ID: S24963 and S24968).

Taxonomy

The proposed new species was compared with the type specimens of all accepted names in the genus, including collections of *Hiptage* deposited in the herbaria KUN, PE, IBSC, and IBK (acronyms according to Thiers 2019). We also downloaded all *Hiptage* specimens from JSTOR Global Plants (http://plants.jstor.org), and Chinese Virtual Herbarium (http://www.cvh.ac.cn) to compare detailed morphological traits between the proposed new species with the currently accepted species of *Hiptage*. The morphological terminology follows Niedenzu (1924), Jacobs (1955), Hô (1992), Anderson et al. (2006), Chen and Funston (2008), Pelser et al. (2011), and Ren (2015).

Results

For the 17 *Hiptage* species, we obtained 36 sequences of ITS in total. Source information and the GenBank accession numbers of the new sequences are listed in Table 1. The dataset had an aligned length of 691 base pairs (bp), containing 128 parsimony-informative characters.

In the ITS tree (Fig. 1) *Hiptage* was recovered as monophyletic, strongly supported (PP/BS=1/100) by both analyses, with *H. stellulifera* from Vietnam as the first diverging lineage in the genus. The remaining species of *Hiptage* sampled formed two separate clades, although with weak support (PP/BS=0.64/52). Most species of *Hiptage* show reflexed petals (red line), with all species bearing erect petals (i.e., *H. lucida*, *H. bullata*, and *H. minor*) being recovered on a single clade, suggesting a single origin of

Table 1. Taxa and GenBank accession numbers for the nrITS sequences used in this study; an asterisk (*) indicates the new species record.

Species	Locality	GenBank accession numbers	Voucher number
Hiptage benghalensis (L.) Kurz	Phatthaya, Thailand	MH718408	K. Tan, S. P. Dong, & M. X. Ren 3344 (HUTB)
	Chiang Mai, Thailand	MH718410	K. Tan, S. P. Dong, & M. X. Ren 3336 (HUTB)
	Singapore	MH718399	T. W. Yam 3334 (HUTB)
	Lekang County, Guizhou, China	MH718415	K. Tan, S. P. Dong, & M. X. Ren 82 (HUTB)
	Yangjie, Yunnan, China	MH718400	M. X. Ren & L. Tang 128 (HUTB)
	Daxin County, Guangxi, China	MH718414	K. Tan & S. P. Dong 95 (HUTB)
	Menglian County, Yunnan, China	MH718422	S. P. Dong 131 (HUTB)
H. bullata Craib	Lampang, Thailand	MH718412	K. Tan, S. P. Dong, & M. X. Ren 3320 (HUTB)
H. candicans Hook. f.	Chiang Mai, Thailand	MH718409	K. Tan, S. P. Dong, & M. X. Ren 3328 (HUTB)
	Chom Thong, Thailand	MH718411	K. Tan, S. P. Dong, & M. X. Ren 3330 (HUTB)
H. detergens Craib	Kui Buri, Thailand	MH718404	K. Tan, S. P. Dong, & M. X. Ren 3328 (HUTB)
	Sam Roi Yot, Thailand	MH718405	K. Tan, S. P. Dong, & M. X. Ren 3326 (HUTB)
H. ferruginea Y.H.Tan & Bin Yang	Xishuangbanna, Yunnan, China	MH718402	S. P. Dong 116 (HUTB)
	Xishuangbanna, Yunnan, China	MH718403	S. P. Dong 117 (HUTB)
H. incurvatum 1*	Pingpo Town, Yunnan, China	MK967956	K. Tan, H. L. Zheng, & M. X. Ren 201903309 (HUTB)
H. incurvatum 2*	Pingpo Town, Yunnan, China	MK967957	K. Tan, H. L. Zheng, & M. X. Ren 201903310 (HUTB)
H. incurvatum 3*	Pingpo Town, Yunnan, China	MK967958	K. Tan, H. L. Zheng, & M. X. Ren 201903305 (HUTB)
H. incurvatum 4*	Pingpo Town, Yunnan, China	MK967959	K. Tan, H. L. Zheng, & M. X. Ren 201903306 (HUTB)
H. lucida Pierre	Phatthaya, Thailand	MH718406	K. Tan, S. P. Dong, & M. X. Ren 38 (HUTB)
	Xishuangbanna, Yunnan, China	MH718418	Z. N. Qian & S. P. Dong120 (HUTB)
H. luzonica Merr.	Luzon Island, Philippines	MH718425	K. Tan, W. Q. Xiang & M. X. Ren 20191181436 (HUTB)
	Cebu Island, Philippines	MH718431	K. Tan & W. Q. Xiang 3301(HUTB)
	Palawan Island, Philippines	MH718432	K. Tan, W. Q. Xiang & M. X. Ren 3305 (HUTB)
H. marginata Arènes	Hue, Vietnam	MH718413	K. Tan & Q. Yang 3363 (HUTB)
H. minor Dunn	Lushui City, Yunnan, China	MH718401	K. Tan, S. P. Dong, & M. X. Ren 88 (HUTB)
	Lekang County, Guizhou, China	MH718398	K. Tan, S. P. Dong, & M. X. Ren 79 (HUTB)
	Wenshan City, Yunnan, China	MH718423	K. Tan, S. P. Dong, & M. X. Ren 94 (HUTB)
H. monopteryx Sirirugsa	Phatthaya, Thailand	MH718407	K. Tan, S. P. Dong, & M. X. Ren 3337 (HUTB)
H. multiflora F.N.Wei	Nonggang Natural Reserve, Guangxi, China	MH718424	K. Tan & S. P. Dong 52 (HUTB)
H. pauciflora Y.H.Tan & Bin Yang	Menglian County, Yunnan, China	MH718420	S. P. Dong 73 (HUTB)

Species	Locality	GenBank accession numbers	Voucher number
H. stellulifera Arènes	Nha Trang, Vietnam	MH718429	K. Tan & S. J. Ling 3376 (HUTB)
H. subglabra Arènes	Nui Chua National Park, Phan Rang, Vietnam	MH718427	K. Tan & S. J. Ling 3364 (HUTB)
H. tianyangensis F.N.Wei	Liulian Town, Tianyang, Guangxi, China	MK967960	K. Tan & S. P. Dong 50 (HUTB)
H. umbellulifera Arènes	Nui Chua National Park, Phan Rang, Vietnam	MH718428	K. Tan & S. J. Ling 3385 (HUTB)
	Cana, Phan Rang, Vietnam	MH718426	K. Tan & S. J. Ling 3386 (HUTB)
	Phan Rang, Vietnam	MH718430	K. Tan & S. J. Ling 3399 (HUTB)
Mascagnia australis C.E. Anderson	South America	KR092931	A. Francener 1177 (SP)
M. divaricata (Kunth) Nied.	South America	KR092932	R. F. Almeida 547 (HUEFS)

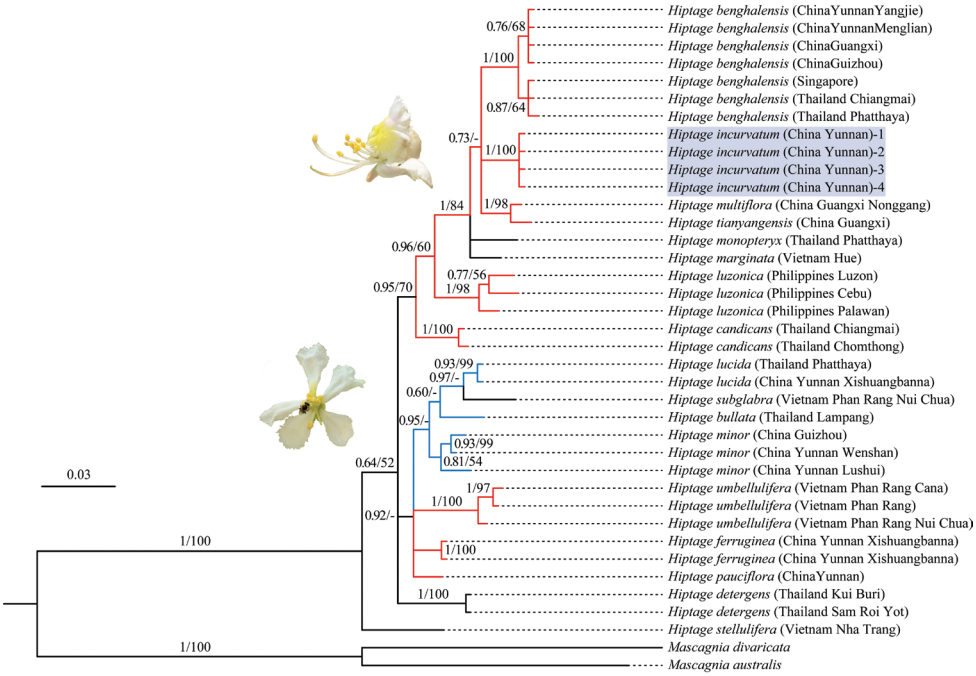

Figure 1. Molecular phylogeny for 17 species of *Hiptage* and two Neotropical outgroups based on ITS sequences. Bayesian posterior probability (PP) and MP bootstrap values (BS) are showed above branches as PP/BS (only shown if BS > 50%). *H. incurvatum* was shown in grey. The red, blue, black clades indicate reflexed petals, erect petals, and unknown, respectively. Inserted photos indicate petal-reflexed flowers (red branches) and petal-plat flowers (blue branches). Black branches represent the unclear mode.

erect petals in the genus (Fig. 1). The four specimens of the proposed new species, *H. incurvatum*, coalesced in a clade with strong support (PP/BS=1/100%) (Fig.1). This clade was recovered in a polytomy, the *H. multiflora* + *H. tianyangensis* clade, from Guangxi Province, and the widespread *H. benghalensis* clade, from Southwest China and Indo-China Peninsula (Fig. 1). Relationships among these four species are mostly poorly supported (PP/BS=0.73/-) with the exception of the strongly supported *H. multiflora* + *H. tianyangensis* clade (PP/BS=1/98).

Taxonomy

***Hiptage incurvatum* K.Tan & M.X.Ren, sp. nov.**
urn:lsid:ipni.org:names:77203327-1
Figs 2, 3

Type. China. Yunnan Province: Pingpo Town, Mt. Cangshan, Dali City, 25°35'N, 100°02'E, 1400 m altitude. 31 Mar 2019, *K. Tan and M.X. Ren 2019033110* (holotype: HUTB!, isotypes: HUTB!, KUN!)

Diagnosis. Similar to *H. tianyangensis* in ovate leaf shape, suborbicular petals; but differing from this species by sepal glands twice big as *H. tianyangensis* (vs. sepal gland, ~ 3 × 1 mm), the elevation ca. 400 m (vs. 1379–1724 m), the short inflorescence 1–4 cm (vs. 4–10 cm).

Description. Woody lianas; stems 20–30 (–200) mm diam. Branches round, lenticels white or greenish, tomentose to glabrous, with white to grey hairs. Leaves opposite; stipules absent; petioles ca 0.5 cm long, round, tomentose, with white hairs, eglandular; leaf blades 6–12 × 2.5–4.5 cm, elliptic, base cuneate, margin plane, apex attenuate, both surfaces sericeous, 10–16-glandular dots abaxially near margin, lateral veins 5–8 pairs, prominent on both surfaces. *Thyrses*, solitary, axillary or terminal; main axis 4–10 cm long, tomentose, with white hairs; peduncles 1.5–2.5 cm long, tomentose; bracteoles inserted below the apex of peduncles, 0.3–0.5 cm long, lanceolate. *Flowers* with pedicels 1.5–2.5 cm long, sericeous, with white hairs; sepals 5, ca. 0.5 cm long, elliptic to oblong, margin slightly revolute, apex rounded, adaxial surface glabrescent, abaxial surface white tomentose; glands 4 (–6), 0.5–3 × 0.5–1 mm, prominent, rounded, restricted to sepals, two large, basally fused glands on the dorsal sepals, remaining glands small and free; petals white to light pink, yellow at the base, ca. 1 × 0.8 cm, suborbicular, extremely reflexed, claws ca. 1 mm long. *Stamens* 10, filaments white or light yellow, free or basally fused, 7–13 mm long, glabrous; anthers ca. 0.5 × 0.3 cm, ovate, pubescent, with yellow hairs; pollen sacs dehiscing longitudinally. *Ovary* ca. 2 mm diam.; styles 1, light pink, ca. 13 mm long, curved upwards, deflected either to the left or right side, glabrous; stigma apical. *Mericarps* 3, each flower developing up to three mericarps, detaching from a pyramidal torus; individual mericarps three-winged (laterally placed in the nut), wings pink with greenish base, the posterior wing ca. 3.6 × 1.3 cm, ovoid, apex round or slightly lobed, with white or brown hairs; anterior lateral wings ca. 2.3 × 0.7 cm, lanceolate, arcuate back to the middle; nut ca. 0.2 cm, round or slight ovate, glabrous; areole ca. 0.3–0.6 cm, roughly triangular. *Seeds* angular-globose, ca. 3–5 mm, dark yellow or brown.

Additional specimens examined (paratypes). China. Yunnan Province: Pingpo Town, Mt. Cangshan, Dali City. 31 Mar 2019, *K. Tan and M.X. Ren 2019033109* (HUTB), *K. Tan and M.X. Ren 2019033108* (KUN).

Phenology. Flowering from April to May, and fruiting in May.

Distribution and habitat. *Hiptage incurvatum* is only known from two localities near Mt. Cangshan, Pingpo Town, Dali City, North Yunnan, growing on soil slopes or forest margins and river valleys, at 1400–1700 m. In China, a total of 13 species of

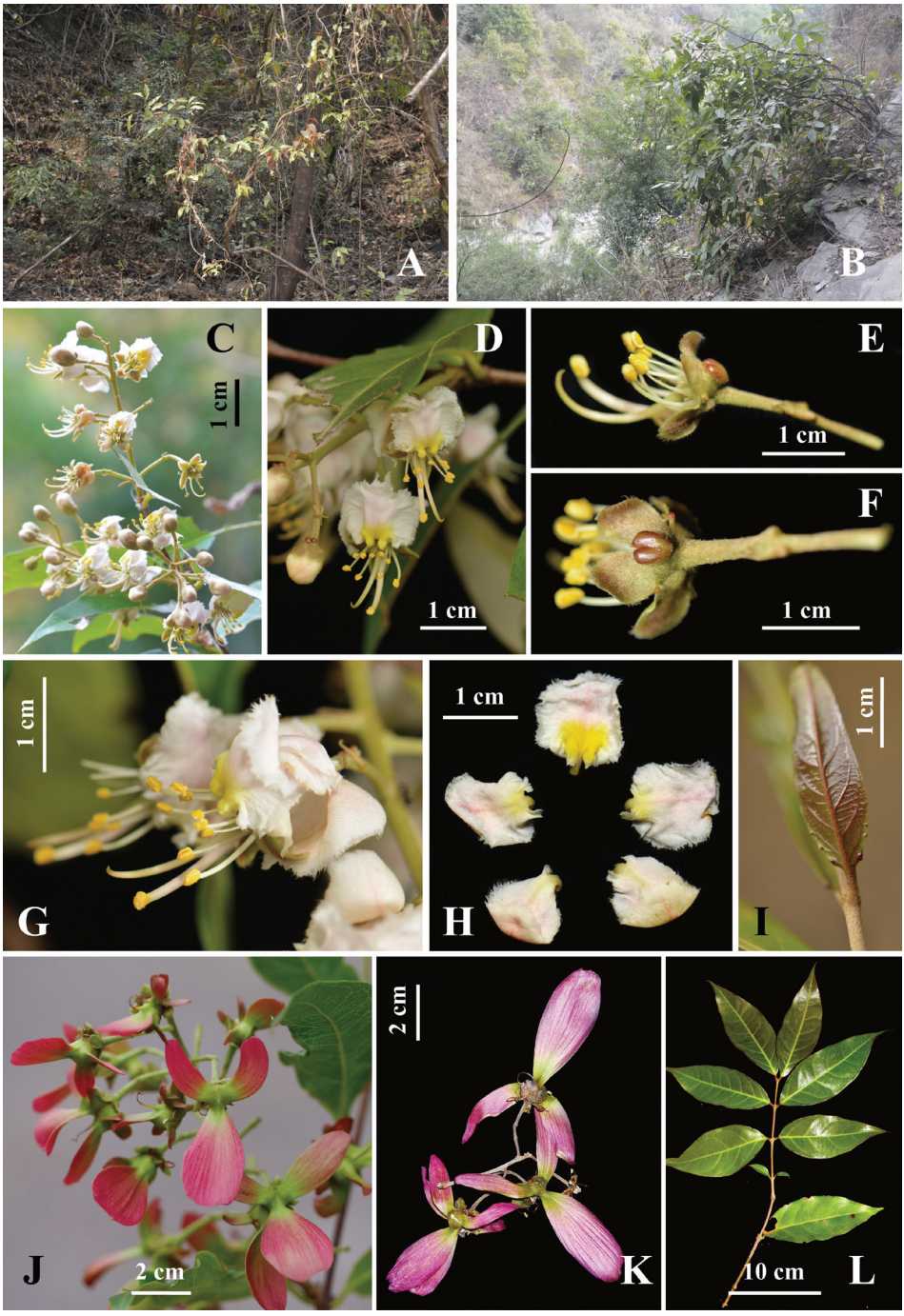

Figure 2. *Hiptage incurvatum* K.Tan & M.X.Ren, sp. nov. **A, B** habit **C** flowering branch **D** flower in frontal view **E** flower with petals removed in sideview **F** flower with petals removed in dorsal view showing two large glands on the dorsal sepals) **G** flowers in sideview **H** detached petals **I** young leaf in adaxial view **J** young samaras **K** mature samaras **L** leaf branch in adaxial view. Photos **A–C** by M. X. Ren, **J, K** by H. L. Zheng and **D–I, L** by K. Tan.

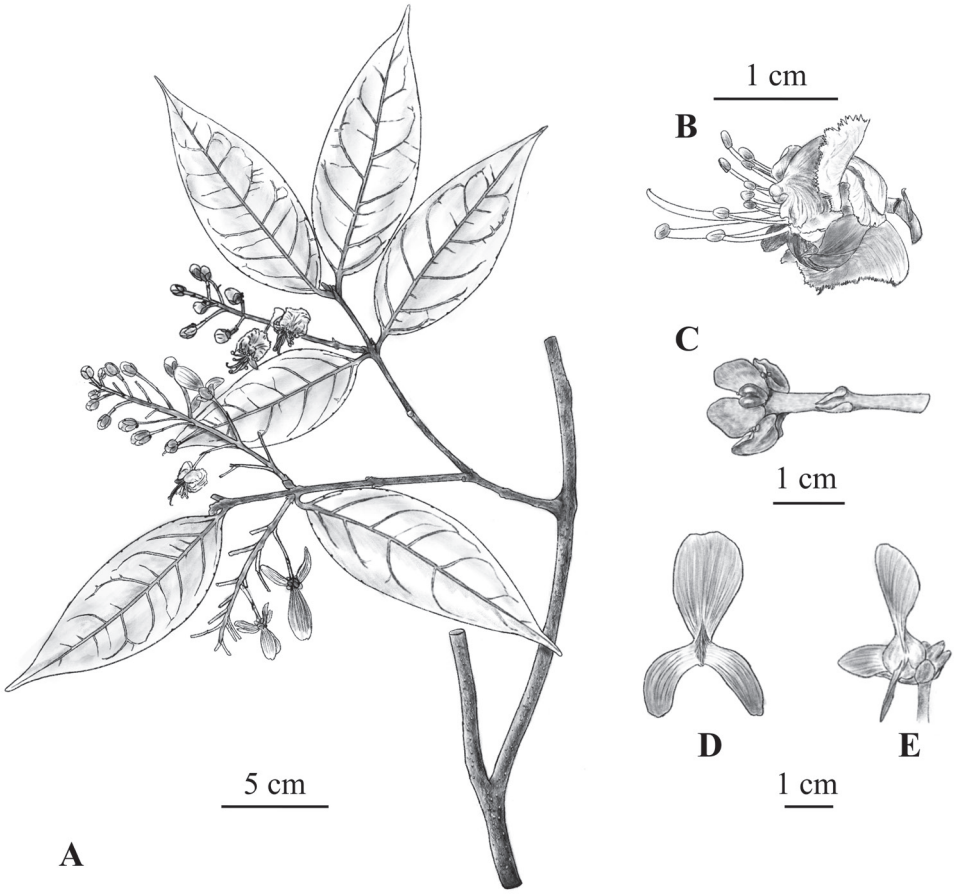

Figure 3. Line drawing of *Hiptage incurvatum* K.Tan & M.X.Ren, sp. nov. **A** flowering branches **B** flower (in sideview) **C** sepals showing two large glands on the dorsal sepals and small glands on the remaining sepals **D** samara in dorsal view, showing the curved lateral wings **E** samara in sideview. Drawings by Ya-Jing Zhang based on K. Tan and M.X. Ren 2019033109 (HUTB).

Hiptage now have been recorded, 10 of which, including the new species, are endemic to the country (Chen and Funston 2008; Ren 2015; Yang et al. 2018).

Etymology. The specific epithet reflects the arcuate and curved anterior lateral wings of the three-winged samara.

Vernacular name. Chinese: 弯翅风筝果 (wān chì fēng zhēng gǔo). The name 'wān chì' means arcuate wing, 'fēng zhēng gǔo' is the Chinese name of *Hiptage*.

Conservation status. The only two known populations of *Hiptage incurvatum* are in Pingpo Town of Dali City, in a river valley near Mt. Cangshan. These two populations have about 50 individuals in total along the woodland margins or slopes of the valley near a road. Very limited information is known about the new species. There-

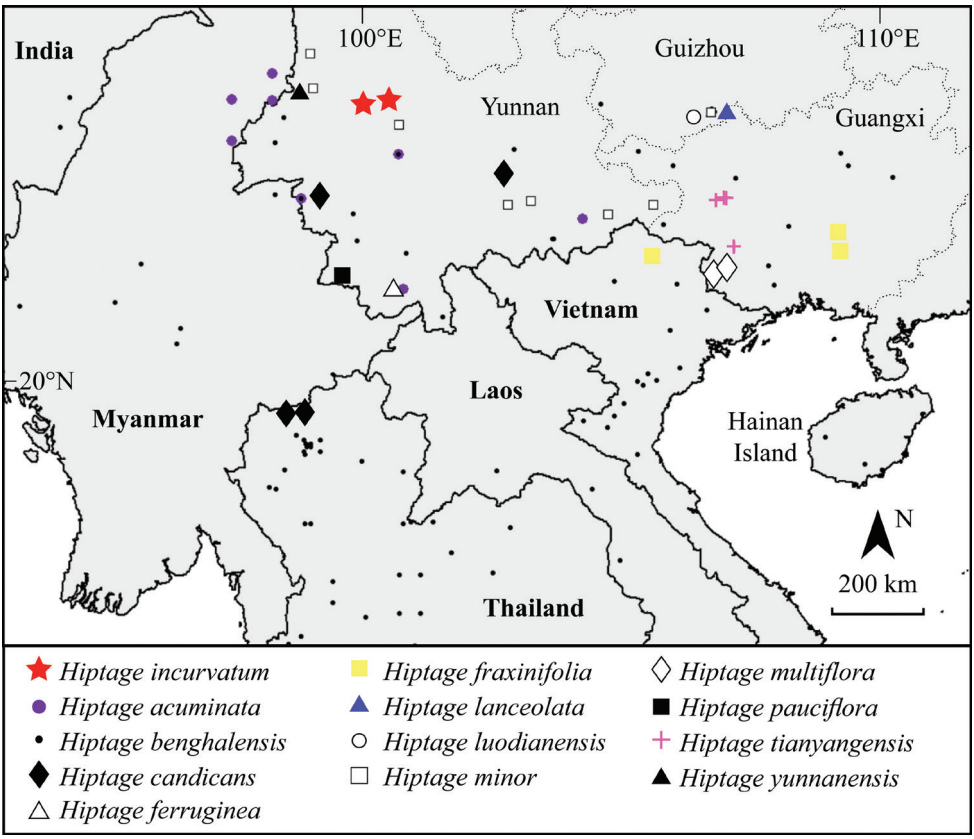

Figure 4. Distribution map of the new species *Hiptage incurvatum* and the other 12 species of the genus known in China and nearby regions.

fore, *H. incurvatum* can be treated as Near Threatened (NT, close to being at high risk of extinction in the near future under the criterion [B1ab(iii) + 2ab (iii)] according to the IUCN Red List criteria (IUCN 2013).

Notes. The new species can be clearly identified from three similar species (*H. benghalensis*, *H. multiflora*, *H. tianyangensis*) from the following traits. Leaf: *H. incurvatum* (ovate, 6–12 × 2.5–4.5 cm) is smaller than all the three species, i.e. *H. tianyangensis* (ovate, 7–12× 3–5.5 cm), *H. multiflora* (oblong, 12–13 × 5–5.5 cm), *H. benghalensis* (elliptic, 9–18 × 3–7cm). Petal color: *H. incurvatum* (white with light pink), *H. tianyangensis* (white), *H. multiflora* (pink), *H. benghalensis* (white with yellow on the vexillum). Calyx glands: *H. incurvatum* (2 large and fused at the lower part, not decurrent to the pedicel; sometimes 2 or 4 smaller glands can be seen on other sepals), *H. tianyangensis* (2, small, clearly isolated, not decurrent to the pedicel), *H. multiflora* (1, large, not decurrent to the pedicel), *H. benghalensis* (1, very large, 1/2 adnate to the pedicel).

Key to the species of *Hiptage* in China (modified from Chen and Funston 2008)

1 Calyx eglandular **2**
– Calyx glandular **4**
2 Leaf blades eglandular ..... ***H. lanceolata***
– Leaf blades with 1 pair of marginal glands near base **3**
3 Inflorescence covered in yellow-brown appressed hairs; leaf blades ovate, ovate-lanceolate, or elliptic, apex acuminate, base cuneate; petals white, erect ..... ***H. minor***
– Inflorescence covered in rust-colored hairs; leaf blades elliptic or elliptic-oblong, apex acute to attenuate, base cuneate to obtuse; petals pink to light pink, reflexed ..... ***H. ferruginea***
4 Two or more sepals glandular **5**
– Only 1 sepal glandular **6**
5 Two sepal glands, elliptic; glands slightly adnate to pedicel ..... ***H. luodianensis***
– 4 (-6) sepal glands; rounded; glands restricted to the sepals ..... ***H. incurvatum***
6 Sepal glands rotund or oblong, not decurrent onto the pedicel **7**
– Sepal glands oblong, oblong-lanceolate, or ovate-oblong, ± decurrent onto the pedicel **10**
7 Leaf blade oblong, base cordate, apex acute; posterior lateral wing obovate . **8**
– Leaf blade elliptic to ovate, base cuneate or rounded, apex acuminate; posterior lateral wing oblong **9**
8 Basal dotted glands of leaves absent; inflorescence with < 10 flowers, pedicels 1.8–2.9 cm, calyx ovate or sub-orbicular to cordate ..... ***H. pauciflora***
– Basal dotted glands of leaves present, inflorescence with >10 flowers, pedicels ca. 1 cm, calyx oblong ..... ***H. multiflora***
9 Thyrses terminal, ca. 11 cm; sepal oblong; leaf base cuneate ..... ***H. fraxinifolia***
– Thyrses axillary, ca. 3 cm; sepal ovate; leaf base rounded or broadly cuneate .. ***H. tianyangensis***
10 Leaf blade abaxially yellow-brown or gray-white tomentose; sepal glands oblong-lanceolate, base decurrent onto the pedicel ..... ***H. candicans***
– Leaf blade glabrous to base of midrib sparsely pubescent abaxially; sepal glands oblong or ovate-oblong, 1/4–1/2 decurrent onto the pedicel **11**
11 Nut shortly sericeous, wings glabrous; leaf blade oblong, elliptic-oblong, or ovate ..... ***H. benghalensis***
– Nut and wings pubescent; leaf blade lanceolate, oblong, ovate, or elliptic . **12**
12 Leaf blades lanceolate, oblong, or ovate, 7.5–12 × 3–4.5 cm; posterior lateral wing obovate-oblong, 2.5–3 × ca. 1.2 cm, anterior lateral wing linear-lanceolate, ca. 13 × 5–6 mm ..... ***H. acuminata***
– Leaf blades elliptic, 12.5–17 × 4–7 cm; posterior lateral wing oblanceolate, ca. 3 × 1 cm, anterior lateral wings linear, ca. 15 × 3 mm ..... ***H. yunnanensis***

Discussion

We provide here the first well-sampled phylogenetic study for the Asian endemic *Hiptage*, although this phylogeny is based on a single marker and most clades are not highly supported. *Hiptage* is one of the largest Old-World genera of Malpighiaceae, being adapted to various habitats such as forest edges, river valleys and limestone hills in Asia (Sirirugsa 1991; Ren 2015; Yang et al. 2018). Our phylogenetic tree shows that two species from South Vietnam and Thailand (i.e. *H. stellulifera* and *H. detergens*) were recovered as basal groups, suggesting the genus might have evolved at the southern part of Indo-China Peninsula (Fig. 1).

Based on the phylogeny tree, the most widespread species in the genus, *H. benghalensis*, might have appeared late in the evolution of the genus, although we are not providing divergence time estimates. *H. benghalensis* is well-known for its extremely reflexed petals and single oversized calyx gland secreting nectar, attracting both pollinators and herbivory-defending ants (Ren et al. 2013). Such floral syndromes indicate generalized pollination by pollen-collecting bees (Ren et al. 2013; Qian et al. 2016), which can explain the widespread distribution of *H. benghalensis*. Moreover, both our data and the results of Davis and Anderson (2010) demonstrated the polyphyly in *H. benghalensis* (Fig. 1), suggesting this most widespread species might be treated as two taxa. Further studies are still needed to properly address this question with more extensive sampling.The petal shape and calyx glands are diagnostic traits of the family Malpighiaceae, being used for species identification and taxonomic study (Niedenzu 1924; Anderson et al. 2006; Chen and Funston 2008; Ren 2015). The phylogeny indicates that reflexed petals may be common in both basal and nested clades, and flat petals probably evolved only once (Fig. 1). Normally there are ten oil secreting calyx glands in Neotropical Malpighiaceae, with two glands on each sepal (Anderson et al. 2006). In *Hiptage*, however, most species show a single calyx gland, but secreting nectar instead of oil (Ren et al. 2013). In the basal *H. stellulifera*, five calyx glands were found (i.e., each sepal shows a single gland) (Hô 1992). Therefore, one of the evolutionary trends in *Hiptage* appears to be the numeric reduction of calyx glands (Anderson et al. 2006; Ren et al. 2013; Ren 2015).

The multiple accessions of the proposed new species were recovered as a strongly supported clade (Fig. 1), separated from closely related taxa by weak molecular, but several morphological traits (Fig. 2). Specifically, the new species is distinctive in having two large glands on the dorsal sepals and two small glands on the remaining sepals (Figs 2E–F, 3C). Interestingly, the lower parts of the two large glands are fused (Figs 2F, 3C), indicating a possible explanation for the evolution of the single oversized calyx gland in *H. benghalensis* (Anderson et al. 2006; Ren et al. 2013). The similar evolutionary trend was also found in the Paleotropical genus *Acridocarpus* (Malpighiaceae) (Guesdon et al. 2019), in which the adjacent sepal glands show different degrees of fusion in several species and the single sepal gland in some species shows sagittate-acute shape, shared secretory tissues and vascular bundles, providing strong evidence of the fusion of two glands on adjacent anterior sepals (Vogel 1990; Guesdon et al. 2019).

Molecular data showed that *H. incurvatum* is closely related to *H. tianyangensis*, *H. multiflora* and *H. benghalensis*. These species, however, differ significantly in habitat type, and in calyx gland and mericarp morphology (see Key). The new species grows along a river valley at very high latitudes (>1300 m) in North Yunnan, while *H. multiflora* and *H. tianyangensis* normally grow at the top of limestone mountains in Guangxi and *H. benghalensis* is widespread in Asia in forest margins and riversides (Anderson et al. 2006; Ren 2015).

The most distinctive trait in the new species is the arcuate anterior lateral wings of the three-winged mericarp (Figs 2J–K, 3D). Winged mericarps are an adaption for wind dispersal of fruits (Tan et al. 2018) and the striking diversity of winged mericarps types in Malpighiaceae indicates that this morphology played a role in long-distance dispersals and speciation (Davis et al. 2001, 2002; Qian and Ren 2016; Tan et al. 2018). Pingpo Town is located at the northern edge of the distribution range of the genus *Hiptage*. The surrounding mountains and gorges form a unique isolated habitat, which might be the main reason for the evolution and maintenance of the new species.

Conclusions

We presented the first well-sampled phylogeny of *Hiptage*, based on the ITS region, suggesting that the southern part of Indo-China Peninsula may be the area of origin of the genus. It also indicates that the erect petals have probably evolved only once in the genus. The number of calyx glands in *Hiptage* seems to have decreased during the genus evolutionary history. And specimens from Mt. Cangshan in North Yunnan were treated as a new species due to forming a highly supported clade in our phylogenetic study and being morphologically distinct from all accepted species in *Hiptage*.

Acknowledgements

We are grateful to Ding Song and Min Li for providing locality information and all reviewers (Augusto Francener, Kenneth Wurdack, Rafael Felipe de Almeida, Ricarda Riina and Wen-Heng Zhang) for their insightful comments. This study was financially supported by the Innovative Team Program of Hainan Natural Science Foundation (2018CXTD334), National Natural Science Foundation of China (31670230) and Postgraduate Innovative Grant of Hainan Province (Hyb2016-06).

References

Aguilar JF, Feliner GN (2003) Additive polymorphisms and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). Molecular Phylogenetics and Evolution 28(3): 430–447. https://doi.org/10.1016/S1055-7903(02)00301-9

Anderson WR, Anderson C, Davis CC (2006-onwards) Malpighiaceae. http://herbarium.lsa.umich.edu/malpigh/index.html [accessed on 19 November 2018]

Chen SK, Funston AM (2008) Malpighiaceae. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol.11). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 132–138.

Davis CC, Anderson WR (2010) A complete generic phylogeny of Malpighiaceae inferred from nuclear sequence data and morphology. American Journal of Botany 97(12): 2031–2048. https://doi.org/10.3732/ajb.1000146

Davis CC, Anderson WR, Donoghue MJ (2001) Phylogeny of Malpighiaceae: Evidence from chloroplast *ndhF* and *trnL-F* nucleotide sequences. American Journal of Botany 88(10): 1830–1846. https://doi.org/10.2307/3558360

Davis CC, Bell CD, Mathews S, Donoghue MJ (2002) Laurasian migration explains Gondwanan disjunctions: Evidence from Malpighiaceae. Proceedings of the National Academy of Sciences of the United States of America 99(10): 6833–6837. https://doi.org/10.1073/pnas.102175899

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Guesdon IR, Amorim AM, Meira RMSA (2019) Functional role and evolutionary contributions of floral gland morphoanatomy in the Paleotropical genus *Acridocarpus* (Malpighiaceae). PLoS One 14(9): e0222561. https://doi.org/10.1371/journal.pone.0222561

Hô PH (1992) Malpighiaceae. Câycó Viêtnam [Illustrated flora of Vietnam] 2(1): 424–434.

IUCN (2013) Red List of Threatened Species, Version 2013.1. http://www.iucnredlist.org [accessed on 26 September 2013]

Jacobs M (1955) Malpighiaceae. In: Steenis CGGJ (Ed.) Flora Malesiana Ser. 1, 5(2). Noordhoff-Kolff, Djakarta, 125–145.

Jesson LK, Barrett SCH (2002) Enantiostyly: Solving the puzzle of mirror-image flowers. Nature 417(6890): 707. https://doi.org/10.1038/417707a

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874.

Morariu VI, Srinivasan BV, Raykar VC, Duraiswami R, Davis LS (2008) Automatic online tuning for fast Gaussian summation. Advances in Neural Information Processing Systems (NIPS).

Müller K, Müller J, Quandt D (2010) PhyDE: Phylogenetic Data Editor, version 0.9971. http://www.phyde.de/index.html

Niedenzu F (1924) Malpighiaceae palaeotropicae II. Verzeichnis der Vorlesungen an der Akademie zu Braunsberg im Sommer 1924, 1–19.

Pelser PB, Barcelona JF, Nickrent DL (2011–onwards) Co's Digital Flora of the Philippines. http://www.philippineplants.org [accessed on 11 November 2018]

Qian ZN, Ren MX (2016) Floral evolution and pollination shifts of the "Malpighiaceae route" taxa, a classical model for biogeographical study. Biodiversity Science 24: 95–101. https://doi.org/10.17520/biods.2015195 [in Chinese with English abstract]

Qian ZN, Meng QW, Ren MX (2016) Pollination ecotypes and herkogamy variation of *Hiptage benghalensis* (Malpighiaceae) with mirror-image flowers. Biodiversity Science 24: 1364–1372. https://doi.org/10.17520/biods.2016248 [in Chinese with English abstract]

Ren MX (2015) The upper reaches of the largest river in Southern China as an "evolutionary front" of tropical plants: Evidences from Asia-endemic genus *Hiptage* (Malpighiaceae). Collectanea Botanica 34: e003. https://doi.org/10.3989/collectbot.2015.v34.003

Ren MX, Zhong YF, Song XQ (2013) Mirror-image flowers without buzz pollination in the Asia-endemic *Hiptage benghalensis* (Malpighiaceae). Botanical Journal of the Linnean Society 173(4): 764–774. https://doi.org/10.1111/boj.12101

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inferenceunder mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sirirugsa P (1991) Malpighiaceae. In: Smitinand T, Larsen K (Eds) Flora of Thailand (Vol.5, Part 3). The Forest Herbarium, Royal Forest Department, Bangkok, 272–299.

Srivastava RC (1992) Taxonomic revision of the genus *Hiptage* Gaertner (Malpighiaceae) in India. Candollea 47: 601–612.

Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theoretical and Applied Genetics 89(1): 26–32. https://doi.org/10.1007/BF00226978

Tan K, Dong SP, Lu T, Zhang YJ, Xu ST, Ren MX (2018) Diversity and evolution of samara in angiosperm. Chinese Journal of Plant Ecology 42: 806–817. https://doi.org/10.17521/cjpe.2018.0053 [in Chinese with English abstract]

Thiers B (2019) [and continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed on 19 October 2019]

Vogel S (1990) History of the Malpighiaceae in the light of pollination ecology. Memoirs of the New York Botanical Garden 55: 130–142.

Yang B, Ding HB, Li JW, Tan YH (2018) Two new species of *Hiptage* (Malpighiaceae) from Yunnan, Southwest of China. PhytoKeys 110: 81–89. https://doi.org/10.3897/phytokeys.110.28673

Zhang W, Kramer EM, Davis CC (2016) Differential expression of CYC2 genes and the elaboration of floral morphologies in *Hiptage*, an Old-World genus of Malpighiaceae. International Journal of Plant Sciences 177(7): 551–558. https://doi.org/10.1086/687225

PhytoKeys 135: 105–117 (2019)
doi: 10.3897/phytokeys.135.46624
http://phytokeys.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Rediscovering two *Isoetes* species in the Brazilian Amazon and Cerrado after 167 years

Jovani B. S. Pereira[1], Ana Maria Giulietti[1,2], Vali J. Pott[3], Maurício T. C. Watanabe[1]

1 *Instituto Tecnológico Vale, Rua Boaventura da Silva, 955, 66055-090, Belém, PA, Brazil* **2** *Depto, Botânica da Universidade Estadual de Feira de Santana, Feira de Santana-BA, Brazil* **3** *Universidade Federal de Mato Grosso do Sul, Campo Grande – MS, Brazil*

Corresponding author: *Jovani B. S. Pereira* (jovanibio@gmail.com)

Academic editor: *A. Troia* | Received 16 September 2019 | Accepted 12 November 2019 | Published 5 December 2019

Citation: Pereira JBS, Giulietti AM, Pott VJ, Watanabe MTC (2019) Rediscovering two *Isoetes* species in the Brazilian Amazon and Cerrado after 167 years. PhytoKeys 135: 105–117. https://doi.org/10.3897/phytokeys.135.46624

Abstract

Isoetes amazonica and *I. gardneriana* were the first two species of the genus to be collected from Brazil. *Isoetes amazonica* was gathered by Richard Spruce in the Amazon basin near Santarém in the state of Pará in 1850. *Isoetes gardneriana* was collected by George Gardner in the current Dianópolis in Tocantins State in 1843. Despite being known for a long time by botanists, these species have not been recollected since then, which raised questions about their taxonomic recognition, current distribution ranges and conservation status. Fieldwork efforts led to the rediscovery of *I. amazonica* and *I. gardneriana* after 167 years. These collections enrich our understanding of their habitats and morphologies. We provide here re-descriptions for these species. Based on IUCN criteria, *Isoetes amazonica* and *I. gardneriana* should be assigned as data deficient (DD) and endangered (EN), respectively. The rediscovery of these species raises hopes that other areas in Amazon and Cerrado biomes harbour *I. amazonica* and *I. gardneriana*, respectively. This study will serve as a basis towards the conservation of these species.

Keywords

Aquatic plants, conservation status, endemic species, fieldworks, Isoetaceae, taxonomy

Introduction

Brazil presents the greatest diversity of plants in the world (Forzza et al. 2012), which partially reflects its large quantity of habitats. Particularly, habitats of its two largest biomes − Amazon and Cerrado − are undergoing a rapid reduction due to deforesta-

tion and large scale agriculture, including soybean and cattle farming and construction of hydroelectric dams (Laurance et al. 2000; Carvalho et al. 2009). At the same time, these areas remain largely unexplored botanically (Sousa-Baena et al. 2014), which raises conservation concerns about the numerous "lost plant species" that have been known only from type specimens.

The lycophyte genus *Isoetes* L. is globally distributed with an estimated 250 species (Troia et al. 2016), 22 of them being endemic to Brazil (Prado et al. 2015). The genus is frequently overlooked by botanists due to its resemblance to grasses or sedges (Taylor and Hickey 1992) and due to its aquatic habitat occurring semi- to fully submerged up to 6–7 m deep in water (Middelboe and Markager 1997). As a result, many species are known only from type specimens (e.g. Pereira et al. 2016, 2017; Hickey et al. 2009).

Isoetes amazonica A. Braun and *I. gardneriana* Kunze ex A. Braun were the first two *Isoetes* species to be collected and described from Brazil. *Isoetes amazonica* was first collected by Richard Spruce in September 1850 from inundated shores of the Tapajós river near Santarém municipality in the state of Pará (Kuhn 1884). *Isoetes gardneriana* was first found by George Gardner (1849: 236) in 1843 in a marsh by the side of the river Preto, Mission of Duro, in the state of Goiás (currently on the border between Tocantins and Bahia in the municipalities of Dianópolis and Formosa do Rio Preto, respectively). *Isoetes amazonica* was published in 1880, *I. gardneriana* in 1862 (see Troia et al. 2016) and further information about them was compiled in the "*Flora Brasiliensis*" of Martius by Kuhn (1884). Despite having been collected and known for a long time, *I. amazonica* and *I. gardneriana* have not been recollected for 167 years. Our lack of knowledge about these species raises questions about their taxonomic recognition, current distribution ranges and their conservation status.

Motivated by these issues, we embarked on an attempt to rediscover these species in both the type localities and other similar environments in Amazon basin and Brazilian Cerrado.

Material and methods

For *Isoetes amazonica*, fieldwork was carried out along both banks of the Tapajós river, near the district Alter do Chão, municipality of Santarém, in the state of Pará, Brazil, in September 2016 and July 2017. For *Isoetes gardneriana*, fieldwork efforts were carried out along the margins of the Preto river in Formosa do Rio Preto (Bahia) in January 2018. Additional efforts to find this species took place in other Brazilian Cerrado areas: Ondas river, Barreiras (Bahia) − ca. 200 km away from the type location − in January 2018; Parque Nacional Serra da Mesa (Maranhão) − 500 km away from the type location − in November 2017; Parque Nacional da Serra do Cipó − 900 km away from the type location − in June 2018; Fazenda Modelo, Campo Experimental da Embrapa, Terenos (Mato Grosso do Sul) − 1200 km away from the type location − in November 2017.

Besides field trips, specimens from the following herbaria were consulted to check for previous records of these species (acronyms following Thiers 2018): CGMS, MG,

RB and UPCB (Brazil); B, E, M, HBG, P and K (Europe). These materials were compared to type specimens of *I. amazonica* (Spruce 1081, K [K000574506]) and *I. gardneriana* (Gardner 3563, B and E [E00429095]).

We checked the total monthly precipitation and average monthly maximum and minimum temperatures of the environments of these species' localities to understand the influence of both flooding and drought in their habitats and life forms. For *I. amazonica*, the climatic data were collected from the meteorological station located in Belterra in the state of Pará and made available by "Instituto Nacional de Meteorologia" (INMET 2019). The climatic data for *I. gardneriana* were obtained from Campo Grande in the state of Mato Grosso do Sul (MS) and made available by the "Centro de Monitoramento do Tempo e Clima, MS" (CEMTEC/MS 2019).

Habitat, life form, colour, size and ornamentation of the mega- and microspores, the proportion of the sporangium wall covered by the velum and the sporangial wall colouration were used in the identification of the species. The megaspores and microspores were analysed using scanning electron microscopy (SEM). Images of the spores were made by transferring the spores to aluminium stubs coated with a carbon adhesive. The stubs were then coated with gold-palladium-alloy in a sputter-coater for 180 s and then digitally imaged using a Zeiss SIGMA VP.

Since megaspore ornamentations are essential for the correct species identification, the absence of detailed images of spores during the determination process may have potentially led to the name *I. gardneriana* being misused for several collections of *I. panamensis* Maxon & C.V.Morton *sensu lato*. We consulted these materials to check whether the identification was correct or not in these cases. Amongst these materials were collections from: Paraguay in 1878 (Balansa 3294, P [P00170381, P00573953, P04459456]); municipality of Barreiras in Bahia, Brazil, in 1971 (Irwin 31615, P [P01591973]); an area next to type location of *I. gardneriana* in the municipality of Formosa do Rio Preto in Bahia, Brazil, in 2015 (Labiak 5783, UPCB with duplicates in NY [NY2697584]). In this step, megaspores of these materials were removed, images were taken using SEM and then compared with the type of *I. gardneriana*. We used both qualitative and quantitative characters to identify the species. The terminology used for the description of the spores follows that of Punt et al. (2007), with some modification using Pereira et al. (2016). Boxplots of the megaspore macro-ornamentation projects were generated using an R script (v. 3.0.2; R Core Team 2013).

Results

Rediscovering *I. amazonica* after 167 years and re-description of the species

***Isoetes amazonica* A. Braun, J. Bot. 18: 109. 1880.**

Description. Stems globose, 0.35−0.7 cm wide, 3-lobate. Leaves 0.45−1 mm wide at mid length, 4−17 cm long, 9−23 per individual, filiform, straight, ascending, apex

acute; alae 0.8–4.5 cm long, extending from the base 1/10 – 1/4 of total leaf length, hyaline, membranaceous, attenuate. Subula present, olive green, trigonal. Labium present, 0.5–0.7 × 0.9–1.1 mm long, cordate. Ligule 2.5–3 × 1–1.2 mm, hyaline, triangular. Velum 0.1–0.2 mm along the lateral edges of the sporangium, rudimentary. Sclerified phyllopodia absent. Sporangium at the base of the leaf, 2–2.5 × 1.8–2.5mm, elliptic, hyaline, light brown, brown dots present or absent. Megaspores white, 420–512 (–590) μm in equatorial diameter (average = 490 μm), trilete; laesurae as wide as high or higher than wide, 40–53 × 35–47 μm; proximal surface verrucate, projections 24–41.4 × 24–46 μm; equatorial ridges arched, slightly sinuous; distal surface verrucate, macroscuptural projections 25–45 × 25–46 μm. Microspores 28–32 μm long (average = 30 μm), proximal surface echinate, distal surface sparsely echinate.

Type. Brazil. Province of Pará: inundated places near Santarém, Sept 1850, Spruce 1081, (holotype: B! [B200107121]; isotype: K! [K000574506], P! [P00573942; P00573943]).

Remarks. *Isoetes amazonica* was rediscovered at its type location in July 2017 after 167 years (Pereira 1015, MG). This species was found in a single area at approximately 2.5 km from the left bank of the Tapajós river at the geographical coordinates 2°24'15.15"S, 55°3'1.89"W (Figure 1A). This location was a marsh area between a flooded forest and cattle farming. The plants were found as terrestrials in wet clay and sandy soils (Fig. 1B, C). None of the individuals was completely submerged (but see discussion). The monthly precipitation was 42.4 mm and it was the lowest recorded value for July between the years of 2008 and 2017 (INMET 2019). The average monthly maximum and minimum temperatures were 32 and 22 °C, respectively (INMET 2019). This species occurred in association with other plant groups such as Cyperaceae, Poaceae, Mayacaceae and Eriocaulaceae. The newly rediscovered population showed typical characteristics of *I. amazonica*, such as 10–20 leaves per individual, ascending leaves, rudimentary velum, hyaline sporangia with or without brown spots, verrucate megaspores with 470–590 μm diameter, sparsely echinate microspores 28–32 μm long (Fig. 2).

New record of *I. gardneriana* at about 1200 km away from its type location and re-description of the species

Isoetes gardneriana **Kunze ex A. Braun, Verh. Bot. Vereins Prov. Brandenburg 4: 330. 1862.**

Description. Stems globose, 2.5–4 cm wide, 3 or 4-lobate. Leaves 1.0–1.8 mm wide at mid length, 32–45 cm long, 30–90 per individual, linear, straight, ascending, apex acute; alae 7–15 cm long, extending from the base 1/5–2/5 of total leaf length, hyaline or light brown, chartaceous, attenuate. Subula present, olive green, trigonal. Labium present, 2.5–3.5 × 4–6 mm, cordate. Ligule not observed. Velum > 0.4 mm along the lateral edges of the sporangium, rudimentary. Sclerified phyllopodia absent. Sporangium at the base of the leaf, 8–18 × 4.3–7 mm, oblong, hyaline, brown dots absent. Megaspores grey, 490–650 μm in equatorial diameter

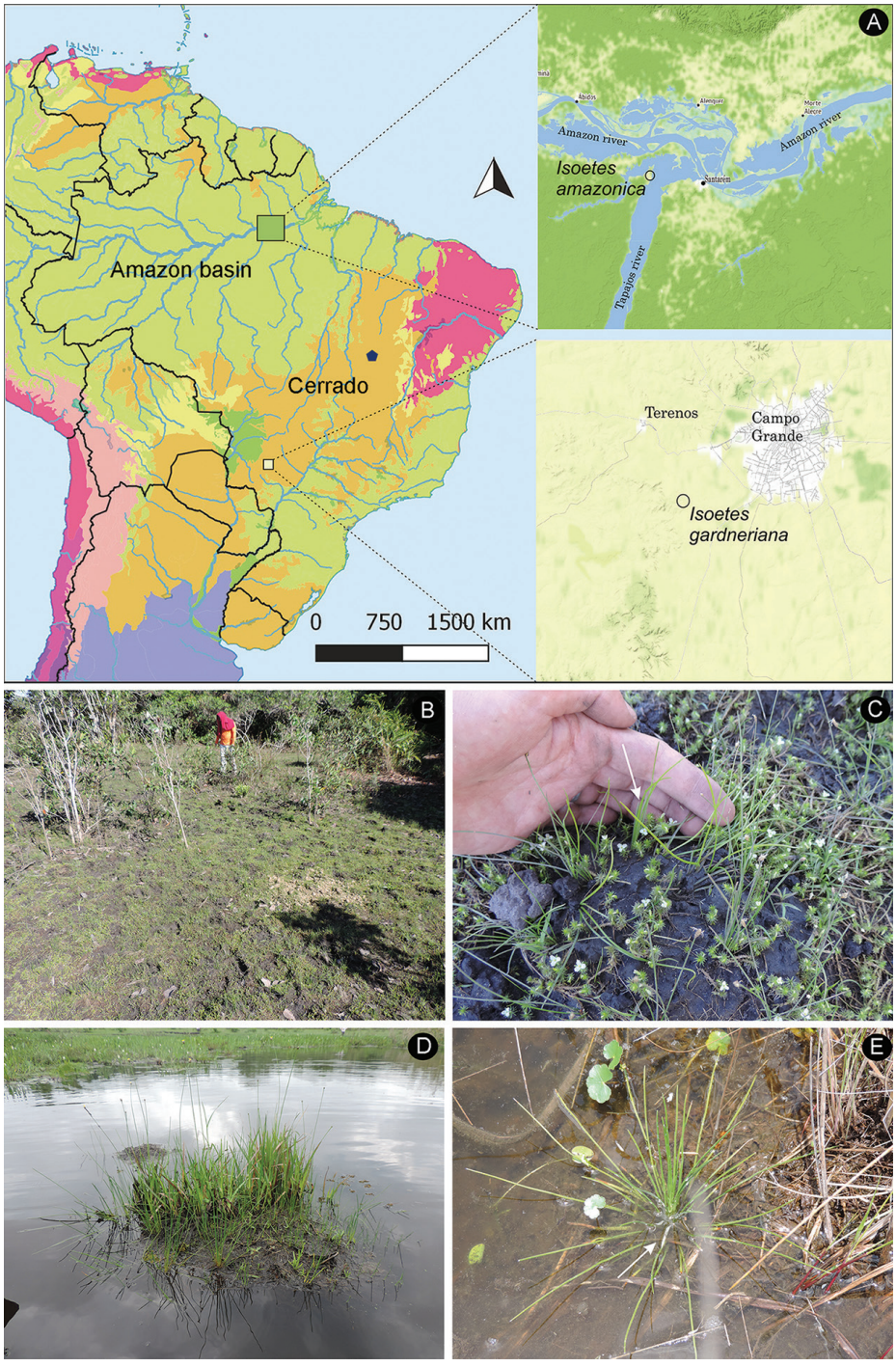

Figure 1. Geographic distribution, habit and habitat of *Isoetes amazonica* and *I. gardneriana* **A** location where *Isoetes amazonica* and *I. gardneriana* were rediscovered in Brazil (type location of *I. gardneriana* in blue pentagon) **B–C** *Isoetes amazonica* (*Pereira 1015*, MG): **B** habitat **C** habit **D–E** *Isoetes gardneriana* (*Pereira 1028*, MG): **D** habitat **E** habit.

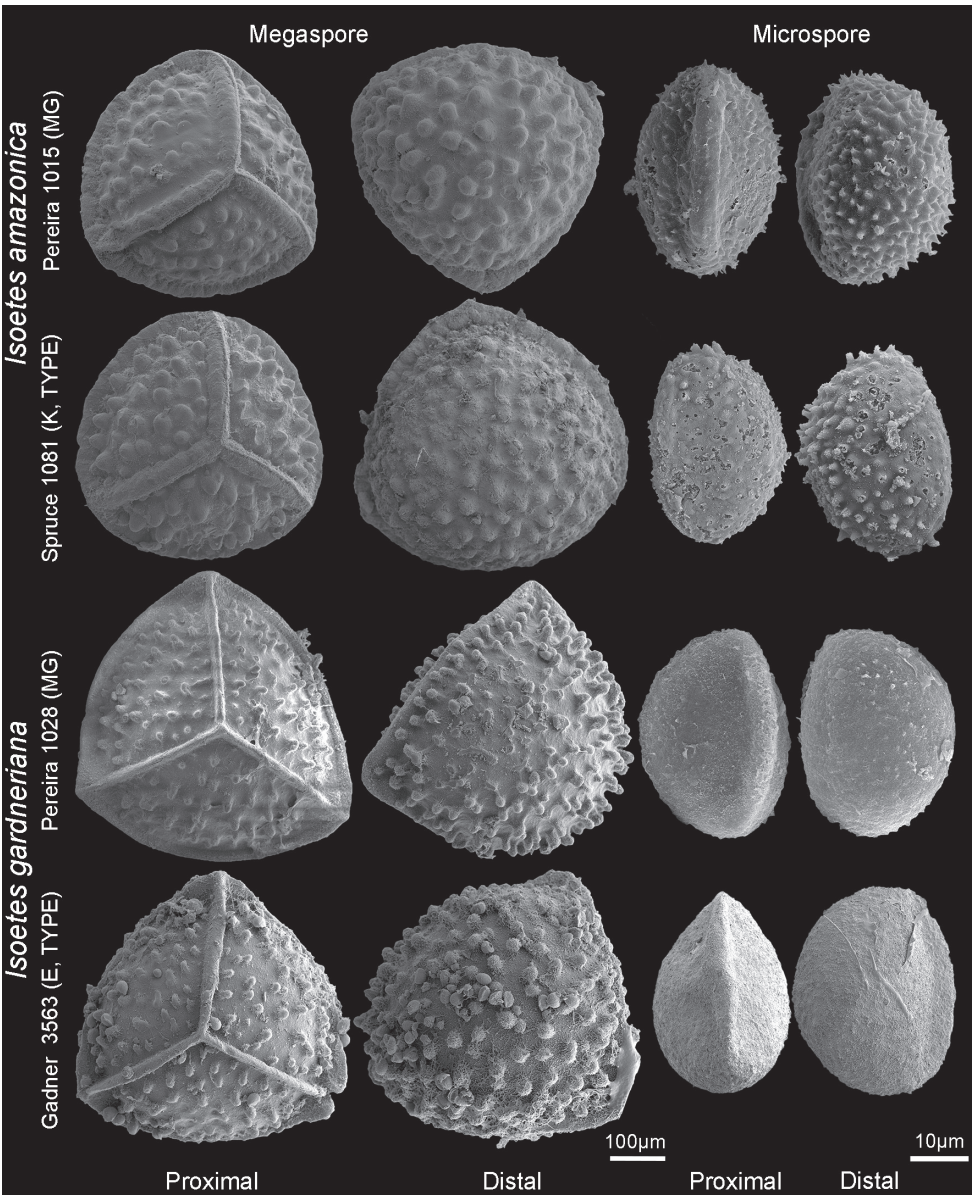

Figure 2. Mega- and microspores of *Isoetes amazonica* and *I. gardneriana*.

(average = 540 µm), trilete; laesures higher than wide, 40–50 × 11–16 µm; proximal surface tuberculate, macrosculptural projections 20–39 × 13–24 µm; equatorial ridges arched, straight; distal surface tuberculate, projections 24–44 × 17–34 µm. Microspores 33–40 µm long (average = 37 µm), proximal and distal surface smooth or sparsely microechinate.

Type. Brazil. Province of Goyaz: Missiones Duro, Sept 1841, Gardner 3563, (holotype: B! [B200107577]; isotype: BM [BM000097912,]E! [E00429095], K! [K000574505]).

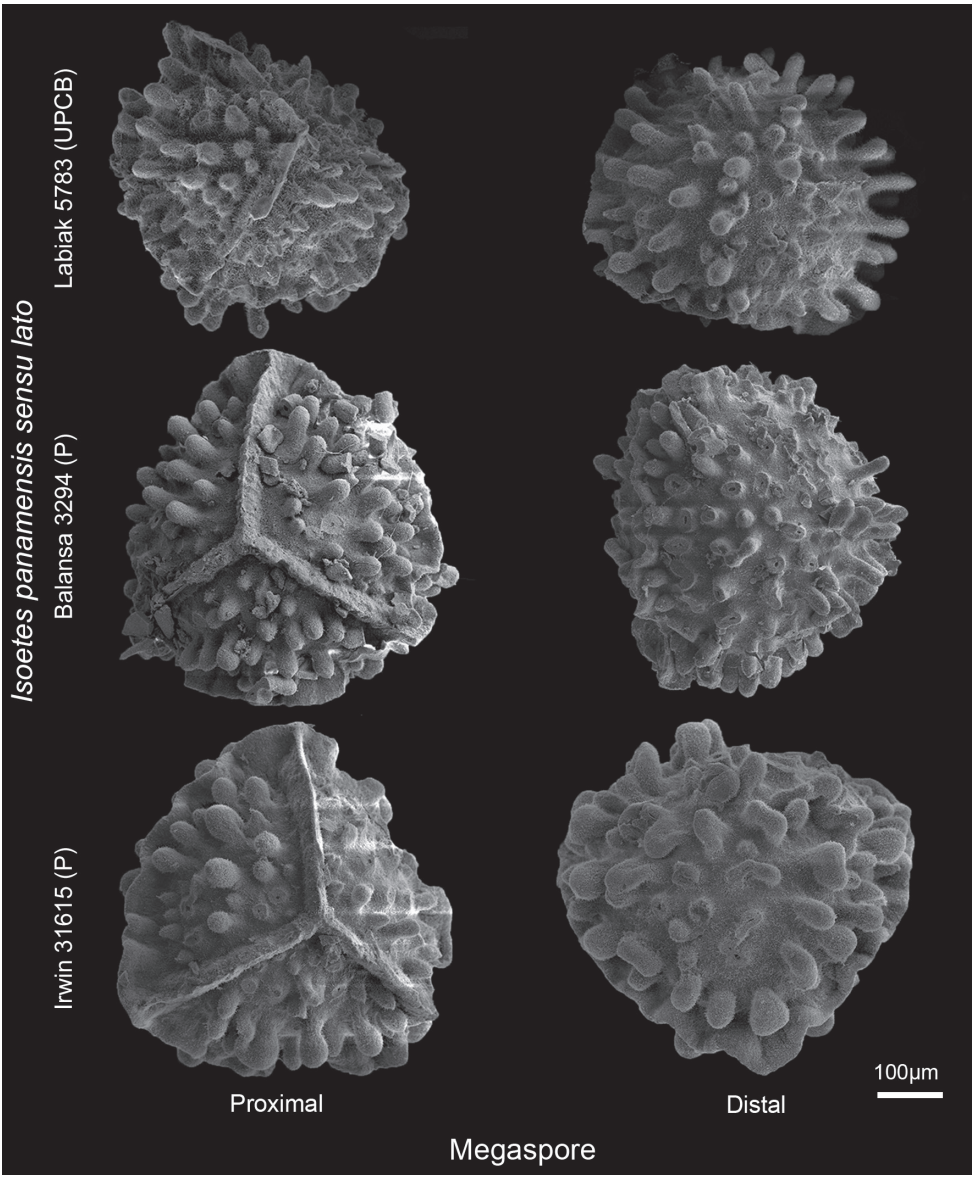

Figure 3. Megaspores of the variants of *Isoetes panamensis sensu lato.*

Remarks. Despite our intensive fieldwork efforts in the Brazilian Cerrado, *I. gardneriana* was only rediscovered in Terenos in the state of Mato Grosso do Sul at the geographical coordinates 20°33'32"S, 54°47'23"W. This area is located at about 1200 km away from its type location (Fig. 1A). It was collected there by both Vali Pott in September 2010 (Pott 11018, CGMS) and Jovani Pereira in November 2017 (Pereira 1028, MG) after 167 and 175 years, respectively. Although these records were far from the type location, habitat and morphology of this newly collected population are almost identical to the type.

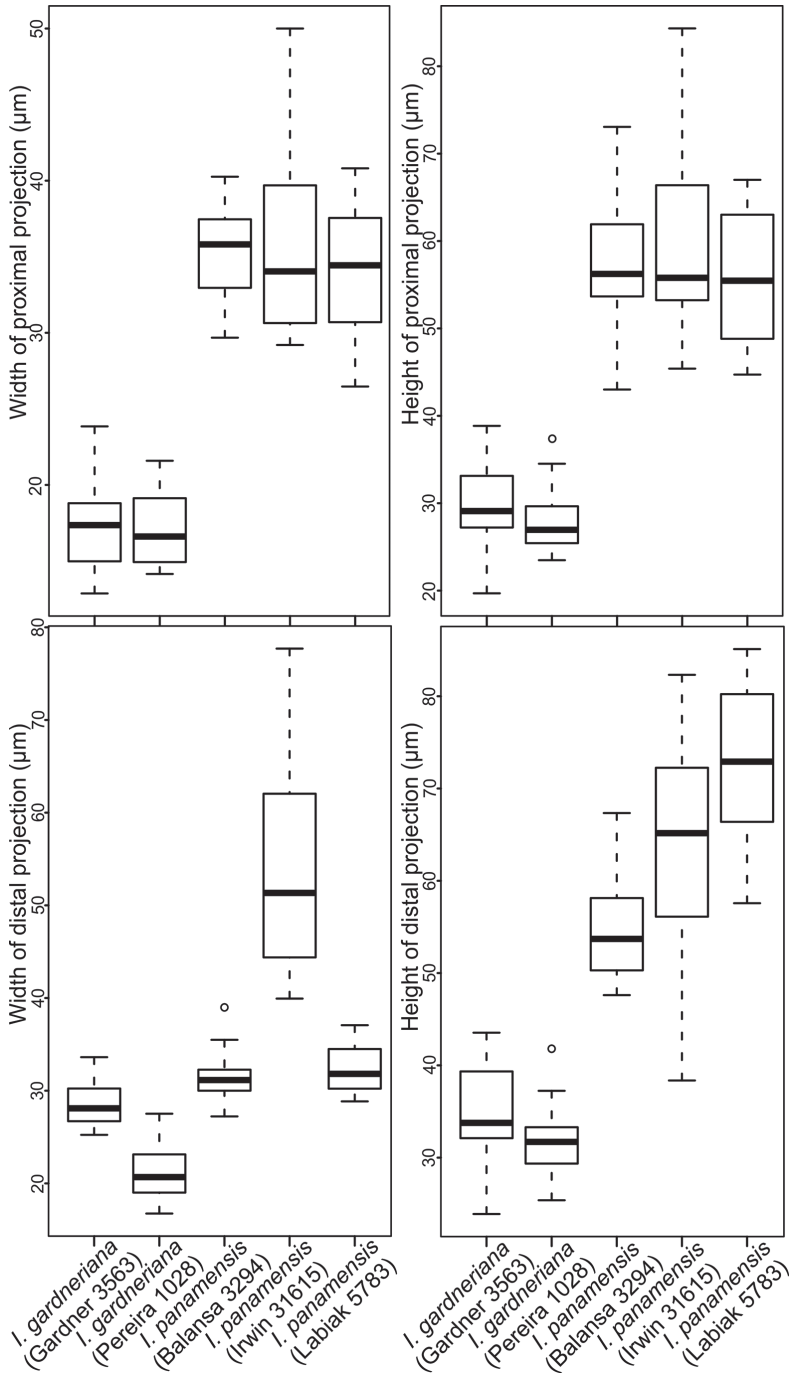

Figure 4. Boxplots showing quantitatively the variation in the size of the macro-ornamentation projections of the megaspores of *I. gardneriana* and *I. panamensis sensu lato*. In the proximal surface, the projects of the macro-ornamentation are narrower and shorter in *I. gardneriana* than in *I. panamensis s.l.* In the distal surface, the macro-ornamentation projections are slightly narrower and considerably shorter in *I. gardneriana* than in *I. panamensis s.l.*.

Isoetes gardneriana was found in a pond along with *Rhynchospora corymbosa* (L.) Britton, *Pontederia cordata* L. and *Xyris* spp. (Fig. 1D). This pond occurred by the side of a "vereda", which showed a clay and hydromorphic soil and an open vegetation physiognomy with the presence of numerous "buriti" palms (*Mauritia flexuosa* L.f.) that grow over a dense herbaceous stratum. The life form of *I. gardneriana* was of a partially submerged aquatic in September, although in November, plants were found both partially and totally submerged (Fig. 1E). The total monthly precipitations were 127 and 315.8 mm in September 2010 and November 2017, respectively (CEMTEC/MS 2019). The average monthly maximum and minimum temperatures were 32 and 22 °C, respectively (CEMTEC/MS 2019). The maximum and minimum temperature averages were 32.7 and 19.6 °C, respectively, in September 2010. In November 2017, the average monthly maximum and minimum temperatures were 30.7 and 20 °C, respectively (CEMTEC/MS 2019).

Morphologically, the individuals have ascending leaves, rudimentary vela, elliptic sporangia and 3-lobate corms (or more rarely 4). The megaspores are brown, sparsely verrucate, 490−650 μm diameter (vs. 548−615 μm), with knife-like laesurae (Fig. 2). The microspores are echinate, 33−40 μm long (vs. 34−38 μm) (Fig. 2).

On the other hand, none of the analysed herbarium collections appeared (Balansa 3294, Irwin 31615 and Labiak 5783) to be *I. gardneriana*. The megaspores of these collections are both qualitatively and quantitatively distinct from the type of *I. gardneriana*. The Balansa and Labiak collections have baculate-tuberculate megaspores and Irwin's collection revealed baculate-clavate megaspores (Fig. 3), which confirm that these materials represent variants of *I. panamensis s.l.* Additionally, the macro-ornamentation projections of the megaspores of *I. gardneriana* are, in general, narrower and shorter than those found in *I. panamensis s.l.* (Fig. 4).

Discussion

Although fieldwork investigation is fundamental to improve our understanding about how human impacts on biological systems can be recognised, mitigated or averted, fieldwork has considerably decreased in the past decades with negative implications for global biodiversity conservation (Ríos-Saldaña et al. 2018). The rediscovering of these species was only possible due to intense fieldwork; otherwise, they would have remained little known to science.

Both proper habitat and taxonomic identification of species are the first steps towards conserving biodiversity. Amongst the aquatic macrophytes, *Isoetes* is one of the most threatened groups (Murphy et al. 2019). However, difficulties related to finding species in the field, identifying them morphologically and, consequently, establishing their geographical distribution, hamper efforts to assess their current conservation status. *Isoetes amazonica* and *I. gardneriana* were known only from their type materials collected 167 years ago, which raised questions about their current occurrences and morphological distinction. Our rediscoveries provide a basis for a better understanding of the distribution and taxonomy of these species, which will help develop a plan to conserve these plants.

Even though *I. amazonica* was collected only during the dry season, we can make inferences about its habitat conditions and life forms during the year, using climatic data (see INMET 2019). *Isoetes amazonica* was collected as a terrestrial at the beginning of the dry season in July. However, its life form may oscillate between terrestrial and completely aquatic due to the alternating flooding and drought conditions in the Amazon basin during the year (see Marengo and Espinoza 2016). Additionally, during the driest and hottest period in August-November, its habitat may entirely dry out and this species may lose its leaves due to the combination of low precipitation, decreasing of the water table above the surface and high temperature. On the other hand, during the peak of the rainy season in March, its habitat is flooded and *I. amazonica* may become a completely submerged aquatic. Similarly, *I. gardneriana* occurs in an area which undergoes dry and rainy seasons (see CEMTEC/MS 2019 for climatic data). However, *I. gardneriana* grows in the deepest part of a small pond just by the side of the "vereda" grassland, which stays waterlogged year-round and feeds this pond in the dry season (see Moreira et al. 2011). This factor leads its habitat to be marshy and flooded throughout the year and *I. gardneriana* may rarely be found as terrestrial.

Despite the importance of habitat data for species characterisation, they provide a limited amount of information for species distinction if two or more similar species occupy the same habitat and/or show morphological convergence due to habitat adaptation (e.g. Taylor and Hickey 1992; Jiménez-Mejías et al. 2017). Both *Isoetes gardneriana* and *I. panamensis s.l.* are found in areas of "veredas" in Cerrado, which partially contribute to taxonomic difficulties involving these two species. However, they can be distinguished by qualitative and quantitative characters of megaspores.

Additionally, an *Isoetes* population from Itaparica lake in Xique-Xique (Bahia State) in north-eastern Brazil was tentatively identified as *I. amazonica* (Harley 19109, K). However, despite its resemblance to *I. amazonica* by size of megaspores and number and size of leaves, the presence of brown sporangium (vs. hyaline) and its occurrence in Caatinga (vs. Amazon) leads us to believe that this population is either a variant of *I. luetzelburgii* U. Weber or an undescribed species.

The geographical distribution of the species is crucial in assessing their conservation status (IUCN 2016). In *Isoetes*, the proportion of species with narrow-range distributions is remarkably high (Prado et al. 2015). The same extreme restricted distribution patterns are also found in several other aquatic macrophytes, such as Podostemaceae, Araceae, especially *Cryptocoryne* spp., Cyperaceae and Eriocaulaceae (Murphy et al. 2019). However, in several cases, it appears unclear whether this pattern occurs due to endemism (driven by biological factors) or collection deficiency. Although more fieldwork efforts are needed to address this question in *I. amazonica*, this study revealed that *I. gardneriana* shows a much wider distribution than previously known.

Isoetes amazonica is currently known from a single locality next to a cattle farm and, thus, it is prone to the effects of human activities within a short time. However, given its potential occurrence in other areas in the Amazon basin and the lack of current knowledge about its distribution range, *I. amazonica* should be assessed as data deficient (DD), according to IUCN criteria (IUCN 2016). On the other hand, *I. gardneriana* – which is endemic to Cerrado – is clearly undergoing a population size re-

duction due to the loss of suitable habitats. The agri-business expansion, infrastructure development, weak legal protection and limited conservation incentives have led to the loss of 46% of Cerrado native vegetation and, by 2050, Cerrado may lose up to 34% of its remaining area (Strassburg et al. 2017). This habitat reduction will have a direct impact on *I. gardneriana* and the population size of this species may likely substantially decrease in the next years. Thus, *I. gardneriana* should be assigned as endangered (EN), according to IUCN criteria (IUCN 2016).

In conclusion, the rediscovering of these species raises hopes that other areas in Amazon and Cerrado biomes still harbour *Isoetes amazonica* and *I. gardneriana*, respectively. We hope that these rediscoveries spark research towards a deeper understanding of the life history of *Isoetes* and provide information for any future efforts to protect *Isoetes amazonica* and *I. gardneriana* from extinction.

Acknowledgements

We thank Daniela Zappi, Diego Pinangé, Paulo Labiak, Raymond Harley and Thaís Almeida for assistance in fieldworks. We are also grateful to Jim Hickey, Alexandre Salino and Angelo Troia for their valuable comments on the manuscript. ICMbio provided licence permits to JBSP (35897). CNPq provided a Senior Grant to AMG. This study was supported through a fellowship from Capes/ITV (88887.130616/2016) to JBSP.

References

Carvalho FM, Marco P, Ferreira LG (2009) The Cerrado into-pieces: Habitat fragmentation as a function of landscape use in the savannas of central Brazil. Biological Conservation 142(7): 1392–1403. https://doi.org/10.1016/j.biocon.2009.01.031

CEMTEC/MS (2019) Centro de Monitoramento do Tempo e Clima no estado do Mato Grosso do Sul, Brasil. http://www.cemtec.ms.gov.br/boletins-meteorologicos [accessed 10.06.2019]

Forzza RC, Baumgratz JFA, Bicudo CEM, Canhos DAL, Carvalho Jr AA, Coelho MAN, Costa AF, Costa DP, Hopkins MG, Leitman PM, Lohmann LG, Lughadha EN, Maia LC, Martinelli G, Menezes M, Morim MP, Peixoto AL, Pirani JR, Prado J, Queiroz LP, Souza S, Souza VC, Stehmann JR, Sylvestre LS, Walter BMT, Zappi DC (2012) New Brazilian floristic list highlights conservation challenges. Bioscience 62(1): 39–45. https://doi.org/10.1525/bio.2012.62.1.8

Gardner G (1849) Travels in the Interior of Brazil, Principally Through the Northern Provinces, and the Gold and Diamond Districts, During the Years 1836–1841. Reeve Benham & Reeve, London, 428 pp.

Hickey RJ, Macluf CC, Link-Pérez M (2009) *Isoetes maxima*, a new species from Brazil. American Fern Journal 99(3): 194–199. https://doi.org/10.1640/0002-8444-99.3.194

INMET (2019) Instituto Nacional de Meteorologia, Brasil. http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep [accessed 4.08.2019]

IUCN (2016) The IUCN red list categories and criteria, version 3.1. IUCN Red List Unit, Gland, Switzerland and Cambridge, UK. http://www.iucnredlist.org/technical-documents/categories-and-criteria [accessed 4.02.2019]

Jiménez-Mejías P, Benítez-Benítez C, Fernández-Mazuecos M, Martín-Bravo S (2017) Cut from the same cloth: The convergent evolution of dwarf morphotypes of the Carex flava group (Cyperaceae) in Circum-Mediterranean mountains. PLoS One 12(12): e0189769. https://doi.org/10.1371/journal.pone.0189769

Kuhn FAM (1884) Isoetaceae. In: Martius CFP (Eds) Flora Brasiliensis v. 1, part 2., 645–648. http://florabrasiliensis.cria.org.br/search?taxon_id=1403 [accessed 4.02.2019]

Laurance WF, Vasconcelos HL, Lovejoy TE (2000) Forest loss and fragmentation in the Amazon: Implications for wildlife conservation. Oryx 34(1): 39–45. https://doi.org/10.1046/j.1365-3008.2000.00094.x

Marengo JA, Espinoza JC (2016) Extreme seasonal droughts and floods in Amazonia: Causes, trends and impacts. International Journal of Climatology 36(3): 1033–1050. https://doi.org/10.1002/joc.4420

Middelboe AL, Markager S (1997) Depth limits and minimum light requirements of freshwater macrophytes. Freshwater Biology 37(3): 553–568. https://doi.org/10.1046/j.1365-2427.1997.00183.x

Moreira SM, Pott A, Pott VJ, Damasceno GA (2011) Structure of pond vegetation in a vereda in the Brazilian Cerrado. Rodriguésia 62(4): 721–729. https://doi.org/10.1590/S2175-78602011000400002

Murphy K, Efremov A, Davidson TA, Molina-Navarro E, Fidanza K, Crivelari TCB, Chambers P, Tapia JG, Varandas SM, Springuel I, Kennedy M, Mormul RP, Dibble E, Hofstra D, Lukács BA, Gebler D, Baastrup-Spohr L, Urrutia-Estrada J (2019) World distribution, diversity and endemism of aquatic macrophytes. Aquatic Botany 158: 103–127. https://doi.org/10.1016/j.aquabot.2019.06.006

Pereira JBS, Salino A, Arruda A, Stützel T (2016) Two new species of *Isoetes* (Isoetaceae) from northern Brazil. Phytotaxa 272(2): 141–148. https://doi.org/10.11646/phytotaxa.272.2.5

Pereira JBS, Stützel T, Schulz C (2017) *Isoetes nana*, a new species from the coastal mountains of southeastern Brazil. PhytoKeys 89: 91–105. https://doi.org/10.3897/phytokeys.89.20171

Prado J, Sylvestre LS, Labiak PH, Windish PG, Salino A, Barros ICL, Hirai RY, Almeida TE, Santiago ACP, Kieling-Rubio MA, Pereira AFN, Ollgaard B, Ramos CGV, Mickel JT, Dittrich VAO, Mynssen CM, Schwartsburd PB, Condack JPS, Pereira JBS, Matos FB (2015) Diversity of ferns and lycophytes in Brazil. Rodriguésia 66(4): 1073–1083. https://doi.org/10.1590/2175-7860201566410

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143(1–2): 1–81. https://doi.org/10.1016/j.revpalbo.2006.06.008

R Core Team (2013) A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Wien. http://www.R-project.org/ [accessed 10.08.2018]

Ríos-Saldaña CA, Delibes-Mateos M, Ferreira CC (2018) Are fieldwork studies being relegated to second place in conservation science? Global Ecology and Conservation 14: e00389. https://doi.org/10.1016/j.gecco.2018.e00389

Sousa-Baena MS, Garcia LC, Peterson AT, Brotons L (2014) Completeness of digital accessible knowledge of the plants of Brazil and priorities for survey and inventory. Diversity & Distributions 20(4): 369–381. https://doi.org/10.1111/ddi.12136

Strassburg BBN, Brooks T, Feltran-Barbieri R, Iribarrem A, Crouzeilles R, Loyola R, Latawiec AE, Oliveira Filho FJB, Scaramuzza CAM, Scarano FR, Soares-Filho B, Balmford A (2017) Moment of truth for the Cerrado hotspot. Nature Ecology & Evolution 1: 0099. https://doi.org/10.1038/s41559-017-0099

Taylor WC, Hickey RJ (1992) Habitat, evolution, and speciation in *Isoetes*. Annals of the Missouri Botanical Garden 79(3): 613–622. https://doi.org/10.2307/2399755

Thiers BM (2018) The World's Herbaria 2017: A summary Report Based on Data from Index Herbariorum (2nd ed.). The New York Botanical Garden Press, 19 pp. https://doi.org/10.3897/biss.2.26440

Troia A, Pereira JBS, Kim C, Taylor WC (2016) The genus *Isoetes* (Isoetaceae): A provisional checklist of the accepted and unresolved taxa. Phytotaxa 277(2): 101–145. https://doi.org/10.11646/phytotaxa.277.2.1

PhytoKeys135: 119–131 (2019)
doi: 10.3897/phytokeys.135.46946
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
Launched to accelerate biodiversity research

Zahora, a new monotypic genus from tribe Brassiceae (Brassicaceae) endemic to the Moroccan Sahara

Marcus A. Koch[1], Claude Lemmel[2]

1 *Centre for Organismal Studies, Dept. Biodiversity and Plant Systematics, Heidelberg University, Heidelberg, Germany* **2** *Atlas Sahara, Boudenib, Morocco*

Corresponding author: *Marcus A. Koch* (marcus.koch@cos.uni-heidelberg.de)

Academic editor: *P. Lange* | Received 30 September 2019 | Accepted 15 November 2019 | Published 5 December 2019

Citation: Koch MA, Lemmel C (2019) *Zahora*, a new monotypic genus from tribe Brassiceae (Brassicaceae) endemic to the Moroccan Sahara. PhytoKeys 135: 119–131. https://doi.org/10.3897/phytokeys.135.46946

Abstract

Zahora ait-atta Lemmel & M.Koch, a new species from the Moroccan Sahara, is described and documented here and constitutes a monotypic new genus. The new taxon belongs to the tribe Brassiceae (Brassicaceae), and cytogenetic and phylogenetic analyses reveal that this diploid species has a remote status of Miocene origin in the northwestern Sahara Desert. We examined the morphological differences between morphologically related genera and provide photographs of the new species. The new genus may play a key role in future *Brassica-Raphanus* crop research since it is placed phylogenetically at the base of a generically highly diverse clade including *Raphanus sativus*, and it shows affinities to various *Brassica* species.

Keywords

Brassiceae, Brassicaceae, flora of the Sahara, Morocco, new genus, *Zahora ait-atta*

Introduction

The tribe Brassiceae is among the most complex monophyletic lineages within Brassicaceae. The tribe underwent an early genome triplication (Lysak et al. 2005) affecting subsequent diversification (Arias et al. 2014) and gave rise to approximately 50 genera comprising 250 species (Huang et al. 2019; Koch et al. 2017). The entire tribe started to evolve about 23 million years ago, and centers of origin and diversity are the entire

circum-Mediterranean region (Arias et al. 2014). Phylogenic analyses have identified eight clades within the tribe Brassiceae: *Vella* L., *Zilla* Forssk., *Henophyton* Coss. & Durieu, *Crambe* L., *Cakile* Mill., *Savignya* DC., "*Nigra*", and "*Oleracea*" (Arias and Pires 2012), but several genera remain poly- and paraphyletic such as *Brassica* L. and *Diplotaxis* DC. (Arias and Pires 2012), *Raphanus* L. (Ziffer-Berger et al. 2014), or *Sinapis* L. (Arias and Pires 2012). The tribe Brassiceae is not only characterized by an ancient triploidization, but extensive hybridization and reticulate evolution may have been involved while forming numerous polyploids. The actual amount of polyploids in the entire tribe is of about 28%; and approximately 43% of the species are monocarpic (Hohmann et al. 2015), which may coincide with arid and high-temperature environments preferred by numerous species of the tribe.

The new taxon was (re)discovered in 2015 by Claude Lemmel at isolated stands near the national road between Merzouga and Taous (Morocco) close to the border with Algeria. Since then the species has been continuously monitored by the second author and has been found at various places in that region. It is likely that in February 1951 Ph. Guinet and Ch. Sauvage might have noticed the same plant species near Tafilalet, but the plants were in bloom only and fruits were missing, therefore the botanists listed the species as *Brassica* spec. and putatively unknown (Guinet and Sauvage 1954). Originally, we thought that this plant could be of recent hybrid origin, because in this area there are many wild or cultivated cabbage-related species belonging to the genera *Brassica*, *Eremophyton* Bég., *Eruca* Mill., *Moricandia* DC., *Erucaria* Mill. and *Diplotaxis*, and hybrids are often observed between genera of Brassiceae (reviewed in Warwick et al. 2009). However, the species also occurs at sites with no other Brassicaeae nearby; and Aït-atta nomadic herders, who roam with their sheep and goats in this area, told us that they knew this plant as «Zizaou n'oudad» meaning «Barbary-sheep's cabbage» and that it grows more or less frequently in the region in some oueds (a stream-bed that remains dry except during the rainy season) depending on local and seasonal rainfall. This field evidence encouraged us to analyze in greater detail this taxon unknown to science before.

Morphological characters of the new species do not match any known generic circumscription within Brassicaceae; although the new taxon combines characters, which are typically found in members of tribe Brassiceae. Therefore, we also analyzed chromosome number and genome size to compare results with known karyotypes, and we obtained DNA sequence information for phylogenetic placement analysis and phylogenetic reconstructions.

Material and methods

Morphological observations and measurements of the new species were carried out based on living plant material, either from the wild or cultivated at Heidelberg Botanical Garden (HEID), as well as prepared voucher specimens. Characters were measured using a dissecting microscope. Seeds were collected in the wild from the type locality

(Meknés-Tafilalet/Drâa-Tafilalet: Border region with Algeria. Near Errachidia. Oued Bou-Ibourine), and grown for subsequent analysis of chromosome number (root tips) and genome size (leaf material) following protocols provided in detail with earlier studies (Hohmann et al. 2015).

Molecular analysis following the procedure of (i) DNA extraction, (ii) PCR amplification of nuclear encoded ribosomal DNA (ITS1-ITS2 region), and (iii) direct sequencing of the PCR product as it has been described earlier in detail (Karl and Koch 2013). For DNA extraction we used leaf material from the herein presented holotype.

ITS sequence information was added to a tribal-wide alignment of Brassiceae (Huang et al. 2019) and analyzed using maximum-likelihood inferences (Stamatakis 2014) with the same settings as described earlier (Huang et al. 2019). In total 193 taxa from tribe Brassiceae plus two additional outgroups have been combined with the new ITS sequence for phylogenetic analysis. The sampling, therefore, covers approximately 77% of all known species. The entire alignment is presented with Suppl. material 2. Initial phylogenetic tests have been conducted using the phylogenetic placement tool for Brassicaceae as implemented in *BrassiBase* (https://brassibase.cos.uni-heidelberg.de/; Koch et al. 2012).

In addition, plastid DNA markers *trn*L intron and *trn*L-*trn*F intergenic spacer were amplified and sequenced (Koch et al. 2017), and results have been subsequently used for megaBLAST searches (high similarity) in GenBank to identify taxa with similar plastid (maternal) genotypes.

Temporal inferences about divergence time and age of the new species have been analyzed using BEAST (Drummond et al. 2012). Here we also used the same setting as presented earlier (Huang et al. 2019) analyzing the ITS alignment (Suppl. material 2), and details can be found with Huang et al. (2019).

Taxonomic treatment

Zahora ait-atta **Lemmel & M.Koch, gen. et sp. nov.**
urn:lsid:ipni.org:names:77203326-1

Type. Morocco. Meknés-Tafilalet/Drâa-Tafilalet: Border region with Algeria. Near Errachidia. Oued Bou-Ibourine, « Zizaou n´oudad », gps 31.4114, -3.7220, 900 m a.s.l., 11th March 2019, C. Lemmel s.n. (Holotype, HEID 505689; Isotype, G00394714, Conservatoire et jardin botanique de Genève; Paratype, HEID 505749, 505750, ex. cult. Botanical Garden Heidelberg 2019). Figure 1.

Description. Herbs, woody at base, monocarpic, simple trichomes; rhizome fleshy, 2–3 cm in diam. Stems 80–140(-180) cm tall, robust, up to 1.4 cm in diam, erect, simple at base, often alternately branched in lower part. Basal leaves rosulate, fleshy; leaves lyrate, distal lobecordate, (10-)15–25(-40) cm, margin entire to distantly dentate, numerous simple trichomes on lower surface mostly along veins, upper side loosely covered with simple trichomes; cauline leaves similar but apex obtuse to weakly subacute, 10–15 ×

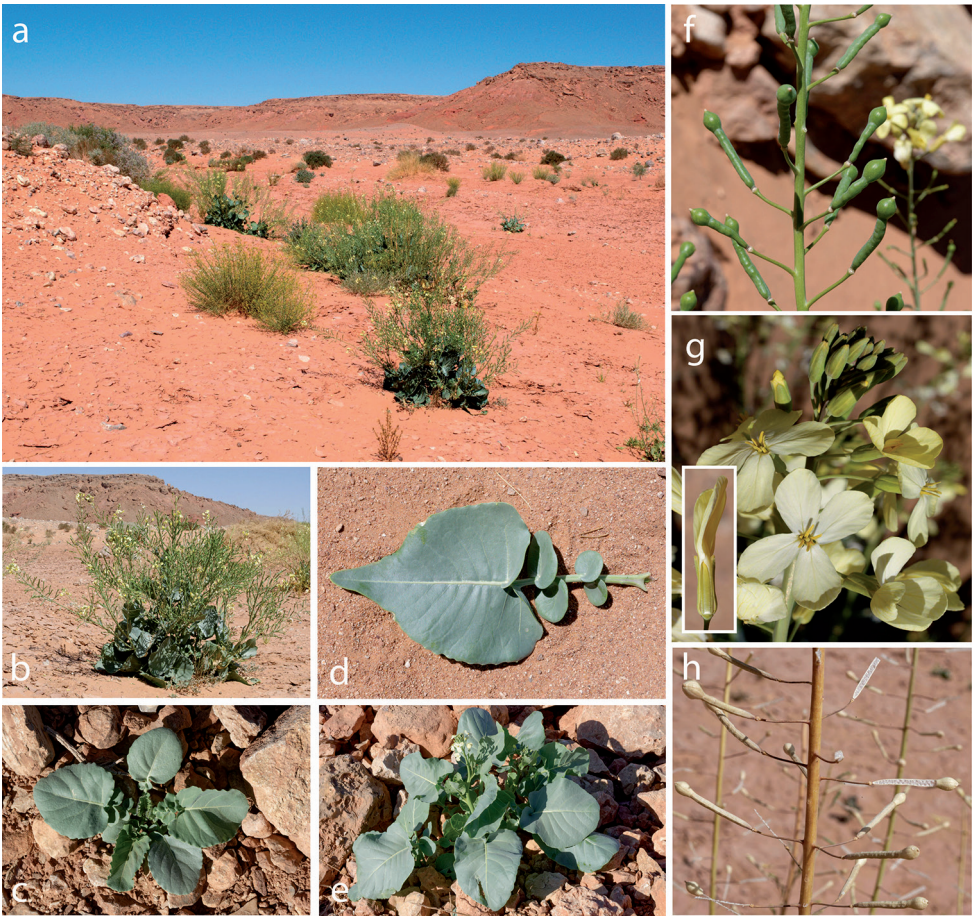

Figure 1. *Zahora ait-atta* in its natural environment. Border region with Algeria. Near Errachidia. Oued Bou-Ibourine – type locality **a** sandy habitat **b** flowering plant **c** rosette during winter **d** lyrate leaf from lower part of the plant **e** rosette starts building the inflorescence **f** ripening heteroarthrocarpic fruits **g** flowers and detailed view on sepals **h** siliques releasing seeds from dehiscent distal part of fruit. Images taken by C. Lemmel and Z. Attioui.

5–7 cm. Raceme ebracteate, elongating in fruit, 40–100 cm; often branched. Sepals erect, saccate ca. 8 mm long, with few simple trichomes; petals pale-yellow,1.5–1.7 cm long, 6–7 mm wide, petal claw 8 mm long, obtuse at apex, glabrous. Filaments tetradynamous, ca. 9 mm long; nectar glands 4, rounded, elateral pair larger. Stigma entire. Infructescence with up to 100(-200) siliques, (30-)40–45(-48) mm, petiolate (9–11 mm). Fruits heteroarthrocarpic with a distal indehiscent balloon-like structure with two viable seeds (3.5–5 × 6–8 mm); proximal part dehiscent, terete (30–45 mm); 20–40 ovules; septum complete. Seeds biseriate, mucilaginous, 1.3–1.4 × 1.4–1.5 mm.

Etymology. Zahora means “flower” in Arabic, indicating the attractive and peculiar appearance of the plant. “Aït-atta” are a Berber tribal confederation of south eastern Morocco who locally know the plant under the name «Zizaou n’oudad» (Barbary-sheep’s cabbage).

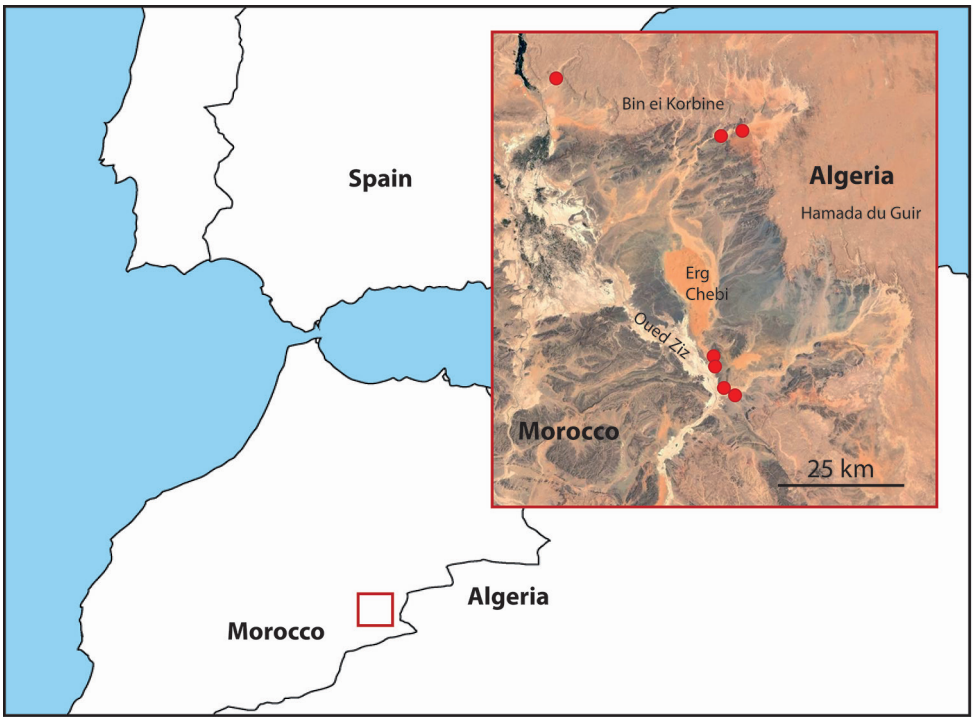

Figure 2. Distribution of known localities (red dots) of *Zahora ait-atta* documented from 2015 to 2019 (satellite map was taken from image metadata Copernicus/Landsat).

Distribution and habitat. The species is a local endemic and was observed at the following and additional places at given dates. From these localities no additional vouchers have been collected, and to our knowledge the species has never been sampled before:

Begaa: [27th January 2015] – gps 30.9453, -3.8767; 680 m a.s.l.
Khamlia: [02nd February 2015] – gps 30.9895, -3.9863; 680 m a.s.l.
Oued-Bou-Ibourine: [09th March 2017] – gps 31.4146, -3.7537; 900 m a.s.l.
Oued-Bou-Ibourine: [04th April 2017] – gps 31.4062, -3.7353; 900 m a.s.l.
Oued-Bou-Ibourine: [02nd December 2017] – gps 31.4115, -3.7214; 900 m a.s.l.
Khamlia: [08th February 2018] – gps 30.9906, -3.9918; 680 m a.s.l.
Taous: [10th October 2018] – gps 30.9286, -3.9753; 680 m a.s.l.
Khamlia: [08th February 2019] – gps 30.99879, -3.9875; 680 m a.s.l.
Oued-Bou-Ibourine: [11th March 2019] – gps 31.4114, -3.7220; 900 m a.s.l.
Oued-Bou-Ibourine: [11th March 2019] – gps 31.4127, -3.7419; 890 m a.s.l.
Begaa: [12th March 2019] – gps 30.9293, -3.9740; 680 m a.s.l.

Habitat. All places are in sandy beds of oueds flowing from the base of the kreb (cliff) of the Hamada du Guir or the Bin el Korbine.

Phenology. The species flowers in spring (February to March). Seeds germinate in late summer and autumn if soil moisture is sufficient and rosettes are formed persisting throughout the winter. After fruit stage seeds are dispersed and monocarpic plants are dying.

Ecology. Greenhouse and pollination experiments showed that the species is largely self-compatible. At its natural stands the plant is annual and monocarpic. However, in cultivation the plant species can be kept growing when cutting frutescence. There are two different options of seed release, either directly into a local soil seed bank from the dehiscent part of fruit or via the distal indehiscent part carrying two seeds, which may allow distributing effectively with water in the wadi systems at rare and occasional events.

Provisional IUCN conservation assessment. The extent of occurrence is less than 10,000 km^2 and falls within the limits of "Vulnerable" (VU) category under criterion B1. Since populations are of small sizes and occur at unique habitat types only, we assign an IUCN conservation status of VU B1.

Description of characters and its discussion

In Brassicaceae there are hardly any apomorphic characters (or character states) defining genera sufficiently. This resulted in numerous poly- and paraphyletic taxa (Al-Shehbaz 2012). Therefore, we used our results from phylogenetic analysis (see below) to identify those genera «widely» associated phylogenetically with the new taxon. In total, there are 13 genera: *Brassica*, *Cordylocarpus* Desf., *Crambella* Maire, *Diplotaxis*, *Erucastrum* Gaertn., *Guiraoa* Coss., *Hirschfeldia* Moench, *Raffenaldia* Godr., *Rapistrum* Crantz, *Sinapis*, *Trachystoma* O.E.Schulz, *Morisia* J.Gray and *Otocarpus* Durieu. For entire Brassicaceae we developed a morphomatrix scoring 37 characters to deliminate genera (« morphology tool » in *BrassiBase*; https://brassibase.cos.uni-heidelberg.de/; Koch et al. 2018). This matrix builds upon an interactive key presented earlier by Ihsan Al-Shehbaz and now integrated into *BrassiBase*. We used the character matrix to identify corresponding genus-level discriminative characters (Suppl. material 1). The combination of 37 characters allow to separate the genus from 12 out of the 14 genera with the following character states found in *Zahora*: (1) annual, (2) herb, (3) glandular hairs are absent, (4) trichomes are simple, (5) stem thorns are absent, (6) basal leaves are rosette-forming, (7) leaf margin is pinnately lobed, (8) stem leaves are present, and (9) petiolate, (10) stem leaves are entire to sinuate, (11) leaf thorns are absent, (12) raceme is ebracteate, (13) petals are distinctly longer than sepals (14) petals are (pale) yellow, (15) petal are wide-shaped in upper part, (16) petal apex is obtuse, (17) petal margin is entire, (18) sepals are erect, and (19) free, (20) stamen number is 6, (21) lower part of filaments and petal claws are without any structure, and (22) filaments are free, (23) flower symmetry is actinomorphic, (24) fruit type is a silique, (25) fruits are terete, (26) fruit wall is thin and leathery, (27) fruit is dehiscent (at least in proximal part), (28) gynophore in fruit is absent, (29) septum in mature fruits is complete, (30) stigma is entire, (31) fruit appendices are missing, (32) there are more than 20 seeds per fruit, (33) seeds are arranged biseriate in middle of fruit, (34) seed wing is missing, (35) cotyledons are conduplicate, (36) seed mucilage is present, and (37) fruit orientation is spreading. The combination of 37 character states does not distinguish between *Brassica* and *Diplotaxis*, two polyphyletic genera. And the new genus shows the same general generic morphotype, too.

However, *Zahora* is different from both, *Brassica* and *Diplotaxis*, because of its peculiar fruit type. Occurrence of heteroarthrocarpic fruits with seeded beak have been described for *Brassica* (Gómez-Campo and Prakash 1999), but *Diplotaxis* shows some trend only towards this feature.

But in none of these cases functional seeds are constantly developed in the distal part. Neither *Brassica* nor *Diplotaxis* have been shown to contain species with heteroarthrocarpic fruits with disarticulation of the joint (Hall et al. 2011). In various heteroarthrocarpic species, a joint forms a novel separation layer such that the distal segment may separate and is dispersed independently of seeds from the proximal segment, a phenomenon referred to as disarticulation. *Zahora ait-atta* variant of heteroarthrocarpy can be defined as "proximal segment dehiscent with disarticulation of the indehiscent distal part".

Results and discussion

Cytogenetics, phylogeny and biogeography

All molecular results refer to the voucher of the holotype, which served as source for the material.

The new species is diploid with $2n$=18, and the haploid genome size (1C value) is 0.71 pg (+/- 8%). This corresponds to a genome size of approximately 553 MBp (Hohmann et al. 2014).

MegaBlast searches of plastid DNA sequences (*trn*L intron and *trn*LF intergenic spacer) searching for related maternal lineages as defined earlier (Arias and Pires 2012) revealed that the new genus belongs to the Oleracea-lineage: A query search with the *trn*LF intergenic spacer (GenBank submission ID2268434) (350 bp) revealed a query cover of 100% and 96.4% sequence similarity with *Brassica villosa* Raimondo & Mazzola and *B. oleracea* L., and 95.5% with *Raphanus raphanistrum* L.. A query search with the *trn*L intron (GenBank submission ID2268434) (318 bp) revealed a query cover of 98% and 99% sequence similarity with *Brassica napus* L., *B. rapa* L., *B. juncea* (L.) Crantz, *B. oleracea* and *Erucastrum gallicum* (Willd.) O.E.Schulz. There was no sequence identity match indicating any further close relation to known species and genera; and sequence identity is not high enough (>99%) to match any known sequence-based documented species or genetically defined genus.

There are no comprehensive phylogenetic hypotheses of the entire tribe Brassiceae based on the nuclear genome and discussed further taxonomically (e.g., Hall et al. 2011). However, it is known that plastid and nuclear phylogenies are largely incongruent to each other (Hall et al. 2011). Our herein presented ITS phylogeny (GenBank submission ID6359700) indicates a congruent pattern with plastid DNA data when referring to the phylogenetic position of the new taxon.

The new genus and species is placed at the base of a clade consisting of species from different genera, such as *Raphanus raphanistrum* L., *Sinapis pubescens* L., *Diplotaxis brachycarpa* Godr., *Erucastrum littoreum* (Pau & font Quer) Maire, *Hirschfeldia incana* (L.)

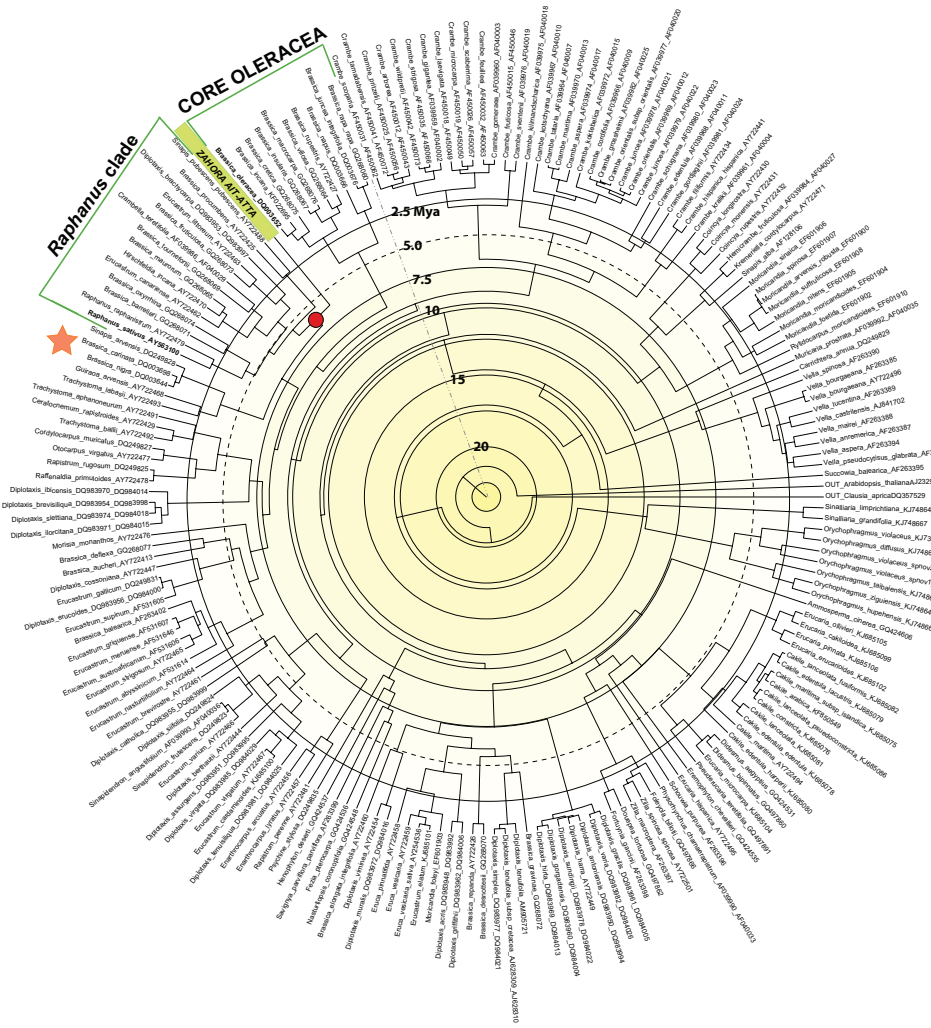

Figure 3. BEAST analysis of tribe Brassiceae based on ITS DNA sequence data (Suppl. material 2). The new genus *Zahora* is highlighted, and its respective stem group node is indicated (red dot). Divergence times are given as Mya (million years ago). Agronomically important species, *Brassica oleracea* and *Raphanus sativus*, are indicated and shown with their respective clades. *Brassica nigra* and *B. carinata* are also indicated with an asterisk (orange).

Lagr.-Foss. or *Crambella teretifolia* (Batt. & Trab.) Maire herein referred to as "*Raphanus* clade" (Fig. 3). Most species of this group are confined to the southwestern Mediterranean region, and some of them are even centered in Morocco (e.g., *Crambella teretifolia*, *Diplotaxis brachycarpa*) and started to diversify approximately 5 Mya.

The sister clade of *Raphanus* and *Zahora* comprises *Brassica nigra* and *B. carinata* (indicated with Fig. 3), an interesting finding since earlier studies split the genus *Brassica* into two evolutionary lineages, the "nigra lineage" and the "rapa/oleraceae lineage" (e.g. Yang et al. 2002). However, *Zahora* is not placed closely with the Core Oleracea

clade (RaXML tree, Suppl. material 3: Fig. S1; BEAST analysis, Fig. 3), which is comprising species such as *B. oleracea*, *B. villosa* or *B. rupestris* Raf.. The Core Oleracea clade within the "rapa/oleraceae lineage" started to diverge at the end of the Miocene appr. 5–6 Mya in the southwestern Mediterranean (Arias et al. 2014). Our herein presented BEAST analysis is in full support of this finding (Fig. 3).

In conclusion, both types of markers (plastid and nuclear genome) clearly indicate (i) a distinct status as a new species, (ii) no phylogenetic affinities with any known genus, and (iii) provides some biogeographical evidence of an old ancestry in the North-Western African region at Late Miocene epoch, which has been shown as a pivotal period for triggering north African aridity and creating the Sahara desert (Zhang et al. 2014). Accordingly, our herein presented BEAST analysis support a stem group age of *Zahora* of approximately 6 Mya.

Haploid genome size is comparable to other related species (*BrassiBase*; Kiefer et al. 2014), and chromosome number of $n = 9$ is a widely found and presumably ancestral situation in several *Brassica* species assemblages (Hohmann et al. 2015). A genome-level comparison of *Raphanus sativus* and *Brassica oleracea* confirmed a $n = 9$ ancestral cytotype to both of the lineages with a split time of 7–14 Mya, which coincides with our results from BEAST analysis (Fig. 3).

Since we found *Zahora* at a basal position to the entire *Raphanus* clade, the new species may play a key role in our future understanding of both genomes, *Brassica oleracea* versus *Raphanus sativus*, representing important crop plant species.

Evolution of heteroarthrocarpic fruit in Brassiceae

Ancestral state reconstructions were unable to determine whether disarticulation precedes or follows loss of dehiscence. Regardless, variation in types among closely related taxa is the rule. For example, within the Nigra (excluding *Coincya* Porta & Rigo ex Rouy and *Muricaria prostrata* (Desf) Desv.) and Rapa/Oleracea lineages of Brassiceae most possible fruit morphologies are present (non-heteroarthrocarpic, fully dehiscent, partially dehiscent, disarticulation, and no disarticulation) (Hall et al. 2011). Therefore, *Zahora* might be an interesting study object to investigate the evolution of dehiscent and indehiscent fruit types in Brassicacae. There is some important progress to unravel the molecular mechanisms of this important trait in Brassicaceae (Carey et al. 2019), and its ecological and evolutionary relevance has been documented recently for crucifer genera *Lepidium* L. (Sperber et al. 2017) and *Aethionema* W.T. Aiton (Lenser et al. 2016, Mérai et al. 2019).

Conclusion

Zahora ait-atta is described as a new species of a new monotypic genus. *Zahora* shows a peculiar fruit feature, namely heteroarthrocarpic fruits, and the species might mediate evolutionary between Core Oleracea clade (e.g. *Brassica oleracea*, *Brassica napus*) and

Raphanus sativus and related genera. Both represent important crop plant groups with seeds playing an enormous agronomical role. The diploid new species might, therefore, serve as important germplasm reservoir to study traits and characters in a number of Brassiceae crop plants.

Acknowledgements

We thank Peter Sack for curation of germplasm, support in the herbarium and cytogenetic analyses. Bärbel Schwarz is curating our living collections and we are grateful for her excellent work. We thank the local ait-atta herders for their hospitality, sharing with us their bread, tea and their knowledge of desert flora, Zahora Attioui for her participation in field work and Dr Abdelmonaim Homrani-Bakali, rangeland ecologist at INRA Morocco for sharing a field trip and his data. Valuable comments from Ihsan Al-Shehbaz and an anonymous reviewer are greatly acknowledged.

References

Al-Shehbaz IA (2012) A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon 61(5): 931–954. https://doi.org/10.1002/tax.615002

Arias T, Pires JC (2012) A fully resolved chloroplast phylogeny of the *Brassica* crops and wild relatives (Brassicaceae: Brassiceae): Novel clades and potential taxonomic implications. Taxon 61(5): 980–988. https://doi.org/10.1002/tax.615005

Arias T, Beilstein MA, Tang M, McKain MR, Pires JC (2014) Diversification times among *Brassica* (Brassicaceae) crops suggest hybrid formation after 20 million years of divergence. American Journal of Botany 101(1): 86–91. https://doi.org/10.3732/ajb.1300312

Carey S, Mendler K, Hall JC (2019) How to build a fruit: Transcriptomics of a novel fruit type in the Brassicaceae. PLoS One 14(7): e0209535. https://doi.org/10.1371/journal.pone.0209535

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Gómez-Campo C, Prakash S (1999) Origin and Domestication. In: Gómez-Campo C (Ed.) Biology of *Brassica* Coenospecies. Elsevier, Amsterdam, 1–486. https://doi.org/10.1016/S0168-7972(99)80003-6

Guinet P, Sauvage C (1954) Botanique. In: Joly F, Poueyto A, Guinet P, Sauvage C, Panouse JB, Vachon M, Kocher L, Reymond A (Eds) Les Hamada sud-marocaines. Travaux de l'Institut scientifique chérifien, Série générale n° 2, 73–168.

Hall JC, Tisdale TE, Donohue K, Wheeler A, Al-Yahya MA, Kramer EM (2011) Convergent evolution of a complex fruit structure in the tribe Brassiceae (Brassicaceae). American Journal of Botany 98(12): 1989–2003. https://doi.org/10.3732/ajb.1100203

Hohmann N, Schmickl R, Chiang TY, Lucanova M, Kolar F, Marhold K, Koch MA (2014) Taming the wild: Resolving the gene pools of non-model *Arabidopsis* lineages. BMC Evolutionary Biology 14(1): e224. https://doi.org/10.1186/s12862-014-0224-x

Hohmann N, Wolf EM, Lysak MA, Koch MA (2015) A time-calibrated road map of Brassicaceae species radiation and evolutionary history. The Plant Cell 27: 2770–2784. https://doi.org/10.1105/tpc.15.00482

Huang XC, German D, Koch MA (2019) Temporal patterns of diversification in Brassicaceae demonstrate decoupling of rate shifts and mesopolyploidization events. Annals of Botany: mcz123. https://doi.org/10.1093/aob/mcz123

Karl R, Koch MA (2013) A world-wide perspective on crucifer speciation and evolution: Phylogeny, biogeography and trait evolution in tribe Arabideae. Annals of Botany 112(6): 983–1001. https://doi.org/10.1093/aob/mct165

Kiefer M, Schmickl R, German D, Lysak M, Al-shehbaz IA, Franzke A, Mummenhoff K, Stamatakis A, Koch MA (2014) BrassiBase: Introduction to a Novel Knowledge Database on Brassicaceae Evolution. Plant & Cell Physiology 55(1): e3. https://doi.org/10.1093/pcp/pct158

Koch MA, Kiefer M, German D, Al-Shehbaz IA, Franzke A, Mummenhoff K, Schmickl R (2012) BrassiBase: Tools and biological resources to study characters and traits in the Brassicaceae – version 1.1. Taxon 61(5): 1001–1009. https://doi.org/10.1002/tax.615007

Koch MA, Michling F, Walther A, Huang XC, Tewes LJ, Müller C (2017) Early-Mid Pleistocene genetic differentiation and range expansions as exemplified by Eurasian invasive *Bunias orientalis* (Brassicaceae) indicates the Caucasus as key region. Scientific Reports 7: 16764. https://doi.org/10.1038/s41598-017-17085-8

Koch MA, German DA, Kiefer M, Franzke A (2018) Database taxonomics as key to modern plant biology. Trends in Plant Science 23(1): 4–6. https://doi.org/10.1016/j.tplants.2017.10.005

Lenser T, Graeber K, Cevik OS, Adigüzel N, Dönmez AA, Grosche C, Kettermann M, Mayland-Quellhorst S, Mérai Z, Mohammadin S, Nguyen TP, Rümpler F, Schulze C, Sperber K, Steinbrecher T, Wiegand N, Strnad M, Mittelsten Scheid O, Rensing SA, Schranz ME, Theißen G, Mummenhoff K, Leubner-Metzger G (2016) Developmental Control and Plasticity of Fruit and Seed Dimorphism in *Aethionema arabicum*. Plant Physiology 172: 1691–1707. https://doi.org/10.1104/pp.16.00838

Lysak MA, Koch M, Pecinka A, Schubert I (2005) Chromosome triplication found across the tribe Brassiceae. Genome Research 15: 516–525. https://doi.org/10.1101/gr.3531105

Mérai Z, Graeber K, Wilhelmsson P, Ullrich KK, Arshad W, Grosche C, Tarkowská D, Turečková V, Strnad M, Rensing SA, Leubner-Metzger G, Mittelsten Scheid O (2019) *Aethionema arabicum*: A novel model plant to study the light control of seed germination. Journal of Experimental Botany 70(12): 3313–3328. https://doi.org/10.1093/jxb/erz146

Sperber K, Steinbrecher T, Graeber K, Scherer G, Clausing S, Wiegand N, Hourston JE, Kurre R, Leubner-Metzger G, Mummenhoff K (2017) Fruit fraacture biomechanics and the release of *Lepidium didymium* pericarp-imposed mechanical dormancy by fungi. Nature Communications 8(1): 1668. https://doi.org/10.1038/s41467-017-02051-9

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Warwick SI, Francis A, La Fleche J (2009) Guide to wild germplasm *Brassica* and allied crops (tribe Brassiceae, Brassicaceae). Agriculture and Agri-Food Canada Research Branch Publication, ECORC, Ottawa, Ontario, Canada.

Yang YW, Tai PY, Li WH (2002) A study of the phylogeny of *Brassica rapa*, *B. nigra*, *Raphanus sativus*, and their related genera using noncoding regions of chloroplast DNA. Molecular Phylogenetics and Evolution 23(2): 268–275. https://doi.org/10.1016/S1055-7903(02)00026-X

Zhang Z, Ramstein G, Schuster M, Li C, Contoux C, Yan Q (2014) Aridification of the Sahara dersert caused by Tethys Sea shrinkage during the Late Miocene. Nature 513: 401–404. https://doi.org/10.1038/nature13705

Ziffer-Berger J, Hanin N, Fogel T, Mummenhoff K, Barazani O (2014) Molecular phylogeny indicates polyphyly in *Raphanus L.* (Brassicaceae). Edinburgh Journal of Botany 72: 1–11. https://doi.org/10.1017/S0960428614000286

Supplementary material 1

Brassiceae genus-level diagnostic morpho-table

Authors: Marcus A. Koch, Claude Lemmel

Data type: DNA sequence data.

Link: https://doi.org/10.3897/phytokeys.135.46946.suppl1

Supplementary material 2

Tribal Brassiceae ITS alignment

Authors: Marcus A. Koch, Claude Lemmel

Data type: DNA sequence data.

Link: https://doi.org/10.3897/phytokeys.135.46946.suppl2

Supplementary material 3

Figure S1. RaXML tree of Brassiceae-wide ITS data

Authors: Marcus A. Koch, Claude Lemmel

Data type: not applicable (phylogenetic reconstruction).

Explanation note: Bootstrap-support from 1000 replicates is indicated.

Link: https://doi.org/10.3897/phytokeys.135.46946.suppl3