

Lithocarpus dahuensis (Fagaceae), a new species from Fujian Province based on morphology and genomic data

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Abstract

Lithocarpus dahuensis, a new Fagaceae species from Fujian Province, China, is described and illustrated. The new species is morphologically similar to *L. konishii*, but its oblanceolate leaf blade has more pairs of acute teeth on the margin, denser lateral veins, smaller cupules enclosing up to 1/4–1/3 of the nut, and its nut is only half as long as those of *L. konishii*. The plastome of *L. dahuensis* was 161,303 bp in length and displayed the typical quadripartite structure. Phylogenetic analyses distinguished *L. dahuensis* from *L. konishii* with strong support based on whole plastome and nrITS, respectively.

Keywords

biodiversity, chloroplast genome, morphology, phylogeny, taxonomy

Introduction

The subfamily Quercoideae, of the family Fagaceae, consists of seven genera, including *Castanea*, *Castanopsis*, *Chrysolepis*, *Lithocarpus*, *Notholithocarpus*, *Quercus*, and *Trigonobalanus*, containing 1,135 species (The plant list 2022). *Lithocarpus* consists of 341 species, making it the second largest genus after *Quercus*. These species are widely

distributed in tropical and sub-tropical broad-leaved evergreen forests throughout East and Southeast Asia, extending to New Guinea (Cannon 2001). The center of diversity is in East to Southeast Asia, with 123 species in China, mainly distributed in Guangdong, Guangxi, and Yunnan (Huang et al. 1999), 58 species in Thailand (Strijk et al. 2014), and 121 species in Vietnam (Ngoc et al. 2022).

Typically, *Lithocarpus* has spirally arranged leaves, which are glabrous, coriaceous, oblong-elliptical to oblong in shape, with the entire margin or with teeth along the margin. Its flowers are white to pale yellow. The male flowers are either solitary or in clusters of three or more, with campanulate or cup-shaped perianths, usually 6-lobed, partially united, and 12 stamens. The female flowers are usually solitary or in clusters of two to five, but only one or two of them are well developed, and they have perianths similar to male flowers, but smaller, and with 12 staminodes (Strijk et al. 2014). The sessile cupules are cup-shaped to discoid, with triangular to rhomboid bracts arranged in a diamond pattern on the cupule surface, enclosing the nuts completely or partially. The nuts are oblate to depressed with a concave or convex scar. Sometimes, the nut scar is concave at the margin but conspicuously convex at the center (Huang et al. 1999).

This paper describes a new species of *Lithocarpus* that was discovered during a field survey in a landscape forest behind the Xuefeng Village in Dahu Town, Minhou County, Fuzhou City in May 2017. It grows in well-preserved native broad-leaved evergreen forests in a valley and has leaves that resemble those of *Quercus engleriana* Seem, which were easily overlooked but were of constant concern. At the end of May 2018, the plants began to bloom and develop erect male inflorescences. By the end of September, fruit-bearing specimens were collected and measured. The newly found species is similar to *L. konishii* from Taiwan but differs in the leaf, cupule, and nut characters, as noted below. Considering the morphological differences, molecular data, and geographical isolation, here we describe it as a new species, *Lithocarpus dahuensis*.

Materials and methods

Morphological description

The morphological description of the new species was based on the study of specimens collected in 2019 from various locations. A Stereoscopic Zoom Microscope (Carl Zeiss, Axio zoom. v.16, Germany), equipped with an attached digital camera (AxioCam), and a Digital caliper were used to record the sizes of the morphological characters. Field observations provided habitats and phenology for the new species.

DNA extraction and sequencing

In this study, total DNA was extracted from fresh leaves of the new species using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Purified total DNA of the new species was fragmented, and genome skimming was performed using next-generation

sequencing technologies on the Illumina Novaseq 6000 platform. The sequencing was conducted by Berry Genomics Co. Ltd. (Beijing, China) using 150 bp paired-end reads with a 480 bp insert size, resulting in 11.58 GB of reads.

Genome assembly, annotation and analysis

The phylogenetic position of the new species was determined through the analysis of nrITS and whole plastome sequences. The nrITS (ITS1-5.8S-ITS2) was assembled using GetOrganelle v1.7.5, with -R of 7 and k-mer set of “35, 85, 115”. The emb-plant_nr library was selected as the reference genome database, then annotated and visualized using Geneious v2021.2.2.

The paired-end reads were filtered and assembled into a complete plastome using a GetOrganelle v1.7.5.0 (Jin et al. 2020) with appropriate parameters, including a K-mer set of “21,45,65,85,105”, and a word size of 0.6. Following previous studies, our workflow includes five key steps as well: 1. Mapping reads to seed and assembling seed-mapped reads for parameter estimation; 2. Recruiting more target-associated reads through extending iterations; 3. Conducting de novo assembly; 4. Roughly filtering fortarget-like contigs; 5. Identifying target contigs and exporting all configurations (Bankevich et al. 2012; Camacho et al. 2009; Jin et al. 2020; Langmead and Salzberg 2012). Graphs of the final assembly were visualized by Bandage (Wick et al. 2015) to assess their completeness. Gene annotation was performed using CPGAVAS2 (Shi et al. 2019) and PGA (Qu et al. 2019). The different annotations of protein coding sequences were confirmed using BLASTx. The tRNAs were checked with tRNAscan-SE v2.0.3. Final chloroplast genome map was created using OGDRAW.

Phylogenetic analysis

The phylogenetic relationship was constructed using Maximum likelihood (ML) analyses with the combined nrITS sequence. In total, 92 samples of *Lithocarpus*, *Morella*, *Corylus* and *Carpinus* were included in our analysis (Suppl. material 1: table S1). Three species of *Morella rubra*, *Corylus fargesii* and *Carpinus cordata* were used as outgroups (Wu et al. 2022). Each individual locus was aligned using MAFFT 7.310 (Katoh and Standley 2013) with default settings. All missing data were treated as gaps. The best nucleotide substitution model according to the Bayesian Information Criterion (BIC) was TNe+R3, which was selected by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE v1.6.8. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the model automatically selected by IQ-TREE (‘Auto’ option in IQ-TREE) for 2000 ultrafast (Minh et al. 2013) bootstraps.

To construct a phylogenetic tree based on plastome sequences, a total of 33 plastome sequences of *Lithocarpus*, *Castanea*, *Castanopsis*, *Cyclobalanopsis*, *Fagus*, *Quercus*, *Trigonobalanus*, *Morella*, *Corylus* and *Carpinus* were included in our analysis (Suppl. material 1: table S2). Three species of *Morella rubra*, *Corylus fargesii* and *Carpinus cordata* were used as outgroups (Wu et al. 2022). Each individual locus was aligned using

MAFFT 7.310 (Katoh and Standley 2013) with default settings. The best nucleotide substitution model according to the Bayesian Information Criterion (BIC) was K3Pu+F+R5, which was selected by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE v.1.6.8. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the model automatically selected by IQ-TREE ('Auto' option in IQ-TREE) for 2000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under the GTR+F+I+G4 model (2 parallel runs, 2000000 generations), in which the initial 25% of sampled data were discarded as burn-in. Phylograms were visualized in iTOLv.5 (iTOL: Interactive Tree Of Life (embl.de)).

Genomic comparison with related species

The online tool IRscope (Amiryousefi et al. 2018) was employed to draw the genetic architecture of the IR/SC junctions. Then the sequences of 12 *Lithocarpus* species were aligned using MAFFT7.310 (Katoh and Standley 2013), the nucleotide diversity (π value) of single copy genes and intergenic regions was estimated by DnaSP v.6 (Rozas et al. 2003).

Results

Characteristics of *Lithocarpus dahuensis* plastome

The complete chloroplast genome of *Lithocarpus dahuensis* is 161,303 bp in length (Fig. 1), which exhibits a typical quadripartite structure, comprising a pair of IR regions (25,894 bp) divided by an SSC region (18,956 bp) and an LSC region (90,559 bp). The overall GC content of the genome was 36.75%, while the GC content of LSC, SSC, and IR regions were 34.58%, 30.79%, and 42.71%, respectively. The whole chloroplast genome of *L. dahuensis* encodes 129 genes, consisting of 85 protein-coding genes, 36 transfer RNA (tRNA), and 8 ribosomal RNA (rRNA) genes. Among 129 genes, 6 protein-coding genes (*ndhB*, *rpl2*, *rpl23*, *rps12*, *rps7*, *ycf2*), 7 tRNA and 4 RNA genes were duplicated in the genome (Table 1). Altogether, 10 protein-coding genes and 5 tRNA genes contained the intron, in which two genes (*clpP* and *ycf3*) harbored a double intron. The annotated plastome was deposited in GeneBank (accession number OP954095).

Comparative analysis of the plastomes

The plastome of *Lithocarpus dahuensis* was compared to those of the other 11 *Lithocarpus* species. The plastome size of these species is very similar (Table 2), ranging from 161,020 bp for *L. balansae* to 161,974 bp for *L. polystachyus*. These genomes displayed a typical circular quadripartite structure consisting of a pair of IR regions (25,606 bp to 25,899 bp) separated by an LSC region (90,407 bp to 90,731 bp) and an SSC region

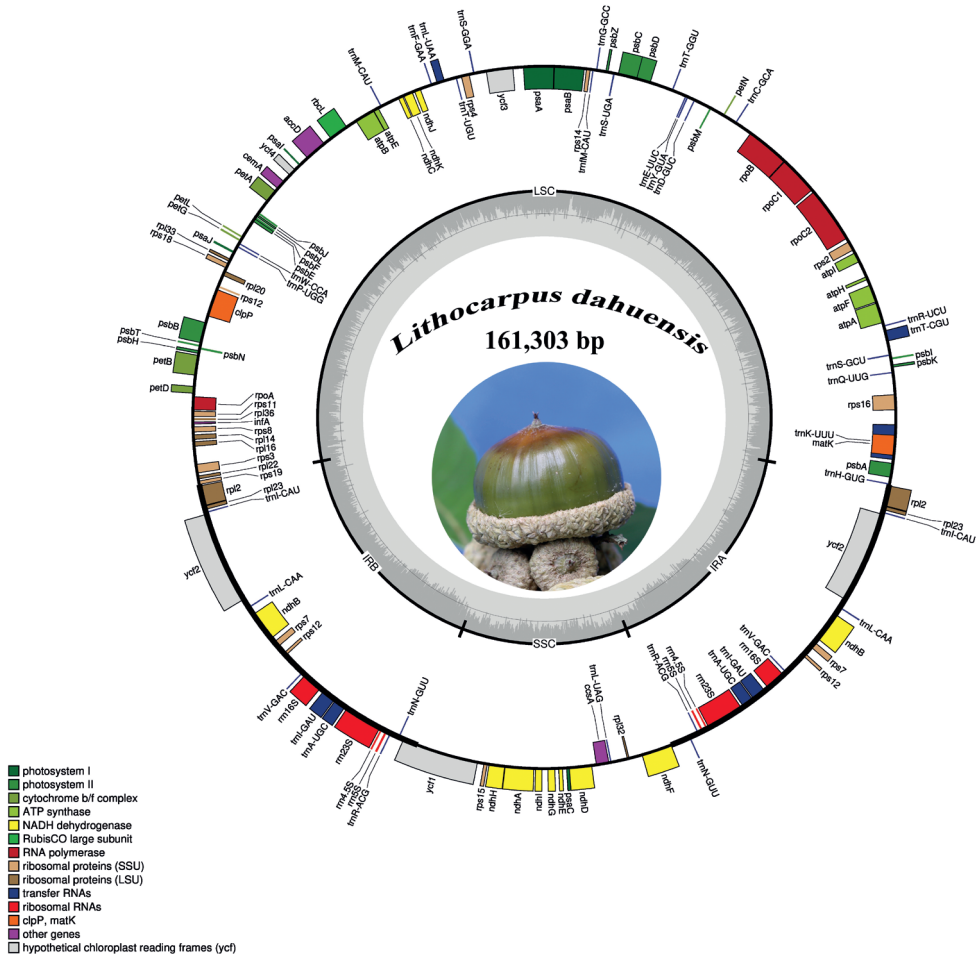


Figure 1. Representative cp genome of *Lithocarpus dahuensis*. Genes drawn inside and outside of the circle are transcribed in clockwise and counter-clockwise directions, respectively. The colored bar indicates chloroplast gene groups. The dark gray bar graphs inner circle shows the GC content, and the light gray bar graphs show the AT content.

(18,239 bp to 19,255 bp) (Table 2). The overall GC content was identical (~36.7%) across all compared plastomes, and was clearly higher in the IR region (~42.7%) than in the other regions (LSC ~34.5% and SSC ~30.8%), possibly because of the high GC content of the rRNA that was located in the IR regions.

A chloroplast genome identification analysis was performed on the 12 *Lithocarpus* species described above, with the *Lithocarpus dahuensis* chloroplast genome used as a reference (Suppl. material 1: fig. S1). We identified a considerable number of variations in the noncoding cp sequences, including *rbcl-accD* (highest, 0.03019), *rpl20-rps12*, *trnK-rps16*, *trnF-ndhJ* and *ccsA-ndhD*, as well as a number of variations in the coding regions, including *accD* (highest, 0.03019), *ycf1*, *rps16*, *ndhF* and *rpl32*. These

Table 1. Gene contents in the plastid genome of *Lithocarpus dahuensis*.

Category, group of genes	Gene names
Photosynthesis:	
Subunits of photosystem I	<i>psaA, psaB, psaC, psal, psaj</i>
Subunits of photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>
Subunits of NADH dehydrogenase	<i>ndhA*, ndhB*(2), ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
Subunits of cytochrome b/f complex	<i>petA, petB*, petD, petG, petL, petN</i>
Subunits of ATP synthase	<i>atpA, atpB, atpE, atpF*, atpH, atpI</i>
Large subunit of rubisco	<i>rbcL</i>
Subunits photochlorophyllide reductase	—
Self-replication:	
Proteins of large ribosomal subunit	<i>rpl14, rpl16, rpl2*(2), rpl20, rpl22, rpl23(2), rpl32, rpl33, rpl36</i>
Proteins of small ribosomal subunit	<i>rps11, rps12*(2), rps14, rps15, rps16*, rps18, rps19, rps2, rps3, rps4, rps7(2), rps8</i>
Subunits of RNA polymerase	<i>rpoA, rpoB, rpoC1*, rpoC2</i>
Ribosomal RNAs	<i>rrn16S(2), rrn23S(2), rrn4.5S(2), rrn5S(2)</i>
Transfer RNAs	<i>trnA-UGC*(2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnH-GUG, trnI-CAU(2), trnI-GAU*(2), trnK-UUU*, trnL-CAA(2), trnL-UAA*, trnL-UAG, trnM-CAU, trnN-GUU(2), trnP-UGG, trnQ-UUG, trnR-ACG(2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-CGU*, trnT-GGU, trnT-UGU, trnV-GAC(2), trnW-CCA, trnY-GUA, trnY-M-CAU</i>
Other genes:	
Maturase	<i>matK</i>
Protease	<i>clpP**</i>
Envelope membrane protein	<i>cemA</i>
Acetyl-CoA carboxylase	<i>accD</i>
c-type cytochrome synthesis gene	<i>ccsA</i>
Translation initiation factor	<i>infA</i>
Genes of unknown function:	
Conserved hypothetical chloroplast ORF	<i>ycf1, ycf2(2), ycf3**, ycf4</i>

Notes: Gene*: Gene with one introns; Gene**: Gene with two introns; Gene(2): Number of copies of multi-copy genes.

Table 2. Statistics on the basic features of the plastid genomes of *Lithocarpus dahuensis* and related taxa.

Species	Accession No.	Number of Genes			Length (bp)				GC Content (%)			
		PCGs	tRNA	rRNA	Total	LSC	SSC	IR	Total	LSC	SSC	IR
<i>Lithocarpus dahuensis</i>	OP954095	79	29	4	161,303	90,559	18,956	25,894	36.75	34.58	30.79	42.71
<i>Lithocarpus konishii</i>	ON422319.1	80	30	4	161,385	90,660	18,927	25,899	36.77	34.61	30.83	42.71
<i>Lithocarpus litseifolius</i>	NC_063927.1	79	29	4	161,322	90,551	18,977	25,897	36.73	34.57	30.71	42.71
<i>Lithocarpus hancei</i>	MW375417.1	80	31	4	161,304	90,585	18,959	25,897	36.72	34.57	30.68	42.70
<i>Lithocarpus longinux</i>	NC_062048.1	80	29	4	161,420	90,407	19,255	25,879	36.76	34.58	31.01	42.71
<i>Lithocarpus dealbatus</i>	NC_063459.1	80	29	4	161,476	90,731	18,987	25,879	36.75	34.58	30.88	42.71
<i>Lithocarpus balansae</i>	KP299291.1	80	31	4	161,020	90,596	19,160	25,632	36.71	34.53	30.83	42.77
<i>Lithocarpus fenestratus</i>	OM112300.1	80	31	4	161,184	90,524	19,052	25,804	36.73	34.55	30.78	42.74
<i>Lithocarpus polystachyus</i>	OL569560.1	75	27	4	161,974	90,523	18,239	26,606	36.70	34.58	30.86	42.30
<i>Lithocarpus cleistocarpus</i>	OM112296.1	80	31	4	161,178	90,558	19,096	25,762	36.78	34.61	30.83	42.78
<i>Lithocarpus obscurus</i>	OM112297.1	80	31	4	161,349	90,616	18,969	25,882	36.79	34.63	30.92	42.71
<i>Lithocarpus glaber</i>	MZ750954.1	81	30	4	161,171	90,466	18,939	25,883	36.75	34.61	30.71	42.71

10 genes or spacer regions were all in the non-IR region, with *rbcL-accD*, *rpl20-rps12*, *trnK-rps16*, *trnF-ndhJ*, *accD*, *rps16* in the LSC region, and *ccsA-ndhD*, *ycf1*, *ndhF*, *rpl32* in the SSC region (Suppl. material 1: fig. S2). This result is consistent with the highest GC content in the IR region (IR42.71>LSC34.58>SSC30.79 (Table 2).

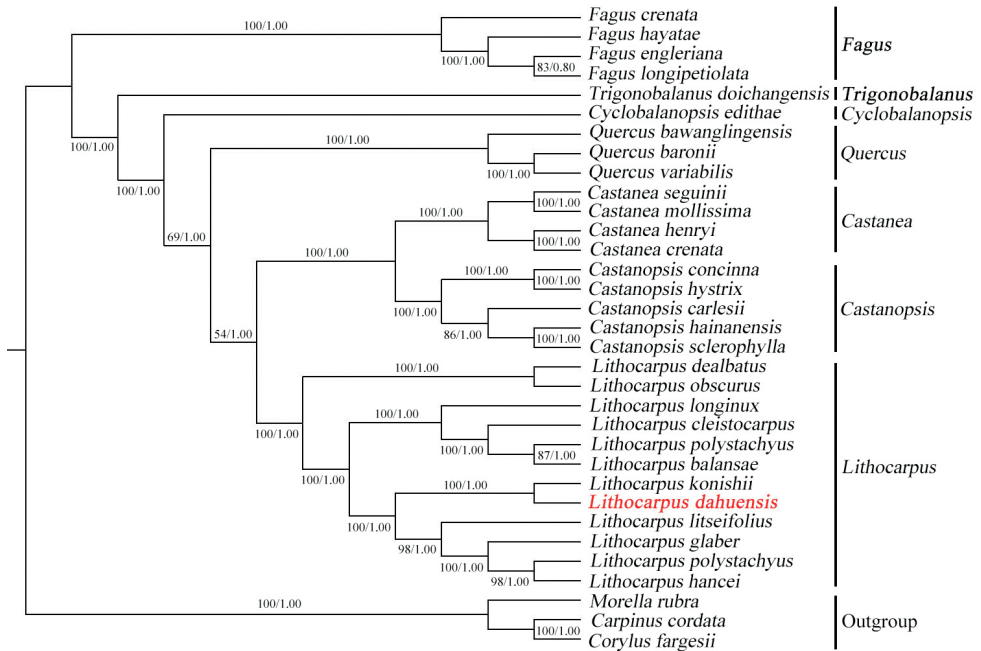


Figure 2. Phylogenetic tree of 33 complete plastid sequences derived from the Fagales species in genus *Lithocarpus*, *Castanea*, *Castanopsis*, *Cyclobalanopsis*, *Fagus*, *Quercus*, *Trigonobalanus*, *Morella*, *Corylus* and *Carpinus*. Numbers above and below branches indicate RAxML (left) bootstrap probabilities (BP) and Bayesian (right) posterior probabilities (PP), respectively. Three species of *Morella rubra* (Myricaceae), *Corylus fargesii* (Betulaceae) and *Carpinus cordata* (Betulaceae) were included as outgroups.

Phylogenetic analysis

The present study confirmed *Lithocarpus dahuensis* as a new species based on phylogenetic analysis based on plastome data, as well as the nrITS sequence. The plastome tree clearly indicated the distinctiveness of *L. dahuensis* from *L. konishii*, with strong support. *Lithocarpus dahuensis* is sister to *L. konishii*, and nested in a clade formed by 4 other *Lithocarpus* species, including *L. litseifolius*, *L. glaber*, *L. polystachyus* and *L. hancei* (Fig. 2). In addition, the phylogenetic analysis based on the nrITS sequence also separates the new species from *L. konishii* with strong support (Fig. 3).

Taxonomic treatment

***Lithocarpus dahuensis* H.X.Su, Miao Zhang & B.Hua Chen, sp. nov.**

urn:lsid:ipni.org:names:77315865-1

Figs 4A–C, F–H, 5, 6A, D–J, 7

Diagnosis. *Lithocarpus dahuensis* differs from *L. konishii* by having an oblanceolate leaf blade with 7–10 pairs of acute teeth on the leaf margin from the second to third lateral

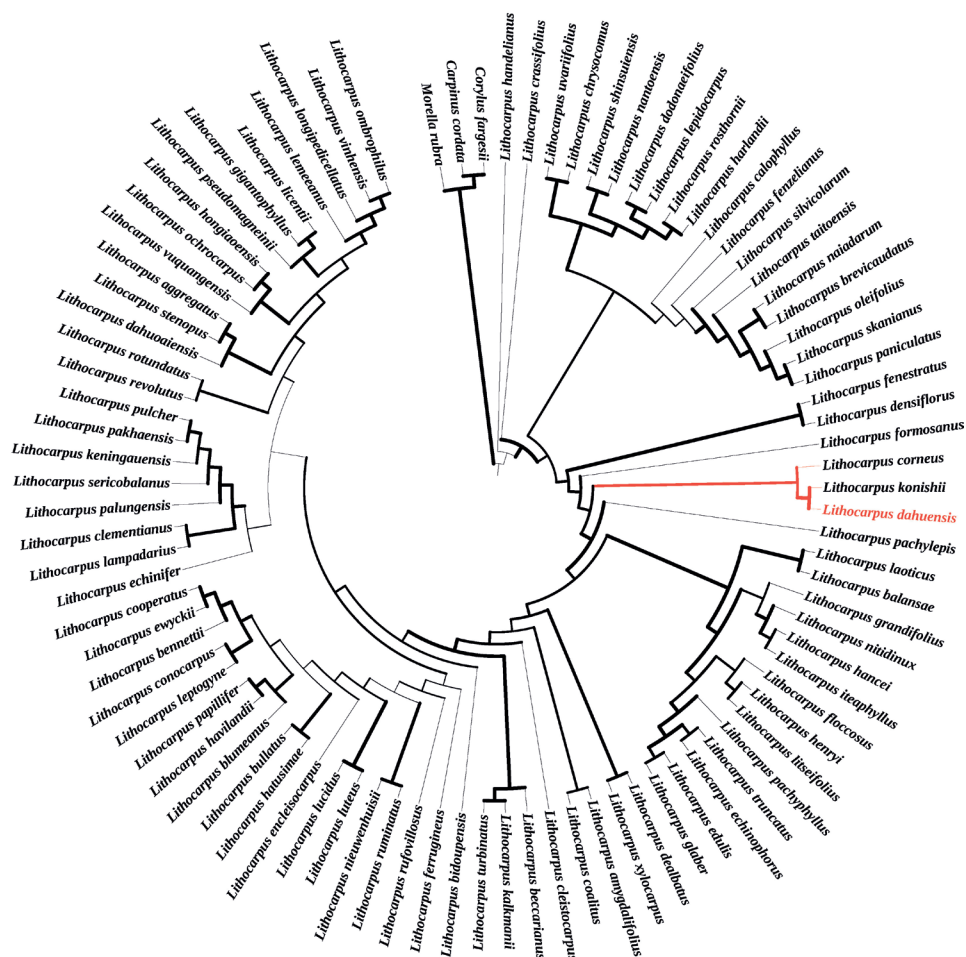


Figure 3. Phylogenetic tree of 92 sequences based on nrITS genes. The boldness of the branches indicates RAXML bootstrap probabilities (BP). Three species of *Morella rubra* (Myricaceae), *Corylus fargesii* (Betulaceae) and *Carpinus cordata* (Betulaceae) were included as outgroups.

veins above the leaf base (compared to 3–6 pairs of obtuse teeth for *L. konishii*), and its lateral veins are numerous and dense, reaching up to 15 pairs; it has 4–10 female flowers, borne singly in the lower part of staminate catkins; the cupules are smaller, encrusting up to 1/4–1/3 of the nut, and the nut is only half as high as those of *L. konishii* (1.4–1.8 vs. 1.8–2.4 cm) (Table 3).

Type. CHINA. Fujian Province, Fuzhou City, Minhou County, Dahu town, Ni-umu Mountain, forest margins, 26°25'N, 119°3'E, elevation 1035 m, 10 Sep. 2017, B. Hua Chen *CBH02292* (Holotype, FNU, barcode FNU0039021; Isotypes, FNU, barcode FNU0038769).

Description. Trees usually less than 8 m tall, evergreen. Branchlets densely grayish yellow tomentose, soon glabrescent. Bud scales compact, densely covered with

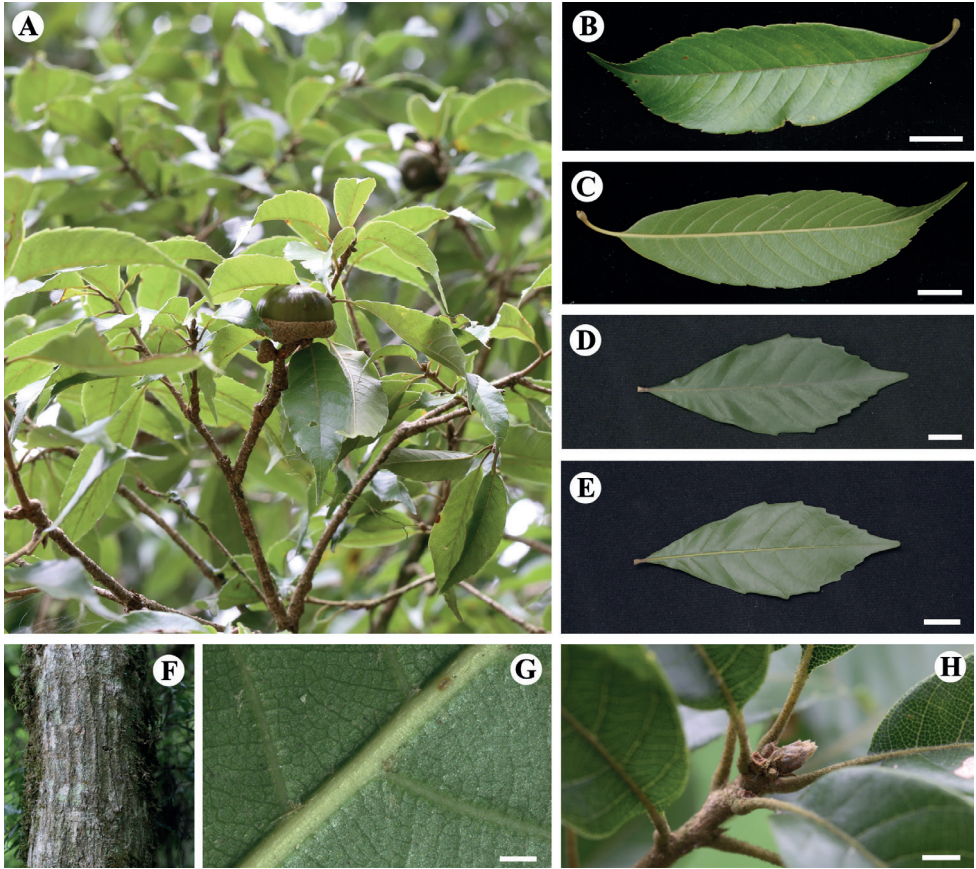


Figure 4. *Lithocarpus dabuensis* H.X.Su, Miao Zhang & B.Hua Chen, sp. nov. **A** fruiting stem with mature cupule **B, C** adaxial and abaxial side of mature leaf, respectively **D, E** adaxial and abaxial side of mature leaf of *Lithocarpus konishii* (Hayata) Hayata, respectively (photographed by Shi Shi) **F** bark **G** lower young leaf surface, showing abaxially hairy **H** petiole and buds, showing grayish yellow short hairs. Scale bars: 1 cm (**B–E**); 1 mm (**G**); 2 mm (**H**).

grayish yellow silky short hairs. Leaf blade oblanceolate or ovate-elliptic, $3.7\text{--}9.8 \times 1.1\text{--}3.2$ cm, leathery, concolorous, apex acuminate to caudate, ca. 9 mm long, base cuneate and inaequilateral, margin with 7–10 acute teeth except basally entire, abaxially with tufts of hairs along veins; midvein adaxially puberulent; secondary veins 7–16 on each side of midvein, adaxially slightly impressed; tertiary veins slender, evident. Petiole 0.5–1.3 cm, tomentose, soon glabrescent. Rachis of inflorescences densely tawny tomentose. Inflorescences male, or androgynous, 2–6, in leaf axils toward base of branchlets or in a dense paniculate cluster on subterminal shoots, erect; rachis of male inflorescences, 5.6 cm long; flowers usually 3 in dichasial clusters; perianth 6-lobed; stamens 12. Female flowers 4–11, borne on basal part of androgynous inflorescences, perianth 6-lobed, styles 3, 3 mm. Infructescences 1.4–4.3 cm; rachis 4.5 mm thick,

Table 3. Morphological differences between *Lithocarpus dahuensis* and *L. konishii*.

Characters	<i>Lithocarpus dahuensis</i>	<i>Lithocarpus konishii</i>
Leaf margin	Acute teeth; 7–10 pairs	Obtuse teeth; 3–6 pairs
Leaf surface	Glabrous on both upper and lower surfaces, only biaxially retaining fascicled hairs on axil of veins	Glabrous on both upper and lower surfaces, only biaxially retaining fascicled hairs on axil of veins
Leaf shape	Oblanceolate, ovate-elliptic	Ovate, obovate, elliptic, or obovate-elliptic
Leaf blade size (cm)	3.7–9.8 × 1.1–3.2	4.0–9.0 × 1.0–4.0
Petiole length	0.5–1.3 cm long	0.5–1.5 cm long
Number of secondary veins	9–13 (–15) pairs	7–10 (–11) pairs
Fruiting stalk length	Almost sessile	Almost sessile
Cupule	Usually solitary, few in clusters of 2 or 3 (–4), 0.5–0.7 cm high by 2.2–2.7 cm in diam.	Solitary (or 2), 0.7–1.1 cm high by 2.3–3.2 cm in diam.
Cupule outside	Very faintly visible hairs	Faintly visible hairs
Scale arrangement	Imbricate	Imbricate
Nut size	1.4–1.8 cm high by 1.5–2.6 cm in diam.	1.8–2.4 cm high by 2.3–3.3 cm in diam.
Nut enclosure by cupule	Enclosing ca. 1/4–1/3 of the nut	Enclosing basal part of nut
Basal scar of the nut	Margin concave but center convex, ca 1.7 cm in diam.	Margin concave but center ± convex, ca 2.0 cm in diam.
Infructescence length	1.5–4.0 cm long	2.0–3.0 cm long

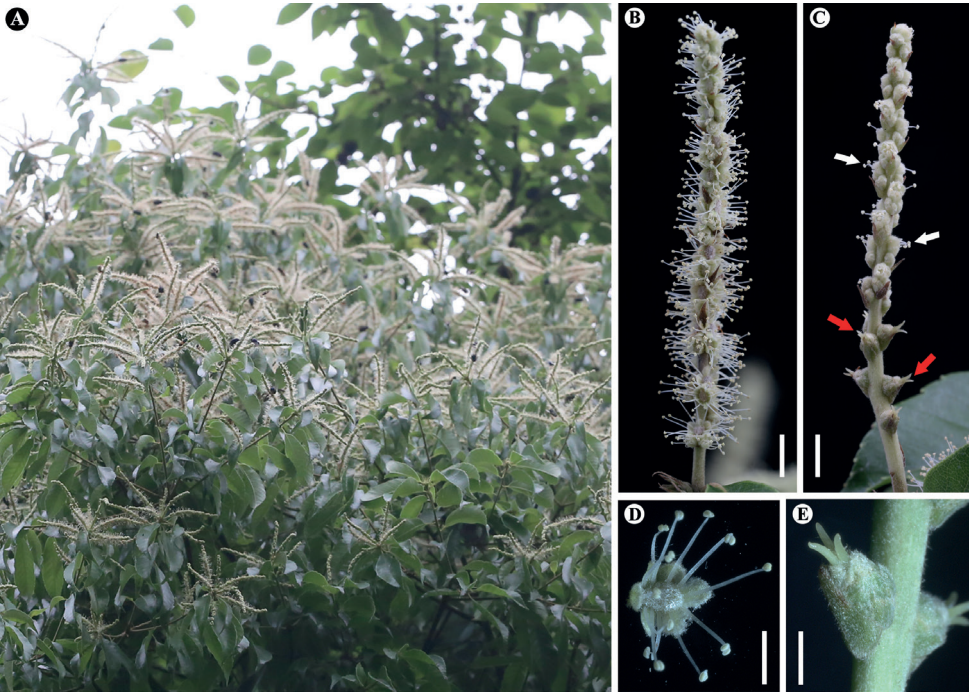


Figure 5. *Lithocarpus dahuensis* H.X.Su, Miao Zhang & B.Hua Chen, sp. nov. **A** branch with mature male and androgynous inflorescences **B** male inflorescences **C** androgynous, showing female flowers borne on basal part of inflorescences (red arrows) and male flowers (white arrows) **D** male flower **E** female flower. Scale bars: 5 mm (**B**, **C**); 2 mm (**D**, **E**).

glabrescent, lenticellate. Cupule usually 1(or in clusters of 2–3–(4)), saucer-shaped, 4.6–7.4 mm × 2.2–2.7 cm, enclosing ca. 1/4–1/3 of nut, wall 1.0–2.5 mm thick; bracts imbricate, broadly triangular, covered with grayish brown, shortly tomentose

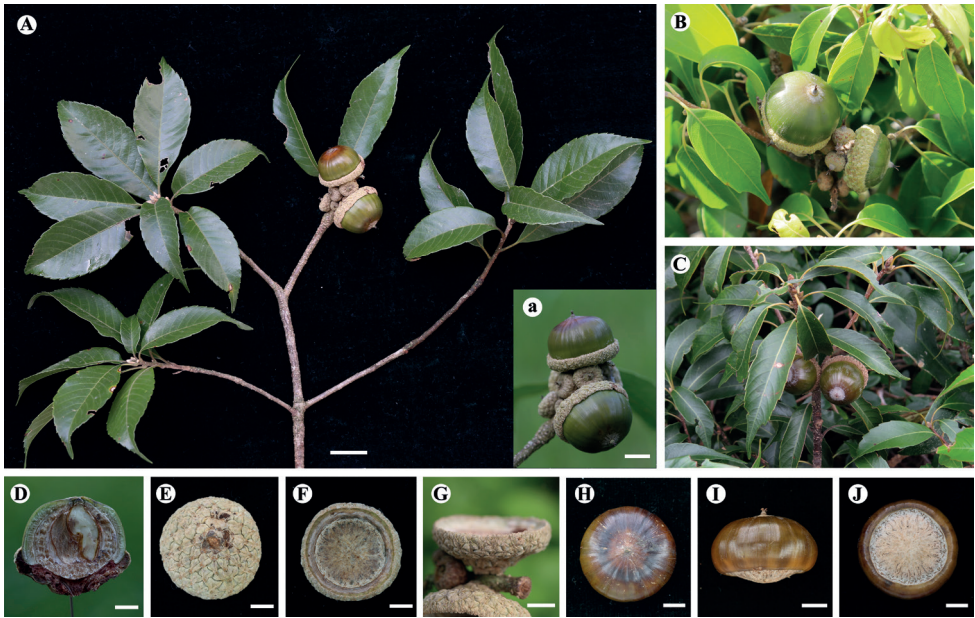


Figure 6. *Lithocarpus dahuensis* H.X.Su, Miao Zhang & B.Hua Chen, sp. nov. **A** branch with mature cupule **a** infructescence, showing mature cupule enclosing 1/4 of nut **B, C** *Lithocarpus konishii* (Hayata) Hayata (photographed by Shi Shi and Jin-Long Zhang from Hainan and HongKong, respectively) **D** vertical section of mature fruit **E–G** outside, inside and side view of the cupule, respectively **H–J** top, side and bottom view of mature nut, respectively. Scale bars: 2 cm (**A**); 1 cm (**a**); 5 mm(**D–J**).

hairs, midvein ridged. Nut depressed globose, $1.0\text{--}1.9 \times 1.5\text{--}2.6$ cm, glabrous, apex flat, wall 3.3–6.9 mm thick and horny; scar 1.5–1.9 cm in diam., margin concave but center convex.

Distribution and habitat. *Lithocarpus dahuensis* is only found in Dahu town, Minhou County, Fujian, China (Fig. 8), where it grows in valleys of subtropical evergreen broad-leaved forest. Many other plants grow in the surrounding habitat, whose tree layer includes *Castanopsis eyrei* (Champ. & Benth.) Tutcher (Fagaceae), *Quercus sessilifolia* Blume (Fagaceae), *Schima superba* Gardner & Champ. (Theaceae), *Semiliquidambar chingii* (F.P.Metcalf) H.T.Chang (Altingiaceae), *Ilex elmerrilliana* S.Y.Hu (Aquifoliaceae), *Dendropanax dentiger* (Harms) Merr. (Araliaceae) and others; the shrub layer includes *Rhododendron ovatum* (Lindl.) Planch. (Ericaceae), *Syzygium buxifolium* Hook. & Arn. (Myrtaceae), *Lindera aggregata* (Sims) Kosterm. (Lauraceae), *Symplocos stellaris* Brand (Symplocaceae), *Ardisia crispa* (Thunb.) A. DC. (Primulaceae), *Ilex asprella* (Hook. & Arn.) Champ. ex Benth. (Aquifoliaceae), *Cleyera japonica* Thunb. (Pentaphylacaceae), *Pyrularia edulis* (Wall.) A. DC. (Santalaceae), *Oligostachyum oedogonatum* (Z.P. Wang & G.H.Ye) Q.F.Zhang et K.F.Huan (Poaceae), *Ilex serrata* Thunb. (Aquifoliaceae), *Rubus buergeri* Miq. (Rosaceae), *Rubus impressinervus* F.P.Metcalf (Rosaceae), *Ligustrum sinense* Lour. (Oleaceae), *Clerodendrum cyrtophyllum* Turcz. (Lamiaceae), *Erythroxylum sinense* Y.C.Wu (Erythroxylaceae) and others; the vegetation layer includes *Dicranopteris pedata* (Hout.) Nakaike (Gleicheniaceae), *Hypolepis punctata* (Thunb.) Mett. (Dennstaedtiaceae),

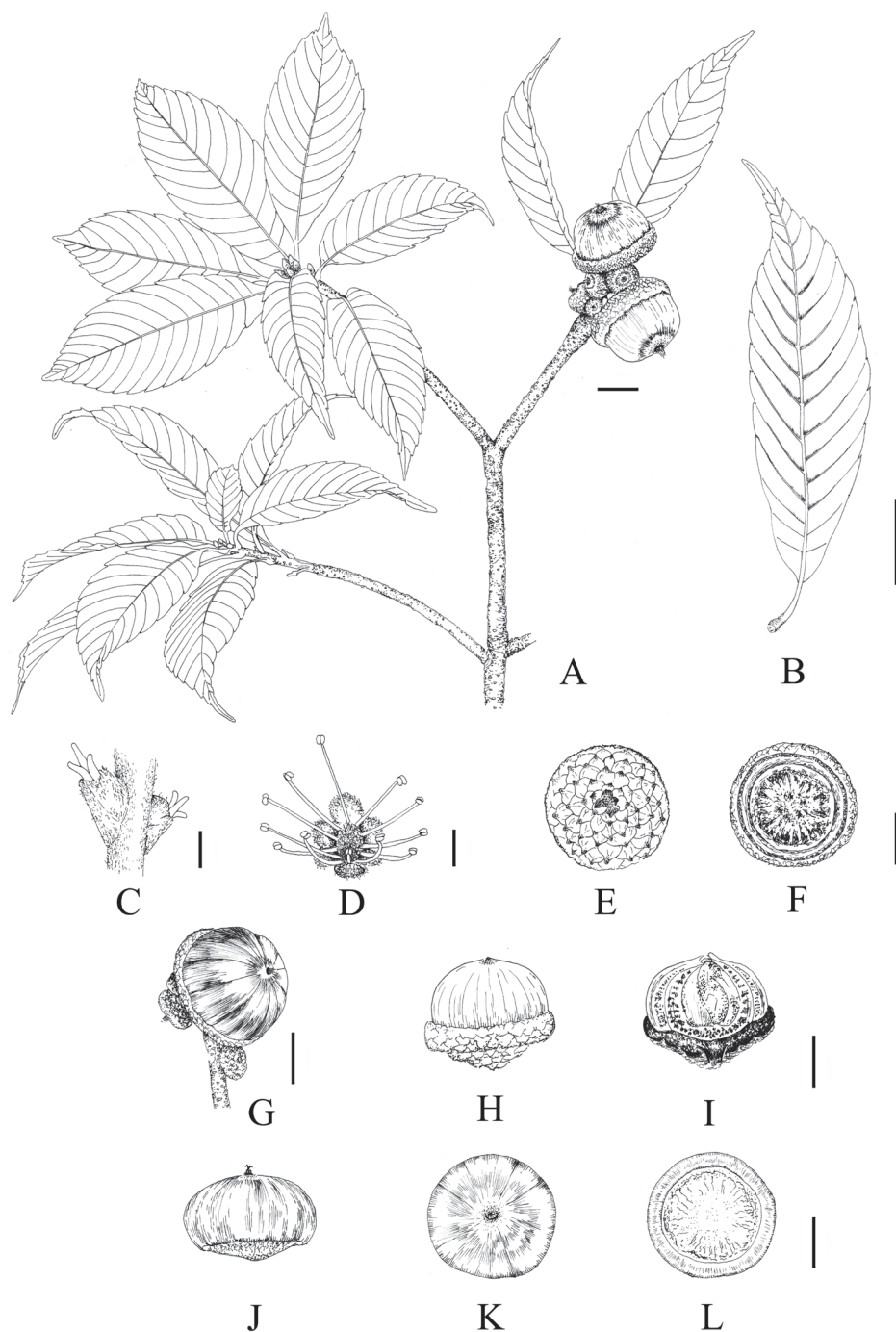


Figure 7. *Lithocarpus dahuensis* H.X.Su, Miao Zhang & B.Hua Chen, sp. nov. **A** fruiting branch with mature cupule **B** abaxial side of mature leaf **C** female flower **D** male flower **E**, **F** outside and inside view of the cupule, respectively **G** infructescence, mature cupule usually solitary **H** mature cupule enclosing 1/4 of nut **I** vertical section of mature fruit **J**–**L** side, top and bottom view of mature nut, respectively. Scale bars: 1 cm (**A**, **B**, **E**–**I**); 1 mm (**C**, **D**).

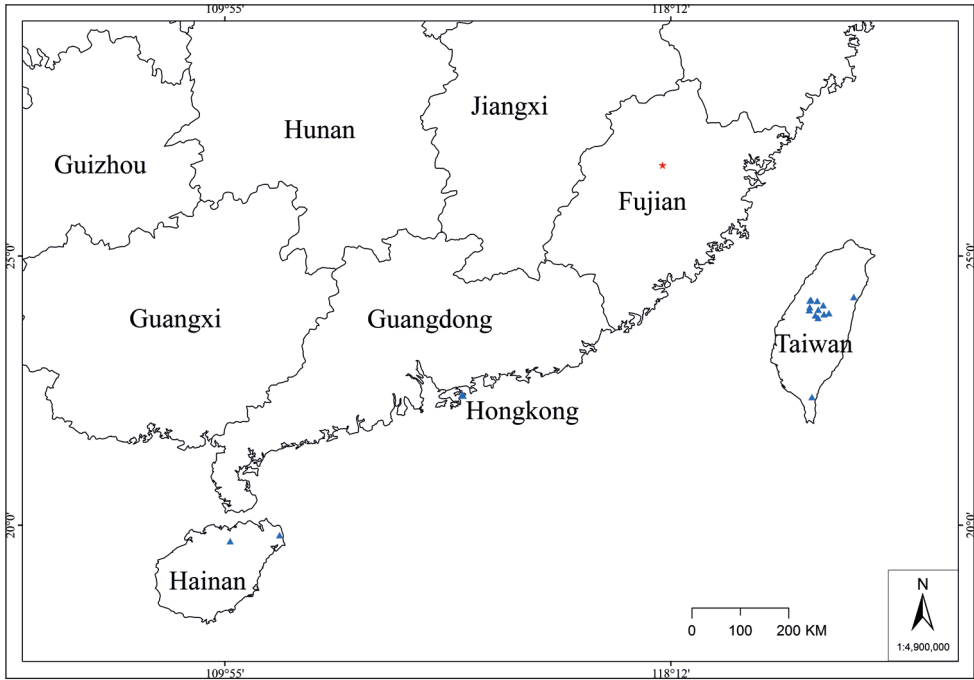


Figure 8. Distribution of *Lithocarpus dahuensis* and *L. konishii* in China. Legend: (red star) *L. dahuensis*, (blue triangle) *L. konishii*.

Diplopterygium chinense (Rosenst.) De Vol (Gleicheniaceae), *Woodwardia japonica* (L. f.) Sm. (Blechnaceae), *Lobelia sessilifolia* Lamb. (Campanulaceae), *Oenanthe linearis* Wall. & DC. (Apiaceae), *Melastoma dodecandrum* Lour. (Melastomataceae), *Carex perakensis* C.B. Clarke (Cyperaceae), *Anoectochilus roxburghii* (Wall.) Lindl. (Orchidaceae), *Tainia dunnii* Rolfe (Orchidaceae) and others; the interlayer plants include *Trachelospermum brevistylum* Hand.-Mazz. (Apocynaceae) and others.

Phynology. Florescence May to June, fruiting season September to October of the following year.

Etymology. Chinese name: 大湖柯 (da hu ke). The epithet *dahuensis* (大湖) refers to Dahu town, Minhou County, Fujian Province where this new species was found.

Taxonomic notes. The following morphological characteristics were used to classify the species, including the acute toothed leaf blade margins, a concave nut scar, and cupules that do not completely enclose the nut. There are four other plants share similar characteristics with *L. dahuensis*, including cupule encrustation (the cupule base is sessile, encasing the base of the nut or about half of it) and a fruit umbilicus (the surrounding margin of the fruit umbilicus is clearly concave), the differences between which are shown in the key.

Conservation status. During our fieldwork from 2017 to 2022, fruit-bearing large trees of *Lithocarpus dahuensis* were only found in the landscape forest of the Xuefeng village valley, Dahu town, Minhou County, Fujian Province, China. They were also found in the surrounding secondary coniferous and broad-leaved mixed forest,

but these were mostly small trees that sprouted after the large trees were felled and did not bear fruit. As the location was discovered to be the only known position, we suggest its placement in the Data Deficient category of IUCN (2022).

Discussion

Phylogenetic analysis was completed on the whole chloroplast genomes, and nrITS sequences of the Fagaceae species. Based on the well-supported phylogenetic trees (Figs 2, 3), *Lithocarpus dahuensis* is a new species most closely related to *L. konishii*. It is worth noting that *L. konishii* is found mainly in the central and southern regions of Taiwan, the eastern regions of Hainan and Hong Kong, all of which are islands. Nevertheless, *L. dahuensis* was found to be endemic in the mountains of central Fujian, separated by a strait and over 385 km from *L. konishii*. *Lithocarpus konishii* is found at altitudes between 100–1150 m, usually 500–700 m (Huang et al. 1999), whereas *L. dahuensis* only occurs at altitudes above 1000 m.

The new species has an overall morphology similar to *Lithocarpus konishii* from Taiwan (Huang et al. 1999). However, there are some obvious differences, especially in the morphology of the leaf, cupules and nuts, such as the oblanceolate leaf blade of *L. dahuensis* has up to 15 pairs of dense lateral veins and 7–10 pairs of acute teeth on the leaf margin from the 2nd–3rd lateral veins above the leaf base, whereas *L. konishii* has 5–8 pairs of obtuse teeth from the 3rd lateral vein above the leaf base. *L. dahuensis* has a smaller cupule that encloses up to 1/4–1/3 of the nut, and its nut is only half as long as those of *L. konishii* (1.4–1.8 vs. 1.8–2.4 cm).

As a result of its simple, stable genetic structure and ease of sequencing, the chloroplast genome has become increasingly popular for species identification, phylogeny reconstruction, demographic history tracing and species divergence studies (Liu et al. 2020). However, genomic information on *Lithocarpus*, particularly the complete chloroplast genome in the NCBI database was very limited. In the current study, we sequenced and assembled the whole chloroplast genome of the new *Lithocarpus* species and found that the chloroplast genome of *L. dahuensis* was 161,303 bp in length, within the expected range (107–218 kb) of most angiosperm chloroplast genomes (Heslop-Harrison 2017), and it had the typical quadripartite structure (Jansen et al. 2005; Daniell et al. 2016). The GC content of *L. dahuensis* chloroplast genome was low (36.75%), which is similar to that reported from other Fagaceae genomes (Yang et al. 2017; Hinsinger and Strijk 2019; Pang et al. 2019).

The chloroplast genome of the new species was compared with the other members of *Lithocarpus* to understand its structural variations and rearrangements. The results showed that all 12 *Lithocarpus* plastomes were remarkably similar in terms of size, genes, and genome structures. Genomic comparison between the species revealed a relatively higher level of divergence in non-coding regions than in coding regions, similar to what has been reported for the genus *Quercus* and *Carya* from the family Fagaceae (Li et al. 2018; Shen et al. 2022). We also identified a considerable number of variations in the

noncoding chloroplast genome sequences, such as *rbcL-accD*, *rpl20-rps12*, *trnK-rps16*, *trnF-ndhJ* and *ccsA-ndhD*. These noncoding sites may be useful in understanding the ecological significance of the species in terms of spatial distribution and adaptability besides the evolutionary relationship of the new species within Fagaceae.

Key to the related species of *Lithocarpus dahuensis*

- 1 Female flower solitary **2**
- Female flowers in clusters of (2 or)3(–5) **4**
- 2 Nut covered with appressed minute hairs..... ***L. quercifolius***
- Nut glabrous..... **3**
- 3 Leaf blade margin with 7–10 acute teeth; cupules enclosing 1/4–1/3 of nut...
..... ***L. dahuensis***
- Leaf blade margin with 3–6 obtuse teeth; cupules enclosing the bottom of
nut..... ***L. konishii***
- 4 Young leaf blade abaxially with tufts of stellate hairs at axils of veins, wall
6–10 mm thick..... ***L. carolineae***
- Young leaf blades covered with appressed minute hairs, wall 10–14 mm
thick **5**
- 5 Leaf blade margin lobate-dentate ***L. cyrtocarpus***
- Leaf blade margin entire or rarely with 1–3 teeth near apex..... ***L. gymnocarpus***

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Supplementary material I

Supplementary data

Authors: Miao Zhang, Xiao-Hui Zhang, Shi Shi, Bing-Hua Chen

Data type: tables and figures

Explanation note: The information of the phylogenetic analysis of the species used in the present study (nrITS). Note: “-” indicate the data is not yet public. The information of the phylogenetic analysis of the species used in the present study (plastid genome). Comparison of the LSC, SSC and IR regions among twelve chloroplast genomes of *Lithocarpus* species. Genes are denoted by colored boxes. The gaps between the genes and boundaries are proportional to the distances in bps. Gene nucleotide variability (pi) values of twelve *Lithocarpus* species. The Y-axis shows the pi values; the X-axis shows the genes.

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A new species of *Hoya* R.Br. (Apocynaceae, Asclepiadoideae) from the Philippines

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Abstract

Hoya medusa M.D.De Leon, Cabactulan, Cuervo & Rodda, **sp. nov.** (Apocynaceae, Asclepiadoideae) is described from the Philippines. Even though numerous taxa with a shrubby habit from this area are known, it can be immediately separated because of its urceolate corolla and prominent elongated corona lobes. No other species in the genus possesses such a combination of characters.

Keywords

Hoya edeni, *Hoya linavergarae*, *Hoya odorata*, Luzon, Marsdeniaceae

Introduction

Hoya R.Br. is a megadiverse genus occurring from the Himalayan foothills to Okinawa (Japan) and through Southeast Asia and Malesia to Australia and the Fiji Islands. Despite being fairly well known in Thailand (Thaithong et al. 2018), Vietnam (Tran 2017), Singapore (Middleton and Rodda 2019), Borneo (Lamb and Rodda 2016;

Rodda and Rahayu 2022) and New Guinea (Cámara-Leret et al. 2020; Rodda and Simonsson 2022), much is still needed to complete larger revisions and incorporate in keys the wealth of new species which keep being described at a fast pace, making published revisions quickly outdated.

Based on the available data (Pelser et al. 2011 onwards; Lamb and Rodda 2016; Cámara-Leret et al. 2020; Rodda and Rahayu 2022; Rodda and Simonsson 2022) the centres of diversity of *Hoya* are Borneo, New Guinea and the Philippines. Species numbers for Borneo and New Guinea will likely keep increasing as new species are described (Rodda and Rahayu 2022; Rodda and Simonsson 2022) but since most species are well known we do not foresee large numbers of synonyms. In the Philippines, Pelsner et al. (2011 onwards) list as many as 202 *Hoya* taxa, but state that ‘The number of *Hoya* species in the Philippines is doubtlessly much lower than suggested by this account’. We agree that many names will become synonyms once the genus is revised. Based on IPNI [<https://www.ipni.org>, accessed on 2 Dec 2022], since 2010, 352 new *Hoya* taxa were published, of which 165 from the Philippines. However, most of these names have been published in non-peer-reviewed publications and their types shall be carefully examined before they can be considered accepted taxa. For most of these names, descriptions are brief, the illustrations provided with the publication are insufficient, and type citation is generally limited to a herbarium sheet number which upon closer examination might be a collector number or even the collection date of the specimen (Rodda 2015; Rodda 2017).

Within the past 20 years numerous species with a shrubby or pendulous (non-twining) habit have been published in *Hoya* (Rahayu and Rodda 2020), and many of them come from the Philippines: *Hoya amrita* Kloppenb., Siar & Ferreras, *H. cembra* Kloppenb., *H. cumingiana* Decne., *H. densifolia* Turcz., *H. densifolia* Turcz. subsp. *panchoi* Kloppenb., *H. golamcoana* Kloppenb., *H. irisiae* Ferreras, Kloppenb. & Tandang, *H. lazaroi* Kloppenb. & Siar, *H. linapauliana* Kloppenb., *H. linavergarae* Kloppenb. & Siar, *H. maquilangensis* Kloppenb., *H. multiflora* Blume, *H. nova* Kloppenb., *H. odorata* Schltr. (with six recently published subspecies), *H. paziae* Kloppenb., and *H. platycaulis* Simonsson & Rodda.

Many of these names will require extensive investigations to verify if they are applicable.

Yet, extraordinary novelties can still be found in the Philippines. In 2018, plants of a shrubby species were found in cultivation. Based on photographs of the flowers, the last author initially thought that this species might be a member of Apocynaceae subfamily Periplocoideae due to the elongated corona lobes (which were mistaken for anther appendages). Upon closer examination of the corona and pollinarium it became clear that it nevertheless belongs to *Hoya* as the corona lobes have basal revolute margins, and the pollinia have the distinct sterile edges diagnostic of *Hoya* (Wanntorp and Kunze 2009). The urceolate corolla combined with prominent elongated corona lobes make this taxon unique among all species of *Hoya*. Comparison with previously described species of *Hoya* was carried out in person or via loans or photographs from A, BM, BO, FI, K, LAE, MO, P, PNH, SING, and UC herbaria (Thiers 2023).

Species treatment

***Hoya medusa* M.D.De Leon, Cabactulan, Cuervo & Rodda, sp. nov.**

urn:lsid:ipni.org:names:77315879-1

Figs 1, 2

Diagnosis. Similar to *Hoya edeni* King & Hook.f. in its shrubby habit and caudate and curved inner corona lobe processes, distinct by the corona processes (wavy to serpentine in *H. medusa* versus linear and curved at the tip in *H. edeni*), corolla shape (urceolate in *H. medusa* versus rotate with reflexed lobes in *H. edeni*) and size (8.5–10 mm in *H. medusa* versus c. 20 mm in *H. edeni*).

Type. PHILIPPINES. Luzon, Cagayan Province, Mt. Cetaceo 500 to 1,000 m, vouchered in cultivation, 07 Feb 2022, *M.D. De Leon s.n.* (holotype: PNH, sheet no. 258696).

Description. *Plant* epiphytic pendent shrub, with white exudate in all vegetative parts. *Roots* basal, fibrous. *Stems* slender, terete, 1.5–7 mm in diam., green, sparsely puberulent when immature, turning lignified, brown and glabrescent when mature, *internodes* 2.8–6 cm long. *Leaves:* petiole stout, usually curved, canaliculate above, flattened towards lamina base, 3–7 mm long, 1.5–2.0 mm in diameter, dark green, sparsely pubescent turning glabrescent when old; blades coriaceous, stiff, flat to slightly curved, variable in shape, oblong, ovate, to elliptic-ovate, 55–90 mm long × 20–34 mm wide; base acute to obtuse, apex acute to acuminate, with a caudate tip, adaxially mid-green, abaxially light green; margins entire, occasionally slightly undulate; adaxially and abaxially sparsely pubescent to glabrescent in older leaves; venation pinnate-brochidodromous, with 4–8 lateral veins on each sides of mid vein, prominent (very pale green) on younger leaves and obscure on older leaves yet clearly visible on dried specimens; colleters one at each lamina base, conical, c. 0.20 mm long, grayish brown. *Inflorescence* extra-axillary, umbelliform, convex, with up to 11 flowers (occasionally up to 16 flowers in particularly strong cultivated specimens). *Peduncle* short stout or sometimes sessile, cylindrical, 1.0–3.0 mm long and 1.0–2.0 mm in diam., perennial, sparsely pubescent. *Pedicels* terete, outer pedicels slightly curved, otherwise straight, 8–10 mm long and 0.5–0.8 mm in diam., light green, lenticellate except the base of the calyx which is pubescent. *Calyx* lobes oblong, 1.8–2 mm long, c. 0.50 mm wide, reddish, abaxially strigose, adaxially glabrous, basal colleters 1 between each calyx lobes, oblong, c. 0.02 mm long × c. 0.02 mm wide. *Corolla* basally urceolate, with flat spreading lobes, white, corolla lobes spreading, 8.5 to 10 mm in diam. across the corolla lobes, corolla tube 2.0–3.0 mm long, 4–5 mm in diameter, lobes triangular ovate, 2.5–3.0 mm long, 2.5–3.5 mm wide, apex acute, slightly revolute, margins revolute, corolla lobes adaxially densely strigose, densely hirsute towards the rim and interior of the tube and the column, abaxially glabrous; *gynostegium* stipitate; *column* cylindrical, 0.50–0.70 mm high × c. 0.50 mm in diam.; *corona* staminal 4–5 mm high, c. 3 mm in diam.; *corona lobe* bulbous-obpyriform, c. 3.5 mm long × 0.5–0.7 mm wide, inner (apical) process caudate, upright, curved, wavy to serpentine, meeting at

the center and overlapping, erect above the gynostegium, outer (basal) process obovate, with basal revolute margins, guide rail raised, laterally compressed, prominent at the base of the corona lobes c. 0.80 mm long and extending c. 0.20 mm laterally. **Pollinia** erect, elliptic-oblong, c. 0.50 mm long, c. 0.22 mm wide; **caudicle** obovoid, c. 0.10 mm diam; **corpusculum** oblong, c. 3.0 mm long by c. 1.0 mm wide; **ovary** ovoid, c. 1.5 mm long, each carpel c. 0.9 mm wide at the base, glabrous. **Fruit and seed** not seen. Flowers vespertine lasting up to 15 days in cultivation, drying of the inner corona processes begins on the second day causing brown to black discoloration, flowers slightly fragrant, fragrance floral, powdery scent or mild jasmine persistent throughout the day and night.

Etymology. The specific epithet refers to the serpentine processes of the inner corona reminiscent of the snake headdress of Medusa in Greek mythology.

Distribution and ecology. *Hoya medusa* was collected by local collectors in Luzon Island, Mt. Cetaceo and has been in cultivation, circulated by local plant nurseries and plant hobbyists. It was first collected in low montane forest at 500 to 1,000 m where it was growing as an epiphyte in disturbed primary broadly leaf and mossy forest in full sun to part shade.

Conservation status. The forested area where *Hoya medusa* was collected is threatened by habitat destruction due to extensive harvesting of trees for local housing and wood-fuel use, destructive farming practices such as “*kaingin*” (slash and burn farming in Filipino), land conversion and illegal commercial logging. The type locality is a heavily logged forest. Parts of the deforested areas had been converted to agricultural land for crops such as corn, cassava, and vegetables. The western portion of the area is near a protected forested area and remains intact but it is endangered by rapid expansion of agricultural lands by the lowland settlements.

Hoya medusa is only known from a single collection and we do not have information on the extant population size; therefore, the conservation status is proposed as Data Deficient (DD, IUCN Standards and Petitions Committee (2022)) until more information is known about its area of occurrence.

Notes. *Hoya medusa* displays an unusual combination of characters, in particular shrubby habit and corolla urceolate, with flat spreading lobes. This sets it apart from all other shrubby species of *Hoya*. In the diagnosis we compared it to *Hoya edeni* because both species are shrubs and present somewhat similar inner corona lobe processes, yet they present very different corolla morphology. The only other species with a similar corolla shape (salverform, with tube narrowly campanulate) is *Hoya kachinensis* Rodda & K.Armstr. from Myanmar, which is a climber with oblanceolate, ca. 25 long leaves and therefore clearly distinct from *H. medusa*. Other species that have a somewhat similar corolla shape are *Hoya telosmoides* Omlor from Borneo, and *H. versteegii* Simonsson & Rodda from New Guinea, which are both twining climbers. The only species with a tubular/urceolate corolla, somewhat spreading but much reduced corolla lobes as well as a shrubby habit is *Hoya manipurensis* Deb from India, Myanmar, China and Thailand. *Hoya manipurensis* has obcordate to triangular laminas and has corolla lobes much shorter than corolla tube, making it once again very easily separated from *H. medusa*.

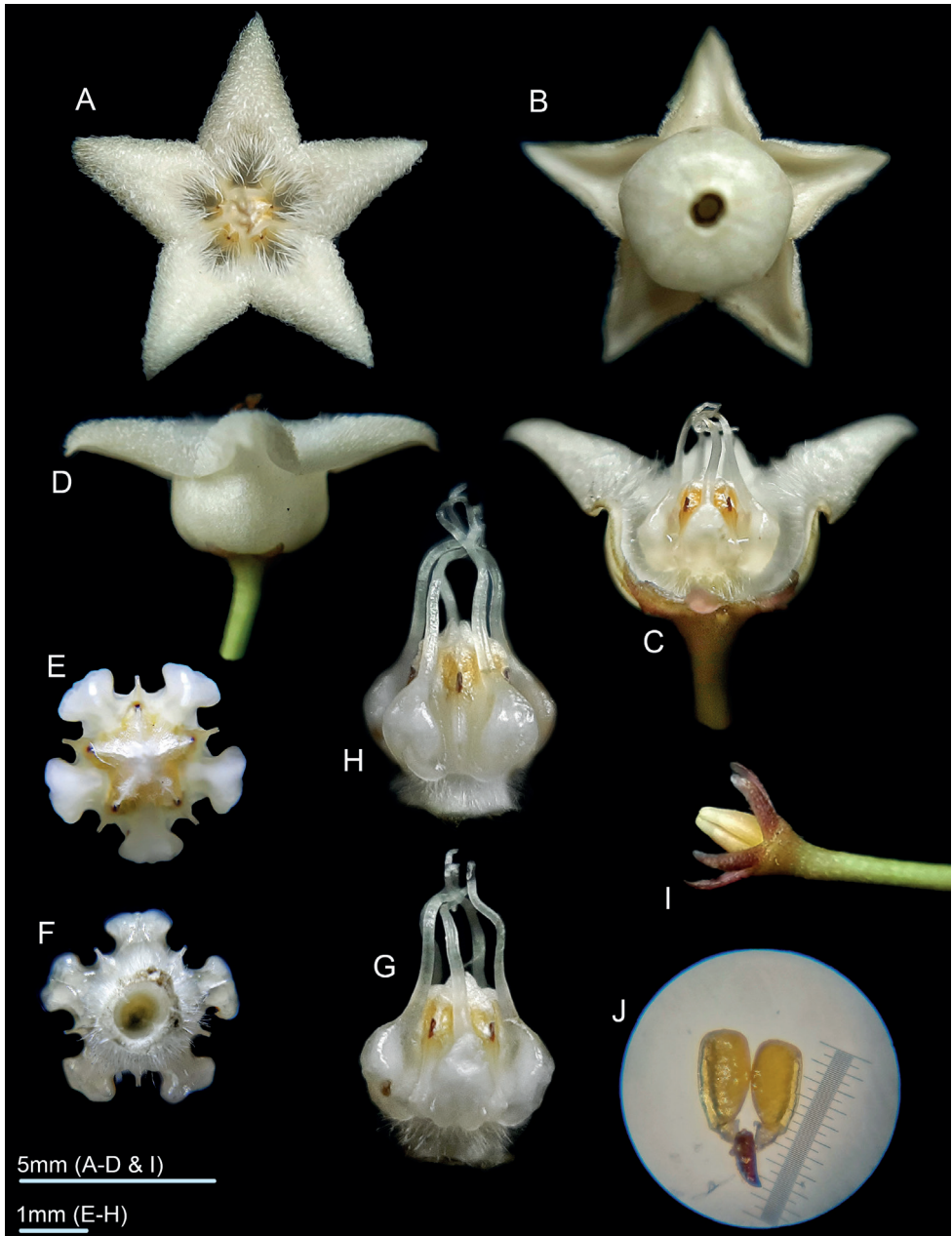


Figure 1. *Hoya medusa* photographed in cultivation **A** flower, top view **B** corolla, from underneath **C** flower, side view two corolla lobes removed **D** flower, side view **E** corona, top view **F** corona, from underneath **G, H** corona, side view **I** calyx and ovaries **J** pollinarium. (Photographs by R.D. Cuervo).

Among other species of *Hoya* occurring in the Philippines *Hoya medusa* is somewhat similar to *Hoya linavergarae* and *Hoya odorata*. The leaf margins of *H. medusa* are slightly undulate, whereas undulate in *H. linavergarae*, and flat in *H. odorata*.



Figure 2. *Hoya medusa* flowering branch. (Photograph by M.D. De Leon).

The flowers of *H. medusa* are smaller (8.5 to 10 mm) and basally urceolate with spreading lobes, whereas *H. linavergaræ* and *H. odorata* have larger flowers (15–23 mm and 13–18 mm respectively), and basally campanulate (*H. linavergaræ*), or rotate (*H. odorata*) with inflexed corolla lobes. Further, the corolla of *H. medusa* is densely hirsute inside, whereas puberulent in *H. linavergaræ* and *H. odorata*. The inner corona processes of *H. medusa* have a caudate, upright, curved, wavy to serpentine appendage, whereas the inner corona processes of *H. linavergaræ* and *H. odorata* do not have an appendage.

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Resurrection of *Leucobryum scalare* Müll.Hal. ex M.Fleisch. (Bryophyta, Leucobryaceae) based on phylogenetic and morphometric evidence

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Abstract

Leucobryum scalare was described in 1904 but its taxonomic status has been disputed, being reduced to a variety of *Leucobryum aduncum* or synonymized with *Leucobryum aduncum*. The taxonomic confusion of this taxon has remained unresolved. Hence, we revisited the taxonomic status of the taxon using phylogenetic and morphometric approaches. A total of 27 samples from *Leucobryum aduncum* var. *aduncum* and *Leucobryum aduncum* var. *scalare* were used to generate data from four markers, including ITS1, ITS2, *atpB-rbcL* spacer, and *trnL-trnF*. The concatenated dataset was used to reconstruct a phylogenetic tree. Both qualitative and quantitative morphological characters were measured and analyzed with Principal Component Analysis (PCA) and PERMANOVA. The results showed that the two taxa are closely related but they are reciprocally monophyletic. Both qualitative and quantitative characters could also separate *Leucobryum aduncum* var. *scalare* from *Leucobryum aduncum* var. *aduncum* as shown with PCA and PERMANOVA. We propose the resurrection of the species rank for *Leucobryum scalare* as separate from *Leucobryum aduncum*. This work highlights the need for a more thorough revision of *Leucobryum* to clarify the actual level of diversity in this genus.

Keywords

Bryophytes, classification, mosses, revision, tropical biodiversity

Introduction

Bryophytes are small land plants with simple morphology and tend to be widely distributed (Schofield and Crum 1972). Many recent phylogenetic studies have demonstrated that taxa with overlapping distributions and indistinct morphologies often consist of two or more cryptic taxa (Shaw et al. 1988; Miwa et al. 2009; Hedenäs 2020). Cryptic species in bryophytes have been attributed to the recent divergences, stasis, parallelisms, reductions, and convergences in morphological characters (Vanderpoorten and Shaw 2010; Renner 2020). With increased access to molecular data, more cases of cryptic species have been identified and have led to the description of new or resurrection of previously disregarded taxa (Shaw 2001; Renner 2020). Recognizing cryptic species is essential for understanding species diversity and speciation rates, which are essential for understanding evolutionary processes and developing effective conservation strategies (Struck et al. 2018).

Similar to many moss genera, the moss genus *Leucobryum* Hampe has been shown to include several cryptic species (Oguri et al. 2006; Oguri et al. 2010; Oguri et al. 2013; Bonfim Santos and Stech 2017). The genus currently includes about 80–100 species worldwide with predominantly temperate and tropical distribution (Eddy 1990; Enroth 1990; Klazenga 2012). The important characters are white to whitish green in color and forming cushion-like colonies. Leaves are packed in a spiral arrangement and are composed of one median row of chlorophyllose cells alternated with two-row hyalocytes in the cross-section. Sporophytes have an inclined cylindrical capsule and long-rostrate operculum. (Gradstein et al. 2001; Yamaguchi 1993). Although several taxonomic studies on *Leucobryum* are available for several countries in Southeast Asia (Gangulee 1971; Eddy 1990; Enroth 1990; Yamaguchi 1993; Lin and He 1999), species classification and identification of the species remain difficult. Many species in this genus exhibit a high degree of morphological variations and overlaps among species, causing taxonomic confusion (Enroth 1990).

Among *Leucobryum* species in Southeast Asia, *Leucobryum aduncum* Dozy & Molk. and *L. scalare* Müll.Hal. ex M.Fleisch. are the most problematic due to their broadly overlapping morphologies and distributions (Fig. 1). *Leucobryum aduncum* was first described in 1854 based on a type specimen collected in Java, Indonesia (Dozy and Molkenboer 1854). The name *L. scalare* appeared in 1900 in Édouard Gabriel Paris's Index Bryologicus, ascribed to Karl Müller, who cited specimens from the Philippines. Max Fleischer later officially described the name in 1904 (Paris 1900; Fleischer 1904). In 1990, Alan Eddy examined specimens from Malaysia and reduced the name to the variety rank as *L. aduncum* var. *scalare* (Müll.Hal. ex Fleisch.) A. Eddy (Eddy 1990). In the same year, however, the name *L. scalare* was also synonymized with *L. aduncum* by Johannes Enroth, who studied Leucobryaceae in the Huon Peninsula, Papua New Guinea. He noticed that the relative length of the inner perichaetial leaves around the sporophytes of *L. scalare* was around the same size as that of *L. aduncum* (Enroth

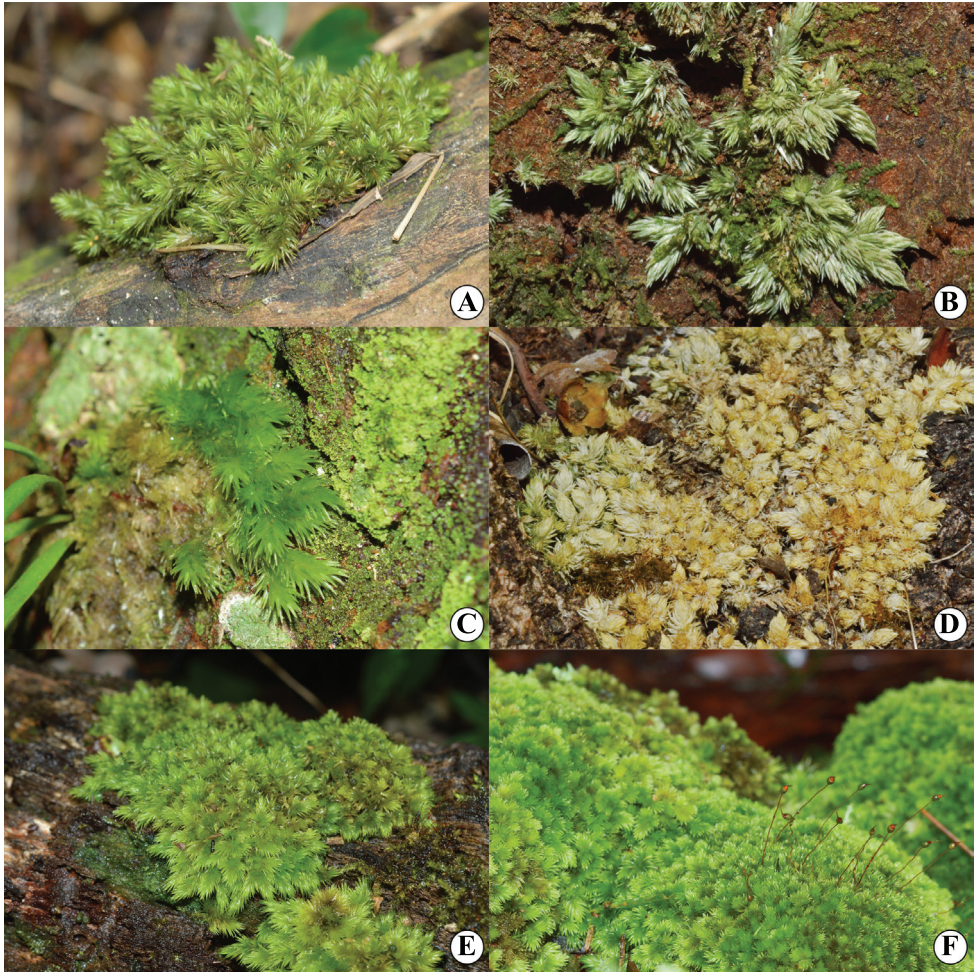


Figure 1. Variation of different population in *Leucobryum aduncum* and *L. scalare* **A, C, E** *Leucobryum aduncum* **B, D, F** *Leucobryum scalare*.

1990). Both classifications (as variety or as synonym) have been used since then interchangeably. No detailed morphological study or molecular work has been conducted to clarify the position of the name *L. scalare*.

Therefore, we aim to clarify the taxonomic status of *Leucobryum aduncum* var. *scalare* based on morphology and molecular phylogeny. We obtained detailed morphological data from the herbarium collection and new samples of *L. aduncum* var. *aduncum* and *L. aduncum* var. *scalare* from Southeast Asia to perform statistical classification. We also generated DNA sequences from these specimens to reconstruct a molecular phylogeny to better understand the taxonomic status of *L. scalare*.

Materials and methods

Plant samples

A total of 27 samples of *L. aduncum* var. *aduncum* (13 samples) and *L. aduncum* var. *scalare* (14 samples), collected from various locations from 2015–2020 (Appendix 1), were used for reconstructing phylogeny and morphometrics. The specimens were identified based on their morphology as described in relevant taxonomic literature (Eddy 1990; Yamaguchi 1993; Lin and He 1999).

Morphology and morphometrics

Gametophytes were investigated for qualitative and quantitative characters using a Motic SMZ-171 stereomicroscope and Motic BA310E biological microscope. The terminology of morphological characters primarily followed those from Yamaguchi (1993) and Malcolm and Malcolm (2006). The quantitative characters (Fig. 2) included gametophyte height (cm), stem diameter (μm), leaf length (mm), leaf width (mm), leaf ratio (length to width ratio), lamina width (μm), lamina cell length (μm), lamina cell width (μm), border cell length (μm) and border cell width (μm). A Canon EOS500D digital camera and the EOS Utility V. 3 software for the automatic image were used to take images of leaves and cells. Measurements were taken from these leaf and cell images using the Fiji V. 1.53s software (Schindelin et al. 2012).

Each quantitative character of the two taxa was compared visually with a boxplot and statistically using a Wilcoxon's test (David 1972). Then, all quantitative data were subjected to a Principal Component Analysis (PCA) to determine whether the combined information corresponded with the two taxa. PERMANOVA was used to test for differences between the two taxa with a multivariate dataset. All morphometric analyses were performed in the R program V. 3.6.1 (R Core Team 2019).

Phylogenetic analyses

Genomic DNA was extracted from the samples using the NucleoSpin Plant II Kit (Macherey-Nagel GmbH & Co. KG, Germany) following the manufacturer's user manual. A sample was homogenized by grinding dried samples in liquid nitrogen. Four regions, including ITS1, ITS2, *atpB-rbcL* spacer, and *trnL-trnF*, were amplified with Polymerase Chain Reactions (PCR) using the primers and conditions in Bonfim Santos and Stech (2017) (Appendix 2). The cleaned PCR products were sent to Macrogen Inc. (www.macrogen.com, Seoul, South Korea) to perform Sanger sequencing. The chromatograms and nucleotide sequence data were then sent back for manual assembly using the Geneious Prime v.2022.0.1 (www.geneious.com).

The corresponding sequences of *L. candidum* (Brid. ex P. Beauv.) Wilson (*HIRO* 203728: AB285170, AB288196, AB742389) and *L. chlorophyllosum* Müll. Hal. (*HIRO* 140710: AB125291, AB124792, AB742390; *HIRO* 140820: AB763361, AB739636, AB742391; *MAK* B119208: AB763362, AB739637, AB742392), avail-

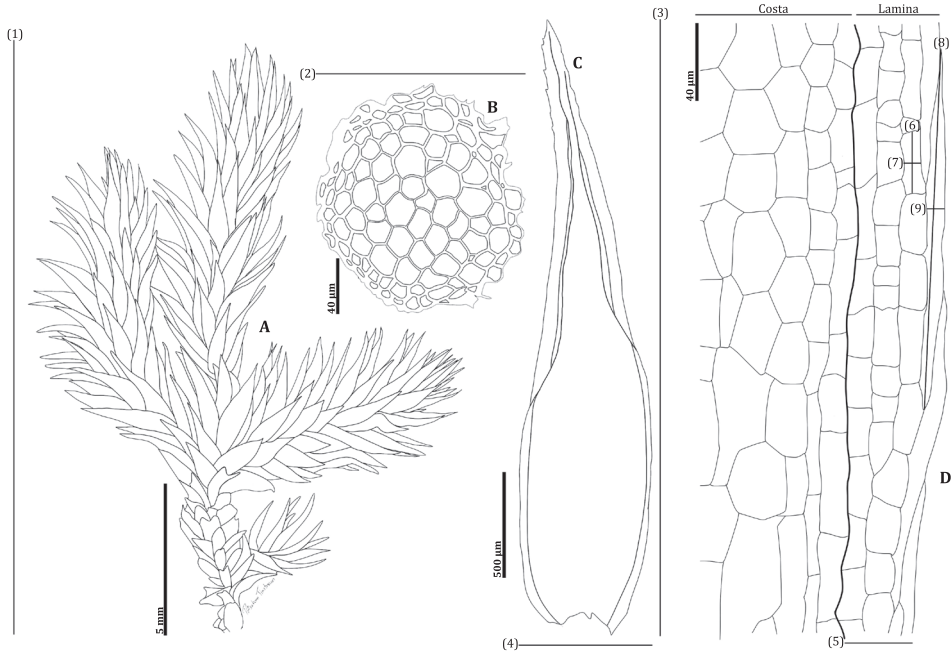


Figure 2. Diagram showing the morphological characteristics **A** gametophyte **B** cross-section of stem **C** leaf **D** costa and lamina. Measuring the quantitative characteristics (1) gametophyte height (2) stem diameter (3) leaf length (4) leaf width (5) lamina width (6) lamina cell length (7) Lamina cell width (8) border cell length (9) border cell width.

able in the NCBI database, were selected as an outgroup based on Bonfim Santos and Stech (2017). The sequences of *L. aduncum* in the NCBI database, the newly generated *L. aduncum* sequences of two varieties and the outgroup sequences (Appendix 1) were aligned to their corresponding homologous position using the MUSCLE algorithm (Edgar 2004) available in Geneious Prime v.2022.0.1 (Biomatters Ltd., Auckland, New Zealand) (<https://www.geneious.com>). Phylogenetic trees were constructed using the maximum likelihood (ML) and Bayesian inference (BI) methods available on HPC, Faculty of Science, Kasetsart University, Thailand. ML trees were constructed using RAxML v. 8.2.12 (Stamatakis 2014), and the branch support value of the ML tree was estimated by the bootstrap algorithm with 1,000 bootstrap replicates. BI tree was constructed by MrBayes v.3.2 (Ronquist et al. 2012) with the Bayesian posterior probabilities calculated using the Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) method. Four chains (three heated and one cold) with the temperature set to 0.2 were run for 20,000,000 generations, with chains sampled every 1000 trees. Twenty-five percent of the posterior trees were discarded as burn-in. The phylogenetic trees were then visualized, adjusted, and produced using Figtree ver. 1.4.4 (Rambaut 2018). Bootstrap support (BS) of 70 or greater from the ML analysis and posterior probability (PP) of 0.9 or greater from the BI analysis were considered strong support for a clade.

Results

Morphology and morphometrics

Variation of Qualitative Characters – Gametophytes of *L. aduncum* var. *scalare* are relatively small and often form a compact cushion with dense branches. The habitat is in open sites on tree trunks or logs, rarely on branches or rocks. Meanwhile, the tuft form of *L. aduncum* var. *aduncum* is small- to medium-sized with little branching, but usually, several branches can be found in small gametophytes. The habitat is in shaded sites on logs, tree trunks, humus, or rocks. Both taxa lack a central strand. When dry, the plants are yellowish green to whitish green and brown (Figs 6–9). *Leucobryum aduncum* var. *aduncum* and *L. aduncum* var. *scalare* are readily distinguished by their leaf arrangement, orientation, and shape. The leaves of *L. aduncum* var. *scalare* are spirally arranged and closely imbricate, forming a conical point at the shoot apex, especially when dry. The leaves are erect but sometime falcate-secund when growing near the substrate. The leaf shape is lanceolate to narrowly lanceolate with an oblong to ovate base (Figs 8, 9). In contrast, the leaves of *L. aduncum* var. *aduncum* are not spirally arranged and do not form a conical point. The leaves are markedly falcate-secund and sometimes slightly erect. The leaf shape is lanceolate with an ovate to oblong base (Figs 6, 7).

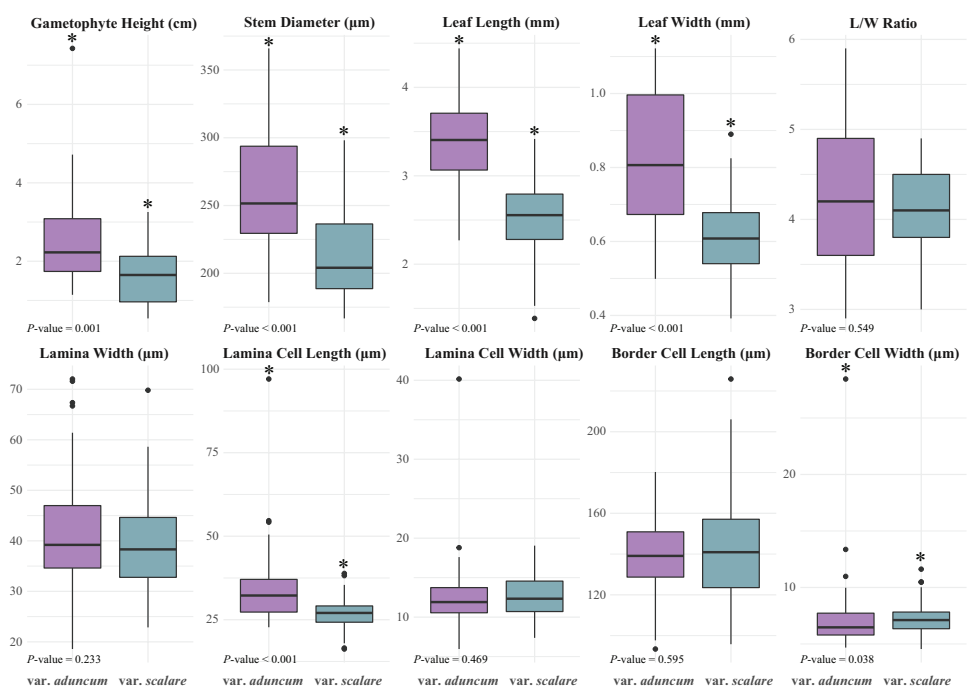


Figure 3. Boxplot showing variation on the ten quantitative characters in the two varieties of *Leucobryum aduncum*. The boxes represent the data from the 25th to 75th percentiles, with the median at the line in the box. The black dots outside the bar represent the “outlier” data points. All P-values are from Wilcoxon’s test between the two varieties, and the two varieties are considered significantly different in that trait at $P\text{-value} \leq 0.05$.

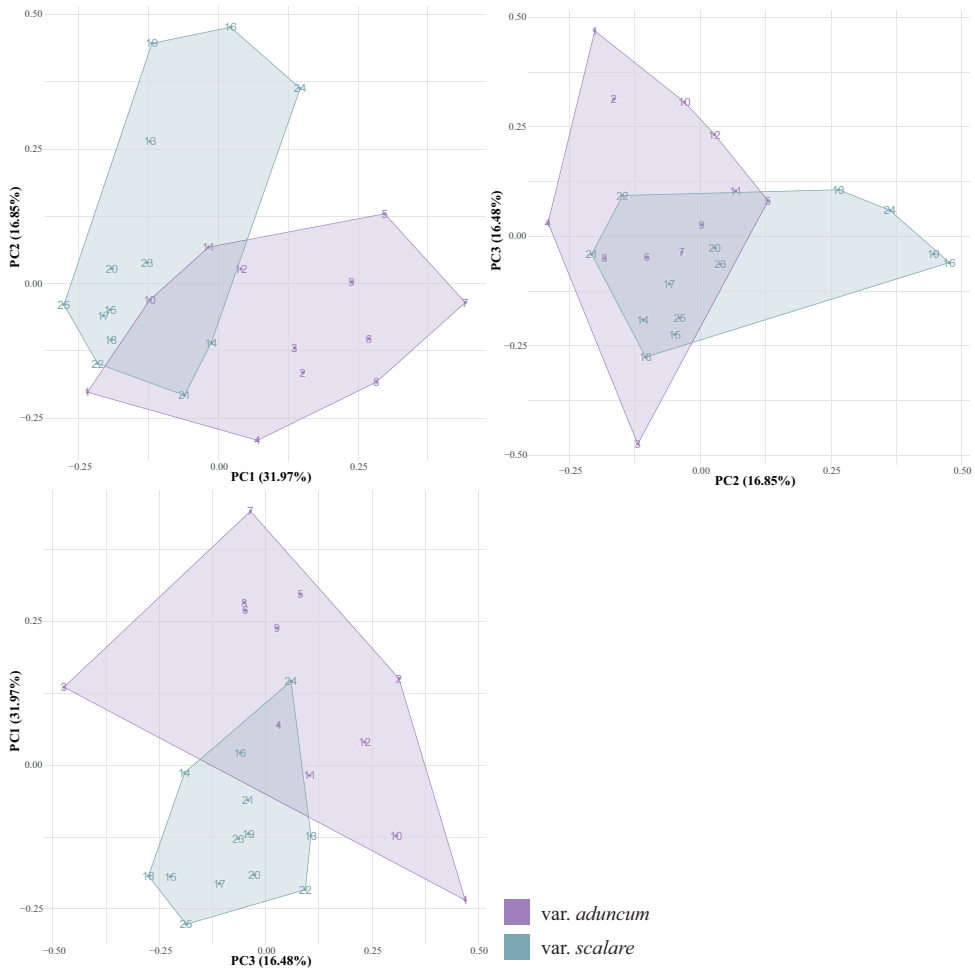
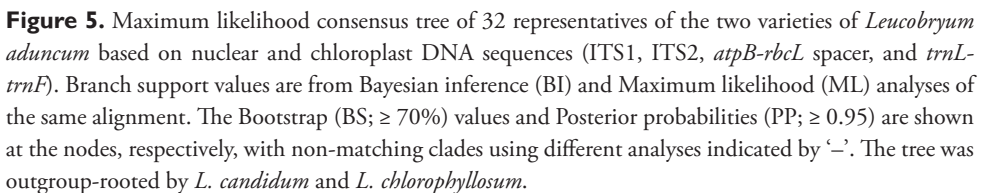


Figure 4. Principal component analysis (PCA) plot based on the ten quantitative characters of the two varieties of *Leucobryum aduncum*. The groups are significantly different at P -values = 0.001 (PERMANOVA).

Variation of Quantitative Characters – *Leucobryum aduncum* var. *scalare* and *L. aduncum* var. *aduncum* were significantly different in the following six morphological characters: gametophyte height, stem diameter, leaf length, leaf width, lamina cell length, and lamina cell width (Fig. 3). Gametophyte height, stem diameter, leaf length, leaf width, and lamina cell length of *L. aduncum* var. *scalare* were smaller than those of *L. aduncum* var. *aduncum*. The other four quantitative characters (leaf ratio, lamina width, lamina cell width, and border cell length) showed no significant differences between the two taxa.

PCA Analysis – The first three principal components (PC I, II, III) from the analysis with ten morphological characters accounted for 31.97%, 16.85%, and 16.48% of the variance, respectively (Fig. 4). All combinations of the first three principal components showed that *L. aduncum* var. *scalare* were separated



Phylogenetic analyses

A total of 106 new sequences from nuclear and chloroplast markers (ITS1, ITS2, *atpB-rbcL* spacer, and *trnL-trnF* regions) were generated in the current study and aligned with existing sequences of *L. aduncum* sequences (five samples) and the outgroup (*Leucobryum candidum* and *L. chlorophyllosum*) available in the NCBI database (Appendix 1). A matrix of 1,644 nucleotide characters was aligned, and

1,310 characters (79.7%) in the alignment were conserved sites. For the nuclear regions, the aligned sequences of ITS1 and ITS2 had a length of 376 base pairs with 219 constant characters (58.2%) and 283 base pairs with 232 constant characters (82%), respectively. For the chloroplast regions, the aligned sequences of *atpB-rbcL* spacer and *trnL-trnF* had a length of 579 base pairs with 563 constant characters (97.2%) and 406 base pairs with 376 constant characters (92.6%), respectively. Because the topologies of the phylogenetic trees constructed from the nuclear and chloroplast regions did not show any strongly supported conflicts in both the ML and BI analyses, only the topology of the ML consensus tree was shown here with the posterior probability of the BI analysis added (Fig. 5). The samples of *L. aduncum* were split into two well-supported sister clades: *Leucobryum aduncum* var. *aduncum* clade and *L. aduncum* var. *scalare* clade (BS 86%, PP 0.99). This result demonstrated that *L. aduncum* var. *scalare* and *L. aduncum* var. *aduncum* were reciprocally monophyletic and could be considered two separate species. Given the support from morphological and molecular data, we propose the resurrection of the name *Leucobryum scalare* Müll.Hal. ex M.Fleisch. at the species level with the revised descriptions as follows.

Taxonomic treatment

***Leucobryum aduncum* Dozy & Molk., Pl. Jungh. 3: 319. 1854.**

Figs 6, 7

Type. Indonesia. Java: *Junghuhn s.n.* (lectotype, designated by Yamaguchi 1993, pg. 31: L; isolectotype: L).

Description. Gametophytes usually form tufts, small to medium size, 1–8 cm long with leaves, yellowish green to whitish green, brown when dry. Stems erect, less branched, usually with several branches in small gametophytes; central strand absent in cross-section of stems. Leaves falcate-secund, sometimes slightly erect, 2.3–4.4 mm long, 0.5–1.1 mm wide, lanceolate, gradually narrowed to subtubulous point from ovate to oblong base, cuneate, margin entire, acute at the apex, undulate and spinosely prorate on abaxial surface; laminae consisting 1–4 rows, lamina cells quadrate to narrowly rectangular, thin-walled; borders consisting 1–3 rows, border cells linear to fusiform, thin-walled; in cross-section of leaf base hyalocytes in 1–2 rows on the adaxial side and 2–3 rows on abaxial side, if 3 rows, usually consisting 1 large row and 2 small rows; adaxial and abaxial side of median leaves consisting 1 row. Dioicous. Perichaetia terminal on short branches; perichaetial leaves around sporophytes shorter than ordinary leaves, ovate to lanceolate, abruptly narrowed to the point, cucullate, acuminate at apex. Sporophytes dicranoid. Setae elongate, erect, 1.7–2.1 cm long. Capsules ovoid to ellipsoid, inclined, 1–2 mm long, 0.5–0.6 mm diameter; opercula long rostrate; peristomes dicranoid. Calyptra cucullate.

Habitat. Usually found in more shaded sites with high moisture, on logs, tree trunks, humus, and rocks.



Figure 6. Gametophyte variation of different populations of *Leucobryum aduncum* Dozy & Molk.

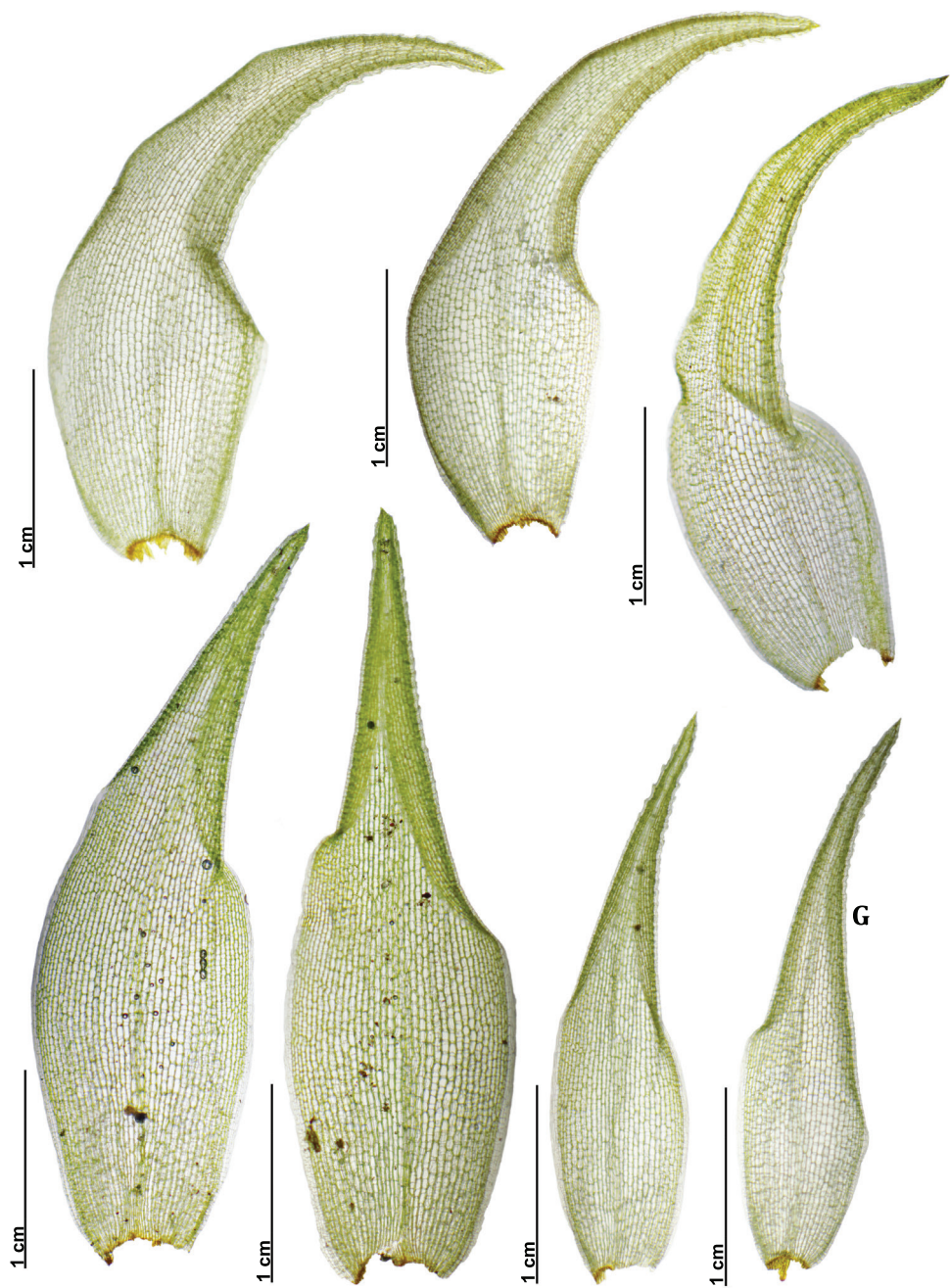


Figure 7. Leaf shape and size variation from different populations of *Leucobryum aduncum* Dozy & Molk.

Distribution. Mainland China, India, Nepal, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, Peninsular Malaysia, Singapore, Philippines, Borneo, Sulawesi, Sumatra, Java, Lesser Sunda Islands, Seram, New Guinea (Yamaguchi 1993; He 1995).

Illustrations. Eddy 1990 (fig. 169A–F); Yamaguchi 1993 (Pls. XV, 1–16; XVI, 1–11).

***Leucobryum scalare* Müll.Hal. ex M.Fleisch., Musci Buitenzorg 1: 143. 1904.**

Figs 8, 9

Leucobryum aduncum var. *scalare* (Müll.Hal. ex M.Fleisch.) A.Eddy, Handb. Males. Mosses 2: 11. 1990, syn. nov.

Type. The Philippines. Luzon, Benguet: 5000 ft. alt., *W. Micholitz 173* (lectotype, designated by Yamaguchi 1993, pg. 33: FH! [00290301]).

Leucobryum perichaetiale Dixon, J. Siam Soc., Nat. Hist. Suppl. 9(1): 11. 1932. Type: Thailand. Northern, Doi Suthep, ca. 1500 m alt., 6 Sept. 1914. *Kerr s.n. (in herb. Dixon, ref. no. 8)* (holotype: BM [BM000866895]).

Leucobryum microleucophanoides Dixon ex A. Johnson, Gard. Bull. Singapore 20: 333. f. 9: m, 12. 1964. Type: Peninsular Malaysia. Kedah, Inchang Estate, on the decaying trunk, 24 Apr. 1940. *Spare s.n. (in herb. Dixon, ref. no. 2941)* (holotype: BM [BM000866907]).

Description. Gametophytes usually form a small compact cushion, 0.5–3.3 cm long with leaves, yellowish green to whitish green, and brown to dark brown when dry. Stems erect, with many short branches, usually very dense; central strand absent in cross-section of stems. Leaves spiral and closely imbricate, forming a conical point at shoot apex when dry, 1.4–3.4 mm long, 0.4–0.9 mm wide, lanceolate to narrowly lanceolate, gradually or abruptly narrowed to subtubulous point from oblong to ovate base, cuneate, margin entire, acute at the apex, undulate and spinosely prorate on abaxial surface, sometimes undulate and papillosely prorate; laminae consisting 1–3 rows, lamina cells quadrate to rectangular, thin-walled; borders consisting 1–3 rows, border cells linear to narrowly fusiform, thin-walled; in cross-section of leaf base hyalocytes in 1–2 rows on the adaxial side and 2–3 rows on abaxial side, if 3 rows, usually 2 large rows and 1 small row; adaxial and abaxial side of median leaves consisting 1 row. Dioicous. Perichaetia terminal on short or long lateral branches; perichaetial leaves around sporophytes longer than ordinary leaves, ovate to lanceolate, abruptly slender to the point, cucullate, acuminate at apex. Sporophytes dicranoid. Setae elongate, erect, 1.5–1.7 cm long. Capsules subglobose to ovoid, inclined, 1.0–1.5 mm long, 0.4–0.5 mm diameter; opercula long rostrate; peristomes dicranoid. Calyptra cucullate.

Habitat. Usually found in more open sites, on logs, tree trunks, tree bases, branches, and rocks.

Distribution. Mainland China, India, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Peninsular Malaysia, Philippines, Borneo, Sumatra, Java, Seram, New Guinea, and New Caledonia (Yamaguchi 1992; He 1995).



Figure 8. Gametophyte variation of different populations of *Leucobryum scalare* Müll.Hal. ex M.Fleisch.

Illustrations. Eddy 1990 (fig. 170A–E); Yamaguchi 1993 (Pls. XIX, 1–28; XX, 1–11; XXI, 1–15; XXII, 1–26).

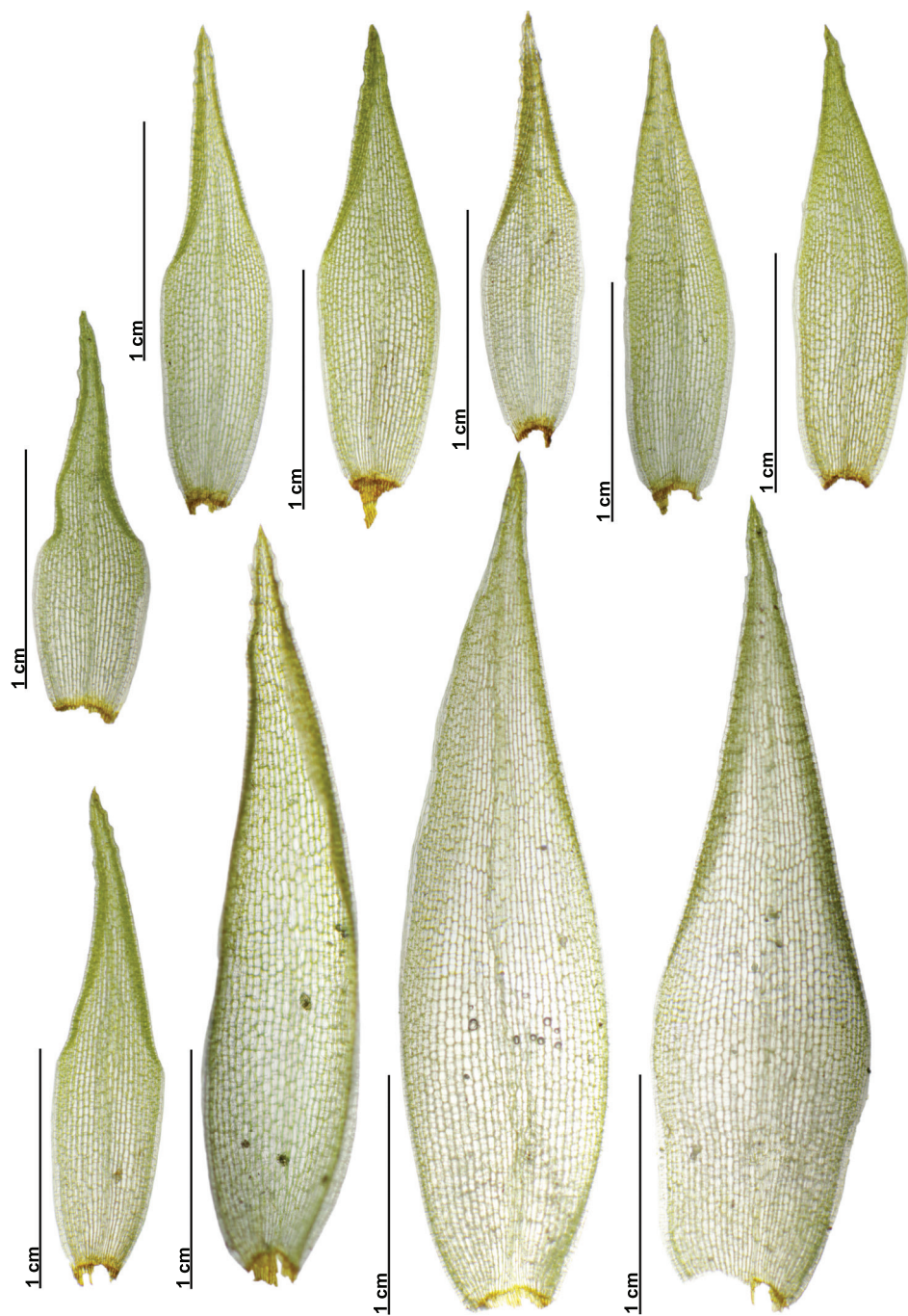


Figure 9. Leaf shape and size variation of different populations of *Leucobryum scalare* Müll.Hal. ex M. leisch

Key to the species

- 1 Gametophytes small to medium-sized, usually forming tufts, less branched, usually several branching in small gametophytes. Leaves falcate-secund, sometimes slightly erect, not forming a conical point at the shoot apex when dry ***Leucobryum aduncum***
- Gametophytes small sized, usually forming a compact cushion, with many short branches, usually very dense. Leaves erect, sometimes falcate-secund when close to the substrate, arranged in spiral and closely imbricate, forming a conical point at shoot apex when dry ***Leucobryum scalare***

Discussion

Leucobryum aduncum and *L. scalare* have long been problematic taxa due to their overlapping geographical distributions and indistinct morphological characters (Fig. 1). Chopra (1975) noticed that these taxa were very similar and needed more detailed study. In 1990, Alan Eddy regarded the specimens of *L. scalare* as the ‘scalare’ phenotype from the environment with long exposures to light and periodic desiccation. Then, Eddy reduced *L. scalare* to a variety of *L. aduncum* (Eddy 1990). This treatment of *L. scalare* as a variety was later accepted by Yamaguchi (1993). He reported that the inner perichaetial leaves around sporophytes of *L. scalare* were longer than vegetative leaves, while *L. aduncum* have inner perichaetial leaves as long as, or a little shorter than, vegetative leaves. This slight difference was recognized as a difference between varieties and insufficient to separate *L. scalare* at the species level (Yamaguchi and Iwatsuki 1987; Yamaguchi 1993). In the same year that Alan Eddy reduced *L. scalare* to the variety level, Johannes Enroth also reported a study of Leucobryaceae in Indonesia and Papua New Guinea. He noticed that the relative length of the inner perichaetial leaves around the sporophytes of *L. scalare* was similar in size to those in *L. aduncum* (Enroth 1990). With this observation, Enroth treated *L. scalare* as a synonym with *L. aduncum*. Enroth’s treatment of *L. scalare* as a synonym of *L. aduncum* has been accepted by many bryologists (Gao 1994; Crosby et al. 1999; Thouvenot and Bardat 2010). Despite the general adoption of Enroth’s concept of *L. aduncum* (with *L. scalare* as a synonym), *L. aduncum* var. *scalare* following Eddy is still widely used today, especially in floras and reports from South and South-east Asia (Lin and He 1999; Mamalo and Supremo 2007; Biju and Daniels 2017), due to somewhat recognizable morphological characters. Still, no taxonomic revision since Yamaguchi in 1993 has attempted to clarify the position of this taxon.

We here propose reinstating the name *L. scalare* at the species level following our morphological and phylogenetic analyses. Our morphological and morphometric studies showed that *L. aduncum* was generally larger than *L. scalare* (Fig. 3). However, many quantitative characters still overlapped between the two taxa and were unsuit-

able as taxonomic characters. Other environmental factors and the age of plants may influence these variable characters. The PCA and PERMANOVA tests showed that these two taxa were separate in their morphological space and should be recognized as separate taxonomic units of the same rank. The recognition of *L. scalare* as a distinct taxon was consistent with the previous observation by Eddy. He noticed that the leaf characters were sufficiently different from other taxa but decided that *L. scalare* should be a variety of *L. aduncum* (Eddy 1990). As for the use of inner perichaetial leaves in Yamaguchi and Iwatsuki (1987) and Enroth (1990), the current study showed that the inner perichaetial leaves were not the most reliable character, as they were hard to find in the specimens. We could not definitively conclude whether these traits differ between the two taxa. Our limited data on perichaetial leaves were consistent with those from Yamaguchi and Iwatsuki (1987). Even though Enroth (1990) found the inner perichaetial leaves to be similar in these two taxa, this character is quite difficult to verify in most specimens. Other than the perichaetial leaves, the other gametophytic characters showed consistent differences between *L. aduncum* and *L. scalare*, allowing bryologists to make a clear, unequivocal identification of these taxa.

The previous confusion over the taxonomic status of *Leucobryum scalare* could be the result of cryptic species within the species complex. Cryptic species are taxonomic groups that are similar in morphology due to their short divergence times despite a clear genetic distinction (Struck et al. 2018; Renner 2020). Many cases of cryptic species have been reported in bryophytes, vascular plants, fungi, and lichens (Shaw 2001; Bickford et al. 2007; Crespo and Pérez-Ortega 2009; Renner 2020). The discoveries of genetically distinct groups within the morphologically similar complex have driven more detailed morphological studies to find the defining characteristics of the observed genetic groups, which subsequently enhance our ability to perform taxonomic revision (Renner 2020). Several species complexes in *Leucobryum* have been recognized (Patterson et al. 1998; Vanderpoorten et al. 2003; Oguri et al. 2006; Oguri et al. 2008; Oguri et al. 2013). For example, *L. glaucum* (Hedw.) Ångstr. and *L. albidum* (Brid. ex P.Beauv.) Lindb. from eastern North America have similar morphological characters and broadly overlapping geographical distributions (circumboreal for *L. glaucum* and amphiatlantic to North America and Europe for *L. albidum*). *L. glaucum* and *L. albidum* do not require significantly different environmental conditions. These are common species in various woodland habitats ranging from xeric, sandy sites to swamp forests. The only difference between the two species was the size. However, RFLP analyses of nuclear ribosomal DNA showed that *L. albidum* is genetically distinct from *L. glaucum* (Patterson et al. 1998). The case of *L. albidum*-*L. glaucum* complex demonstrated that morphological and ecological differences were not the sole determinants of the species boundary. Additional data from molecular markers and detailed morphological work can help identify different taxonomic units within the complex.

In this case, *Leucobryum scalare* and *L. aduncum* could be the results of a recent divergence because of their morphological overlapping (Figs 3, 4) and short genetic distance (Fig. 5). From personal observations, variations in environmental conditions might be responsible for the difference between species. *Leucobryum aduncum* is often found growing in the shade of trees and with high moisture, while *L. scalare* is found

growing on the substrate with prolonged exposure to light and periodic desiccation. The difference in the ecological niche may become one of the reproductive barriers leading to the speciation of *L. scalare* and *L. aduncum*. Further studies on their ecological differences should be conducted to ascertain the mechanisms behind the speciation event.

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PT collected and examined the specimens and measured morphological data. PT provided photographs and illustrations. KC performed DNA extraction and initial analyses. PT performed the analyses. HTL and EK provided suggestions for phylogeny and data analysis. All authors contributed to the final version of the manuscript.

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Appendix I

Voucher Specimens and GenBank numbers for ITS1, ITS2, *atpB-rbcL* spacer, and *trnL-trnF* regions in this study.

Leucobryum aduncum, INDONESIA: Borneo, HIRO 140862, (HIRO), GenBank: AB125287, AB124781, AB742374; HIRO 140934, (HIRO), GenBank: AB763349, AB739623, AB742375. THAILAND: Nakhon Nayok, Khao Yai National Park. Kog Kaeo Waterfall, *Tiwutanon* 57, (BKF), GenBank: OQ556892, OQ557103, OQ581043; *Tiwutanon* 58, (BKF), GenBank: OQ556890, OQ557101, OQ576673, OQ581052; Pha Kluai Mai Waterfall, *Tiwutanon* 53, (BKF), GenBank: OQ556891, OQ557102, OQ576674, OQ581051. Northeast s.n. *Tiwutanon* 16, (BKF), GenBank: OQ556885, OQ557096, OQ576668, OQ581045; *Tiwutanon* 18, (BKF), GenBank: OQ556886, OQ557097, OQ576669, OQ581048; *Tiwutanon* 19, (BKF), GenBank: OQ556887, OQ557098, OQ576670, OQ581046; *Tiwutanon* 20, (BKF), GenBank: OQ556882, OQ557093, OQ576665, OQ581044; *Tiwutanon* 21, (BKF), GenBank: OQ556883, OQ557094, OQ576666, OQ581053; *Tiwutanon* 11, (BKF), GenBank: OQ556880, OQ557091, OQ576663, OQ581047; *Tiwutanon* 13, (BKF), GenBank: OQ556881, OQ557092, OQ576664, OQ581056. Phangnga, Si Phang-Nga National Park. Khao Dan Trail [14°22.5'N, 101°24.54'E], *Chantanaorrapint & Suwanmala* 2634, (PSU), GenBank: OQ556888, OQ557099, OQ576671, OQ581050. Phangnga, Khao Lam-pi-Hat Thai Mueang National Park, Thai Mueang District. [8°29.1'N, 98°13.68'E], *Suwanmala* 98, (PSU), GenBank: OQ556889, OQ557100, OQ576672, OQ581049; *Suwanmala* 100, (PSU), GenBank: OQ556884, OQ557095, OQ576667, OQ581054. ***Leucobryum candidum***, NEW ZEALAND: HIRO 203728, (HIRO), GenBank: AB285170, AB288196, AB742389. ***Leucobryum chlorophyllosum***, INDONESIA: Borneo, HIRO 140710, (HIRO), GenBank: AB125291, AB124792, AB742390; HIRO 140820, (HIRO), GenBank: AB763361, AB739636, AB742391. PHILIPPINES: MAK B119208, (MAK), GenBank: AB763362, AB739637, AB742392. ***Leucobryum***

scalare, MALAYSIA: Malay Peninsula, HIRO 138507, (HIRO), GenBank: AB763350, AB739624, AB742376., SRI LANKA: Nuwara Eliya District, HIRO 166266, (HIRO), GenBank: AB763351, AB739625, AB742377; HIRO 166267, (HIRO), GenBank: AB763352, AB739626, AB742378. THAILAND: Chiang Mai, Chiang Dao Wildlife Sanctuary, Den Ya Khad. [19°22.38'N, 98°50.04'E], *Chantanaorrapint & Suwanmala* 3355, (PSU), GenBank: OQ556894, OQ557105, OQ576676, OQ581064. Chiang Mai, Chiang Mai Royal Agricultural Research Center (Khun Wang) Botanical Garden. [18°45.18'N, 98°55.5'E], *Tiwutanon* 7, (BKF), GenBank: OQ556906, OQ557117, OQ581055. Lampang, Doi Khun Tan National Park, Doi Khun Tan. Tat Mei Khun Tan Waterfall [18° 30.84'N, 99° 17.46'E], *Tiwutanon* 8, (BKF), GenBank: OQ556895, OQ557106, OQ576677, OQ581065; *Tiwutanon* 9, (BKF), GenBank: OQ556896, OQ557107, OQ576678, OQ581066. Loei, Phu Ruea National Park. *Tiwutanon* 45, (BKF), GenBank: OQ556901, OQ557112, OQ576683, OQ581058; *Tiwutanon* 48, (BKF), GenBank: OQ556902, OQ557113, OQ576684, OQ581057; *Tiwutanon* 50, (BKF), GenBank: OQ556903, OQ557114, OQ576685, OQ581060. Loei, Phu Kradueng National Park. Nong Pakbung [16°50.64'N, 101°41.52'E], *Ajintaiyasil* 429, (BCU), GenBank: OQ556904, OQ557115, OQ576686, OQ581059. Nakhon Nayok, Khao Yai National Park, Khao Kheow. [14°22.5'N, 101°24.54'E], *Kraichak* 1732, (BKF), GenBank: OQ556899, OQ557110, OQ576681, OQ581063; *Tiwutanon* 23, (BKF), GenBank: OQ556900, OQ557111, OQ576682, OQ581061; *Tiwutanon* 28, (BKF), GenBank: OQ556898, OQ557109, OQ576680, OQ581068; Deaw Dai Cliff. [14°21.96'N, 101°24.36'E], *Tiwutanon* 34, (BKF), GenBank: OQ556897, OQ557108, OQ576679, OQ581067. Phayao, Doi-Luang National Park. The way up to Doi Luang [19°7.86'N, 99°45.42'E], *Chantanaorrapint & Suwanmala* 3948, (PSU), GenBank: OQ556893, OQ557104, OQ576675, OQ581062; *Chantanaorrapint & Suwanmala* 3945, (PSU), GenBank: OQ556905, OQ557116, OQ576687, OQ581069.

Appendix 2

Table A1. Primers for the ITS1, ITS2, *atpB-rbcL* spacer, and *trnL-trnF* regions in this study.

Region	Primers	References
ITS1		
Bryo18SF	5'- GGT GAA GTT TTC GGA TCG CG -3'	Hartmann et al. 2006
Bryo5.8SR	5'- TGC GTT CTT CAT CGT TGC -3'	Hartmann et al. 2006
ITS2		
Bryo5.8SF	5'- GAC TCT CAG CAA CGG ATA -3'	Hartmann et al. 2006
Bryo26SR	5'- AGA TTT TCA AGC TGG GCT -3'	Hartmann et al. 2006
<i>atpB-rbcL</i> spacer		
atpB-2	5'- AGC GTT GTA AAT ATT AGG CAT CTT -3'	Hsu et al. 2013
rbcL-2	5'- ATC TTT AAC ACC AGC TTT GAA TCC AAC -3'	Hsu et al. 2013
<i>trnL-trnF</i>		
C _(M)	5'-CGA AAT CGG TAG ACG CTA CG- 3'	Taberlet et al. 1991
F _(M)	5'-ATT TGA ACT GGT GAC ACG AG- 3'	Frey et al. 1999

Numerical analyses of seed morphology and its taxonomic significance in the genus *Oxytropis* DC. (Fabaceae) from northwestern China

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Abstract

The lack of diagnostic taxonomic characteristics in some species complexes leave the species delimitation of *Oxytropis* DC. unresolved. Seed morphological features have proved to be useful diagnostic and taxonomic characteristics in Fabaceae. However, there are few systematic studies on the seed characteristics of *Oxytropis*. Here, we used scanning electron and stereoscopic microscopy to investigate the seed characteristics of 35 samples obtained from 21 *Oxytropis* species from northwest China. Our examination showed two main types of hilum positions, terminal and central, and five different types of seed shapes: prolonged semielliptic, reniform, prolonged reniform, quadratic, and cardiform. Seven different sculpturing patterns were identified: scaled, regulated, lophate with stellated testa cells, simple reticulate, rough, compound reticulate, and lophate with rounded testa cells. The seeds ranged from 1.27 to 2.57 mm in length and from 1.18 to 2.02 mm in width, and the length-to-width ratio ranged from 0.89 to 1.55 mm. The seed shape was constant within species and was useful for species delimitation within the genus *Oxytropis* when combined with other macroscopic traits. In contrast, the sculpturing patterns were highly variable at the species level and could not be used for species identification. Results of the cluster analysis and principal component analysis (PCA) indicated that the seed traits of *Oxytropis* species are useful for taxa identification at the species level, but have low taxonomic value at the section level.

Keywords

China, cluster analysis, *Oxytropis*, PCA, seed morphology, SEM, taxonomy

Introduction

The genus *Oxytropis* DC. belongs to the tribe Galegeae (Fabaceae: Papilionoideae). It has been reported to be one of the largest groups of angiosperms, comprising approximately 330 species. The genus is distributed mainly in the cold mountainous regions of Asia, Europe, and North America (Polhill 1981; Zhu et al. 2010). It is thought to have been derived from *Astragalus* L. approximately 12–16 Ma, with which it shares many morphological features (Wojciechowski 2005). The genus *Oxytropis* is distinguished from *Astragalus* by beaked keels, asymmetrical leaflets, and acaulescent habit (Barneby 1952). Likely because of its relatively recent diversification, many taxonomic relationships within *Oxytropis* remain problematic (reviewed in Welsh 2001).

The genus *Oxytropis* was first established in 1802 by De Candolle (De Candolle 1802). It included 33 species, and he divided them into three groups according to whether stipules are adherent to stems or not and whether leaflets are opposite, verticillate, or neither. Bunge's (1874) comprehensive treatment of *Oxytropis* species in Eurasia identified four subgenera, 19 sections, and 181 species. His research also marked the beginning of modern *Oxytropis* research. Vassilczenko (1948) revised the work on *Oxytropis* in the Flora of USSR and separated the genus into six subgenera, 21 sections, and 276 species. Pavlov (1961) divided *Oxytropis* into four subgenera, 15 sections, and 124 species in the Flora of Kazakhstan. Leins and Merxmüller (1968) compiled 24 species, three subspecies, one variety, and two suspected species from Europe and divided them into two groups. Zhang (1998) recognized six subgenera, 146 species, 12 varieties, and three forms in Flora Reipublicae Popularis Sinicae. In contrast, Zhu and Ohashi (2000) recognized 125 species and four varieties in China. Welsh (2001) revised the genus *Oxytropis* in North America to include 57 taxa in only 22 species. Later, Zhu et al. (2010) taxonomically revised the genus in China and reported that it comprises three subgenera and 20 sections containing 133 species. These previous treatments of *Oxytropis* clarified many taxonomic problems. However, the lack of diagnostic taxonomic characteristics in some *Oxytropis* complexes has led to difficulties and differences in species delimitation, leaving the internal classification of *Oxytropis* unresolved.

Seed morphological features, such as seed shape, hilum shape, sculpturing pattern, and size, have been proven to be useful diagnostic and taxonomic characteristics in some genera of Fabaceae and other families (Lersten and Gunn 1981; Solum and Lockerman 1991; López et al. 2000; Al-Gohary and Mohamed 2007; Salimpour et al. 2007; Vural et al. 2008; Venora et al. 2009; Zorić et al. 2010; Celep et al. 2012; Kaya and Dirmenci 2012; Lantieri et al. 2013; Kamala and Aydin 2018; Rashid et al. 2018; Shemetova et al. 2018; Rashid et al. 2020). In the genus *Trifolium* L., Salimpour et al. (2007) reported that seed characteristics such as sculpturing pattern, shape, size, and hilum position, can be used as taxonomic markers within the section *Lotoidea*. In contrast, Zorić et al. (2010) concluded that the seed characteristics do not support infrageneric classification of *Trifolium*. Similarly, Shemetova et al. (2018) reported that seed shapes, colours, sizes, surface sculptures, and hilum positions are very diverse in *Astragalus*, and they emphasized that the systematic importance of seed characteristics

needs to be evaluated in a phylogenetic context. However, Vural et al. (2008) found that *Astragalus* seed sculpturing pattern and seed shape can be used as taxonomically significant characteristics at the species level, if supported by other macromorphological characteristics. López et al. (2000) found that seed colour, weight, shape, and size, presence of an aril, and hilum position can be used as diagnostic characteristics for segregating two subtribes and delimiting lower taxonomic levels in the tribe Genisteae. Similarly, Rashid et al. (2020) concluded that seed shape, sculpturing pattern, and size are valuable characteristics for the identification and delimitation of species in the tribes Astragaleae and Trifolieae. Kamala and Aydin (2018) and Rashid et al. (2018) also reported that seed characteristics (coat, shape, colour, seed size, etc.) can be used to identify taxa in the tribe Viciae.

Seeds of *Oxytropis* species were first studied by Solum and Lockerman (1991), who documented the seed coat patterns of *Oxytropis riparia* Litv. and *Oxytropis campestris* (L.) DC. Bojňanský and Fargašová (2007) studied the seeds of four European *Oxytropis* species and recorded their size, colour, and other information. Farrington et al. (2008) studied the morphological properties of seeds of 15 Alaskan *Oxytropis* taxa and found that seed coat micromorphology and anatomy can distinguish it from the genus *Astragalus*. Meyers et al. (2013) analysed the seed characteristics of 22 *Oxytropis* species in Alaska and concluded that seed coat types are highly variable at the species level and cannot be used for species identification. Erkul et al. (2015) studied the morphological properties of seeds of 13 Turkish *Oxytropis* taxa and found that seed characteristics have low taxonomic value in distinguishing subgenera, sections, and species. The infraspecific variation in seed traits has not been well addressed in most of the abovementioned studies because of sampling limitations. Only Meyers et al. (2013) studied whether seed traits were stable within species, along with studying the correlation between seed traits and the environment; however, they did not conduct any systematic analyses, such as cluster analysis.

Numerical taxonomy, also known as phenetics, mathematical taxonomy and multivariate morphometrics (Singh 2019), is mainly based on the overall affinity (similarity) at any taxonomic level. Quantitative traits have long been overlooked in taxonomic studies until numerical methodologies, such as cluster analysis, started to be widely applied in species delimitation (Thien et al. 1975). Recently, dendrograms and cladograms have been used instead of subjective analyses in many studies on the seed morphology of Fabaceae (Erkul et al. 2015; Fayed et al. 2019; Abusaief and Boasoul 2021). However, quantitative seed traits of the genus *Oxytropis*, such as length, width, length/width ratio, and weight, have not received much attention in taxonomic studies, possibly because these traits are considered fluctuating, and this fluctuation is random or excessive.

Northwest China is one of the main distribution regions of the genus *Oxytropis* (Zhang 1998; Zhu et al. 2010), but there is little research on the seed characteristics of *Oxytropis* in this area. Here, we carried out the first numerical analysis and microscopic investigation of 35 samples belonging to 21 *Oxytropis* species from northwest China using scanning electron and stereoscopic microscopy to elucidate the taxonomic significance of their seed micromorphology.

Materials and methods

The present study was mainly based on seeds collected in the field, with only a few seeds obtained from herbarium vouchers housed at the herbarium of Northwest Normal University (HWTC; Table 1). Voucher specimens collected from wild seeds are also kept at HWTC. The investigated species and their sources are listed in Table 1, and the classification of genera by Zhu et al. (2010) was adopted. Seed morphology was examined using a stereoscopic microscope (Leica M205 FA). For measuring seed length and width among the samples from the field, 80 mature and representative seeds per population were measured, while among the samples from herbarium specimens, 30 seeds per specimen were measured. The minimum–maximum range, mean, standard deviations in seed length and width, and length/width ratio were calculated. For SEM, the selected representative material was directly mounted onto aluminium stubs with double adhesive tape and coated with gold prior to observation with a HITACHI-450 scanning electron microscope (NWNNU University) at 25 kV.

Seed shapes and surface sculpturing were classified according to previous studies on the microscopic morphology of Fabaceae seeds (Bojňanský and Fargašová 2007; Vural et al. 2008; Al-Ghamdi 2011; Meyers et al. 2013; Erkul et al. 2015). Based on previous studies and observations of seed morphology in the genus *Oxytropis*, seven seed traits, including four quantitative and three qualitative traits, were selected for morphometric analysis in the present study (Erkul et al. 2015). The selected characteristics and their states for cluster analysis were as follows: 1. seed length (mm); 2. seed width (mm); 3. seed length/width ratio; 4. seed shape: cardiform (0), prolonged (1), reniform (2), quadratic (3), prolonged semi-elliptic (4); 5. seed surface sculpturing: scaled (0), rugulate (1), lophate with stellated testa cells (2), simple reticulate (3), rough (4), compound reticulate (5), lophate with rounded testa cells (6); 6. hilum position: central (0), terminal (1); 7. seed weight (g). For the seeds collected in the field, 300 mature and full seeds were randomly selected and their 100-seed weight was determined. For the seeds collected from a few specimens, we randomly selected 30 seeds and weighed the 10-seeds. The 100-seed weights determined from the seeds of the specimens in the cluster analysis were expressed as 10-seed weights multiplied by 10.

Numerical analysis

Cluster analysis and principal component analysis (PCA) were performed using the Origin 2022 software (OriginLab Corporation 2022). The raw data matrix included quantitative traits, such as length, width, L/W ratio and weight, and qualitative characteristics, such as shape, sculpturing, and hilum position. The qualitative characteristics were coded using a presence/absence (0/1) matrix. Ward's method was used for cluster analysis using Euclidean distance to interpret the morphological similarities among species. In the cluster analysis, Euclidean distance is one of the most commonly used distance measurements in hierarchical clustering, which can reflect the absolute

Table 1. List of examined taxa with collection details.

Section	Code	Species	Locality	Coordinates	Voucher	Collection date
Section <i>Xerobia</i>	1	<i>O. ciliata</i> Turcz.	Yueliang Mountain	36°25'41.85"N, 105°42'23.71"E	X. Zhao 1947	2019
Section <i>Polyadena</i>	2	<i>O. muricata</i> (Pall.) DC.	Maxian Mountain	35°47'46.48"N, 103°58'12.64"E	X. Zhao 1903	2019
	3	<i>O. muricata</i> (Pall.) DC.	Tiemu Mountain	35°58'32.21"N, 104°46'31.40"E	X. Zhao 1970	2019
Section <i>Falcicarpa</i>	4	<i>O. falcata</i> Bunge	Awangcang wetland park	33°45'32.85"N, 101°41'6.58"E	X. Zhao 1842	2018
	5	<i>O. falcata</i> Bunge	Beach of Maqu section of Yellow River	Unknown	Gannan Grassland Team 60B	Unknown
Section <i>Baicalia</i>	6	<i>O. ochrantha</i> Turcz.	Xinglong Mountain	35°45'52.41"N, 104°2'21.66"E	X. Zhao 1813	2018
	7	<i>O. ochrantha</i> Turcz.	North mountain of Pingliang	35°33'49.11"N, 106°41'2.34"E	X. Zhao 1837	2018
	8	<i>O. bicolor</i> Bunge	Unknown	Unknown	Unknown 790043	Unknown
	9	<i>O. bicolor</i> Bunge	Tiemu Mountain	35°58'32.21"N, 104°46'31.40"E	X. Zhao 1927	2019
	10	<i>O. racemosa</i> Turcz.	Yanchi	37°43'52.02"N, 107°23'55.77"E	X. Zhao 1946	2019
	11	<i>O. myriophylla</i> (Pall.) DC.	Erdaogou	35°25'19.39"N, 106°40'6.25"E	X. Zhao 1831	2018
	12	<i>O. myriophylla</i> (Pall.) DC.	Anguo	35°38'49.75"N, 106°28'54.92"E	X. Zhao 1833	2018
	13	<i>O. myriophylla</i> (Pall.) DC.	Maxian Mountain	35°47'46.48"N, 103°58'12.64"E	X. Zhao 1836	2018
	14	<i>O. neimongolica</i> C.W.Chang & Y.Z.Zhao	Helan Mountain	38°39'37.76"N, 105°48'34.42"E	X. Zhao 1948	2019
Section <i>Eumorpha</i>	15	<i>O. imbricata</i> Kom.	Liancheng National Nature Reserve	36°36'24.65"N, 102°49'34.30"E	X. Zhao 1809	2018
	16	<i>O. imbricata</i> Kom.	Taohe River	34°33'28.66"N, 102°34'53.99"E	X. Zhao 1940	2019
	17	<i>O. coerulea</i> (Pall.) DC.	Taitong Mountain	35°30'8.94"N, 106°35'54.90"E	X. Zhao 1832	2018
	18	<i>O. coerulea</i> (Pall.) DC.	Erdaogou	35°25'19.39"N, 106°40'6.25"E	X. Zhao 1833	2018
	19	<i>O. holanshanensis</i> H.C.Fu	Helan Mountain	38°39'37.76"N, 105°48'34.42"E	X. Zhao 1949	2019
Section <i>Mesogaea</i>	20	<i>O. xinglongshanica</i> C.W.Chang	Maxian Mountain	35°46'46.16"N, 103°59'19.19"E	X. Zhao 1913	2019
	21	<i>O. xinglongshanica</i> C.W.Chang	Xinglong Mountain	35°46'20.53"N, 104°1'2.49"E	X. Zhao 1910	2019
	22	<i>O. glabra</i> (Lam.) DC.	Rabah Lake National Nature Reserve	37°42'3.19"N, 107°2'33.46"E	X. Zhao 1950	2019
	23	<i>O. kansuensis</i> Bunge	Azi Test Station of LZU	33°39'57.96"N, 101°52'22.44"E	X. Zhao 1819	2018
	24	<i>O. kansuensis</i> Bunge	Charlie temple	32°45'7.95"N, 102°3'26.83"E	X. Zhao 1820	2018
	25	<i>O. taochensis</i> Kom.	Liupan Mountain	35°33'21.81"N, 106°25'21.54"E	X. Zhao 1838	2018
	26	<i>O. ochrocephala</i> Bunge	Nanhuang Mountain	36°22'42.67"N, 105°39'26.20"E	X. Zhao 1952	2019
	27	<i>O. ochrocephala</i> Bunge	Xinglong Mountain	35°47'5.17"N, 104°0'0.67"E	X. Zhao 1828	2018
	28	<i>O. ochrocephala</i> Bunge	Maxian Mountain	35°46'46.60"N, 103°59'19.33"E	X. Zhao 1953	2019
	29	<i>O. ochrocephala</i> Bunge	Jinqiang River	37°13'36.45"N, 102°41'3.46"E	X. Zhao 1840	2018
	30	<i>O. ochrocephala</i> Bunge	Hougou Village	35°48'47.34"N, 103°57'53.83"E	X. Zhao 1954	2019
	31	<i>O. qinghaiensis</i> Y.H.Wu	Labrang Monastery	35°11'8.91"N, 102°30'37.00"E	X. Zhao 1822	2018

Section	Code	Species	Locality	Coordinates	Voucher	Collection date
Section <i>Oxytropis</i>	32	<i>O. latibracteata</i> Jurtz.	Helan Mountain	38°39'46.59"E, 105°49'20.25"N	X. Zhao 1951	2019
	33	<i>O. qilianshanica</i> C.W.Chang & C.L.Zhang	Jinqiang River	Unknown	J.Q. Wang 710113	Unknown
Section <i>Lycotriche</i>	34	<i>O. aciphylla</i> Ledeb.	Jiji Spring Nature Reserve	38°59'43"N, 101°55'39"E	X. Zhao 1924	2019
Section <i>Leucopodia</i>	35	<i>O. squammosa</i> Candolle	Shaochagou	35°42'57.20"N, 105°2'21.20"E	X. Zhao 1928	2019

differences of individual numerical characteristics, and were applied to analyze differences in the numerical size of dimensions (Raymond and Sylvia 1993; Farhana and Safwana 2018). The Ward error sum of squares method applies the concept of ANOVA to classification, resulting in richer clustering information that is rarely affected by abnormal data (Ward 1963; Szekely and Rizzo 2005). In the present study, to test the validity of the seed macro-and micromorphological traits, PCA was used to select taxonomically relevant qualitative and quantitative characteristics. It is usually used to distinguish between species within a given genus.

Results

Seed morphology

The studied seeds, all from the genus *Oxytropis*, had two main types of hilum positions, terminal and central, and five different types of seed shapes: prolonged semielliptic, reniform, prolonged reniform, quadratic, and cardiform (Table 2; Figs 1, 2). Hilum position was observed as terminal in *O. racemosa*, *O. neimonggolica*, *O. imbricata* (LC, TR), *O. coerulea* (TT, EDG), *O. xinglongshanica* (MX, XL), *O. glabra*, *O. taochensis*, and *O. ochrocephala* (NH, XL, MX, JQ, and HG). Hilum position was observed as central in *O. ciliata*, *O. muricata* (MX, TM), *O. falcata* (AWC, MQ), *O. ochrantha* (XL, NMP), *O. bicolor* (U, TM), *O. myriophylla* (EDG, AG, and MX), *O. holanshanensis*, *O. kansuensis* (AZ, CT), *O. qinghaiensis*, *O. latibracteata*, *O. qilianshanica*, *O. aciphylla*, and *O. squammosa*. In addition, seed shapes could be separated into five groups (Table 2): a cardiform seed was found in *O. ciliata*, *O. muricata* (MX, TM), *O. falcata* (AWC, MQ), *O. ochrantha* (XL, NMP), *O. bicolor* (U, TM), *O. holanshanensis*, *O. kansuensis* (AZ, CT), and *O. squammosa* (Table 2; Figs 1, 2); a prolonged reniform seed was observed in *O. racemosa*, *O. neimonggolica*, *O. imbricata* (LC, TR), *O. coerulea* (TT, EDG), *O. xinglongshanica* (MX, XL), *O. glabra*, and *O. taochensis* (Table 2; Figs 1, 2); a reniform seed was found in *O. myriophylla* (EDG, AG, MX), *O. qinghaiensis*, *O. latibracteata*, and *O. qilianshanica*; a quadratic seed was only found in *O. ochrocephala* (NH, XL, MX, JQ, HG); and finally, a prolonged semielliptic seed was only found in *O. aciphylla* (Table 2; Fig. 2).

The seeds ranged from 1.27 mm (*O. kansuensis* (AZ)) to 2.57 mm (*O. coerulea* (EDG)) in length and from 1.18 mm (*O. qinghaiensis*) to 2.02 mm (*O. holanshanensis*)

Table 2. Seed morphological features of *Oxytropis* under scanning electron microscopy.

Section	Code	Species	Shape of seed	Sculpturing	Hilum position
Section <i>Xerobia</i>	1	<i>O. ciliata</i>	Cardiform	Scaled	Central
Section <i>Polyadena</i>	2	<i>O. muricata</i> (MX)	Cardiform	Rugulate	Central
	3	<i>O. muricata</i> (TM)	Cardiform	Rugulate	Central
Section <i>Falcicarpae</i>	4	<i>O. falcata</i> (AWC)	Cardiform	Rugulate	Central
	5	<i>O. falcata</i> (MQ)	Cardiform	Rugulate	Central
Section <i>Baicalia</i>	6	<i>O. ochrantha</i> (XL)	Cardiform	Lophate with stellated testa cells	Central
	7	<i>O. ochrantha</i> (NMP)	Cardiform	Rugulate	Central
	8	<i>O. bicolor</i> (U)	Cardiform	Rough	Central
	9	<i>O. bicolor</i> (TM)	Cardiform	Simple reticulate	Central
	10	<i>O. racemosa</i>	Prolonged Reniform	Rough	Terminal
	11	<i>O. myriophylla</i> (EDG)	Reniform	Rugulate	Central
	12	<i>O. myriophylla</i> (AG)	Reniform	Rough	Central
	13	<i>O. myriophylla</i> (MX)	Reniform	Rugulate	Central
Section <i>Neimonggolicae</i>	14	<i>O. neimonggolica</i>	Prolonged Reniform	Scaled	Terminal
Section <i>Eumorpha</i>	15	<i>O. imbricata</i> (LC)	Prolonged Reniform	Rugulate	Terminal
	16	<i>O. imbricata</i> (TR)	Prolonged Reniform	Rough	Terminal
	17	<i>O. coerulea</i> (TT)	Prolonged Reniform	Rugulate	Terminal
	18	<i>O. coerulea</i> (EDG)	Prolonged Reniform	Rugulate	Terminal
	19	<i>O. holanshanensis</i>	Cardiform	Compound reticulate	Central
Section <i>Mesogaea</i>	20	<i>O. xinglongshanica</i> (MX)	Prolonged Reniform	Lophate with stellated testa cells	Terminal
	21	<i>O. xinglongshanica</i> (XL)	Prolonged Reniform	Lophate with stellated testa cells	Terminal
	22	<i>O. glabra</i>	Prolonged Reniform	Rugulate	Terminal
	23	<i>O. kansuensis</i> (AZ)	Cardiform	Rugulate	Central
	24	<i>O. kansuensis</i> (CT)	Cardiform	Rugulate	Central
	25	<i>O. taochensis</i>	Prolonged Reniform	Lophate with stellated testa cells	Terminal
	26	<i>O. ochrocephala</i> (NH)	Quadratic	Rugulate	Terminal
	27	<i>O. ochrocephala</i> (XL)	Quadratic	Rugulate	Terminal
	28	<i>O. ochrocephala</i> (MX)	Quadratic	Rugulate	Terminal
	29	<i>O. ochrocephala</i> (JQ)	Quadratic	Rugulate	Terminal
	30	<i>O. ochrocephala</i> (HG)	Quadratic	Rugulate	Terminal
	31	<i>O. qinghaiensis</i>	Reniform	Compound reticulate	Central
Section <i>Oxytropis</i>	32	<i>O. latibracteata</i>	Reniform	Rugulate	Central
	33	<i>O. gilianshanica</i>	Reniform	Rough	Central
Section <i>Lycotriche</i>	34	<i>O. aciphylla</i>	Prolonged Semielliptic	Simple reticulate	Central
Section <i>Leucopodia</i>	35	<i>O. squamulosa</i>	Cardiform	Lophate with rounded testa cells	Central

in width (Table 3). The lowest length/width ratio (0.89) was observed in *O. ochrocephala* (JQ), while the highest (1.55) was found in *O. imbricata* (LC). The lightest seeds were measured in *O. qinghaiensis* at 0.1058 g, while the heaviest seeds were measured in *O. ciliata* at 0.3521 g (Table 3).

Surface sculpturing

Seven different seed surface sculpturing patterns were observed: scaled, regulate, lophate with stellated testa cells, simple reticulate, rough, compound reticulate, and lophate with rounded testa cells (Table 2; Figs 3, 4). The regulate sculpturing pattern was common in most taxa and was predominant in *O. muricata* (MX, TM), *O. falcata* (AWC, MQ), *O. ochrantha* (NMP), *O. myriophylla* (EDG, MX), *O. kansuensis* (AZ, CT), *O. latibracteata*, *O. imbricata* (LC), *O. coerulea* (TT, EDG), *O. glabra*, and

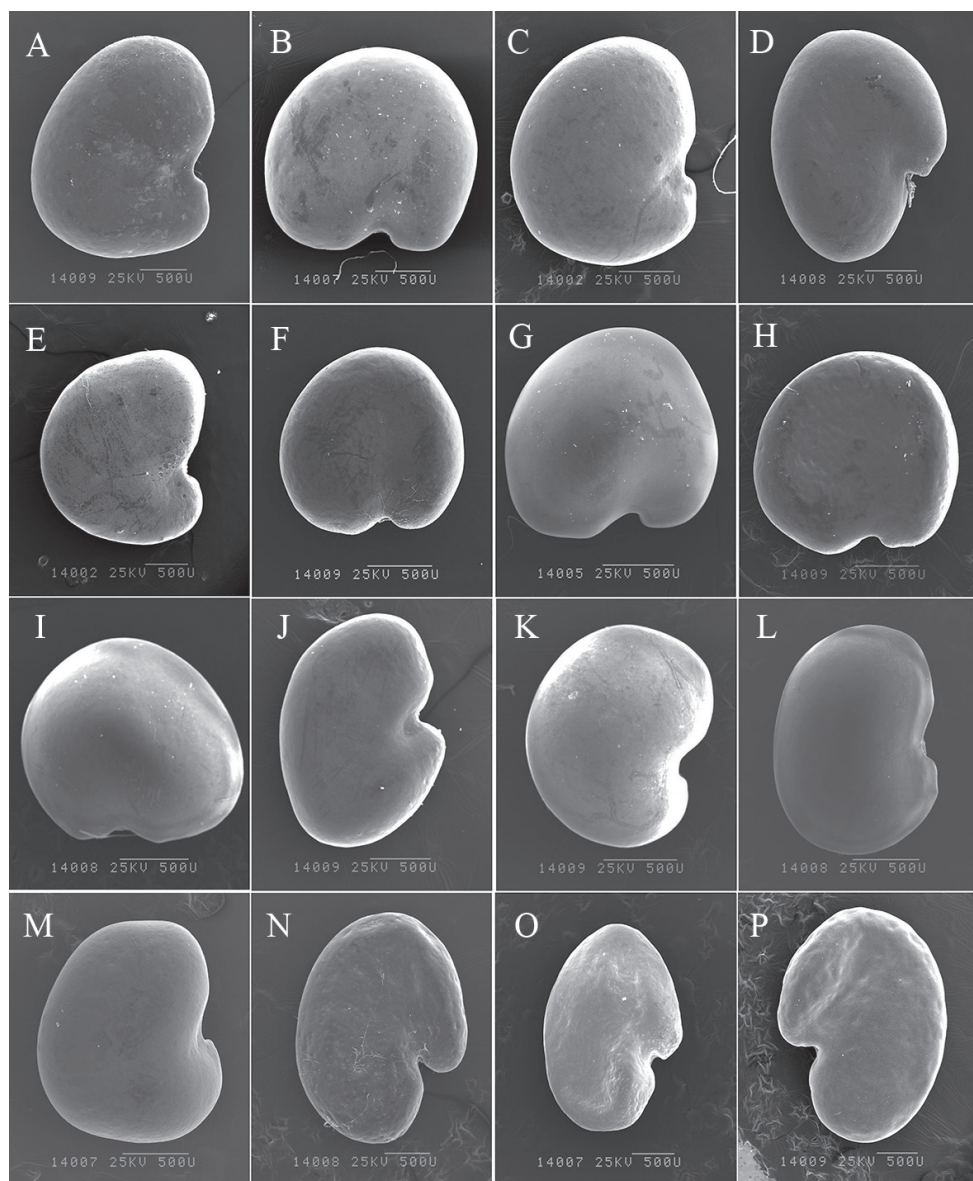


Figure 1. Seed shape of the studied species **A** *O. ciliata* **B** *O. muricata* (MX) **C** *O. muricata* (TM) **D** *O. falcata* (AWC) **E** *O. falcata* (MQ) **F** *O. ochrantha* (XL) **G** *O. ochrantha* (NMP) **H** *O. bicolor* (U) **I** *O. bicolor* (TM) **J** *O. racemosa* **K** *O. myriophylla* (EDG) **L** *O. myriophylla* (AG) **M** *O. myriophylla* (MX) **N** *O. neimonggolica* **O** *O. imbricata* (LC) **P** *O. imbricata* (TR). Scale bar: 500 μ m.

O. ochrocephala (NH, XL, MX, JQ, and HG) (Table 2; Figs 3, 4). The simple reticulate sculpturing pattern was predominant in *O. bicolor* (U) and *O. aciphylla*, while the compound reticulate sculpturing pattern was predominant in *O. holanshanensis* and *O. qinghaiensis* (Table 2; Figs 3, 4). The scaled sculpturing pattern was predominant in *O. ciliata* and *O. neimonggolica*, while the rough sculpturing pattern was predominant

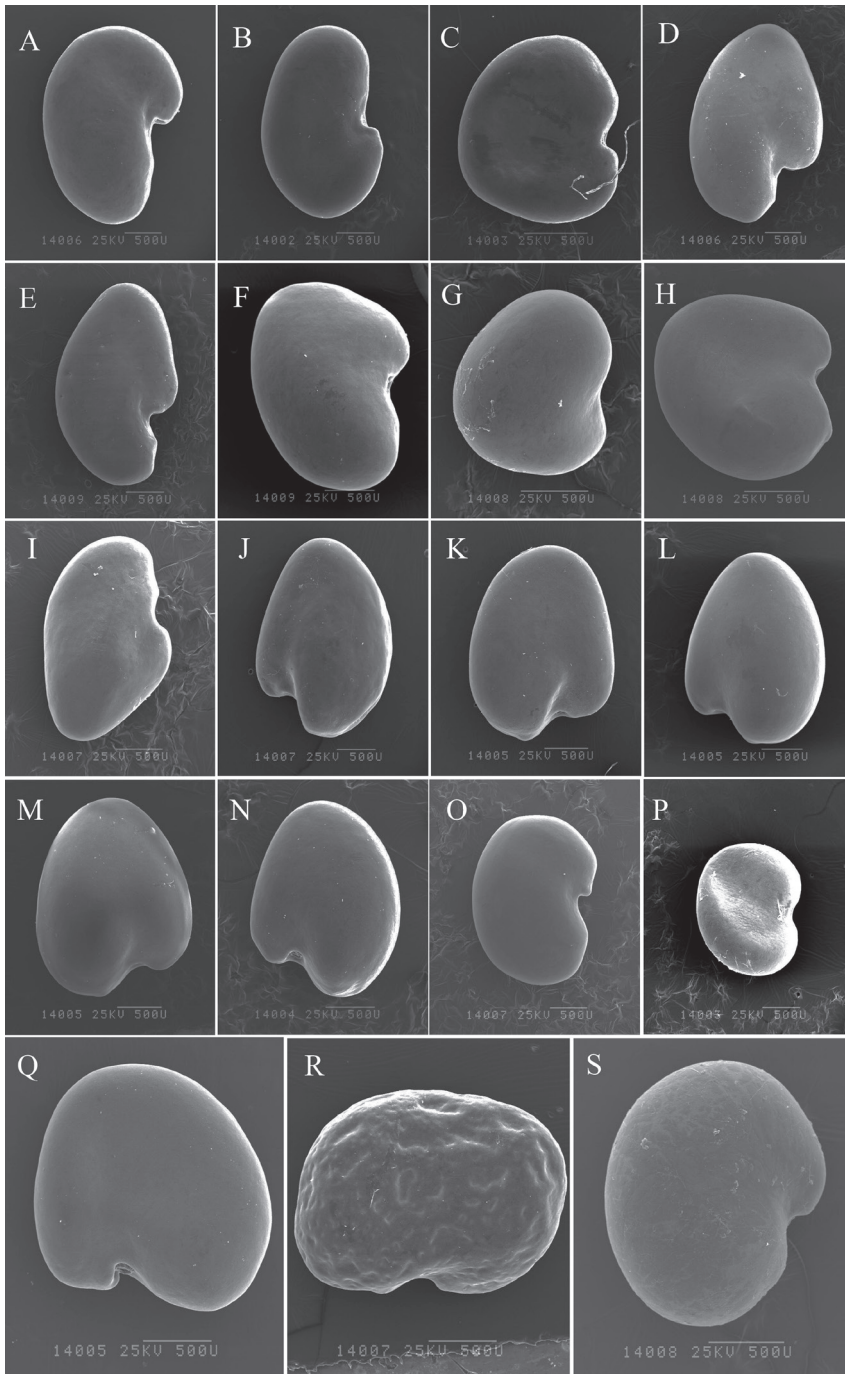


Figure 2. Seed shape of the studied species **A** *O. coerulea* (TT) **B** *O. coerulea* (EDG) **C** *O. holanshanensis* **D** *O. xinglongshanica* (MX) **E** *O. xinglongshanica* (XL) **F** *O. glabra* **G** *O. kansuensis* (AZ) **H** *O. kansuensis* (CT) **I** *O. taochensis* **J** *O. ochrocephala* (NH) **K** *O. ochrocephala* (XL) **L** *O. ochrocephala* (MX) **M** *O. ochrocephala* (JQ) **N** *O. ochrocephala* (HG) **O** *O. qinghaiensis* **P** *O. latibracteata* **Q** *O. qilianshanica* **R** *O. aciphylla* **S** *O. squammulosa*. Scale bar: 500 µm.

Table 3. Seed morphological features of *Oxytropis* under stereoscopic microscopy.

Section	Code	Species	Length Min. (mean ± SD) max./mm	Width Min. (mean ± SD) max./mm	L/W ratio	Seed weight/g
Section <i>Xerobia</i> ection <i>Polyadena</i>	1	<i>O. ciliata</i>	1.54(2.43±0.36)3.28	1.34(2.05±0.28)2.8	1.19±0.07	0.3521±0.0236
	2	<i>O. muricata</i> (MX)	1.38(2.02±0.3)2.91	1.1(1.78±0.26)2.38	1.14±0.07	0.2627±0.0041
	3	<i>O. muricata</i> (TM)	1.41(2.04±0.29)2.66	1.15(1.66±0.23)2.03	1.24±0.18	0.248±0.013
Section <i>Falcicarpae</i>	4	<i>O. falcata</i> (AWC)	1.59(2.14±0.25)2.79	1.42(1.87±0.18)2.35	1.15±0.13	0.2981±0.0106
	5	<i>O. falcata</i> (MQ)	1.7(2.09±0.14)2.5	1.45(1.86±0.17)2.15	1.13±0.13	0.323±0.005
Section <i>Baicalia</i>	6	<i>O. ochrantha</i> (XL)	1.29(1.65±0.17)2.09	1.18(1.53±0.16)2	1.07±0.07	0.2148±0.0091
	7	<i>O. ochrantha</i> (NMP)	1.16(1.49±0.17)1.84	1.15(1.35±0.12)1.6	1.1±0.07	0.1732±0.0021
	8	<i>O. bicolor</i> (U)	1.09(1.57±0.23)1.98	1.21(1.49±0.17)1.88	1.05±0.06	0.146±0.013
	9	<i>O. bicolor</i> (TM)	1.32(1.74±0.21)2.31	1.23(1.65±0.23)2.32	1.06±0.08	0.1326±0.0086
	10	<i>O. racemosa</i>	1.24(1.71±0.17)2.19	0.77(1.23±0.14)1.5	1.4±0.12	0.1668±0.0128
	11	<i>O. myriophylla</i> (EDG)	1.05(1.56±0.21)2.14	0.81(1.25±0.19)1.64	1.26±0.1	0.1290±0.0004
	12	<i>O. myriophylla</i> (AG)	1.26(1.59±0.13)1.93	0.94(1.32±0.16)1.63	1.22±0.09	0.1231±0.0007
	13	<i>O. myriophylla</i> (MX)	1.06(1.67±0.23)2.1	1.04(1.45±0.16)1.9	1.15±0.07	0.1349±0.0043
Section <i>Neimonggolicae</i>	14	<i>O. neimonggolica</i>	1.85(2.11±0.12)2.32	1.54(1.72±0.12)1.98	1.23±0.04	0.326±0.01
Section <i>Eumorpha</i>	15	<i>O. imbricata</i> (TR)	1.77(2.44±0.27)2.93	1.1(1.59±0.2)2.05	1.54±0.11	0.3188±0.0054
	16	<i>O. imbricata</i> (LC)	1.79(2.36±0.31)3.13	1.08(1.56±0.27)2.34	1.52±0.12	0.3264±0.0112
	17	<i>O. coerulea</i> (TT)	1.66(2.39±0.25)2.96	1.22(1.69±0.21)2.11	1.43±0.12	0.2799±0.0016
	18	<i>O. coerulea</i> (EDG)	1.99(2.57±0.16)2.92	1.44(1.86±0.15)2.2	1.39±0.09	0.2986±0.0013
	19	<i>O. holanshanensis</i>	1.81(2.21±0.19)2.66	1.43(2.02±0.22)2.58	1.1±0.09	0.3264±0.0062
Section <i>Mesogaea</i>	20	<i>O. xinglongshanica</i> (MX)	1.56(2.32±0.32)3.22	1.29(1.93±0.29)2.69	1.21±0.1	0.2914±0.0038
	21	<i>O. xinglongshanica</i> (XL)	1.47(2.23±0.23)2.7	1.42(1.77±0.16)2.24	1.26±0.11	0.2763±0.0103
	22	<i>O. glabra</i>	0.93(1.78±0.33)2.63	0.84(1.53±0.27)2.23	1.16±0.09	0.1892±0.0066
	23	<i>O. kansuensis</i> (AZ)	0.87(1.27±0.2)1.9	0.91(1.28±0.17)1.69	0.99±0.09	0.1074±0.0057
	24	<i>O. kansuensis</i> (CT)	0.95(1.38±0.13)1.65	1.05(1.44±0.17)1.77	0.97±0.11	0.1260±0.0044
	25	<i>O. taochensis</i>	1.54(2.09±0.25)2.73	1.08(1.55±0.18)1.89	1.36±0.11	0.2236±0.0134
	26	<i>O. ochrocephala</i> (NH)	1.22(1.73±0.22)2.23	1.35(1.91±0.23)2.53	0.9±0.06	0.2719±0.0043
	27	<i>O. ochrocephala</i> (XL)	1.1(1.63±0.17)2.03	1.23(1.77±0.24)2.39	0.92±0.07	0.2517±0.0103
	28	<i>O. ochrocephala</i> (MX)	1.28(1.64±0.17)2.01	1.56(1.82±0.12)2.14	0.9±0.07	0.2417±0.0065
	29	<i>O. ochrocephala</i> (JQ)	0.92(1.56±0.23)2.06	1.23(1.75±0.21)2.43	0.89±0.08	0.2506±0.0098
	30	<i>O. ochrocephala</i> (HG)	1.02(1.66±0.26)2.28	1.43(1.8±0.14)2.27	0.92±0.11	0.2854±0.0123
	31	<i>O. qinghaiensis</i>	1.2(1.56±0.18)1.99	0.93(1.18±0.11)1.56	1.33±0.1	0.1058±0.0087
Section <i>Oxytropis</i>	32	<i>O. latibracteata</i>	1.5(2.05±0.25)2.64	1.2(1.69±0.21)2.19	1.22±0.1	0.2368±0.0106
	33	<i>O. qilianshanica</i>	1.38(1.57±0.08)1.71	1.09(1.31±0.1)1.49	1.2±0.05	0.112±0.008
Section <i>Lycotriche</i>	34	<i>O. aciphylla</i>	1.36(1.99±0.28)2.81	1.01(1.43±0.21)1.98	1.39±0.12	0.1822±0.0094
Section <i>Leucopodia</i>	35	<i>O. squammulosa</i>	1.22(1.81±0.25)2.61	0.95(1.62±0.29)2.37	1.13±0.09	0.2070±0.0117

in *O. bicolor*, *O. myriophylla* (AG), *O. qilianshanica*, *O. racemosa*, and *O. imbricata* (TR) (Table 2; Figs 3, 4). Lastly, the lophate pattern with stellated testa cells was predominant in *O. ochrantha* (XL), *O. xinglongshanica* (MX, XL), and *O. taochensis*, while the lophate pattern with rounded testa cells was only found in *O. squammulosa* (Table 2; Figs 3, 4).

Numerical analysis

In the present study, principal components analysis (PCA) indicates three groups of traits, which explain 82.81% of the total variation (Table 4). The first principal component (PC1) exhibited 41.51% of the variability, which had a high loading component of the seed length, width, and weight. The second PC (PC2) accounted for 22.18% of the variation and was strongly associated with L/W ratio and sculpturing, whereas the

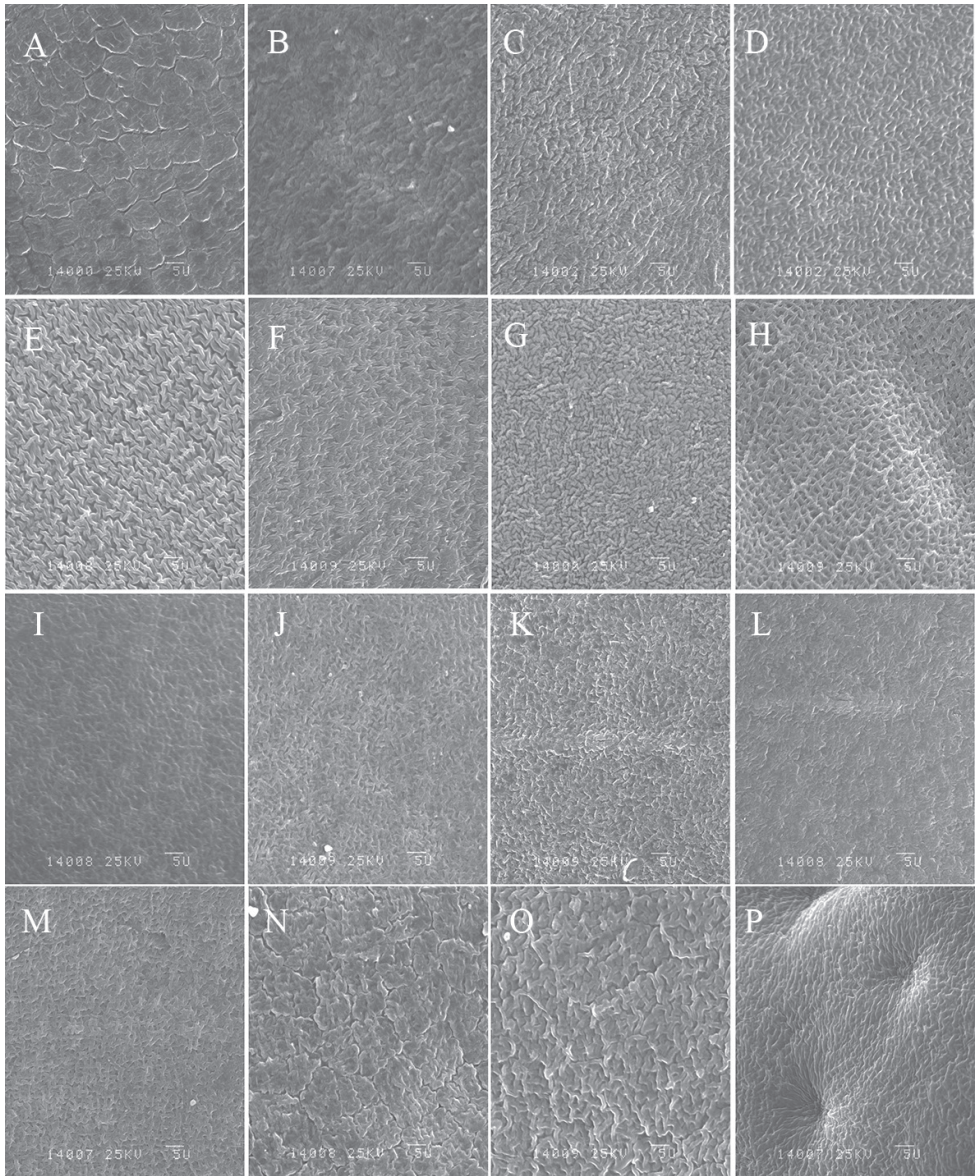


Figure 3. Seed surface sculpturing of the studied species **A** *O. ciliata* **B** *O. muricata* (MX) **C** *O. muricata* (TM) **D** *O. falcata* (AWC) **E** *O. falcata* (MQ) **F** *O. ochrantha* (XL) **G** *O. ochrantha* (NMP) **H** *O. bicolor* (U) **I** *O. bicolor* (TM) **J** *O. racemosa* **K** *O. myriophylla* (EDG) **L** *O. myriophylla* (AG) **M** *O. myriophylla* (MX) **N** *O. neimonggolica* **O** *O. imbricata* (LC) **P** *O. imbricata* (TR). Scale bar: 5 μ m.

third PC (PC3) contained 19.12% of the variability in which hilum position and seed shape were important. As shown in Fig. 5, the scatter points for the same species are closely aggregated, such as the five samples of *O. ochrocephala* (NH, XL, MX, JQ, and HG), indicating that samples from different populations within the same species had

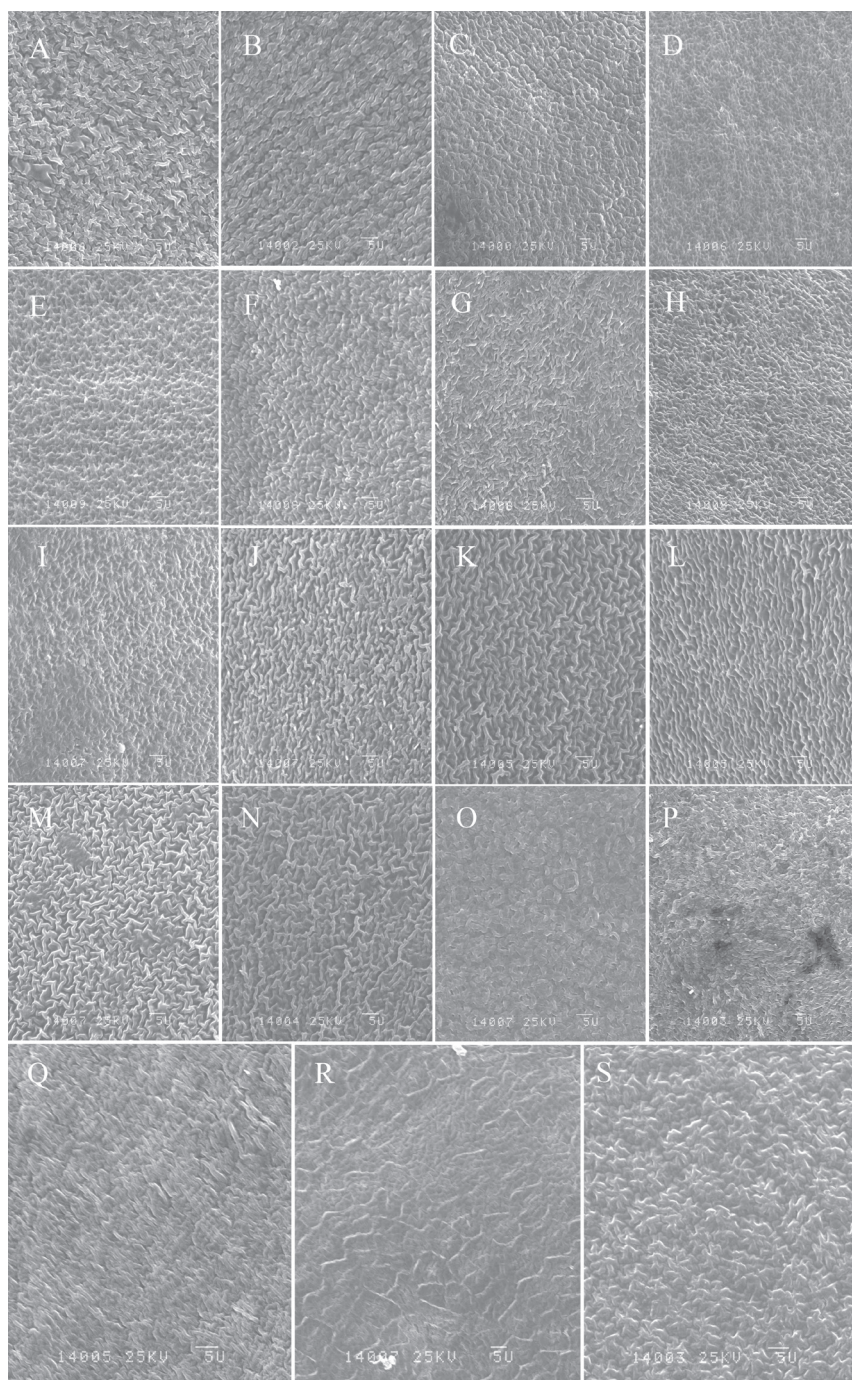


Figure 4. Seed surface sculpturing of the studied species **A** *O. coerulea* (TT) **B** *O. coerulea* (EDG) **C** *O. holanshanensis* **D** *O. xinglongshanica* (MX) **E** *O. xinglongshanica* (XL) **F** *O. glabra* **G** *O. kansuensis* (AZ) **H** *O. kansuensis* (CT) **I** *O. taochensis* **J** *O. ochrocephala* (NH) **K** *O. ochrocephala* (XL) **L** *O. ochrocephala* (MX) **M** *O. ochrocephala* (JQ) **N** *O. ochrocephala* (HG) **O** *O. qinghaiensis* **P** *O. latibracteata* **Q** *O. qilianshanica* **R** *O. aciphylla* **S** *O. squammulosa*. Scale bar: 5 µm.

Table 4. PCA variable loading characters of seed micro-morphology of studied *Oxytropis* species.

PCA variable loadings	PC1	PC2	PC3
Shape of seed	-0.01682	-0.32175	0.7042
Sculpturing	-0.24237	0.44835	0.07685
Hilum position	0.33478	-0.18679	0.5441
Length	0.50744	0.37518	0.00087
Width	0.48498	-0.23663	-0.28718
L/W ratio	0.14335	0.68091	0.32905
Seed weight	0.56183	-0.01324	-0.10682
Eigenvalue	2.90597	1.55275	1.33831
Variability/%	41.51384	22.18208	19.11869
Cumulative/%	41.51384	63.69592	82.81461

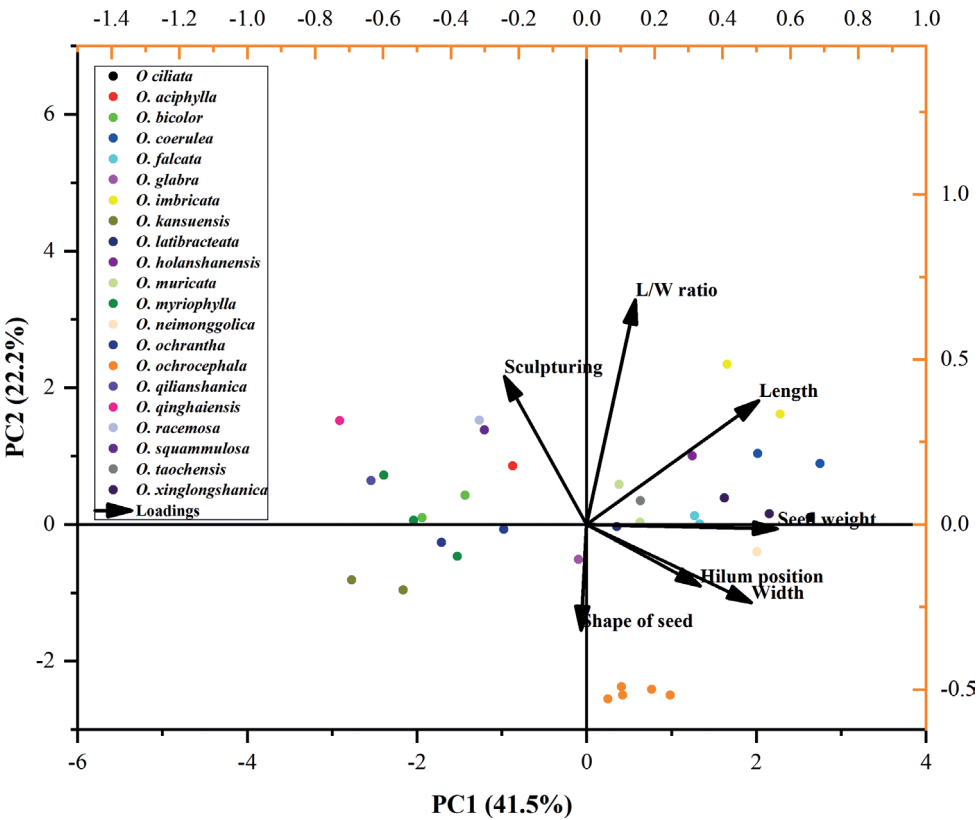


Figure 5. PCA for 35 samples belonging to 21 *Oxytropis* species based on seed morphological characters. Dots of different colors represent different species, and dots of the same color represent different populations of the same species.

similar characteristics. However, the arrangement of 21 species belonging to 10 sections does not show a certain regularity. For example, species belonging to different sections are also arranged together, indicating that the seed morphological characteristics of *Oxytropis* species does not have regularity within the section. Cluster analysis reflects the similarity among species based on the anatomical characteristics and delimitation

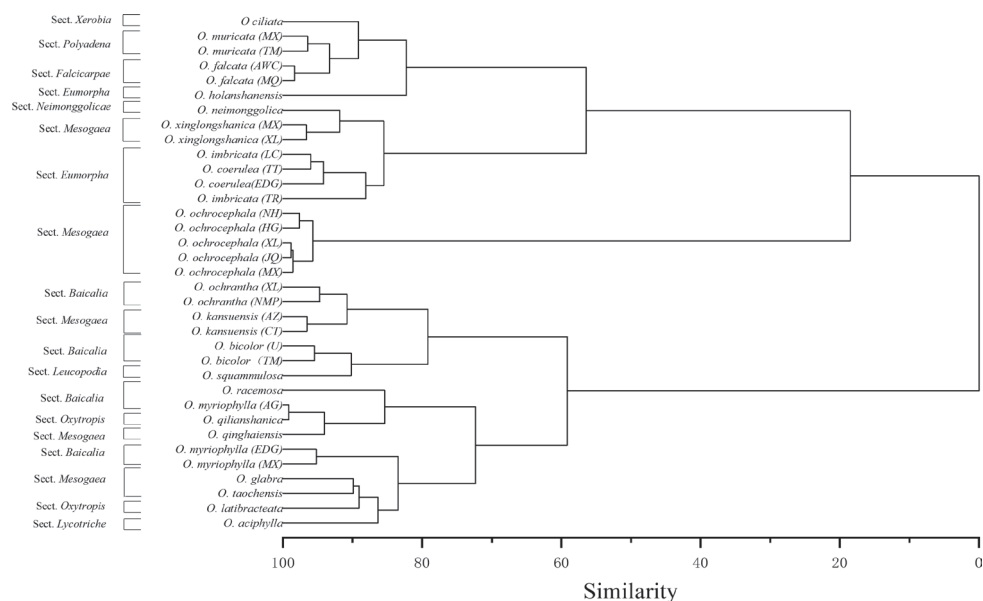


Figure 6. Phenogram for 35 samples belonging to 21 *Oxytropis* species based on based on seed morphological characters.

of these groups. Our phenograms of the quantitative and qualitative data showed three primary clusters (Fig. 6). The first cluster included *O. ciliata*, *O. muricata* (MX, TM), *O. falcata* (AWC, MQ), *O. holanshanensis*, *O. neimonggolica*, *O. xinglongshanica* (MX, XL), *O. imbricata* (LC), *O. coerulea* (TT, EDG), and *O. imbricata* (TR). The second cluster only contained *O. ochrocephala* (NH, XL, MX, JQ, and HG). The third cluster included *O. ochrantha* (XL, NMP), *O. kansuensis* (AZ, CT), *O. bicolor* (U, TM), *O. squammosa*, *O. racemosa*, *O. myriophylla* (AG), *O. qilianshanica*, *O. qinghaiensis*, *O. myriophylla* (EDG, MX), *O. glabra*, *O. taohensis*, *O. latibracteata*, and *O. aciphylla*.

Discussion

Seed morphology of the investigated species was determined for the first time in the present study. Seed characteristics, such as coat pattern, shape, and size, have been shown to be important for the classification within genera of Fabaceae species (Lersten and Gunn 1981; Solum and Lockerman 1991; López et al. 2000; Al-Gohary and Mohamed 2007; Salimpour et al. 2007; Vural et al. 2008; Venora et al. 2009; Zorić et al. 2010; Celep et al. 2012; De-Paula and Oliveira 2012; Kaya and Dirmenci 2012; Lantieri et al. 2013). Previous studies have shown that seed shape and hilum position are taxonomically significant and can therefore be used for the classification of taxa at the genus or even species level (López et al. 2000; Salimpour et al. 2007; Vural et al. 2008). The five main types of seed shapes observed in the present study were consistent with previous findings on *Oxytropis* (Erkul et al 2015). The seed shapes of

different populations of the same *Oxytropis* species were highly consistent, indicating that they were relatively constant within species. Particularly, *O. ochrocephala* and *O. kansuensis* are easily confused, as they are morphologically difficult to distinguish and are both abundant in the northwest China (Zhu et al. 2010). However, our observations demonstrate that these two species can be distinguished based on their seed shape; *O. ochrocephala* has a quadratic seed, whereas *O. kansuensis* has a cardiform seed. These results indicate that seed shape might be a useful taxonomic marker for some *Oxytropis* species. However, similar seed shapes exist in other species of the genus *Oxytropis* and other groups of Fabaceae (Erkul et al. 2015). Thus, they should be considered in combination with other macro-morphological characteristics when applied to species identification within the genus *Oxytropis*.

The sculpturing pattern of seeds is thought to provide useful information for the infrageneric classification of some genera of Fabaceae (Salimpour et al. 2007; Vural et al. 2008; Kamala and Aydin 2018; Rashid et al. 2020). Farrington et al. (2008) proposed that *Oxytropis* seed coat micromorphology and anatomy can be used to distinguish *Oxytropis* from its sister taxon, *Astragalus*. However, studies have shown that the taxonomic value of seed sculpturing patterns in *Astragalus* and *Oxytropis* species is limited. For example, a study that examined 48 species of Turkish *Astragalus* found only two distinct seed coat morphological types (rugulate and rugulate-reticulate) (Vural et al. 2008). Similarly, Shemetova et al. (2018) recognised two main types of seed surface in the genus *Astragalus*: reticulate and indistinct primary sculpture. However, these seed sculpturing patterns have also been observed in the genus *Oxytropis*. Farrington et al. (2008) found that Alaskan *Oxytropis* (15 taxa) has rugulate, rugulate-reticulate, and lophate sculpturing patterns. Consistently, Erkul et al. (2015) reported three types of seed sculpturing patterns in *Oxytropis*, namely rugulate, rugulate-reticulate, and lophate, and proposed that seed characteristics are not useful for separating the genera *Oxytropis* and *Astragalus*. Furthermore, Meyers et al. (2013) proposed that seed coat types among the Alaskan members of *Oxytropis* are highly variable at the species level and cannot be used for species identification. Our results supported this hypothesis because seed sculpturing patterns are variable within some species, including *O. ochrantha* (XL, NMP), *O. bicolor* (U, TM), *O. myriophylla* (EDG, AG, and MX), and *O. imbricata* (LC, TR), suggesting that seed sculpturing pattern has a limited taxonomic value. Interestingly, in the present study, the seed sculpturing pattern appeared to be conserved differently within different sections. Seed coat patterns were stable within some species in the section *Mesogaea*, such as *O. ochrocephala*, *O. kansuensis*, and *O. xinglongshanica*, but highly variable in the species of the sections *Baicalia* and *Eumorpha*. Therefore, the taxonomic significance of seed sculpturing pattern should be comprehensively analysed using a broader sample.

Previous studies on *Oxytropis* have suggested that seed characteristics, such as size (length, width, and length/width ratio), shape, surface sculpturing, and weight have low taxonomic value at the infrageneric level (Solum and Lockerman 1991; Bojňanský and Fargašová 2007; Farrington et al. 2008; Meyers et al. 2013; Erkul et al. 2015). However, most of these studies only subjectively compared their quantitative traits without a systematic analysis such as a cluster analysis. Only Erkul et al. (2015)

systematically analysed the seed traits in 13 *Oxytropis* species from Turkey, but they did not explore the variation in seed traits at the species level because of sampling limitations. In the present study, the results of the cluster analysis showed that, except for *O. myriophylla*, different populations of the same species were clustered into one clade, indicating that the seed traits of *Oxytropis* are useful for the identification of taxa at the species level. However, species belonging to different sections were present in the same clade, indicating that seed characteristics have low taxonomic value at the section level. The results of the PCA also supported the former view that populations within the same species cluster together, while the distribution of samples of different species does not show a certain regularity. Furthermore, the first PC of the PCA provided a highly dominant variability of 41.51%, the characteristics with major scores that contributed to the formation of the groups were quantitative characteristics, such as length, width, and weight of seed. The second and third PCs are mainly qualitative characteristics, accounting for 41.3% of the total variance. These results suggest that even though quantitative traits and some qualitative traits, such as seed sculpturing patterns, are highly variable within species, these traits still play an important role in systematic analysis. Therefore, it is necessary to comprehensively analyse qualitative and quantitative characteristics in future research into *Oxytropis* seed morphology.

To date, a comprehensive phylogenetic study of the genus *Oxytropis* has not been carried out. Moreover, even though several studies have utilized DNA barcodes such as ITS, *trnL-F*, and *psbA-trnH* to investigate the molecular phylogeny of *Oxytropis* in northwest China, the low genetic difference of these barcodes among species makes it difficult to distinguish species within this genus and solve the phylogenetic relationship among its species (Li et al. 2011; Gao et al. 2013; Lu et al. 2014). Therefore, the phylogenetic reliability of seed traits in *Oxytropis* cannot be confirmed. More detailed molecular phylogenetic studies and more extensive taxon sampling are needed to discover the correlation between seed features and genus taxonomy.

Conclusions

Our results suggest that the seed traits of *Oxytropis* are helpful for identifying taxa at the species level, but have low taxonomic value at the section level. Seed shape was constant within species and was useful for species delimitation in the genus *Oxytropis* when combined with other macroscopic traits. The seed sculpturing patterns were highly variable at the species level and could not be used for species identification. Although quantitative traits and some qualitative traits, such as seed sculpturing patterns, are highly variable within species, these traits still play an important role in PCA and cluster analysis. The results of the PCA and cluster analysis showed that different populations of the same species were clustered into one clade, indicating that in *Oxytropis*, seed traits are useful for the identification of taxa at the species level. However, species belonging to different sections also clustered into the same clade, indicating that seed characteristics have low taxonomic value at the section level.

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Hoya spectatissima (Gentianales, Apocynaceae), a new species from Yunnan, China

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Abstract

Hoya spectatissima, a new species from Yunnan Province, China, is described and illustrated. *Hoya spectatissima* is morphologically similar to *H. lyi*, but can be easily distinguished from the latter by its succulent leaves that are 2–4.5 cm long (vs. coriaceous leaves that are up to 9 cm long), corolla that is pink to pale pink (vs. yellowish-white) and corona lobes that are sub-rhombic in top view (vs. ovoid in top view).

Keywords

Asclepiadoideae, Asia, Marsdenieae, morphology, nomenclature, taxonomy

Introduction

Hoya R. Br. is a large genus within the tribe Marsdenieae of Apocynaceae (Wanntorp et al. 2014), comprising 350–450 species of subshrubs or lianas widely distributed in the tropical and subtropical regions of Asia, Oceania and the Pacific Islands (Li et al. 1995; Endress et al. 2018). China possesses more than 40 species (Huang et al. 2021; Huang et al. 2022), with multiple new species or newly-recorded species reported recently, such as *H. yingjiangensis* J.Feng Zhang, L.Bai, N.H.Xia & Z.Q.Peng (Zhang et al. 2015), *H. acuminata* (Wight) Benth. ex Hook.f. (Gui et al. 2017), *H. vangviengensis* Rodda & Simonsson (Zhang et al. 2017), *H. tamdaoensis* Rodda & T.B.Tran (Nong et

al. 2018), *H. burmanica* Rolfe (Ma et al. 2019), *H. longicalyx* Wang Hui & E.F.Huang (Huang et al. 2020), *H. gaoligongensis* M.X.Zhao & Y.H.Tan (Zhao et al. 2020), *H. ny-
ingchiensis* Y.W.Zuo & H.P.Deng (Zuo et al. 2020), *H. pyrifolia* E.F. Huang (Huang
et al. 2021), *H. longlingensis* E.F. Huang and *H. sichuanensis* E.F.Huang (Huang et al.
2022). Recently, we found an unknown *Hoya* species (Fig. 1) in southwest Yunnan
Province, China. After a detailed morphological comparison with all the *Hoya* species
recorded in China and adjacent regions, we concluded that this species is new to sci-
ence. Thus, we formally describe it here.

Taxonomic treatment

***Hoya spectatissima* B.Xue, E.F.Huang, Gang Yao & J.X.Huang, sp. nov.**

urn:lsid:ipni.org:names:77316067-1

Fig. 1

Diagnosis. *Hoya spectatissima* is most similar to *H. lyi* H.Lév, but differs from the lat-
ter by its succulent leaves (vs. coriaceous leaves) that are 2–4.5 cm in length (vs. up
to 9 cm in length) and rounded or rarely obtuse at the apex (vs. mostly acute at the
apex), pink to pale pink corolla (vs. yellowish-white corolla) that is densely sericeous
adaxially (vs. densely pubescent adaxially), ivory white corona lobes (vs. purple towards
the inner angle and ivory white towards the outer angle of corona lobes) that are sub-
rhombic in top view (vs. ovoid in top view) and have an acute outer angle (vs. truncate
to shallowly concave outer angle).

Type. CHINA, Yunnan Province, Gengma Hsien, Sipaishan Town, Guanlong Vil-
lage, Luoshuidong, on rocks in karst habitat, 7 July 2021, *E.F. Huang-RG0017* (Holo-
type: IBSC!).

Description. Lithophytic climber. Stems rounded in cross section, up to 1 m in
length, 1.5–2 mm in diameter, densely pubescent when young, subglabrous when
mature, sometimes 3–5 branches at nodes, internodes 3–8 cm long. Leaves opposite,
wine-red and pubescent when young; mature lamina subglabrous, succulent, elliptic or
oblong, 2–4.5 × 1.3–1.8 cm, 2.5–3 mm in thickness, bright green adaxially, pale green
abaxially, base rounded, apex rounded or rarely obtuse; mid-vein slightly elevated or
obscure adaxially, not elevated abaxially, lateral veins 2–3 pairs or sometimes invisible
in adaxial surface, invisible in abaxial surface; petioles sometimes recurved, 3–7 mm
long, 1–1.5 mm in diameter. Inflorescences axillary pseudo-umbels, many-flowered,
globose; peduncle longer than pedicels, 4.5–5 cm long, papillose to sparsely pubescent;
pedicels 2.5–3 cm long, yellow-green, papillose to sparsely pubescent. Calyx lobes tri-
angular to narrowly triangular, ca. 1.5 × 2 mm, purplish-red, sparsely pubescent or
glabrous. Corolla rotate, pink in early flowering phase, pale pink in late flowering
phase, ca. 2 cm in diameter, densely sericeous adaxially, sub-glabrous abaxially, lobes

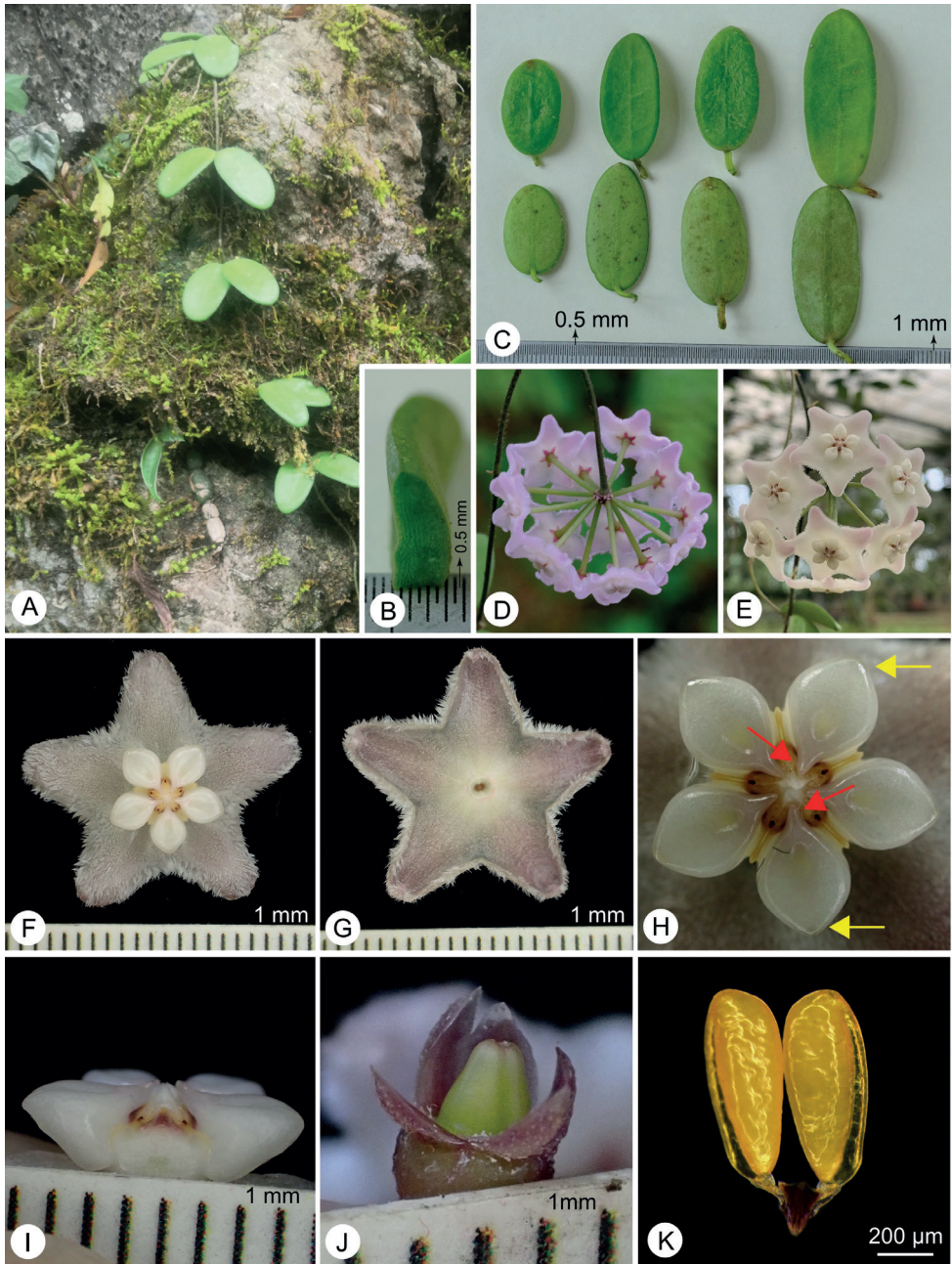


Figure 1. *Hoya spectatissima* **A** habit **B** cross section of lamina **C** leaves **D, E** inflorescence **F** flower (top view) **G** flower (bottom view) **H** corona (top view; Red arrowheads indicate the inner angle of the corona lobes and yellow arrowheads indicate the outer angle of the corona lobes) **I** corona (side view) **J** ovaries **K** pollinarium.

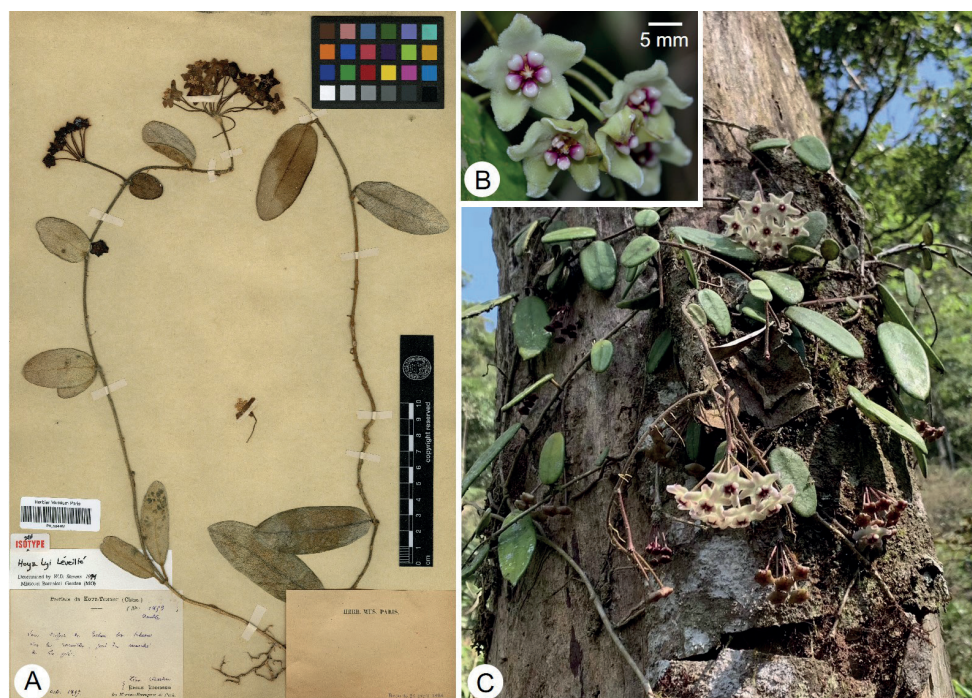


Figure 2. *Hoya lyi* **A** isosyntype (E. Bodinier 1853, P00634485) **B** inflorescence **C** habit.

triangular, 5–6 mm long and 5–6 mm wide, margin recurved, apex rounded, revolute. Corona ivory white, 7–8 mm in diameter, 2.0–2.4 mm high, lobes 5, stellate spreading, ca. 3.5×2.5 mm, sub-rhombic in top view, outer angle acute, inner angle acuminate, the inner tips convex and spreading obviously towards the centre of the flower, ivory white or rarely shallowly pink. Pollinia clavate, ca. 0.73×0.26 mm, base cuneate, apex truncate, narrowing towards the base, sterile edge all along the outer edge of the pollinium, translator arms attached at the centre of the corpusculum. Ovaries 2, triangular-ovate, attached to each other, ca. 2 mm long and 1.5 mm wide, green, glabrous. Fruit and seed not seen.

Distribution and habit. *Hoya spectatissima* is known from at least two localities in Cangyuan Hsien and Gengma Hsien, Yunnan Province, China. It is a lithophytic liana that climbs on rocks in karst habitat. It is a common species in Guanlong Village, Gengma Hsien, but an occasional species in Menglai Town, Cangyuan Hsien.

Etymology. *Hoya spectatissima* is named to reflect its beautiful flowers.

Taxonomic discussion. *Hoya spectatissima* has evidently succulent leaves that are 2.5–3 mm thick (Fig. 1B); this trait is also found in *H. pandurata* Tsiang, which is also endemic in Yunnan Province, China. However, *H. pandurata* is a subshrub and is epiphytic on trees in open or mixed woods and it further differs from *H. spectatissima* by its pandurate or oblong leaves (vs. elliptic or oblong) with acuminate apex (vs. rounded or rarely obtuse), shorter peduncle and pedicels that are ca. 3 mm long (vs.

4.5–5 cm long) and 1.5 cm long (vs. 2.5–3 cm long) respectively and yellow or reddish corolla (vs. pink or pale pink) that is 0.8–1 cm (vs. ca. 2 cm) in diameter (Li et al. 1995). Additionally, *H. spectatissima* is morphologically similar to *H. lyi*, a species recorded from China, Laos and Vietnam (Rodda 2012). Both species have elliptic or oblong leaves (Figs 1A, C, 2A, C), triangular to narrowly triangular calyx lobes, corolla ca. 2 cm in diameter and clavate pollinia. However, *Hoya spectatissima* differs from *H. lyi* by the following characters: succulent leaves that are 2.5–3 mm thick (Fig. 1B) (vs. coriaceous leaves that are less than 1.5 mm thick, Fig. 2A, C); shorter leaves that are 2–4.5 cm long (Fig. 1C) (vs. up to 9 cm long, Fig. 2A); apex of leaves rounded or rarely obtuse (Fig. 1A, C) (vs. mostly acute, Fig. 2A, C); 2–3 paired lateral veins in leaves (vs. 3–5 paired lateral veins); pink to pale pink corolla (Fig. 1D, E) (vs. yellowish-white, Fig. 2B); corolla lobes densely sericeous adaxially (Fig. 1F, G) (vs. densely pubescent adaxially, Fig. 2B); corona lobes sub-rhombic in top view (Fig. 1F, H) (vs. ovoid, Fig. 2B) and ivory white (Fig. 1F, H) (vs. purple towards the inner angle and ivory white towards the outer angle, Fig. 2B); and outer angle of corona lobes acute (Fig. 1H) (vs. truncate to shallowly concave, Fig. 2B).

Paratype. China. Yunnan Province, Cangyuan Hsien, Menglai Town, on rocks in karst habitat, 22 August 2021, *E.F. Huang-RG0033* (IBSC).

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Eighteen new species of Neotropical Costaceae (Zingiberales)

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Abstract

In preparation for a full taxonomic revision of the Neotropical genera of Costaceae (i.e., *Chamaecostus*, *Costus*, *Dimerocostus*, and *Monocostus*), we present the description of 17 new species of Neotropical *Costus* and one new species of the Neotropic endemic genus *Chamaecostus* with notes on their distribution and ecology, vernacular names (when known), and diagnostic characters for identification. Distribution maps are included for all species, and each description is accompanied by photographic plates illustrating diagnostic characters.

Keywords

Chamaecostus, *Costus*, *Dimerocostus*, Monocots, spiral ginger

Introduction

The Neotropical members of the family Costaceae (Zingiberales) comprise two separate evolutionary lineages of species included in genera *Costus*, *Chamaecostus*, *Dimerocostus* and *Monocostus*. Originally considered a subfamily of the Zingiberaceae, they were first treated comprehensively in Floral Neotropica monographs No. 8 Costoideae (Zingiberaceae) and Monograph No. 18 Costoideae (Additions) (Maas 1972, 1977)

and as part of the Flora of Suriname (Maas 1979). Species pertaining to the genus *Chamaecostus*, included in these monographs as *Costus* subgenus *Cadalvena*, were subsequently removed from *Costus* based on phylogenetic analyses demonstrating that they are in fact more closely related to *Monocostus* and *Dimerocostus* than to other species of *Costus* (Specht et al. 2001; Specht 2006a; Specht and Stevenson 2006). Of these genera, *Chamaecostus*, *Monocostus* and *Dimerocostus* are endemic to the Neotropics while *Costus* contains species with African distributions that appear to be ancestral to a clade of Neotropical species (Specht and Stevenson 2006; Specht 2006a, b; Salzman et al. 2015).

Since Maas' monographic work based largely on observations of herbarium collections, novel observations from extensive field work and herbarium research have resulted in the publication of 18 additional species by Paul Maas (Maas 1976) and Hiltje Maas-van de Kamer (Maas and Maas-van de Kamer 1990, 1997): *Costus asplundii*, *C. asteranthus*, *C. beckii*, *C. cordatus*, *C. cupreifolius*, *C. curvibracteatus*, *C. glaucus*, *C. leucanthus*, *C. nitidus*, *C. osae*, *C. plicatus*, *C. plowmanii*, *C. productus* (to be placed in *C. juruanus*), *C. ricus*, *C. Vargasii*, *C. varzearum*, *C. vinosus*, and *C. wilsonii*. A few additional species have been published by other taxonomists, including García-Mendoza and Ibarra-Manríquez (1991), *Costus dirzoi*; Salinas et al. (2007), *Costus fissicalyx*; Salinas and Betancur (2004), *Dimerocostus cryptocalyx*; and the most recent by Pessoa et al. (2020), *Costus atlanticus*.

Recent years have also seen a rise in ecological and evolutionary studies on Costaceae, which have greatly impacted our interpretation of phenotypic patterns and evolutionary processes leading to a better understanding of species delimitations and phylogenetic relationships among taxa (Sakai et al. 1999; Specht et al. 2001; Kay and Schemske 2003; Kay et al. 2005; Kay 2006; Specht 2006a, b; Specht and Stevenson 2006; Araújo and Oliveira 2007; Kay and Schemske 2008; Yost and Kay 2009; Bartlett and Specht 2010; Specht et al. 2012; Surget-Groba and Kay 2013; Almeida et al. 2015; André et al. 2015; Morioka et al. 2015; Salzman et al. 2015; André et al. 2016; Bergamo et al. 2016; Valderrama et al. 2020; Vargas et al. 2020; André et al. 2022). These studies have helped to tease apart species complexes, identify cryptic variation, define biogeographic patterns and pollinator relationships, understand aspects of floral developmental evolution, and explore the tempo and mode of speciation and diversification across the neotropical lineages.

In the present publication, 17 new species of *Costus* and one new species of *Chamaecostus* are described. We include novel character observations made in recent years, including differences in the orientation of the flower relative to the inflorescence, a character that is variable between but consistent within species of *Costus* and thus useful for identification. Floral orientation may be a key feature in defining pollinator preferences and efficiencies (Fenster et al. 2009), and future studies will investigate possible functional significance. In a later study, we will incorporate these new species into a complete revision for all Neotropical Costaceae, including keys to all species, updated distribution maps, photographs with diagnostic characters, a complete index of exsiccatae (comprising ca. 11,000 entries) and a phylogenetic hypothesis to demonstrate evolutionary relationships.

Materials and methods

Material from ca. 150 herbaria was consulted for this study. A complete list of specimens examined will be provided in the forthcoming revision (Part II).

In addition, extensive field observations and collections have contributed to this study, supporting our understanding of species distributions and providing opportunities to photograph and observe key traits for distinguishing among taxa, including the colour of floral organs, orientation of the flower relative to the inflorescence (abaxial, erect, adaxial), colour of bracts, and colouration patterns of vegetative material. Field work was led by Paul and Hiltje Maas, with all co-authors participating. Dave Skinner visited most Neotropical countries and made essential observations on this group, particularly visiting type localities and taking detailed photographs, contributing to our understanding of the distribution of characters among taxa. Skinner's observations have been recorded in iNaturalist and are available at www.inaturalist.org/observations/selvadero. Living material is available in cultivation at the Fairchild Tropical Gardens in Miami, Florida, USA.

Taxonomy

Chamaecostus manausensis Maas & H.Maas, sp. nov.

urn:lsid:ipni.org:names:77316081-1

Diagnosis. *Chamaecostus manausensis* sp. nov. (Fig. 1) looks quite similar to *C. congestiflorus* and has been regularly confused with that species but can be distinguished by a narrowly cylindric (instead of broadly obovoid) inflorescence and a non-fimbriate labellum.

Type. BRAZIL. Amazonas: Mauá Road, 22 Mar 1971, *Prance, Coêlho, Kubitzki & Maas 11529* (holotype: U 2 sheets: U1219716 & U1219717; isotypes: BR, COL128233, CR, DAV, E, F175333, F1842269, GH, INPA, K, MO, NY, P, S, US, VEN, W).

Description. *Herb* 0.4–1 m tall. *Leaves* sheaths 2–15 mm diam; ligule 1–2 mm long; petiole 0–5 mm long; sheaths, ligule and petiole densely puberulous; lamina 13–25 × 3–6 cm, narrowly elliptic, adaxially sparsely puberulous to glabrous, abaxially sometimes reddish-purple, sparsely to densely puberulous, particularly the primary vein, base obtuse, apex acuminate, acumen 5–15 mm long. *Inflorescence* 4–10 × 1.5–3 cm, up to ca. 13 × 4 cm in fruit, narrowly cylindric, terminating a leafy shoot; bracts, bracteoles, calyx, ovary, and capsule rather densely to densely puberulous; bracts 2.5–3.5 × 0.3–0.5 cm, narrowly triangular, green, chartaceous, apex often mucronate, callus 3–6 mm long; bracteole 15–20 mm long, tubular, often deeply split on the abaxial side, 2-lobed, lobes 3–7 mm long, deltate. *Flowers* calyx 20–33 mm long, green, tube often deeply split during anthesis, lobes 5–10 mm long, subulate; corolla 50–65 mm long, cream, densely puberulous, lobes 30–40 mm long narrowly, obovate; labellum ca. 35 × 40 mm, cream, distal edge horizontally spreading, broadly angular-obovate, margin crenulate; stamen 25–30 × 10 mm, cream, apex reflexed, irregularly dentate, anther 5–6 mm long. *Capsules* 11–13 mm long, ellipsoid.



Figure 1. *Chamaecostus manausensis* Maas & H.Maas **A** plant in habitat Floresta Nacional do Tapajós, Brazil **B** flower showing the non-fimbriate margin of the labellum **C**, **E** inflorescence narrowly cylindric instead of broadly obovoid **D** sheath and ligules. Photos **A–E** by Thiago André.

Distribution. Brazil (Amazonas, Pará) (Fig. 21A).

Habitat and ecology. In terra-firme rainforests or campinarana vegetation, on sandy or clayey soils, at elevations of about sea level. Flowering and fruiting: October to March, July.

Etymology. This species is named “manausensis” after the city of Manaus, State of Amazonas, Brazil, where this species is most commonly distributed.

Paratypes. BRAZIL. Amazonas: Reserva Florestal Ducke, km 26 of Manaus-Itacoatiara road, 1 Dec 1994, *Assunção 107* (INPA, U), 5 Jan 1995, *Costa et al. 73* (INPA, U), 12 Dec 1996, 568 (INPA, U), 10 Oct. 1995, *Miralha & Maas 296* (INPA, U), 26 Jan 1995, *Nascimento & Silva 726* (INPA, U); Cachoeira Alta do Tarumã, Manaus, 7 Feb 1930, *Ducke RB 25622* (U); Rio Maués-Acú, across from Maués, Mun. Maués, 21 Jul 1983, *Hill et al. 13138* (INPA, MG, NY, RB, US). **Pará** Floresta Nacional do Tapajós, comunidade Jamaraquá, *Mortati 92* (HSTM).

Notes. For additional field data, see Costa et al. (2011), where this species has been treated under the name *Costus congestiflorus*.

***Costus alfredoi* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316083-1

Diagnosis. *Costus alfredoi* sp. nov. (Fig. 2) is recognizable by an extremely small ligule (<1 mm long), a “basal” inflorescence (inflorescence terminates a short, leafless shoot and thus is positioned at the base of the plant) with red, unappendaged bracts and yellow, tubular flowers, an almost complete lack of indument, and very narrow, grass-like leaves. It shares the last two features with *C. vargasii* but differs by a shorter ligule (<1 mm vs. 5–35 mm long) and a much longer calyx (15–16 vs. 6–10 mm) and bracteole (25–27 vs. 12–22 mm).

Type. BOLIVIA, La Paz: Prov. Bautista Saavedra, Area Natural de Manejo Integrado Apolobamba, between Paujeyuyo and Calzada, 1140 m, 16 Nov 2003, *Fuentes Claros, Cayola, Whitehead & Cuevas 6216* (holotype: MO2098346; isotypes: U1212213 & U1212214).

Description. *Herb* ca. 2 m tall. *Leaves* sheaths 4–5 mm diam; ligule obliquely truncate, <1 mm long; petiole 5–7 mm long; sheaths, ligule and petiole glabrous; lamina linear, 15–23 × 1.5–2.5 cm, adaxially and abaxially glabrous, except for some hairs along the margin, base acute to obtuse, apex long-acute. *Inflorescence* ovoid, 11–12 × 5–6 cm, terminating a leafless shoot 50–85 cm long, sheaths obliquely truncate, 6–8 cm long, glabrous; bracts, bracteole, calyx, ovary, and capsule glabrous; bracts red, coriaceous, ovate, 3–4 × 1.5–2.5 cm, apex obtuse, callus 5–7 mm long; bracteole boat-shaped, 25–27 mm long. *Flowers* abaxially oriented; calyx 15–16 mm long, lobes shallowly ovate-triangular, 2–3 mm long; corolla yellow to orange, ca. 50 mm long, glabrous, lobes narrowly elliptic, ca. 30 mm long; labellum yellow, lateral lobes rolled inwards and forming a curved tube ca. 10 mm diam, oblong-elliptic when spread out, ca. 35 × 15 mm, middle lobe with yellow honey mark, 5–7-lobulate, lobules red, 5–6 mm long; stamen yellow, ca. 35 × 7 mm, slightly exceeding the labellum, apex red, slightly dentate, anther length not measured. *Capsule* ellipsoid, ca. 15 mm long.

Distribution. Bolivia (La Paz) (Fig. 21B).

Habitat and ecology. In wet, sub-Andine forests with *Oenocarpus bataua* Mart. (Arecaceae) at an elevation of ca. 1140 m. Flowering in November.

Etymology. *Costus alfredoi* is named after the very active and inspiring Bolivian plant collector Alfredo Fuentes, who we personally met some years ago during a visit to the MO Herbarium and who provided us with additional data on Bolivian species of Costaceae.

Notes. In Bolivia and Brazil, D. Skinner has observed plants with leaves and ligules that match this description. However, they were not in flower. The distribution of this species may be found to extend beyond the department of La Paz, Bolivia.

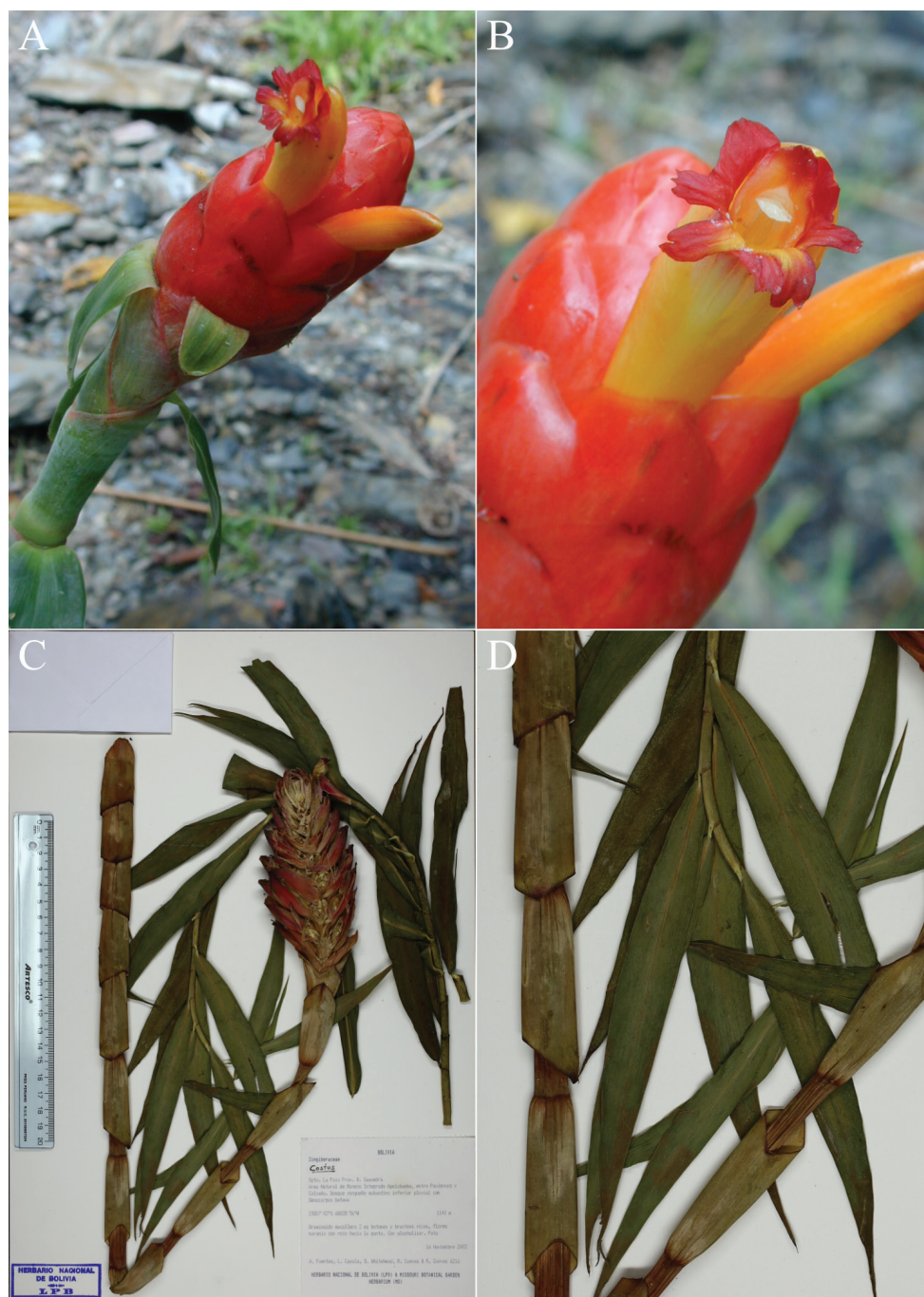


Figure 2. *Costus alfredoi* Maas & H.Maas **A** inflorescence terminal on a leafless shoot **B** flower **C** holotype from Bolivia, La Paz **D** detail from holotype showing narrow, grass-like leaves and short ligule. (A–D from Fuentes et. al. 6216). Photos by Alfredo Fuentes Claros.

***Costus alleniiopsis* Maas & D. Skinner, sp. nov.**

urn:lsid:ipni.org:names:77316084-1

Diagnosis. *Costus alleniiopsis* sp. nov. (Fig. 3) resembles *C. allenii* but differs by distinctly plicate leaves and a longer ligule.

Type. PANAMA, Veraguas: Río Segundo Brazo, 700–750 m, 8 Sep 1974, *Maas & Dressler 1652* (holotype U1231561; isotypes F, MO-2193025).

Description. *Herb* 0.5–1.2 m tall. *Leaves* sheaths 10–20 mm diam; ligule obliquely truncate, 12–20 mm long, often reddish; petiole 10–25 mm long; sheaths, ligule and petiole densely brownish villose; lamina ovate to obovate, 25–32 by 13–16 cm, abaxially sometimes reddish, 10–12-plicate, adaxially and abaxially densely brownish villose, base cordate, acute, or obtuse, apex acuminate (acumen 10–15 mm long). *Inflorescence* ovoid, 6–7 × 3–5 cm, terminating a leafy shoot or rarely terminating a leafless shoot; bracts rather densely to densely brownish villose, bracteole, calyx, ovary, and capsule rather densely to densely puberulous to glabrous. *Flowers* adaxially oriented to erect; bracts green, rarely red, coriaceous, broadly ovate to ovate, 3–5 × 2–5 cm, apex obtuse, callus 5–7 mm long; bracteole boat-shaped, 20–30 mm long; calyx pink to red, 8–10 mm long, lobes shallowly triangular, 2–3 mm long; corolla yellow, 60–70 mm long, glabrous, lobes narrowly elliptic, 50–60 mm long; labellum yellow, distal edge horizontally spreading, broadly obovate, 50–70 × 40–50 mm, lateral lobes striped with red, middle lobe reflexed, with yellow honey mark, margin crenulate; stamen yellow, 40–50 × 12–15 mm, not exceeding the labellum, apex red, obtuse to irregularly dentate, anther 7–10 mm long. *Capsule* ellipsoid, 15–20 mm long.

Distribution. Panama (Coclé, Colón, Veraguas) (Fig. 21C).

Habitat and ecology. In forests, at elevations of 200–1300 m. Flowering in the rainy season.

Etymology. This species strongly resembles *C. allenii*, and the Greek word “*opsis*” (= likeness) refers to the resemblance with that species.

Notes. *Costus alleniiopsis* sp. nov. is restricted to Panama. It is closest to *C. allenii* Maas, differing by a longer ligule (12–20 mm vs. 2–10 mm) and distinctly plicate leaves. They both share adaxial oriented to erect flowers, a feature not often seen in the genus *Costus*.

Paratypes. PANAMA. Coclé: Alto Calvario, above El Copé, ca. 6 km N of El Copé, 23 Jun 1988, *Croat 68796* (MO, U); Alto Calvario, forest along Continental Divide, ca. 5 miles N of El Copé, 900–1000 m, 31 Mar 1993, *Croat 75052* (MO, U); Caribbean side of divide at El Copé, 200–400 m, 4 Feb 1983, *Hamilton & Davidse 2778* (MO); near sawmill, 8 km N of El Copé (28 km NW of Penonomé), 600–750 m, 1 Sep 1977, *Maas et al. 2760* (U); Parque Nacional General Omar Torrijos Herrera, El Copé, Sendero Rana Dorada, 750 m, 31 May 2004, *Maas et al. 9541* (U); above El Potroso sawmill at Continental Divide, N of El Copé, 1200–1300 m, 13 May 1981, *Sytsma & Andersson 4610* (MO, U). Colón: MPSA Concession, around Petaquilla Tower, 23 May 2012, *Hammel et al. 26342* (MO). Veraguas: valley of Río Dos Bocas, on road between Alto Piedra (above Santa Fé) and Calovebora, 350–400 m, 29 Aug



Figure 3. *Costus alleniiopsis* Maas & D. Skinner **A** inflorescence **B** bracts and flower **C** plant in habitat **D** ligule and leaf base **E** ligule and leaf base of *C. allenii* for comparison **F** leaf plicae and indument **G** leaf plicae and indument of *Costus allenii* for comparison. Photos of *C. alleniiopsis* in habitat, Veraguas, Panama by Dave Skinner.

1974, *Croat* 27455 (MO, U); near the Continental Divide, ca. 15 km NW of Santa Fé, 800 m, 22 Oct 1980, *Maas & Dressler* 5006 *p.p.* (K, L, U); 16 km NW of Santa Fé, on road to Calovebora (Panamanian Highway 35), 300–500 m, 16 May 1975, *Mori & Kallunki* 6124 (MO).

***Costus alticolus* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316085-1

Diagnosis. *Costus alticolus* sp. nov. (Fig. 4) can be recognised by its leafless flowering shoots up to ca. 1 m long, combined with large, red and unappendaged bracts, tubular and yellow flowers, and stamen far exceeding the labellum like in *C. pulverulentus* C.Presl.

Type. MEXICO. Oaxaca: Sierra Mazateca, Mun. Santa María Chilchotla, Cuauhtemoc, ca. 4 km NE of Santa María Chilchotla, near Clemencia and Santa Rosa, 1200 m, 16 Jan 1984, *Solheim & Reisfeld* 1367 (holotype WIS).

Description. *Herb* 2–3.5 m tall. *Leaves* sheaths 10–30 mm diam; ligule truncate, 4–12 mm long; petiole 5–20 mm long; sheaths, ligule and petiole densely to sparsely puberulous; lamina narrowly elliptic, 25–47 × 6–14 cm, adaxially glabrous, abaxially densely (particularly along the primary vein) sericeous to almost glabrous, base acute, apex acuminate (acumen 5–10 mm long). *Inflorescence* cylindric, 11–18 × 5–9 cm, terminating a leafless shoot 20–95 cm long, sheaths obliquely truncate, 4–7 cm long, rather densely puberulous to glabrous; bracts, bracteole, calyx, ovary, and capsule glabrous to densely puberulous. *Flowers* abaxially oriented; bracts red to bright crimson, coriaceous, ovate-oblong, 4–6 × 3–4 cm, apex obtuse, callus ca. 10 mm long; bracteole boat-shaped, 30–35 mm long; calyx red, 13–21 mm long, lobes deltate to shallowly triangular, 3–6 mm long; corolla yellow to orange, 50–60 mm long, glabrous, lobes narrowly elliptic, ca. 40 mm long; labellum yellow, lateral lobes rolled inwards and forming a slightly curved tube 18–20 mm diam, obovate when spread out, ca. 60 by 35 mm, irregularly lobulate, lobules recurved, ca. 4 mm long; stamen yellow, ca. 65 × 11 mm, far exceeding the labellum, apex acute, anther ca. 8 mm long. *Capsule* ellipsoid, 17–18 mm long.

Distribution. Mexico (Oaxaca) (Fig. 21D).

Habitat and ecology. In primary, wet montane to premontane cloud forests, with some mosses on trunks, drier on ridge crests, with *Hedyosmum mexicanum* Cordem. Ex Baill., *Liquidambar macrophylla* Oerst., *Siparuna andina* A.DC., *Posoqueria latifolia* (Rudge) Roem. & Schult., *Heliconia* sp., *Deppea* sp., *Chamaedorea* sp., *Solenophora* sp., and *Salvia divinorum* Epling & Játiva, at elevations of 1040–1900 m. Flowering year-round.

Etymology. *Costus alticolus* sp. nov. occurs at quite high elevations up to 1900 m, hence the specific epithet alticolus ('altus' means 'high' in Latin; 'colere' means 'to live' in Latin).

Paratypes. MEXICO. Oaxaca: Distr. and Mun. Ixtlán, near abandoned community of Tarabundi, S side of Río Soyalapán, access via La Luz, primary forest toward Río Langaro, 1230–1260 m, 16 Nov 1993, *Boyle & Massart* 2556 (MO, U); Mun. San Felipe Usila, cuenca del Río Perfume, 4.4 km SE of Santa Cruz Tepetotutla, 1400 m, 29 Mar 1995, *Rincón Gutiérrez et al.* 592 (MO); Distr. Mixe, Mun. Totontepec, Totontepec, 1900 m, 9 Aug 1990, *Rivera Reyes & Martin* 1610 (CAS), Hwy 175, km 169, near La Esperanza, 1075 m, 12 Aug 2017, *Skinner* R3400 (BH).

Notes. We are greatly indebted to Manuel Gutiérrez, who assisted Dave Skinner in obtaining permission from the indigenous people of Santa Cruz Tepetotutla, Oaxaca, Mexico, to study this new species common in the Chinantla region of Oaxaca.



Figure 4. *Costus alticolus* Maas & H.Maas **A** inflorescence with flower, showing stamen (st) extending far beyond labellum, fully exposing the thecae **B, C** plant in habitat **D** flower and bract details **E** flowering shoot. Photos in habitat in Chinantla region near Santa Cruz Tepetotutla, Oaxaca, Mexico by Dave Skinner.

***Costus antioquiensis* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316086-1

Diagnosis. *Costus antioquiensis* sp. nov. (Figs 5, 6) is well recognisable by adaxially oriented flowers, an often present ring of brown, erect and rather stiff hairs at the base of the ligule, and the abaxial leaf lamina and the bracts being often densely villose. It could be confused with *Costus laevis* Ruiz & Pav., but that species has much darker flowers and is often completely glabrous.

Type. COLOMBIA, Antioquia: Mun. San Rafael, along road from Guatapé to San Rafael, Río El Bizcocho, Finca El Castillo, 1059 m, 12 Oct 2013, *Maas, Maas-van de Kamer & Valderrama 10488* (holotype L, 3 sheets; isotypes JAUM, K, MO-3159234, MO-3159235).

Description. *Herb* 0.5–5 m tall. *Leaves* sheaths 10–20 mm diam; ligule truncate, 5–10 mm long, basally densely covered with a ring of brown, erect and rather stiff hairs (villose), sometimes absent; petiole 5–20 mm long; sheaths and petiole sparsely or sometimes densely villose; lamina narrowly elliptic, 30–50 × 9–13 cm, adaxially glabrous, sometimes densely villose, abaxially densely to sometimes sparsely villose or glabrous, base obtuse, apex acuminate (acumen 5–10 mm long). *Inflorescence* ovoid, 8–16 × 5–6 cm, enlarging to ca. 25 by 7–8 cm in fruit, terminating a leafy shoot; bracts, bracteoles, calyx, ovary, and capsule densely puberulous to villose, rarely glabrous. *Flowers* adaxially oriented to erect; bracts green, coriaceous, broadly ovate, 3–4 × 3–4 cm, apex obtuse, callus 8–10 mm long; appendages rarely present; bracteole boat-shaped, 20–27 mm long; calyx (pale) red, 7–11 mm long, lobes deltate to shallowly triangular, ca. 3 mm long; corolla yellow, pale orange, or pink, 60–70 mm long, glabrous, lobes elliptic, 40–60 mm long; labellum yellowish white, distal edge horizontally spreading, broadly obovate, 50–70 × 40–60 mm, lateral lobes striped with red, middle lobe reflexed, with dark yellow honey mark, margin irregularly dentate; stamen yellowish white to pink, ca. 40 × 10–12 mm, not exceeding the labellum, apex dark red, entire to dentate, anther ca. 8 mm long. *Capsule* obovoid, 25–28 mm long.

Distribution. Colombia (Amazonas, Antioquia, Arauca, Boyacá, Caldas, Cauca, Chocó, Córdoba, Cundinamarca, Guajira, Guaviare, Huila, Meta, Norte de Santander, Quindío, Risaralda, Santander, Tolima, Valle del Cauca, Vichada), Venezuela (Mérida), Ecuador (Azuay, Carchi, Pastaza, Zamora-Chinchipe), Peru (Ucayali). (Fig. 21E)

Habitat and ecology. In forests (a.o., “selva baja/alta perennifolia” or wet montane forests) or forested roadsides at elevations of 50–1700 m. Flowering year-round.

Vernacular names. Colombia: Caña agria, Cañagria, Cañaguatate. Peru: Sacha huiro.

Etymology. This species was collected by Paul and Hiltje Maas for the first time in the Colombian department of Antioquia and is therefore named in recognition of that locality.

Paratypes. COLOMBIA. **Amazonas:** Mun. Puerto Nariño, Trapécio Amazónico, orillas del Amazonas, 1 Nov 1945, *Duque-Jaramillo 2251* (COL). **Antioquia:** Mun. Frontino, Corregimiento Nutibara-La Blanquita, 4–7.1 km between Alto de Cuevas and La Blanquita, 1420–1610 m, 26 Jan 1995, *Betancur B. et al. 5970* (COL, U, US); Carauta, Mun. Frontino, Corregimiento Carauta, Parque Nacional Natural “Las Orquídeas”, sector Tres Bocas, quebrada San Francisco, poco antes de la desembocadura al río Carauta, Finca La Pradera, 1640–1680 m, 3 Sep 2012, *Betancur B. et al. 16507* (NY); Mun. Frontino, Corregimiento Carauta, Parque Nacional Natural “Las Orquídeas”, sector Tres Bocas, margen derecha del río Carauta, poco después de Tres Bocas, Finca La Pradera, 1610 m, 12 Sep 2012, *Betancur B. et al. 16651* (NY). **Arauca:** Campo petrolero de Caño Limón, Consorcio Cravo Norte, cerca del relleno sanitario, 280 m, 16 Oct 1997, *Betancur B. 7500* (COL). **Boyacá:** Puerto Boyacá,

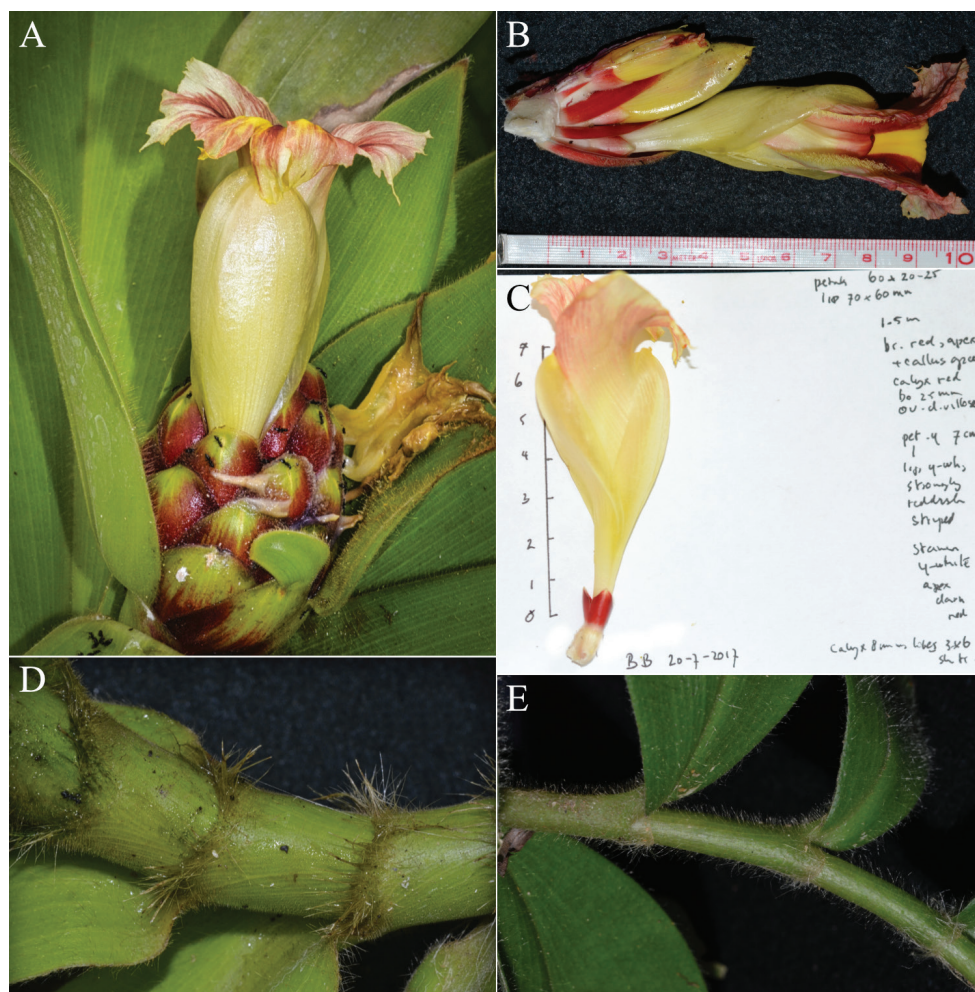


Figure 5. *Costus antioquiensis* Maas & H. Maas **A** inflorescence **B** flower **C** flower details and handwritten notes by Paul Maas **D** detail of hairy ligule **E** sheath. Photos **A–E** of specimen *Maas et al* 10488 taken prior to pressing. Photos by Paul Maas.

Corregimiento Puerto Romero, quebrada alrededores del campamento Techint, 10 Oct 1996, *Balcázar* 49 (COL); Guacamayas, Vereda La Laguna, sector Litargón, 2349 m, 7 Jun 2009, *Beltrán Cuartas* 78 (COL). **Caldas:** Mun. Norcasia, Reserva Natural Ríomanso, ca. 40 km de La Dorada, valle del río Magdalena, 212 m, 14 Oct 2013, *Guevara-Ibarra* 39 (COL, CUV); Mun. Victoria, Vereda Gigante, Hacienda La Española, 314–321 m, 5 Jun 2014, *Vargas-Figueroa et al.* 855 (CUVC). **Cauca:** Mun. Santander del Quilichao, Hacienda Nana Loisa, 1000 m, 2 Nov 2002, *Silverstone-Sopkin et al.* 8963 (CUVC). **Chocó:** Carretera Tutunendo to El Carmén, entre kms 135 y 120, 800–1200 m, 29 Apr 1979, *Forero et al.* 6161 (COL, MO, U); Mun.

San José del Palmar, mouth of Río Torito (affluent of Río Habita), 850–950 m, 16 May 1980, *Forero et al.* 7413 (COL, U). **Córdoba:** Montería, Buenos Aires, Finca La Poderosa, 13 Apr 2005, *Pulido* 107 (COL). **Cundinamarca:** Yacopí, Inspección de policía de Guadualito, Vereda del Lamal, al lo largo de la carretera de El Lamal a El Gramal, 950–1215 m, 26 Oct 1998, *Galeano et al.* 2036 (COL); Yacopí, Churupaco, areas llamadas Peladeros, 1700 m, 8 Feb 1956, *Idrobo* 2027 (COL). **Guajira:** 16 km S of Carraipia, Corregimiento Guajira, 400 m, 6 Aug 1944, *Haught* 4292 (MEDEL, US). **Guaviare:** Mun. San José del Guaviare, Vereda Agua Bonita, Finca El Caimám, 1178 m, 17 Apr 2008, *Vélez et al.* 7073 (HUA). **Huila:** Cordillera Oriental, Vertiente occidental, montes más arriba de Guadalupe, 1600–1700 m, 20 Mar 1940, *Cuatrecasas & Pérez-Arbeláez* 8396 (COL, F); Neiva, Vereda Tamarindo, Finca La Tribuna, camino al pozo San Francisco 67, 550 m, 18 Apr 2004, *Obando* 284 (COL). **Meta:** Carretera Buenavista-Villavicencio, *Duque-Jaramillo* 3912 A (COL, NY); Villavicencio, Reserva Bavaria. 1000 m, 15 Nov 1995, *Lozano-Contreras* 7397 (COL). **Norte de Santander:** Hoya del río Sardinata, Finca La Soledad y carretera a Tibú, 50–70 m, 10 May 1965, *García-Barriga & Lozano-Contreras* 18240 (COL). **Quindío:** Mun. Montenegro, Vereda El Gigante, Finca El Porvenir, ladera del río Roble, 1100–1200 m, 2 May 2003, *Cordero-P. et al.* 91 (COL). **Risaralda:** Mun. Pereira, Hacienda La Alejandria, en la vía Cerritos-La Virginia, 900 m, 24 Jan 1991, *Ramos-Pérez et al.* 3052 (CUVC, U); Mun. Pereira, Hacienda Alejandria, km 6 of road Cerritos-La Virginia, 900 m, 1 Dec 1989, *Silverstone-Sopkin et al.* 5811 (CUVC, U). **Santander:** Puerto Parra-Campo Capote, 24 Jan 1991, *Rentería Arriaga et al.* 2123 (COL, HUA, JAUM, MO). **Tolima:** Melgar, Río Sumapán, Hacienda Corinto, 350 m, 26 Sep 1974, *Maas & Jaramillo Mejía* 1783 (COL); Ibagué, Hacienda Altamira (Laserna), 5 Apr 2014, *Villanueva & Cabezas* 1844 (COL). **Valle del Cauca:** Cordillera Occidental, hoya del río Digua, Quebrada del Cauchal, 300 m, 19 Dec 1942, *Cuatrecasas* 13714 (F, US); Cordillera Central, near Palmira, 1400 m, 3 Jan 1972, *Maas & Escobar* 596 (U); Quebrada Nueva to Cuchilla, E of Zarzal, 1100–1300 m, 21 Jul 1922, *Pennell et al.* 8486 (NY, US); Mun. Zarzal, Hacienda El Medio (Carretera Panamericana between La Paila and Zarzal), 950 m, 15 Apr 1987, *Silverstone-Sopkin et al.* 3085 (CUVC, U). **Vichada:** Cumaribo, Cerro Thomas, 50 m, 26 Aug 2006, *Rojas-Zamora* 136 (COL). **ECUADOR. Azuay:** Highway Cuenca-Cola de San Pablo, Contego encampment, 1640 m, 15 Feb 1977, *Boeke & Loyola* 1009 (L). **Carchi:** Peñas Blancas, 20 km below Maldonado, on the Río San Juan, 900–1000 m, 27 May 1978, *Madison et al.* 4623 (F, QCA, SEL, U). **Pastaza:** valley of the Río Pastaza, near El Topo, 4000–5000 ft, 17 Apr 1945, *Camp E* 2395 (NY, US). **Zamora-Chinchipe:** along road from Namirez to Nambija, just S of Nambija, 1779 m, 23 Jul 2004, *Croat* 92034 (MO, QCNE, U). **PERU. Ucayali:** Prov. Coronel Portillo, Distr. Padre Abad, La Divisoria, near Río Chino, 1500–1600 m, 3 Jun 1978, *Schunke V.* 10195 (MO, U). **VENEZUELA. Mérida:** Carretera Panamericana, 15 km SW of El Vigía, 50–130 m, 6 Jun 1952, *Vareschi & Pannier* 1674 (M), 4 Mar 1968, *Wessels Boer et al.* 2440 (U). **Cultivated Material** Cultivated in Burgers' Bush, Arnhem, the Netherlands, from *Maas et al.* 10488 from Colombia, *P.J.M. & H. Maas* 10631 (L).

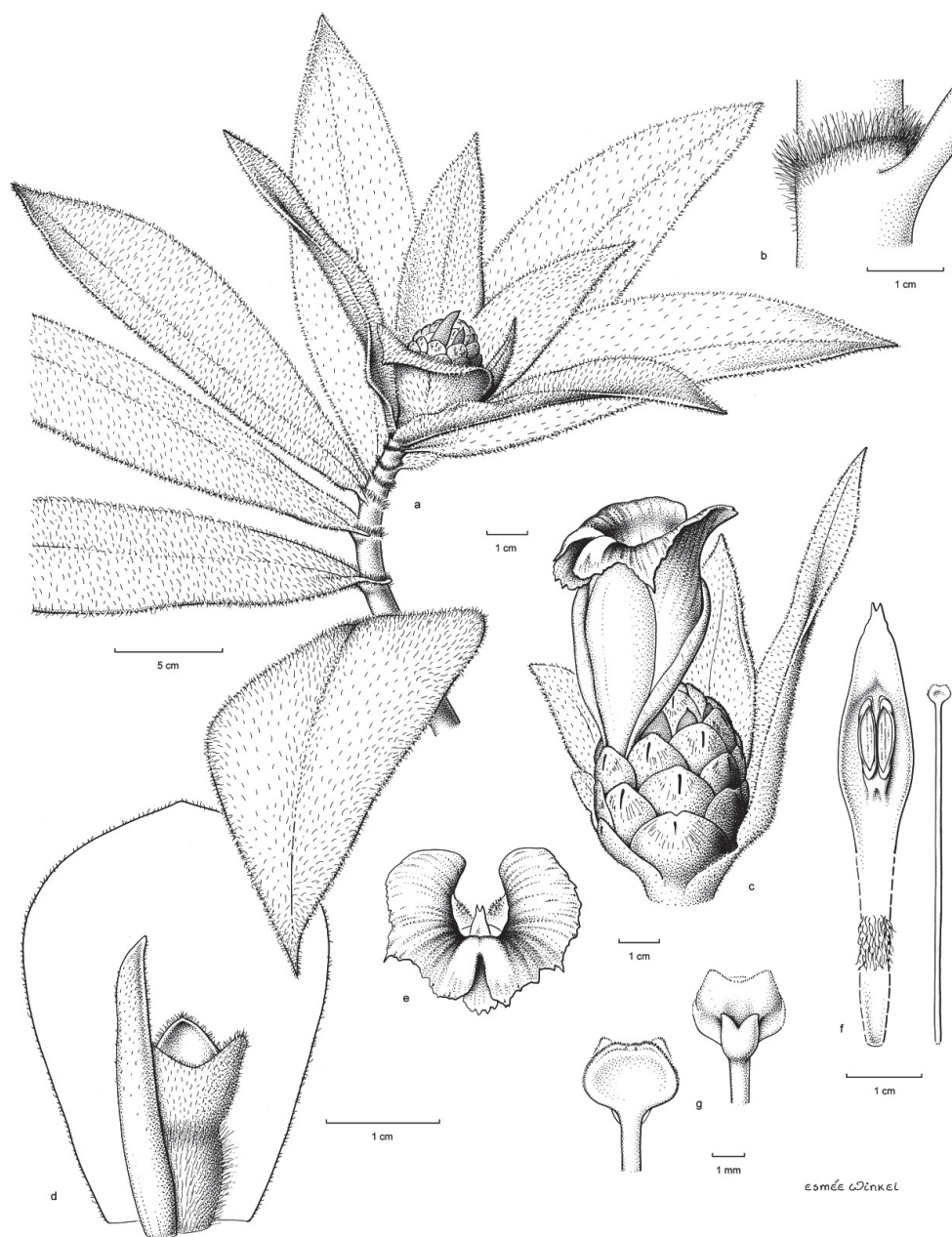


Figure 6. *Costus antioquiensis* Maas & H.Maas **A** inflorescence terminating a leafy shoot **B** ligule showing indument at margin **C** inflorescence close up with flower at anthesis **D** bract (adaxial surface) subtending a bracteole (left), calyx with three lobes, and ovary at the base of the calyx **E** labellum as viewed from front, with the forked tip of the single fertile stamen visible at the center **F** stamen (left) and style (right) **G** stigma close-up, dorsal (left) and ventral (right) views. The forked projection on the ventral surface hooks between the thecae of the fertile stamen to hold the style erect. Drawing by Esmée Winkel.

Notes. *Costus antioquiensis* sp. nov. could be confused with *C. allenii* Maas, but in that species, the entire surface of sheaths, ligule and petiole are villose, while *C. antioquiensis* is highly variable in its indument. Most material has a quite dense villose indument all over the plant, but some specimens of the same population can be almost completely glabrous, especially the adaxial side of the leaves. The ring of erect hairs at the base of the ligule is almost always present in *C. antioquiensis* but consistently lacking in *C. allenii*.

C. antioquiensis sp. nov. also looks similar to *C. laevis* Ruiz & Pav. and was misidentified by us as belonging to that species in the past. It shares with *C. laevis* the adaxially oriented flowers, but the flowers are a much lighter colour in *C. antioquiensis*. Moreover, there are notable differences in indumentum, which is almost completely lacking in *C. laevis* Ruiz & Pav., whereas, in *C. antioquiensis*, the vegetative parts are mostly densely villose.

***Costus callosus* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316087-1

Diagnosis. *Costus callosus* sp. nov. (Fig. 7) is most similar to *C. curvibracteatus*, differing by the inflorescence mostly produced terminally on a leafless shoot, with soon withering sheaths and bracts with a very long callus.

Type. COLOMBIA, Chocó: Mun. San José del Palmar, mouth of Río Torito (affluent of Río Hábita), Finca Los Guadales, 730–830 m, 4 Mar 1980, *E. Forero, Jaramillo-Mejía, Espina Z. & Palacios* 6668 (holotype COL, 3 sheets; isotypes MO-1456278, U1607067, U1607068, U1607069, U1610613).

Description. *Herb* 0.5–3 m tall. *Leaves* sheaths 15–30 mm diam; ligule obliquely truncate, 10–30 mm long, distally black to dark purple, with a minute, salient rim; petiole 5–20 mm long; sheaths, ligule and petiole glabrous, sparsely puberulous, or densely villose; lamina narrowly elliptic, 25–50 × 7–16 cm, adaxially sparsely to rather densely villose to puberulous, abaxially rather densely villose, base acute, apex acuminate (acumen 5–15 mm long). *Inflorescence* ovoid to subglobose, 6–23 × 6–12 cm, terminating a leafless shoot 20–90 cm long, rarely terminating a leafy shoot (*Knapp & Vodicka* 5524), sheaths withering and completely or partly falling off with age, obliquely truncate, 4–10 cm long, glabrous, sparsely puberulous to densely villose; bracts, bracteole, calyx, ovary, and capsule glabrous to rather densely puberulous. *Flowers* abaxially oriented; bracts orange-red, red, or yellow, coriaceous, ovate-triangular, 4–7 × 2.5–4 cm, apex acute to obtuse, more or less curved outwards to patent in living plants, callus 8–25 mm long, margins wooly in living material; bracteole boat-shaped, 20–30 mm long; calyx red, 10–18 mm long, lobes deltate, 2–8 mm long; corolla orange to yellow, ca. 50 mm long, glabrous, lobes elliptic, ca. 30 mm long; labellum yellow, lateral lobes rolled inwards and forming a tube ca. 10 mm diam, elliptic when spread out, ca. 35 × 25 mm, 3-lobulate, lobules red, 5–6 mm long; stamen yellow,

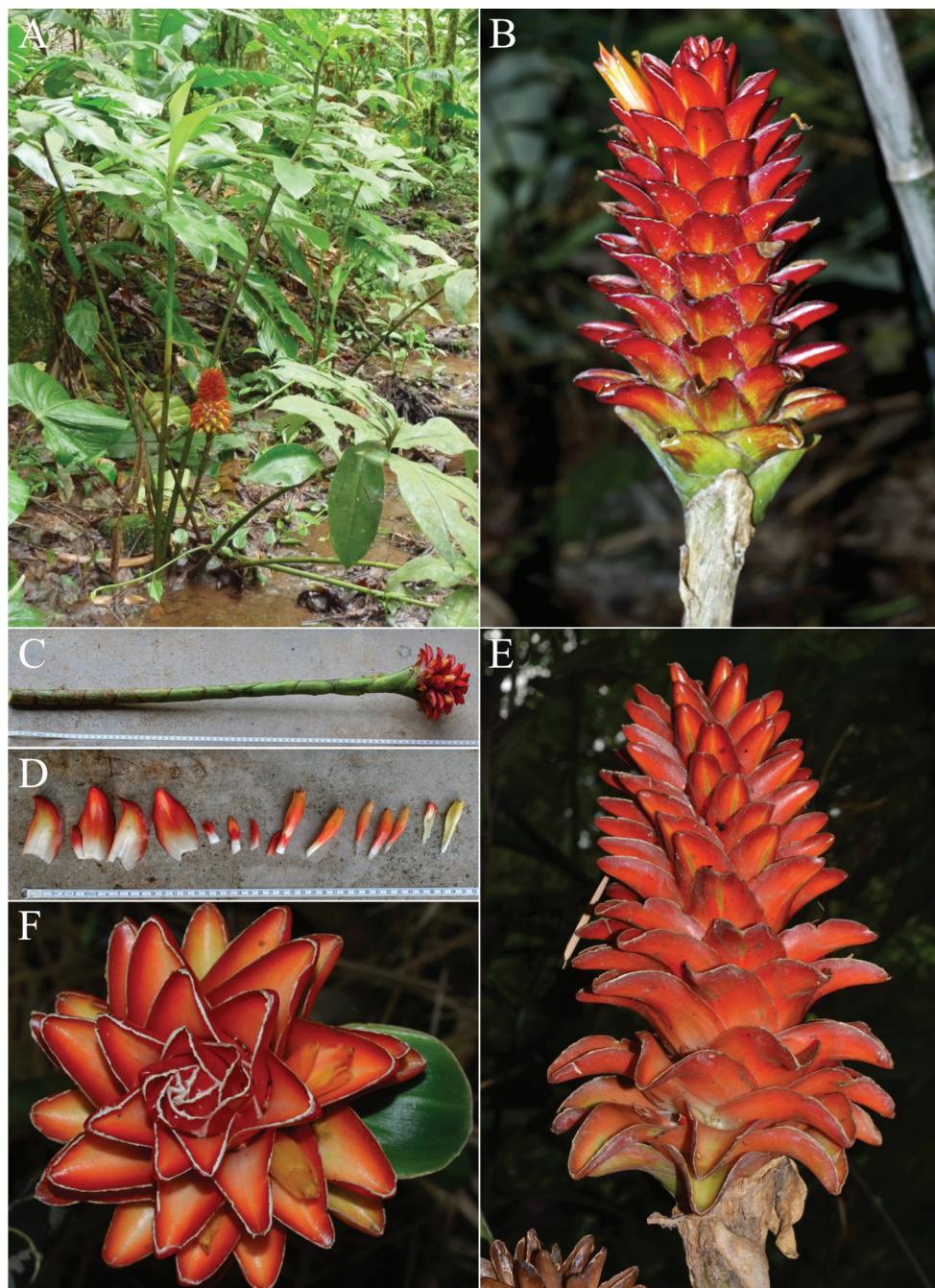


Figure 7. *Costus callosus* Maas & H.Maas **A** plant in habitat **B** inflorescence **C** separate flowering shoot **D** bract and flower details showing very long bract callus **E, F** cultivated plant **A, C, D** observation from Willie Mazu site, Bocas del Toro, Panama **B** plant in cultivation at Lyon Arboretum 2003.0125, collected in Costa Rica by Alan Carle **E, F** plant observed at Reserva Natural Nirvana, Valle del Cauca, Colombia. Photos **A, C, D** by Dave Skinner, **B** by John Mood, **E, F** by Paul Maas.

35–40 × 12–15 mm, slightly exceeding the labellum, apex orange-red, acute, anther 9–10 mm long. **Capsule** ellipsoid to obovoid, 8–15 mm long.

Distribution. Costa Rica, Panama, Colombia (Antioquia, Chocó) (Fig. 21F).

Habitat and ecology. In forests, at elevations of 0–1300 m. Flowering year-round.

Vernacular names. Panama: Caña agria (Kuna).

Etymology. *Costus callosus* sp. nov. has been named after the very conspicuous thickened callose zone on its bracts.

Paratypes. **COSTA RICA.** **Alajuela:** Volcán Arenal, *Funk 10490* (MO). **Heredia:** path beyond Río Sucio, Braulio Carrillo, 400 m, 4 May 1984, *Gómez Pignataro et al. 21201* (MO); Cantón de Sarapiquí, Llanura de Tortuguero, ca. 5 km NE of Puerto Viejo, NE side of Río Sucio, 50 m, 5 Oct 1996, *Hammel et al. 20494* (IN-BIO, MO, U, 2 sheets); Cantón de Sarapiquí, Cuenca del Sarapiquí, 5 km NE of Puerto Viejo, NE side of Río Sucio, 50 m, 22 Sep 1996, *Hammel et al. 20694* (IN-BIO, MO). **Limón:** Hacienda Tapezco-Hacienda La Suerte, 29 air km W of Tortuguero, 40 m, 24 Aug 1979, *Davidson & Donahue 8770* (CR, LAM, U); Parque Nacional Braulio Carrillo, W side of Río Sucio, 450 m, 4 Aug 1989, *Hammel et al. 17668* (MO, U); Braulio Carrillo National Park, Quebrada Gonzales section, ca. 40 km NNE of San José, 500 m, May 1992, *Lücking s.n.* (U). **PANAMA.** **Bocas del Toro:** 2 miles below the divide along main highway, 500 m, 23 Jun 1986, *Kress et al. 86-1978* (MO). **Chiriquí:** behind Vivero Forestal de Boquete, 12 km N of Los Planes de Hornito, IRHE Fortuna, 1100–1200 m, 17 Jun 1982, *Knapp & Vodicka 5524* (U); near Fortuna dam, from Continental Divide to Chiriquí Grande, 1100 m, 22 Oct 1985, *LaFrankie 85-38* (MO, U). **Darién:** Parque Nacional del Darién, along S branch of Río Pucuro, near Tacarcuna, ca. 18 km E of Pucuro, 600–800 m, 25 Oct 1987, *Hammel et al. 16513* (MO, U); 0–2 miles E of Tres Bocas, along the shortest headwater of Río Cuasi, 28 Apr 1968, *Kirkbride & Duke 1180* (MO, U). **Panama:** Altos de Pacora, ca. 20 km NE of Cerro Azul, 750 m, 19 May 1972, *Dressler 4190* (U, 3 sheets); summit of Cerro Jefe, 26 Aug 1967, *Hayden 1020* (MO, 2 sheets); 5–6 hours walk from Chocó village, Serranía de Maje, 650–800 m, 31 Mar 1982, *Knapp et al. 4519* (MO, U); Altos de Pacora, 750 m, 2 Sep 1974, *Maas et al. 1548* (U, 2 sheets). **COLOMBIA.** **Antioquia:** Mun. Amalfi, between Amalfi and Fraguas, 1220–1300 m, 14 Feb 1989, *MacDougal et al. 4020* (HUA). **Chocó:** Carretera Panamericana, between Río San Pablo and Río Pató, 23 Apr 1979, *Forero et al. 5765* (COL, MO, U, 2 sheets). **Cultivated Material.** Colombia, Valle del Cauca, Mun. Palmira, Reserva Natural Nirvana, 13 Jul 2018, *Maas et al. 10688* (L, 3 sheets).

Notes. Maas (1977) already suspected this species differed from *C. curvibracteatus* Maas, although they share many ligule and bract characters. The presence of a separate, leafless flowering shoot with soon withering sheaths and the presence of a distinct callus on the bracts convinced us that it is worthy of recognition as a separate species. Material recently collected (*Maas et al. 10688*) in the Reserva Natural Nirvana, Valle del Cauca, Colombia, made it possible to add all flower details to the description.

***Costus cochabambae* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316088-1

Diagnosis. *Costus cochabambae* sp. nov. (Figs 8, 9) superficially resembles *C. comosus* but differs by greenish appendaged bracts, a subglabrous corolla, a glabrous lower leaf side (except for the hairy primary vein), and a much longer ligule up to 25 mm long (vs. 1–3 mm).

Type. BOLIVIA, Cochabamba: Prov. Chapare, Villa Tunari, 40 km hacia el Beni, por Chipiriri, 400 m, 24 Nov 1981, *Beck 7320* (holotype LPB, specimen lacking a barcode; isotypes L, 2 sheets: L1480367 & L1480368 and spirit collection: L0303088).

Description. *Herb* ca. 1.7 m tall. *Leaves* sheaths 5–8 mm diam; ligule 15–25 mm long, unequally lobed, lobes obtuse; petiole ca. 5 mm long; sheaths, ligule and petiole densely to rather densely puberulous to villose; lamina narrowly elliptic to narrowly obovate, 17–30 × 6–8 cm, adaxially glabrous, abaxially glabrous with primary vein densely villose, strongly raised and prolonged as a prominent rim into the sheaths, base obtuse, apex acuminate (acumen 5–10 mm long). *Inflorescence* ovoid, 5–8 × 3–5 cm, terminating a leafy shoot; bracts, bracteole, and calyx densely to rather densely puberulous, ovary and capsule densely puberulo-sericeous. *Flowers* abaxially oriented; bracts green, coriaceous, broadly ovate, 1.5–2 × 1.5–2 cm; appendages green, foliaceous, reflexed when dry, broadly to shallowly ovate-triangular, 0.5–1.2 × 1.2–2 cm, apex obtuse to acute; bracteole boat-shaped, 15–17 mm long; calyx 9–12 mm long, lobes shallowly ovate-triangular, 2–3 mm long; corolla yellow, 30–40 mm long, subglabrous, lobes elliptic, 20–25 mm long; labellum yellow, lateral lobes rolled inwards and forming a tube ca. 10 mm diam, oblong-elliptic when spread out, ca. 20 × 15 mm, dentate; stamens yellow, ca. 20 × 5 mm, not exceeding the labellum, apex dentate, anther 8–9 mm long. *Capsule* not seen.

Distribution. Bolivia (Cochabamba) (Fig. 21G).

Habitat and ecology. In high forests (“pie de la ladera con bosque alto”), at an elevation of ca. 400 m. Flowering in November.

Etymology. This species is named after the Bolivian department of Cochabamba, where it was collected.

Notes. *Costus cochabambae* sp. nov. resembles *C. comosus* and is likely to be closely related to this species.

***Costus convexus* Maas & D.Skinner, sp. nov.**

urn:lsid:ipni.org:names:77316089-1

Diagnosis. *Costus convexus* sp. nov. (Fig. 10) shares with *C. glaucus* Maas the presence of glaucous shoots and leaves especially in young plants, but differs from that species by a globose inflorescence enveloped by the upper leaves and by the presence of distinctly convex bracts.



Figure 8. *Costus cochabambae* Maas & H.Maas Isotype photographed at Leiden, L.1480367 including the photos enclosed with the specimen, Beck 7320.

Type. COLOMBIA, Putumayo: 15 km S of Villagarzón on road to Puerto Umbria, 360 m, 17 Jul 2018, Maas, Maas-van de Kamer, Valderrama, Specht, Erkens & Rodríguez 10707 (holotype, L, 2 sheets).

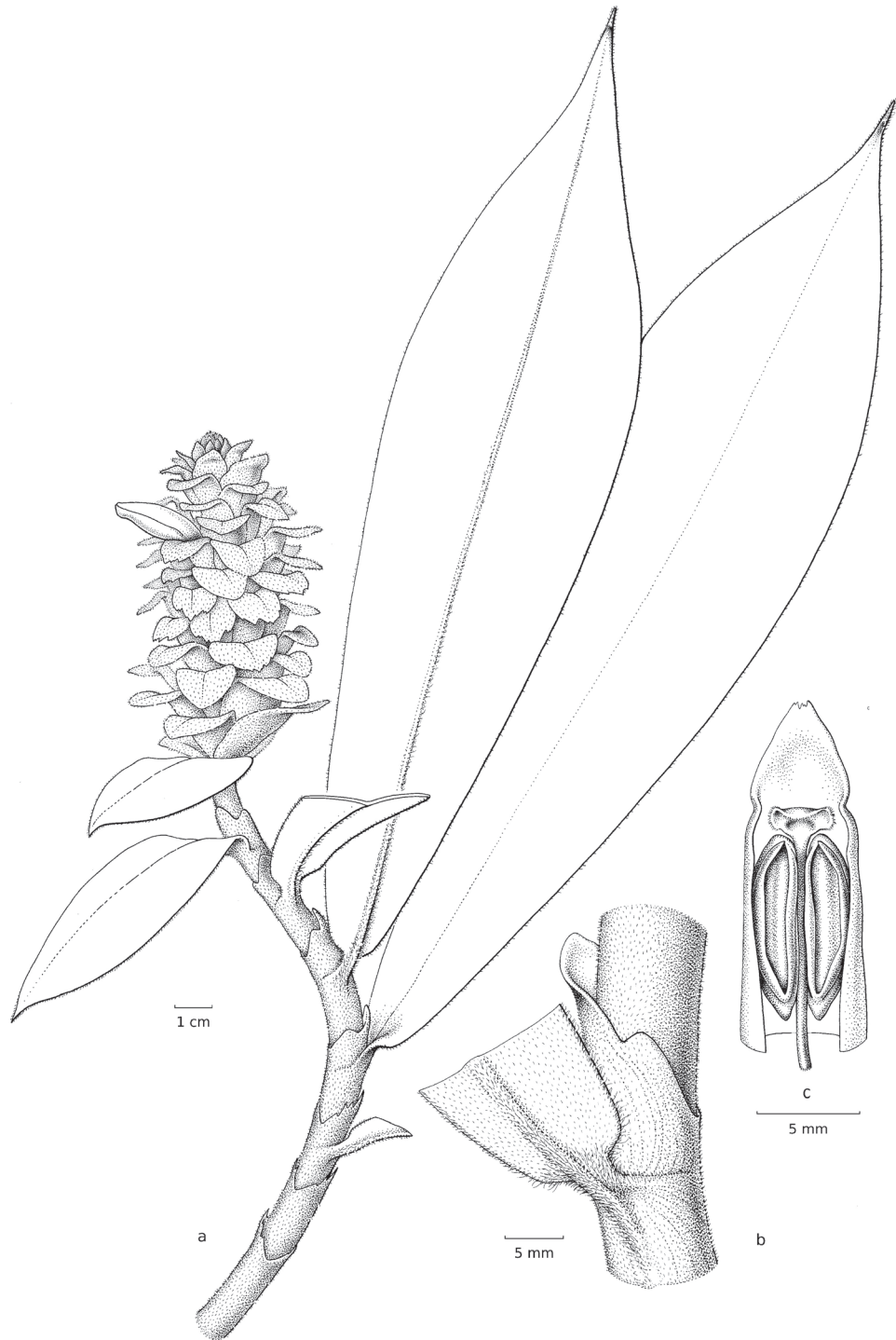


Figure 9. *Costus cochabambae* Maas & H.Maas **A** habit **B** leaf base with ligule **C** stamen. Drawing by Esmée Winkel.

Description. *Herb* 3–5 m tall, young shoots and young leaves glaucous. *Leaves* sheaths ca. 20 mm diam; ligule truncate to obliquely truncate, 20–45 mm long; petiole 20–22 mm long, blushed in pink; sheaths, ligule and petiole glabrous; lamina narrowly elliptic, 31–57 × 12–16 cm, adaxially pale green and glabrous, abaxially green or purple in very young plants, glabrous to sparsely puberulous, base acute to slightly cordate, apex acuminate (acumen ca. 15 mm long). *Inflorescence* globose to ovoid, 8–20 × 5–8 cm, wrapped tightly by the upper leaves, terminating a leafy shoot; bracts, bracteole, calyx, ovary, and capsule glabrous. *Flowers* adaxially oriented to erect; bracts distinctly convex, reddish on the outer margins, greenish at the centre, coriaceous, ovate, 55–65 × 40–45 mm, apex obtuse, callus green, indistinct; bracteole boat-shaped, 40–45 mm long; calyx red, 15–25 mm long, lobes shallowly triangular to deltate, 4–10 mm long; corolla deep pink, 80–85 mm long, glabrous, lobes 50–60 mm long; labellum yellow, distal edge horizontally spreading, broadly obovate, 60–70 × 60–70 mm, lateral lobes strongly striped with pink or red, middle lobe reflexed, with yellow honey mark, margin irregularly dentate to lobulate; stamen dark pink, 30–40 × 12–15 mm, not exceeding the labellum, apex obtuse, anther 12–15 mm long. *Capsule* ellipsoid, 12–30 mm long.

Distribution. Colombia (Putumayo), Ecuador (Morona-Santiago, Zamora-Chinchi) (Fig. 21H).

Habitat and ecology. In forests, in open shade or sunny areas, often found along roadsides and other disturbed areas, at elevations of 360–1500 m. Flowering year-round.

Etymology. *Costus convexus* sp. nov. is named for the form of the bracts, which are distinctly convex; thus, the epithet *convexus*, an adjective meaning “convex or curved outwards” in Latin.

Paratypes. COLOMBIA. **Putumayo:** road to Pto. Guzmán, 300 m, *Skinner R3467*. ECUADOR. **Morona-Santiago:** near Mendez, 856 m, 17 Oct 2009, *Skinner R3196*. **Zamora-Chinchi:** Zamora-Loja road, 1200–1500 m. 3 Feb 1990, *Madsen & Knudsen 86796* (AAU, QCNE); Quebrada del León, affluent of Río Bombuscara, S of Zamora, 1100 m, 5 Feb 1989, *Øllgaard et al. 90385* (AAU, MO), near entrance to P. N. Podocarpus, 970 m, *Skinner R3331*.

Notes. Plants of *Costus convexus* sp. nov. can grow to be huge plants, up to 5 m tall, with large inflorescences and flowers. The younger shoots and leaves usually have a glaucous covering making them appear similar to *C. glaucus* Maas but having an inflorescence with distinctly convex bracts that are reddish or pink instead of the pale green and glaucous bracts in *C. glaucus*. It is also characterized by having upper leaves that wrap tightly around and, in doing so, completely hide the young inflorescence.

This new species is observed to be very common along the eastern flanks of the mountains from Putumayo, Colombia, south through Ecuador to Zamora-Chinchi. Many more recent observations of this new species are recorded on iNaturalist.com in Napo and Pastaza, Ecuador.

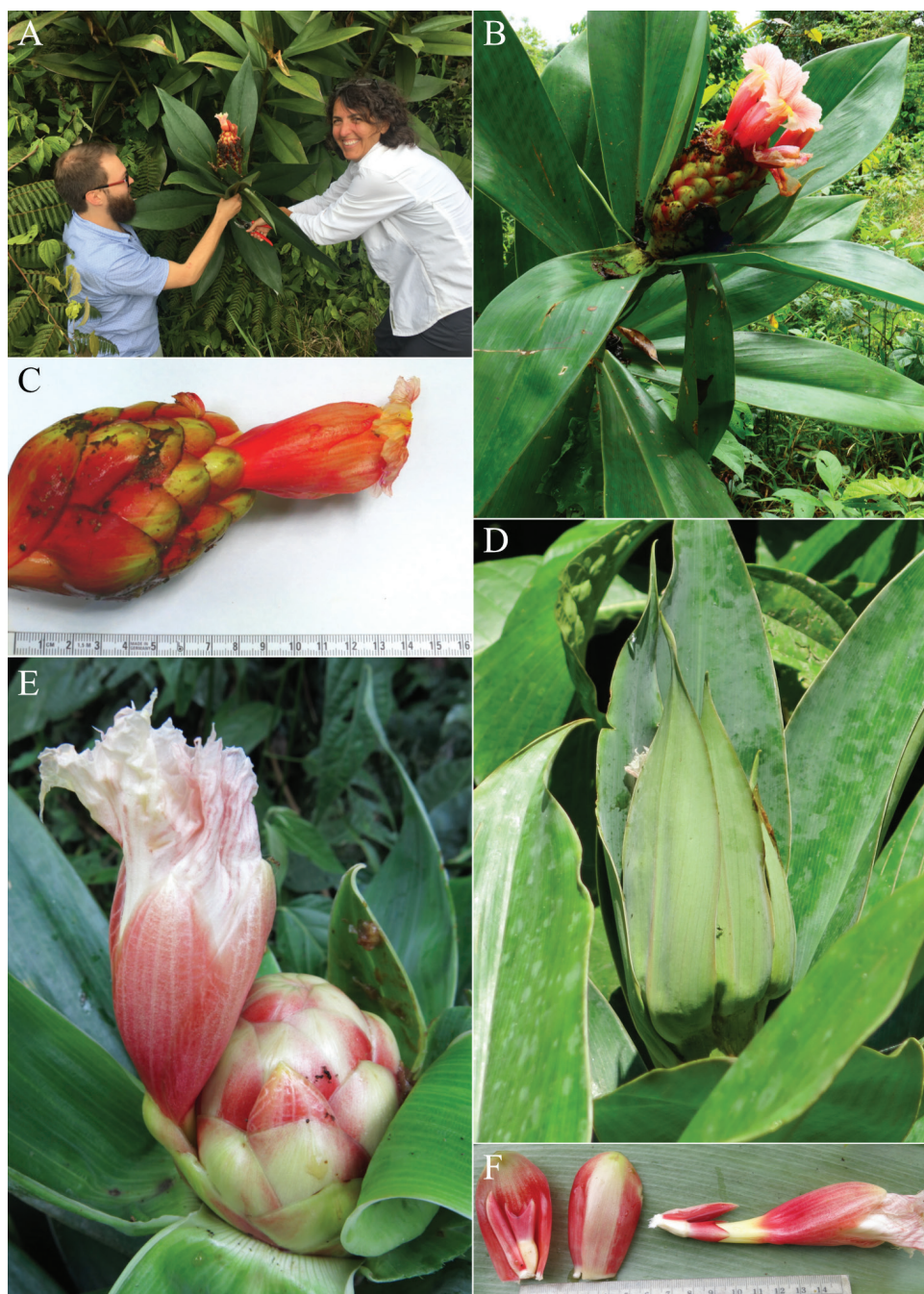


Figure 10. *Costus convexus* Maas & D.Skinner **A** plant in habitat with Eugenio Valderrama (left) and Chelsea Specht (right) **B** inflorescence **C** inflorescence with scale **D** upper leaves wrapping around and hiding the inflorescence **E** close-up of inflorescence **F** bract (left) and flower (right) detail. Photo in **A** taken in the wild prior to pressing *Maas 10707*, Putumayo, Colombia **B, C** plant observed in Putumayo, Colombia **D–F** plant observed in Zamora-Chinchipe, Ecuador. Photo **A** by Paul Maas **B–F** by Dave Skinner.

***Costus douglasdalyi* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316090-1

Diagnosis. *Costus douglasdalyi* sp. nov. (Fig. 11) can be confused with *C. erythrothyrus* Loes., but differs from that species by its very narrow, often almost linear leaves.

Type. BRAZIL Acre: Mun. Tarauacá, Basin of Rio Juruá, Rio Tarauacá, Seringal Sumaré, 19 Nov 1995, *Daly, Silveira, Costa, Oliveira, Lima, Figueiredo & Ehringhaus 8606* (holotype U1225319, isotype NY).

Description. *Herb* 1–4 m tall. *Leaves* sheaths 5–20 mm diam; ligule truncate, 2–3 mm long; petiole 3–8 mm long; sheaths, ligule and petiole glabrous to sparsely puberulous; lamina narrowly elliptic to linear, 20–35 × 2–7 cm, abaxially sometimes purple, glabrous to sparsely puberulous, primary vein sparsely covered with a row of erect hairs, adaxially glabrous, base acute to rounded, apex long-acute. *Inflorescence* ovoid to cylindric, 8–9 × 4 cm, enlarging to 13–17 × 5–7 cm in fruit, terminating a leafless shoot 45–70 cm long or rarely terminating a leafy shoot, sheaths obliquely truncate, 3–5 cm long, glabrous; bracts, bracteole, calyx, ovary, and capsule glabrous, apex of ovary and fruit sometimes sparsely puberulous. *Flowers* abaxially oriented; bracts red to dark red, coriaceous, broadly ovate, 2.5–4 × 2–3 cm, apex obtuse, callus 5–7 mm long; bracteole boat-shaped, 20–25 mm long; calyx red, 9–13 mm long, lobes shallowly triangular, 1–2 mm long; corolla red, pink, or pinkish orange, 45–55 mm long, glabrous, lobes narrowly ovate-elliptic to ovate-elliptic, 35–45 mm long; labellum pink to red, lateral lobes rolled inwards and forming a curved tube ca. 8 mm diam, broadly obovate to elliptic when spread out, 18–40 × 12–30 mm, irregularly 3–7-lobulate, lateral lobes striped with red, middle lobe yellow, 3–5 mm long, irregularly crenulate; stamen pale pink, 15–40 × 7–10 mm, not exceeding the labellum, apex acute, anther 8–10 mm long. *Capsule* ellipsoid, 10–15 mm long.

Distribution. Peru (Ucayali), Brazil (Acre, Rondônia) (Fig. 21I).

Habitat and ecology. In non-inundated (terra firme) forests, campinarana, river margins, or roadsides at elevations of 0–350 m. Flowering year-round.

Etymology. This species is named for our dear colleague and friend Douglas Daly, whom PM and HM met many times in his home institute at the New York Botanical Garden Herbarium (NY) and who enabled us to undertake field work in the Brazilian regions of Acre and Amazonas. He also inspired CDS during her graduate studies at the New York Botanical Garden, providing insights into excellence in field research and tropical botany.

Paratypes. BRAZIL. Acre: along road from Cruzeiro do Sul to Barão do Rio Branco, NW of Cruzeiro do Sul, vicinity of São Francisco, 150 m, 25 Aug 1986, *Croat & Rosas Jr. 62674* (MO, U); Mun. Cruzeiro do Sul, vicinity of Cruzeiro do Sul, between airport and downtown, headquarters of Rondon Project, 10 Nov 2001, *Croat 85008* (MO, NY); Mun. Cruzeiro do Sul, km 6 of road from Cruzeiro do Sul to Boa Fé, Ramal dos Carobas, 16 Oct 2001, *Maas et al. 8999* (NY, U); Porangaba, Rio Juruá-Mirim, 15 May 1971, *Maas et al. P 12972* (INPA, NY, U); Porangaba, Rio Juruá-Mirim, 21 May 1971, *Maas et al. P 13213* (INPA, NY, U); Cruzeiro do Sul, Sub-base

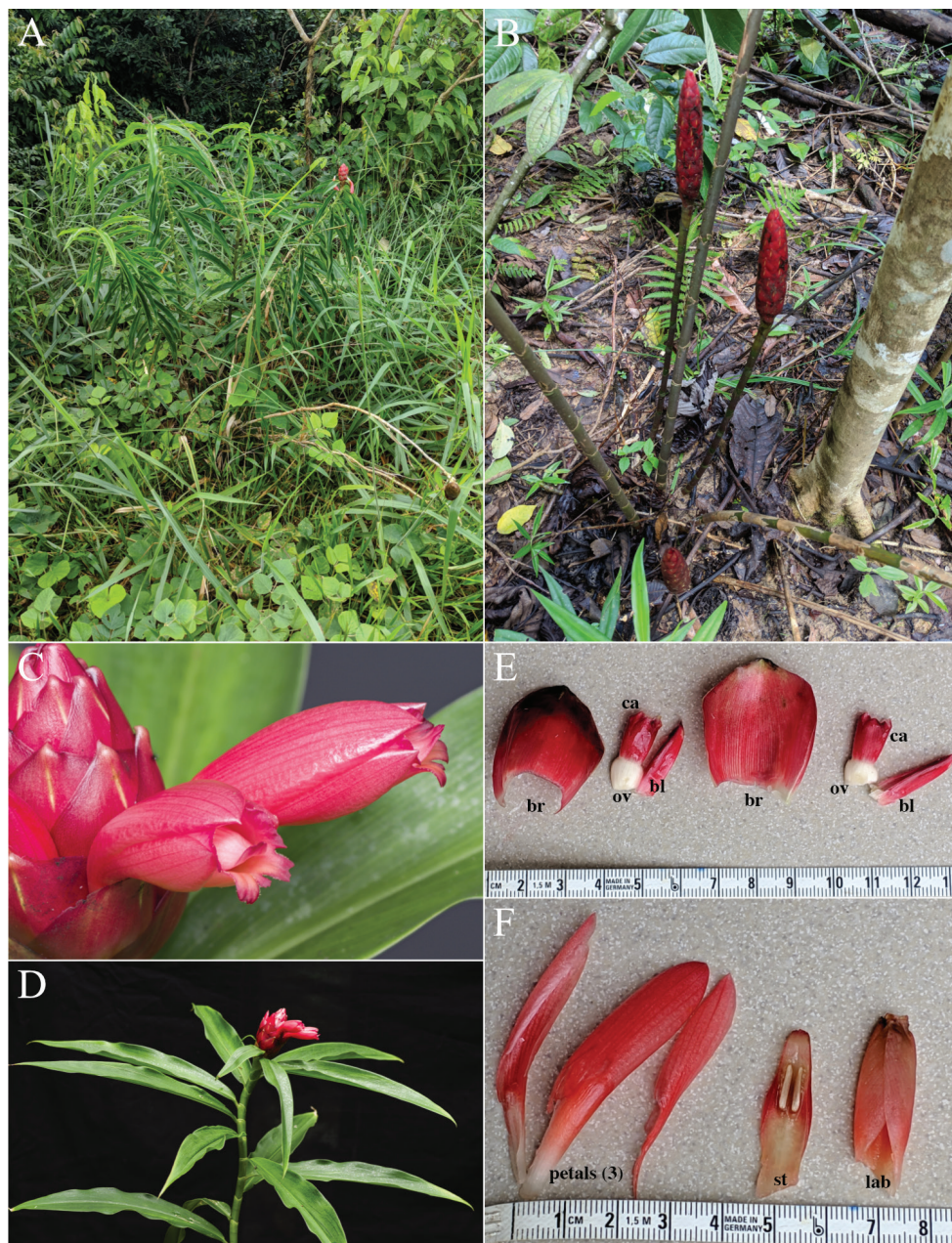


Figure 11. *Costus douglasdalyi* Maas & H.Maas **A** plant in habitat, inflorescence with flowers on a leafy shoot **B** in habitat, inflorescence on separate leafless shoot **C** flowers **D** plant in cultivation showing narrow leaves **E, F** bract and flower details showing bracts (br), bracteoles (bl), calyx (ca), ovaries (ov), three petals (petals), the fertile stamen (st) and the labellum (lab) **A, B** taken in Cruzeiro do Sul, Acre, Brazil **C–F** photos from plant pressed and accessioned as *D.Skinner* R3482 in cultivation. Photos **A–F** by Dave Skinner.

do Projeto RADAM/BRASIL, próximo ao Aeroporto novo, 23 Feb 1976, *Monteiro & Damião 601* (INPA, MG, U); vicinity of Serra da Moa, 22 Apr 1971, *Prance et al. 12230* (INPA, NY, U). **Rondônia:** Mineração Taboca, próximo ao Campo de Pouso da Mineração 10 Oct 1979, *M.G.G. Vieira et al. 369* (L, NY). **PERU. Ucayali:** Prov. Coronel Portillo, Distr. Caleria, Quebrada Pumayaquillo, left margin of Río Utiquina, 150–175 m, 10 Apr 2003, *Schunke V. & Graham 15567* (U); Prov. and Distr. Padre Abad, carretera al caserio San Miguel y Mapuya, 12–17 km de la Aguaytia, 350 m, 1 Oct 2004, *Schunke V. & Graham 16185* (U).

Notes. *Costus douglasdalnyi* looks quite similar to *C. erythrothyrus* Loes. in its floral characters but differs by having very narrow, often linear leaves.

***Costus gibbosus* D.Skinner & Maas, sp. nov.**

urn:lsid:ipni.org:names:77316091-1

Diagnosis. *Costus gibbosus* sp. nov. (Fig. 12) can readily be recognized by the swollen margin of the lower leaf sheaths; the inflorescence is similar to that of *Costus guanaensis* Rusby var. *macrostrobilus* (K.Schum.) Maas, from which it differs by the horizontal orientation of the bract appendages with often incurved apex and the mostly glabrous leaves, bracts, and calyx.

Type. ECUADOR, Pichincha: along the road from Aloag to Santo Domingo, 1150 m (“3500 ft”), 17 Nov 1974, *Plowman & Davis 4455* (holotype U1212018; isotypes GH, USM).

Description. *Herb* 1.5–5 m tall. *Leaves* sheaths 15–35 mm diam, the margin of the lower ones swollen; ligule truncate to slightly 2-lobed, often with a ridge near the apex, 7–14 mm long; petiole 10–15 mm long; sheaths, ligule and petiole glabrous to densely puberulous; lamina narrowly elliptic, 30–50 × 10–20 cm, slightly shiny above, pale green to slightly glaucous below, adaxially glabrous, rarely puberulous, abaxially glabrous, sometimes densely puberulous to villose, base acute to cordate, apex acuminate (acumen 15–20 mm long) to acute. *Inflorescence* ovoid to cylindric, 11–18 × 5–9 cm, terminating a leafy shoot; bracts, bracteole, and calyx glabrous, rarely rather densely puberulous, ovary and capsule glabrous to densely puberulo-sericeous. *Flowers* abaxially oriented; bracts red, coriaceous, broadly ovate, 40–50 × 30–40 mm; appendages green, foliaceous, erect, broadly triangular to triangular, 15–60 × 10–30 mm, often incurved at the apex; bracteole boat-shaped, 22–32 mm long; calyx red to pink, rarely white, (7–)12–20 mm long, lobes deltate to shallowly triangular, 2–5 mm long; corolla pale yellow, white, or pink, 60–70 mm long, glabrous, lobes elliptic, 45–60 mm long; labellum pale yellow, orange, pinkish, or white, distal edge horizontally spreading, broadly obovate, 60–85 × 50–70 mm, lateral lobes erect, more or less striped with pink or red, middle lobe reflexed, with yellow honey mark, margin irregularly dentate to lobulate; stamen pink, red, yellow, or white, 40–50 × 12–15 mm, not exceeding the labellum, apex irregularly dentate, anther 9–13 mm long. *Capsule* ellipsoid to narrowly ellipsoid, 12–20 mm long.

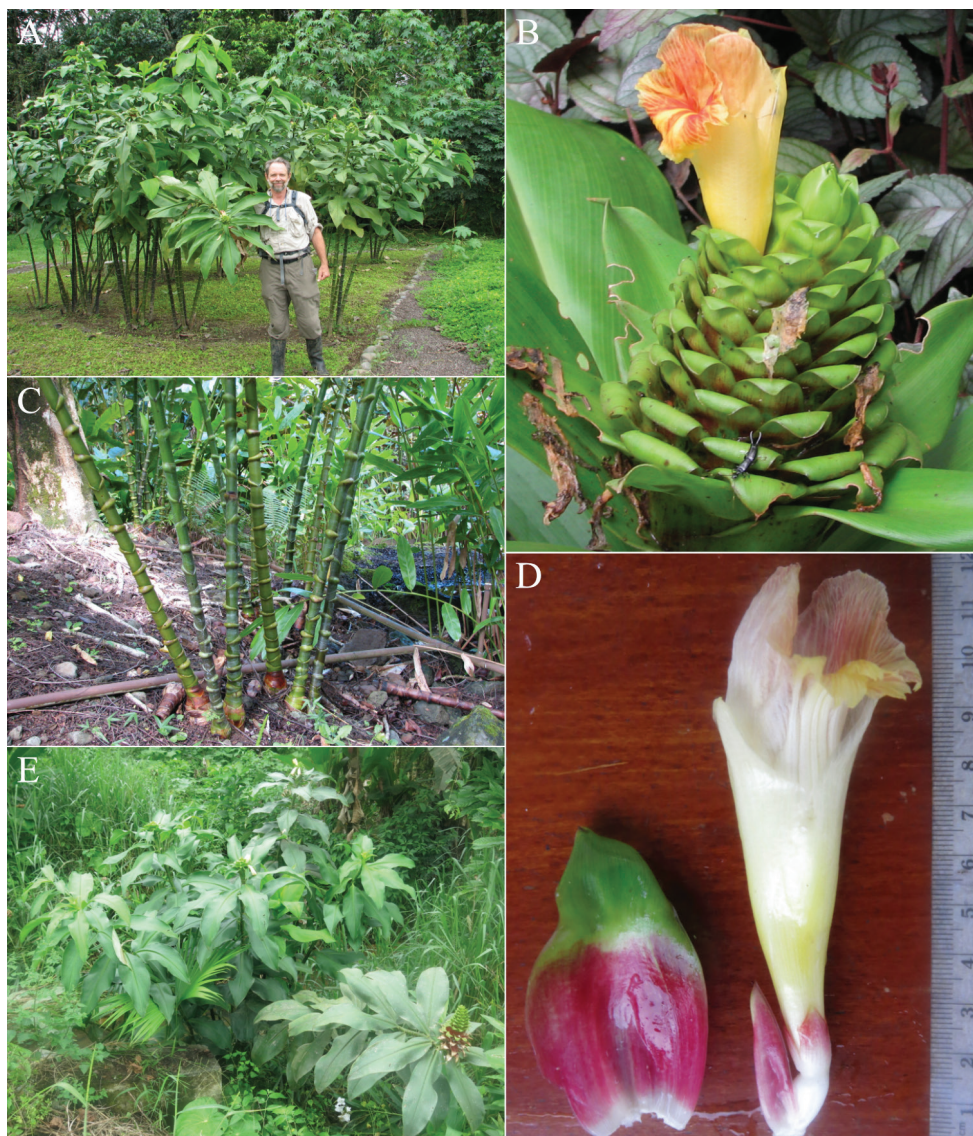


Figure 12. *Costus gibbosus* D. Skinner & Maas **A** plant in cultivation at Rio Palenque Science Center, Ecuador with Dave Skinner **B** inflorescence showing the recurved apices of the bract appendages **C** lower leaf sheaths showing swollen margins **D** close-up of bract (left) and flower (right) **E** plant in habitat near Caluma, El Oro, Ecuador **B**, **C** at Waimea Arboretum, Hawaii 74S2037, grown from seeds collected by Tim Plowman **D**, **E** Photos of plant in the wild prior to collecting seeds for *D. Skinner* R3346, currently in cultivation. Photos **A–E** by Dave Skinner.

Distribution. Ecuador (El Oro, Esmeraldas, Guayas, Loja, Manabi, Pichincha) (Fig. 22A).

Habitat and ecology. In forested roadsides, dry forests, or gallery forests at elevations of 0–1500 m. Flowering year-round.

Etymology. *Costus gibbosus* sp. nov. is named for the swollen upper margin of the lower leaf sheaths.

Paratypes. ECUADOR. Bolivar: Caluma, Pasagua Road, 836 m, *Skinner R3344*, Caluma-El Mirador, 185 m, *Skinner R3346*. **El Oro:** between Piñas and Piedras, 1000 m, *Asplund 18160* (S); Road from Santa Rosa to Piñas, 3 km E of Playón, 8 Feb 1987, 350 m, *Bohlin 1226* (GB, QCA); Camino a Limón-Playa, cerca a Río Dumari, 600 m, 12 Oct 1993, *Cornejo & Bonifaz 425* (AAU, QCNE); Road Zaruma-Santa Rosa, between Piñas and El Placer, 800 m, 6 May 1974, *Harling & Andersson 14354* (GB, U). **Esmeraldas:** Hacienda Timbre, 28 May 1955, *Asplund 16494* (S); along road to Río Tulubí from main San Lorenzo-Lita Highway, 12 July 2000, 59 m, *Croat 83948A* (MO, QCNE); between Esmeraldas and Tabiazo, 0 m, 14 Sep 1977, *Maas et al. 2915* (QCA, U). **Guayas:** Guayaquil, Cerro Azul, *Asplund 15412* (S); Cerro Azul, W of Guayaquil, 25 Mar 1955, *Asplund 15895* (NY, S); Cordillera Chongón-Colonche, Río California, 150 m, 27 Sep 1997, *Cornejo & Bonifaz 5792* (GUAY, U); Cerro Azul Cordillera Chongon-Colonche, 12 km W of Guayaquil, Quebrada Canoa, 17 Jan 1991, 50–300 m, *Gentry & Josse 72332* (F, MO, QCNE); Teresita, 3 km W of Bucay, 270 m, 5 Jul 1923, *Hitchcock 20543* (GH, NY, US); Bosque Protector Cerro Blanco, Carretera Guayaquil-Salinas, km 17, 200–440 m, 31 Oct 1995, *T. Nuñez 306* (MO, QCNE); Cantón Guayaquil, Bosque Protector Cerro Blanco, carretera a Salinas, km 15, 100 m, 19 Aug 1991, *Rubio et al. 2007* (MO, QCNE, U). **Loja:** Macará-Cariamanga Road, ca. 3 km NE of Sabiango, 900–1000 m, 17 Apr 1980, *Harling & Andersson 18358* (S, U). **Los Ríos:** Río Palenque Biological Station, 220 m, 17 Sep 1973, *Dodson & Tan 5347* (F, QCA, US); Río Palenque Science Center, km 56 of Quevedo-Santo Domingo Road, 14 May 1982, *Dodson 13039* (MO, SEL); Río Palenque Field Station, halfway between Quevedo and Santo Domingo de los Colorados, 200 m, 24 Feb 1974, *Gentry 10165* (MO, RPSC, SEL). **Manabi:** 40 km along road from Santo Domingo de los Colorados to Chone, 19 May 1955, *Asplund 16421* (S); El Aromo, 13 km SW to San Mateo, then E 13 km to town, 100 m, 4 Dec 1986, *Hammel & Trainer 15871* (MO); road Chone-El Carmén, at Flavio Alfaro, 350 m, 28 Oct 1980, *Holm-Nielsen et al. 27916* (AAU). **Pichincha:** shore of Río Pilatón, at the bridge of the road from Chiriboga to Santo Domingo de los Colorados, 1000 m, 2 Jul 1955, *Asplund 16763* (S); Santo Domingo de los Colorados, 530 m, 19 Jun 1975, *Gilli 119* (W); new Alluriquín-Quito Road, km 6, 850 m, 10 Oct 1980, *Maas & Cobb 4795* (NY, QCA, U); Santo Domingo de los Colorados, 200 ft, 28 Oct 1960, *Pennington 56 SD* (K, NY); along the road from Aloag to Santo Domingo, Tandape, 1150 m (“5000 ft”), 17 Nov 1974, *Plowman & Davis 4454* (GH, U); Toachi, on road Aloag-Santo Domingo, 750 m, 3 Jan 1967, *Sparre 13862* (S, U); Carretera Quito-Puerto Quito, km 113, near Reserva de ENDESA “Corporación Forestal Juan Manuel Durini”, 800 m, 4 Mar 1984, *Ulloa U. 111* (QCA, U).

Notes. *Costus gibbosus* sp. nov. is easily recognized in the field by the swollen margins of the lower leaf sheaths.

***Costus mollissimus* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316092-1

Diagnosis. *Costus mollissimus* sp. nov. (Fig. 13) shares with *C. dirzoi* Garcia-Mend. & G.Ibarra a distinct velutinous indument on most parts of the plant, but differs by having appendaged bracts and a very short ligule. Moreover, *C. dirzoi* has bracts with a distinct callus, absent in *C. mollissimus*.

Type. MEXICO, Chiapas: Mun. Chilon, 22 km from Temo in the direction of Palenque, 2 km from Patathel, near El Chich, 850 m, 4 Nov 1998, *Ishiki Ishihara, Maas & Maas-van de Kamer 2400* (holotype L, 2 sheets: L4368349 & L436835; isotype ECOSUR).

Description. *Herb* 0.5–2.5 m tall. *Leaves* sheaths 12–15 mm diam; ligule truncate, 1–4 mm long; petiole 10–15 mm long; sheaths, ligule and petiole densely velutinous; lamina narrowly elliptic, sometimes elliptic, 18–36 × 6–13 cm, slightly 5–8-plicate, adaxially shiny, glabrous, abaxially densely velutinous, base acute, obtuse, to cordate, apex acuminate (acumen 5–15 mm long). *Inflorescence* cylindric to subglobose, 5–13.5 × 4–5 cm, terminating a leafy shoot; bracts, appendages of bracts, bracteole, calyx, ovary, and capsule densely velutinous. *Flowers* abaxially oriented; bracts green, red, or pink, coriaceous, broadly elliptic, 2–2.5 × 2–3 cm; appendages green, foliaceous, patent to slightly reflexed, triangular to broadly triangular, 0.5–3.5 × 1.2–2.5 cm, apex acute to obtuse; bracteole boat-shaped, 16–20 mm long; calyx red to pink, 9–10 mm long, lobes broadly to shallowly triangular, 2–5 mm long, with a hairy tuft at the apex; corolla cream, yellow, or pale orange, 50–60 mm long, densely velutinous, lobes narrowly elliptic, 30–40 mm long; labellum yellow, distal edge horizontally spreading, broadly obovate, 30–40 × 40–50 mm, lateral lobes dark red with yellow venation, middle lobe reflexed with yellow honey mark, margin dentate; stamen yellow, 30–35 × 10–12 mm, not exceeding the labellum, apex red, slightly 3-dentate, anther ca. 8 mm long. *Capsule* broadly obovoid to obovoid, 12–17 mm long.

Distribution. Mexico (Chiapas, Oaxaca, Tabasco, Veracruz) (Fig. 22B).

Habitat and ecology. In forests (a.o. “selva baja/alta perennifolia”) with *Brosimum* sp., *Bursera* sp., *Calophyllum* sp., *Cymbopetalum* sp., *Dialium* sp., *Ficus* sp., *Tapirira* sp., and *Sloanea tuerckheimii* Donn.Sm., at elevations of 200–1000(–1500) m. Flowering year-round.

Vernacular name. Mexico: Apagafuego.

Etymology. This species is named after its very soft indument on the abaxial surface of the lamina, the word “mollis” meaning soft in Latin.

Paratypes. MEXICO. **Chiapas:** Mun. Solosuchiapa, 3–5 km above Solosuchiapa, along road to Tapilula, 450 m, 26 Jul 1972, *Breedlove 26463* (MO); Mun. Ixcomitan, 2 km E of Ixcomitan, road from Villahermosa to Tuxtla Gutierrez, 550 m, 11 Feb 1983, *Martinez Salas et al. 3160* (MEXU); Mun. Pichucalco, 10–12 km S of Pichucalco, 8 Mar 1983, *Ramamoorthy et al. 1790* (MEXU); NW end of Valley of Chiapas on road to Mal Paso, 41 km (by road) NW of Ocozocoautla, 350 m,



Figure 13. *Costus mollissimus* Maas & H.Maas **A** inflorescence **B** ligules and sheath, showing short ligule and indument **C, D** cultivated plant growing in Florida, propagated from living accession 1998GR01567 at Utrecht Botanical Garden. Photos **A, B** by Paul Maas **C, D** by Dave Skinner.

4–5 Aug 1965, *Roe et al.* 928 (WIS). **Oaxaca:** Mun. Santa María Chimalapa, Arroyo Milagrito, ca. 6 km SE of Santa Maria, 1 km from Vereda, which passes Arroyo Sanate, 230 m, 30 Aug 1984, *Hernández G.* 387 (MEXU, MO); Mun. Santa María Chimalapa, ca. 15 km ESE of Santa María, Vereda near Arroyo Plata, loma S of Río Milagro, 400 m, 3 Sep 1985, *Hernández G.* 1435 (MEXU, MO). **Tabasco:** near San Manuel, S of village along Río Mezcalapa, 11 Sep 1944, *Gilli & Hernandez Xolocotzi* 97 (MEXU). **Veracruz:** Las Cruces, Las Choapas, 250 m, 14 Jul 1970, *Nevling & Gomez-Pompa* 1526 (F).

Notes. *Costus mollissimus* sp. nov. superficially resembles *C. dirzoi* García-Mend. & G.Ibarra, as both share the velutinous indument all over the plant, but differs in having bracts with foliaceous, green appendages and a shorter ligule (1–4 mm vs. 3–12 mm long). *Costus dirzoi* has bracts with a distinct callus that is absent in *C. mollissimus*.

In Maas' treatment of Costaceae ("Costoideae") for Flora Neotropica (Maas 1972: 57), this species was erroneously included in *C. guanaiensis* Rusby as var. *tarmicus* (Loes.) Maas.

***Costus obscurus* D.Skinner & Maas, sp. nov.**

urn:lsid:ipni.org:names:77316093-1

Diagnosis. *Costus obscurus* sp. nov. (Fig. 14) can be recognized by its large, dark green leaves with dark purple undersides and densely whitish villose sheaths. It could superficially be confused with *C. erythrophyllus* Loes. and *C. acreanus* (Loes.) Maas, but these species never have densely villose sheaths.

Type. PERU, Madre de Dios: Prov. Manu, near Shintuya, on road to Salvación, 600 m, 9 Feb 1974, *Plowman & Davis* 5054 (holotype USM; isotypes GH, L1483069, L1480371, L1480372).

Description. *Herb* 1–1.5 m tall. *Leaves* sheaths 10–20 mm diam; ligule truncate, 10–15 mm long; petiole 8–12 mm long; sheaths, ligule and petiole densely to rather densely whitish villose; lamina narrowly elliptic, 32–45 × 9–17 cm, adaxially dark green, rather densely to densely whitish villose, abaxially dark purple, densely to sparsely whitish puberulous, base acute to cordate, apex acuminate (acumen 5–10 mm long). *Inflorescence* ovoid, 8–13 × 5–7 cm, terminating a leafy shoot; bracts, appendages of bracts, bracteole, calyx, ovary, and capsule rather densely to sparsely puberulous. *Flowers* abaxially oriented; bracts green in the exposed part, red or green in the covered part, coriaceous, broadly ovate-triangular, 35–45 × 30–35 mm; appendages green, erect, broadly ovate-triangular, 15–20 × 18–20 mm, or almost absent, apex rounded; bracteole boat-shaped, 35–37 mm long; calyx red, 25–27 mm long, deeply split on one side, lobes deltate to broadly triangular, ca. 2 mm long; corolla cream to yellow, ca. 75 mm long, glabrous, lobes narrowly elliptic, 55–60 mm long; labellum pale yellow, distal edge horizontally spreading, broadly obovate, ca. 60–70 × 60–70 mm, lateral lobes strongly striped with pink or red, middle lobe reflexed, with yellow honey mark,

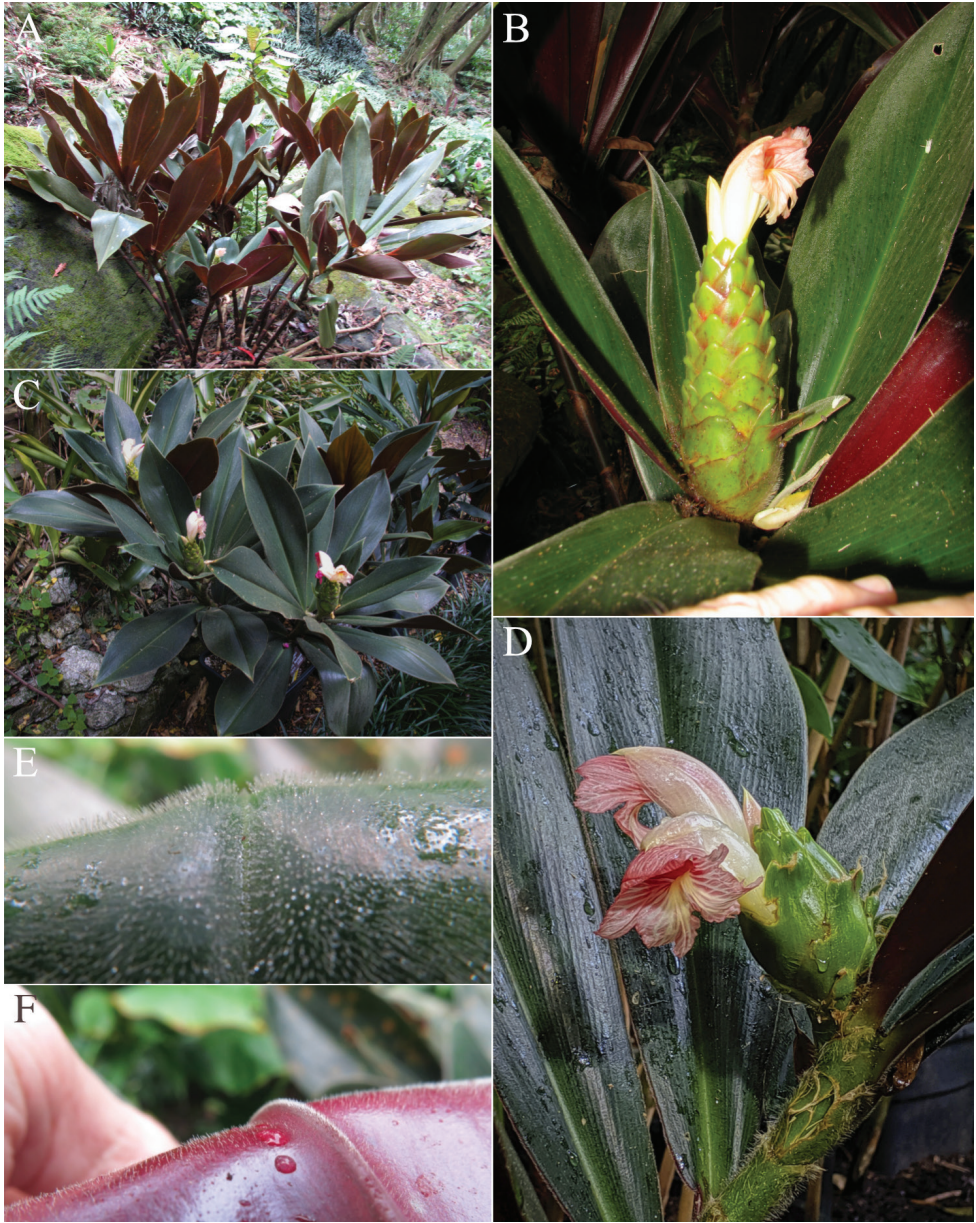


Figure 14. *Costus obscurus* D.Skinner & Maas **A** cultivated plant from Plowman's type collection showing upright orientation of leaf lamina **B** inflorescence **C** cultivated plant showing colours and upright orientation of leaf lamina **D** photo showing villose indument on leaf sheaths **E** Indument on adaxial leaf surface **F** indument on abaxial leaf surface **A, B** cultivated specimen from Lyon Arboretum, Hawaii L-81.0901 grown from seeds wild collected by Tim Plowman from the plant accessioned as *Plowman* 5054 **C–F** photos taken in Pantiacolla, Madre de Dios, Perú prior to collecting seeds for *D.Skinner* R3322, currently in cultivation. Photos **A–F** by Dave Skinner.

margin irregularly dentate to lobulate; stamen white to pink, 35–45 × 12–15 mm, not exceeding the labellum, apex pinkish red, deeply and irregularly lobed, anther 7–8 mm long. **Capsule** ellipsoid, 12–15 mm long.

Distribution. Peru (Cuzco, Huánuco, Madre de Dios, San Martín) (Fig. 22C).

Habitat and ecology. In forests, in wet, shady areas, often in disturbed places, at elevations of 450–1300 m. Flowering in the rainy season.

Etymology. *Costus obscurus* sp. nov. is known for its leaves having a very dark green adaxial surface and dark purple abaxial surface, and for growing in dark and shady places, hence the specific epithet *obscurus* (*obscurus* means ‘dark, shady, “indistinct” in Latin). This species might also be considered “obscure” (in English: unclear, uncertain, unknown, or in doubt) because it has been confused by the authors in the past as either *C. erythrophyllus* Loes. or as *C. acreanus* (Loes.) Maas. Only recently has it been determined to be an undescribed species.

Paratypes. PERU. Cuzco: Prov. La Convención, Distr. Echarati, Cashiriari-3 well site, 5 km S of Camisea River, 700 m, 2 Sep 1998, *Núñez V. et al.* 23785 (F). **Huánuco:** Prov. Leoncio Prado, Puente Pucayaco, Río Pucayaco, road from Tingo Maria to Tocache, 570 m, 30 Mar 1976, *Plowman & Kennedy* 5775 (U); cultivated in Tocache, besides Maior Plaza, originally collected by José Schunke V. at Quebrada Cachiayaco de Lopuna, near Mina de Sal, S of Tocache, 31 Mar 1976, *Plowman & Kennedy* 5791 (GH, U); cultivated in Jardín Botánico de Tingo Maria, from material collected by Plowman in Ramal de Aspusana, 65 km N of Tingo Maria, on the border of Huánuco and San Martín, 5 Jul 1978, *Plowman & Ramírez R.* 7595 (F, U). **Madre de Dios:** Prov. Manu, Cerro de Pantiacolla, Río Palotoa, 10–15 km NNW of Shintuya, 700–1300 m, 16 Dec 1985, *Foster et al.* 10937 (F); Pantiacolla, serranía across Río Alto Madre de Dios from Shintuya, 450–650 m, 28 Oct 1979, *Gentry* 27304 (MO, U). **Cultivated material:** Harvard Forest Greenhouses, Petersham, Massachusetts, USA, cultivated from *Plowman & Davis* 5054, Peru, *Plowman & Davis* 5054 A (F, L); cultivated from Peru, Huánuco, 8 km NW of Tingo Maria, along quebrada on road to Shínchi Roca, 900 m, 14 Nov 2016, *Skinner* R3383 (BH). Peru, Madre de Dios, Pantiacolla, 25 Jan 2013, *Skinner* R3322 (UC).

Notes. Plants of *Costus obscurus* sp. nov. have a compact appearance, with leaves closely spaced together along the shoot and generally pointed upward rather than horizontally or drooping. The leaves’ dark green adaxial surface and dark purple abaxial surface are striking in appearance. *Costus obscurus* can be confused with *C. erythrophyllus* Loes., but can be distinguished from that species by its shorter, truncate ligule instead of a long and deeply 2-lobed ligule as well as by having smooth rather than (mostly) plicate leaves.

This new species has been widely cultivated from the type collection *Plowman* 5054. Plowman’s journal indicates the distribution of live plants to Marie Selby Botanical Garden and Lyon Arboretum, which is accessioned as L-81.0901. It has sometimes been confused with the famous “El Whiskey” plant from Colombia, but it can be easily distinguished by the indumenta on the adaxial and abaxial leaf surfaces.

***Costus oreophilus* Maas & D.Skinner, sp. nov.**

urn:lsid:ipni.org:names:77316094-1

Diagnosis. *Costus oreophilus* sp. nov. (Fig. 15) can be confused with *C. laevis* Ruiz & Pav., with which it shares the mostly green bracts and adaxially oriented flowers, but it is distinguished by the puberulous sheaths and leaves and a smaller ligule (3–10 mm vs. 5–20 mm long); moreover the corolla lobes are often recurved, a feature rarely seen in *Costus*.

Type. ECUADOR, Tungurahua: Río Verde Grande, 1500 m, 30 Mar 1956, *Asplund* 20068 (holotype S, 2 sheets S06-1367 & S06-13643; isotype GB).

Description. *Herb* 3–4 m tall. *Leaves* sheaths 15–20 mm diam; ligule truncate, 3–10 mm long; petiole 5–10 mm long; sheaths, ligule and petiole densely to sparsely puberulous; lamina narrowly elliptic, 20–38 × 7–11 cm, adaxially shiny, glabrous, sometimes rather densely puberulous, abaxially green, densely to sparsely puberulous to velutinous, base cordate to acute, apex acuminate (acumen 5–10 mm long). *Inflorescence* cylindric to ovoid, 7–12 × 3–6 cm, wrapped tightly by the upper leaves, terminating a leafy shoot; bracts, bracteole, calyx, ovary, and capsule sparsely puberulous to glabrous. *Flowers* adaxially oriented to erect; bracts green in the exposed part, red in the covered part, sometimes completely red, coriaceous, broadly ovate, 3.5–4.5 × 3–4 cm, apex obtuse, callus inconspicuous; bracteole boat-shaped, 18–31 mm long; calyx red, 6–12 mm long, lobes shallowly triangular, 3–4 mm long; corolla white to yellow, 50–60 mm long, glabrous, lobes recurved, narrowly obovate, 40–55 mm long; labellum white, distal edge horizontally spreading, broadly obovate, 70–75 × 70–75 mm, lateral lobes striped with red, middle lobe reflexed, with yellow honey mark, margin irregularly crenulate; stamen red to pink, 35–40 × 12–13 mm, not exceeding the labellum, apex red, irregularly dentate, anther 10–12 mm long. *Capsule* ellipsoid, c. 15 mm long.

Distribution. Ecuador (Pastaza, Tungurahua, Zamora-Chinchipe) (Fig. 22D).

Habitat and ecology. In forests, at elevations of 1150–1600 m. Flowering in the rainy season.

Etymology. The species name “*oreophilus*” is derived from the Greek words *oros* (= mountain) and *philos* (= beloved) as this is a mountain-loving species.

Paratypes. ECUADOR. **Pastaza:** road to Puyo, km 45–60, 1300–1400 m, 8 Oct 1961, *Dodson & Thien* 913 (WIS). **Tungurahua:** valley of Río Pastaza, Hacienda Verde Grande, 1500 m, 26 Jul 1939, *Asplund* 7833 (GB, S); between Río Mapoto and Río Margaritas, along Canelos trail, 1225 m, 20 Mar 1939, *Penland & Summers* 179 (BM, F, MO). **Zamora-Chinchipe:** along road Loja-Zamora, km 45–51, 1400–1600 m, 20 Nov 1961, *Dodson & Thien* 1441 (WIS). **Cultivated material:** Ecuador, Zamora Chinchipe, Parque Nacional Podocarpus, 1175 m, 25 Jan 2013, *Skinner* R 3332; Ecuador, Zamora Chinchipe, Río Numbamia, 1270 m, *Skinner* R 3333.

Notes. Most collections of *Costus oreophilus* sp. nov. were placed by Maas (1972) under *Costus laevis* Ruiz & Pav. with a note saying: “Three Ecuadorian collections



Figure 15. *Costus oreophilus* Maas & D.Skinner **A** plant in habitat **B** inflorescence showing clasp upper leaves and recurved corolla lobes on a fresh flower **C** flowering shoot showing narrowly elliptic leaf lamina **D** upper part of inflorescence showing recurved corolla lobes and adaxially oriented to erect labelum. Photos **A–D** by Dave Skinner taken near La Molienda along the Río Estancia, Tungurahua, Ecuador.

deviated by being densely puberulous to velutinous on the lower side [abaxial surface] of the leaves. The flower material being incomplete, I could not determine their taxonomic status, i.e., whether it is a variety of *C. laevis* or a distinct species”. Now, 50 years

later, we recognize these specimens merit specific rank. *Costus oreophilus* differs from *C. convexus* sp. nov. Maas & Skinner by various features such as the hairy instead of glabrous sheaths and leaves, a smaller calyx (6–12 vs. 15–25 mm long), a smaller ligule (3–10 vs. 20–45 mm long), and recurved corolla lobes, a feature quite rare in *Costus*.

The authors are indebted to orchid specialist Marco Jiménez Villata of Zamora, Ecuador, who has explored the forests of Zamora-Chinchipe for over 30 years. He showed Dave Skinner several localities in the province, where he was able to make photographs of this species. Skinner later found the living plants of this new species in Tungurahua, which were consistent with the plants in Zamora-Chinchipe but usually with less dense hairs on the undersides of the leaves. Moreover, in living material, the corolla lobes were found to be sharply recurved, which is very unusual in the genus *Costus*.

***Costus pitalito* C.D. Specht & H. Maas, sp. nov.**

urn:lsid:ipni.org:names:77316095-1

Diagnosis. *Costus pitalito* sp. nov. (Fig. 16) looks superficially like *C. leucanthus* Maas based on the shared light-coloured flowers and a very long ligule, but it differs from *C. leucanthus* by markedly obtuse ligule lobes and bracts with densely brownish villose margins.

Type. COLOMBIA, Cauca: forested roadside from San Juan de Villalobos to Pitalito, 1590 m, 19 Jul 2018, *Maas, Maas-van de Kamer, Valderrama, Specht, Erkens & Rodríguez 10733* (holotype L, 2 sheets).

Description. *Herb* 1.7– 2.5 m tall. *Leaves* sheaths ca. 35 mm diam; ligule deeply 2-lobed, 45–70 mm long, lobes rounded, obtuse; petiole 10–20 mm long; sheaths, ligule and petiole densely, brownish villose; lamina narrowly elliptic, 30–45 × 12.5–14 cm, adaxially glabrous but sparsely brownish villose when young, abaxially densely brownish villose, base acute, apex acuminate (acumen ca. 10 mm long). *Inflorescence* suglobose, 11–12 × 9–11 cm, terminating a leafy shoot; bracts rather densely villose, appendages of bracts sparsely villose to glabrous, its margins densely brownish villose, bracteole and calyx sparsely puberulous, ovary and capsule rather densely sericeous. *Flowers* abaxially oriented to erect; bracts red, coriaceous, broadly ovate, 3–4 × 2–3 cm; appendages green, foliaceous, cup-shaped, erect to patent, broadly ovate-triangular, 1–1.5 × 1–1.5 cm, apex obtuse to acute; bracteole 1- to 2-keeled, 30–32 mm long; calyx red, apex green, 20–22 mm long, lobes ovate-triangular, 10–12 mm long; corolla pale yellow to hyaline, base pinkish, 55–60 mm long, glabrous, lobes narrowly elliptic, 35–40 mm long, with filiform apex; labellum light yellow to cream, darker yellow in throat, distal edge horizontally spreading, broadly obovate, ca. 70 × 70–80 mm, margin irregularly dentate to crenate; stamen cream, ca. 50 × 15 mm, apex reflexed, irregularly dentate, not exceeding the labellum, anther ca. 13 mm long. *Capsule* ellipsoid, ca. 25 mm long.

Distribution. Colombia (Cauca, Putumayo) (Fig. 22E).

Habitat and ecology. Along forested roadsides, at an elevation of ca. 1600 m. Flowering and fruiting: May and July.



Figure 16. *Costus pitalito* C.D.Specht & H.Maas **A** inflorescence as seen from above showing hairs on the margins of the bracts **B** close-up of single flower with attached bracteole (bl) indicating the calyx (ca), petals (pe) and labellum (lab) **C** indument of abaxial leaf surface **D** flower as viewed from the top showing the open labellum (lab) and single fertile stamen (st) **E** close-up of swollen leaf sheaths **F** leaf ligule. Photos of type specimen *Maas et al.* 10733 taken prior to pressing **A–E** by Chelsea Specht, **F** by Paul Maas.

Etymology. This species is named “pitalito” after its type locality near Pitalito, in the Colombian department of Cauca.

Paratypes. COLOMBIA. **Putumayo:** Mun. Villagarzón, carretera a Puerto Asis, 4 May 1994, *Fernández-Alonso et al.* 11440 (COL).

Notes. *Costus pitalito* looks superficially like *C. leucanthus* Maas by its white flowers and a very long ligule, but it differs by obtuse ligule lobes and bracts with densely brownish villose margins.

***Costus prancei* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316096-1

Diagnosis. *Costus prancei* sp. nov. (Fig. 17) looks quite similar to *C. sprucei* Maas (a species restricted to the Brazilian state of Pará) and has been confused with it in the past, both sharing most of the vegetative and floral characters, but this species has yellow to orange flowers whereas they are pinkish red in *C. sprucei*.

Type. BRAZIL, Acre: vicinity of Serra da Moa, 22 Apr 1971, *Prance, Maas, Kubitzki, Steward, Ramos, Pinheiro & Lima* 12265 (holotype U, 2 sheets: U1607120 & U1603130 (spirit collection); isotypes INPA, NY00867011).

Description. *Herb* 1–1.5 m tall. *Leaves* sheaths 8–10 mm diam; ligule truncate, 2–7 mm long; petiole 2–8 mm long; sheaths, ligule, and petiole densely to sparsely villose; lamina narrowly elliptic, 11–25 × 4–7 cm, adaxially densely villose to glabrous with a dense row of hairs along the primary vein, abaxially densely puberulous to villose, base acute, rounded, or cordate, apex acuminate (acumen ca. 15 mm long). *Inflorescence* cylindrical, 3–15 × 1.5–4 cm, terminating a leafy shoot; bracts, bracteole, calyx, ovary, and fruit densely puberulo-villose to glabrous. *Flowers* abaxially oriented; bracts red, coriaceous to chartaceous, broadly to depressed ovate, 1.5–2.5 × 1.5–3 cm, callus 3–5 mm long; bracteole boat-shaped, 9–15 mm long; calyx red to pale orange-red, 6–12 mm long, lobes shallowly triangular, 1– mm long; corolla yellow to orange (2 labels indicated cream and pink to orange!), 20–30 mm long, glabrous, lobes narrowly elliptic, 20–25 mm long; labellum yellow to orange, lateral lobes involute and forming a straight tube 7–8 mm diam, oblong-obovate when spread out, 20–25 × 12–16 mm, irregularly lobulated, lobules 0.5–5 mm long; stamen yellow, 23–25 × 7–8 mm, slightly exceeding the labellum, apex rounded, anther ca. 6 mm long. Capsule ellipsoid to subglobose, 6–7 × 5–7 mm.

Distribution. Brazil (Acre, Amazonas, Rondônia), Peru (Loreto, San Martín) (Fig. 22F).

Habitat and ecology. In non-inundated (terra firme) or periodically inundated (várzea) forests at elevations of 0–260 m. Flowering year-round.

Vernacular names. Brazil: Canafiche.

Etymology. This species is named after our friend and dearest colleague Sir Ghilleen Prance, who invited us (PM and HM) in 1971 to join various of his expeditions into the Brazilian Amazon region and who inspired me (PM) very much to continue working as a Neotropical taxonomist.



Figure 17. *Costus prancei* Maas & H.Maas Photo in habitat taken by Paul Maas at Serra do Moa, Acre, Brazil. Photo corresponds to *Prance* 12265 collected by G.T. Prance in 1971.

Paratypes. BRAZIL. Acre: Mun. Mançio Lima, Upper Rio Moa, top of Serra Azul, 12 Oct 1968, *Campbell 8951* (NY, RB); Mun. Mançio Lima, Serra do Moa, 29 Dec 1998, *Ehrlich 5c* (L, NY); Mun. Mançio Lima, PARNA, Serra do Divisor, trilha para a Cachoeira Formosa, 225 m, 21 Aug 2008, *Fiaschi 3287* (NY). **Amazonas:** Rio Nhamundá, *Assumpção & Coêlho 72* (INPA); Tapurucuara, Rio Negro, 6 Feb 1959, *Cavalcante 540* (MG); Mun. Humaitá, BR 230, Rodovia Transamazônica, 126 km from Humaitá, 12 Apr 1985, *Cid et al 5437* (INPA, NY); Mun. Manaus, c. 80 km NNE of Manaus, Fazenda Esteio, 50–125 m, 23 Jun 1992, *Nee 42842* (NY, U); Tapurucuara, road to the airport, 16 Oct 1971, *Prance et al. 15300* (COL, DAV, F, INPA, NY, U, US, VEN, W); Rio Canumá, 1 Mar 1945, *Proctor Cooper s.n.* (COL, US); Manaus-Itacoatiara Road, Rio Preto, km 80, 14 Nov 1966, *Prance et al. 3151* (F, MO, NY, U, US); Manaus-Itacoatiara Road, Rio Preto, km 90, 19 Jul 1961, *Rodrigues & Lima 2996* (INPA, U); Tapurucuara, 22 Jan 1978, *Steward et al. 476* (INPA, MO, NY, U, US); Lake Canumá, *Von Martius s.n.* (M). **Rondônia:** Estrada Porto Velho-Cuiabá, km 48, 5 Feb 1983, *Bilby et al. 33* (INPA); SE bank of Rio Jaci Paraná, before Jaci Paraná town, 9 Jul 1979, *C.E. Calderón et al. 2986* (NY, US); Porto Velho, Guajará-Mirim, Estrada do Palheta, km 12, foot of Serra Parecis, 28 Jan 1983, *Carreira 315* (INPA, MG); Itapuá de Oeste, Floresta Nacional de Jamari, 4 Dec 2011, *Castro et al. 81* (herbarium unknown); 1 km from BR 364, ca. 1 km N of road to São Sebastião, 24 May 1984, *Frame 171* (NY, U); Ji-Paraná, Reserva Biológica do Jarú, 125 m, 8 Jun 2015, *Labiak 6250* (NY); between Jaci Paraná and Rio Madeira, 26 Jun 1968, *Prance et al. 5218* (INPA, NY, U, US); basin of Rio Madeira, foothill of Serra dos Pacaás-Novos, 12 km NNE of Guajará-Mirim, 1 Aug 1968, *Prance et al. 6672* (COL, INPA, NY, U); 8 km N of Porto Velho, basin of Rio Madeira, 7 Nov 1968, *Prance et al. 8227* (F, INPA, NY, U); road to São Lorenço mines, N bank of Rio Madeira, 10 km above Mutumparaná, 25 Nov 1968, *Prance et al. 8866* (NY, U, US); Porto Velho, Parque Nacional Mapinguari, trilha do parquet, 12–14 Dec 2013, *Silveira 559, 596* (L, NY). **PERU. Loreto:** Prov. Requena, Oyo de Contaya, Serra del Divisor, between Contamana and Río Tapiche, 260 m, 10 Aug 2005, *Uliana et al. 1342* (AMAZ, F, L). **San Martín:** Prov. Alto Amazonas, Yurimaguas-Tarapoto road, 15 km SW of Yurimaguas, 180 m, 10 Oct 1985, *Gentry et al. 52203* (MO, U).

Notes. We have named this species after Sir Ghilleen Prance, who enabled the first author (PM) to undertake his first steps in the Amazonian world and assisted and taught him in a very kind and generous way.

Costus sprucei Maas and *C. prancei* sp. nov. closely resemble a third species, *C. chartaceus* Maas, which occurs in Amazonian Colombia, Ecuador, and Peru. They share many of the vegetative and floral features, but *C. chartaceus* Maas mostly has an unequally lobed ligule of 5–15 mm long, while that of the other two is truncate and 2–7 mm long. The essential difference can be found in the flower colour, which is pinkish-white in *C. chartaceus* (vs. pinkish-red in *C. sprucei* and yellow to orange in *C. prancei*).

The two Peruvian collections (*Gentry et al. 52203* and *Uliana et al. 1342*) look quite similar to this species and agree in most of the floral measurements, but the leaves are quite large for this species (i.e., 25–35 × 9–13 cm).

***Costus pseudospiralis* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316099-1

Diagnosis. *Costus pseudospiralis* sp. nov. (Fig. 18) looks superficially quite similar to *C. spiralis* (Jacq.) Roscoe, but differs by the orientation of the flowers (floral opening facing abaxially v adaxially) and a cordate leaf base. It differs from *C. douglasdalyi* by the inflorescence terminating a leafy shoot instead of terminating a shorter leafless shoot (sometimes referred to as ‘basal’ in the literature), the presence of villose indumentum on most of its vegetative parts (vs. glabrous or sparsely puberulous), and a cordate (vs. acute to rounded) leaf base.

Type. BOLIVIA, Beni: km 4 of road from Riberalta to Santa María, 150 m, 22 Jan 1999, *Maas, Maas-van de Kamer & Apaza 8751* (holotype U, 2 sheets: U1610003 & U1610004; isotypes K, MO, USZ).

Description. *Herb* 1.5–2 m tall. *Leaves* sheaths 10–15 mm diam; ligule obliquely truncate, 5–8 mm long; petiole 3–5 mm long; sheaths, ligule and petiole densely villose; lamina narrowly elliptic, 20–43 × 4–12 cm, adaxially densely to rather densely villose, abaxially densely villose, base cordate, apex acuminate (acumen 10–15 mm long). *Inflorescence* ovoid, 4–12 × 3–6 cm, terminating a leafy shoot; bracts, bracteole, calyx, ovary, and capsule glabrous. *Flowers* abaxially oriented; bracts red to dark red, coriaceous, broadly ovate, 2.5–3.5 × 2.5–3.5 cm, apex obtuse, callus 5–7 mm long, sometimes with a small, deltate appendage c. 5 × 5 mm; bracteole boat-shaped, 15–20 mm long; calyx red, 8–11 mm long, lobes shallowly ovate-triangular to very shallowly ovate-triangular deltate, 1–2 mm long; corolla dark salmon pink, 45–50 mm long, glabrous, lobes narrowly obovate-elliptic, 30–35 mm long; labellum pale salmon pink, apex and centre white, distal part of middle lobe yellowish, lateral lobes rolled inwards and forming a slightly curved tube 6–12 mm diam, oblong-obovate when spread out, 30–35 × 20–25 mm, apex irregularly 5-lobulate; stamen salmon pink, ca. 30 × 7 mm, not exceeding the labellum, apex white, rounded, anther 8–10 mm long. *Capsule* ellipsoid, 10–20 mm long.

Distribution. Bolivia (Beni), Brazil (Rondônia) (Fig. 22G).

Habitat and ecology. In secondary roadside vegetation, at elevations of 150–700 m. Flowering year-round.

Etymology. This species is named “pseudospiralis”, referring to its resemblance to *C. spiralis*.

Paratypes. **BOLIVIA. Beni:** 3 km E of Riberalta on road to Guayaramerín, then 2 km SE of side road, 230 m, 7 Jun 1982, *Solomon 7978* (L, LPB, MO). **Santa Cruz:** Prov. Velasco, Parque Nacional Noel Kempff Mercado, Los Fierros, 200 m, 8 Nov 1993, *Jardim & Quevedo 56* (MO, NY, USZ); Prov. Velasco, Parque Nacional Noel Kempff Mercado, Huanchaca II, 700 m, 4 Jul 1996, *Peña-Chocarro et al. 65* (U, USZ). **BRAZIL. Rondônia:** trail from Fortaleza to Rio Abuña, 20 km above mouth to São Sebastião mines, 15 Nov 1968, *Prance et al. 8459* (NY, U).



Figure 18. *Costus pseudospiralis* Maas & H.Maas **A** plant in habitat near Xapurí, Acre, Brazil with Martin Acosta **B** inflorescences emerging from the same rhizome **C** close-up of inflorescence with flowers showing their abaxial orientation **D** inflorescence with scale, emerging flower showing abaxial orientation **E** abaxial leaf surface showing indument. Photos **A–E** by Dave Skinner.

***Costus rubineus* D.Skinner & Maas, sp. nov.**

urn:lsid:ipni.org:names:77316100-1

Diagnosis. *Costus rubineus* sp. nov. (Fig. 19) looks somewhat similar to *C. scaber* Ruiz & Pav. but differs from that species by a larger calyx (9–15 vs. 3–7 mm), larger bracteole (20–28 vs. 9–12 mm), larger corolla (50–60 vs. 35–40 mm), and a labellum which

is distinctly lobulate at the apex vs. entire in *C. scaber*; furthermore it is restricted to high elevations whereas *C. scaber* is mostly a lowland species; *C. rubineus* also lacks the row of hairs along the adaxial midvein of the lamina, which is so typical for *C. scaber*.

Type. PERU, Pasco: Prov. Oxapampa, Gramazú, 1961 m, 17 Mar 2005, *Rojas G., Vásquez Chávez & Francis 3574* (holotype L4196133; isotypes HUT, MO-2152325, USM).

Description. *Herb* 0.5–4 m tall. *Leaves* sheaths 15–30 mm diam; ligule truncate to slightly lobed, 2–7 mm long; petiole 3–8 mm long; sheaths, ligule and petiole densely sericeous to glabrous; lamina narrowly elliptic, 20–37 × 5–13 cm, adaxially glabrous, abaxially rather densely villose to almost glabrous, base obtuse to slightly cordate, apex acuminate (acumen 10–15 mm long) to acute. *Inflorescence* ovoid, 7–11 × 4–6 cm, terminating a leafy shoot; bracts, bracteole, and calyx rather densely puberulous to glabrous, ovary and capsule densely sericeous. *Flowers* abaxially oriented; bracts dark ruby red to red, coriaceous, broadly ovate, 3–4 × 2.5–4 cm, apex obtuse, callus 6–10 mm long; bracteole boat-shaped, 20–28 mm long; calyx red to pink, 9–15 mm long, lobes very shallowly triangular, 2–3 mm long; corolla pink to orange, 50–60 mm long, glabrous, lobes narrowly elliptic, 40–50 mm long; labellum yellow, distal part red, lateral lobes rolled inwards and forming a curved tube ca. 10 mm diam, oblong-obovate when spread out, 30–50 × 18–20 mm, 5-lobulate, lobules narrowly ovate-triangular, ca. 4 mm long; stamen yellow, 30–45 × 7–10 mm, not or slightly exceeding the labellum, apex red, irregularly dentate, anther 8–9 mm long *Capsule* obovoid, ca. 13 mm long.

Distribution. Peru (Huánuco, Pasco) (Fig. 22H).

Habitat and ecology. In primary or secondary, premontane or montane forests, at elevations of (300–)1400–2200 m. Flowering year-round.

Etymology. *Costus rubineus* sp. nov. is named in reference to the Spanish explorer Hipolito Ruiz who visited in August 1780 the place called Chinchao in the Peruvian department of Huánuco. In his journal, he mentioned the collection of a plant he referred to as “*Costus ruber*”, but his collection was lost in a shipwreck off the coast of Portugal, and this species’ name was never again mentioned in the journal. Since there is already a *C. ruber* C.Wright ex Griseb. (a synonym of *C. pulverulentus* C.Presl), we cannot use that name but are using the very apt name of *C. rubineus* to reflect the beautiful ruby-red colour of the bracts. The first author of this species visited the Chinchao region in 2016 and found at that locality specimen which he believed to be the same species as Ruiz mentioned in his journal (Skinner 2016).

Paratypes. PERU. **Huánuco:** Cordillera Azul, ca. 42.7 km E of Tingo Maria on road to Pucallpa, 5650 ft, 21 Nov 1979, *Davidson & Jones 9395* (U). **Pasco:** Prov. Oxapampa, Distr. Huancabamba, Zona de Amortiguamiento del Parque Nacional Yanachaga-Chemillén, Sector alto tunqui, 1781 m, 10 Dec 2006, *Castillo et al. 642* (AMAZ, HUT, L, MO, USM); Prov. Oxapampa, Pichis Valley, new road between Santa Rose de Chivis and Puerto Bermúdez, 300–400 m, 30 Sept. 1982, *Foster 8950A* (MO); Prov. and Distr. Oxapampa, Carretera Oxapampa-Tsachopem, 1850 m, 15 Nov 2004, *Monteagudo Mendoza et al. 7658* (HUT, MO, U, USM); Prov. Oxapampa, Distr. Huancabamba, Carretera

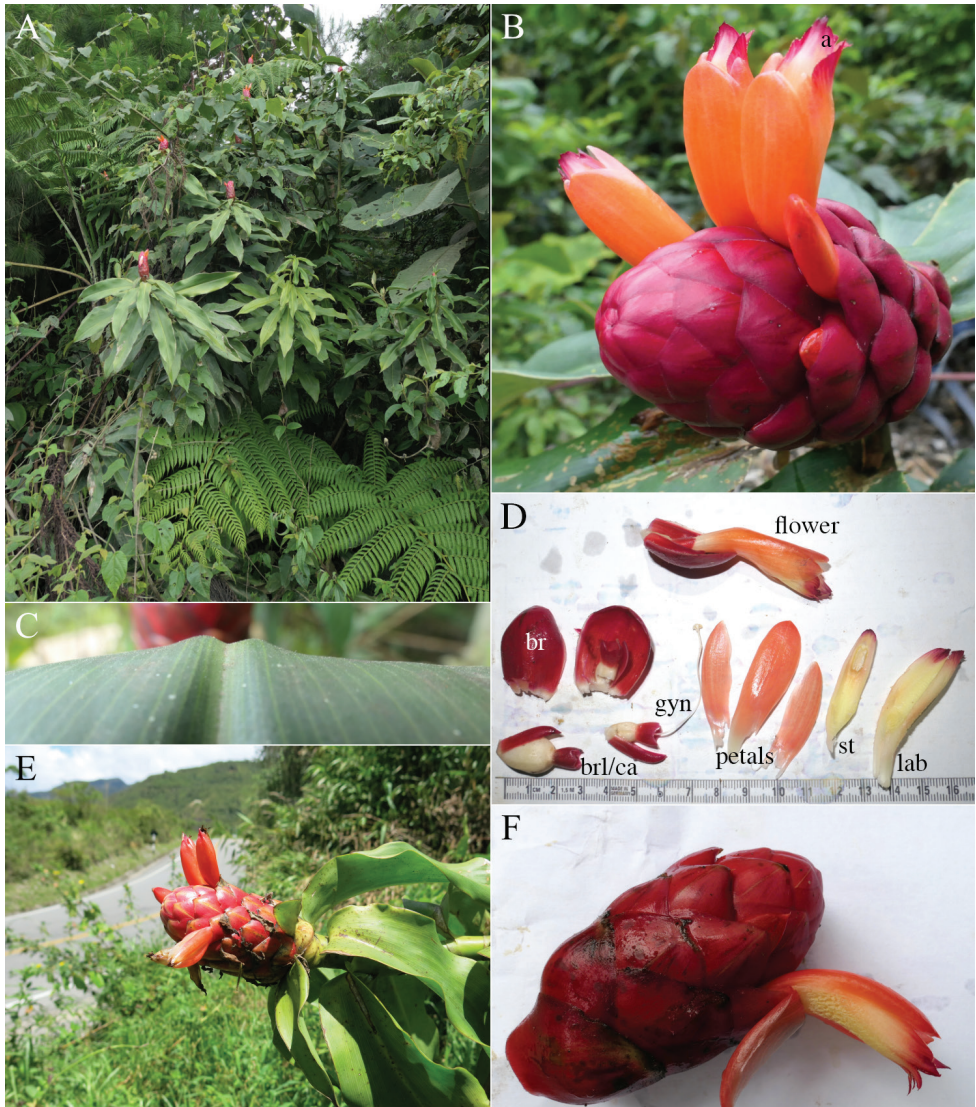


Figure 19. *Costus rubineus* D.Skinner & Maas **A** plant in habitat near Villa Rica, Pasco, Peru **B** inflorescence with flowers showing the labellum with characteristic lobulate apex (a) **C** adaxial leaf midrib showing absence of line of hairs **D** bract and flower detail from plant in habitat at Alto Churumazu, Pasco, Perú showing the full flower with subtending bract (flower), bract (br), bracteole subtending the capsule (ovary plus calyx) (brl/ca), stigma and style as attached to the young capsule (gyn), petals, single fertile stamen (st) and labellum (lab). Seeds from this plant were collected and cultivated as *D.Skinner R3421* **E** plant in habitat at 1980 meters near Chinchao, Huánuco, Perú, the original collection site of “*C. ruber*” as indicated in Ruiz’ journal **F** inflorescence with lower bracts removed and single flower photographed in the wild near Chinchao, Perú. Photos **A–F** by Dave Skinner.

hacia Pozuzo-Zona de Amortiguamiento, Parque Nacional Yanachaga-Chemillén, 1390–1630 m, 22 Mar 2005, *Ortiz V. et al.* 521 (L, MO, USM); Prov. Oxapampa, 5 km SE of Oxapampa, Oswald Müller property, 1850 m, 13–16 Dec 1982, *Smith* 2946 (MO, U), idem, 24 Dec 1983, *Smith* 5360 (HUT, MO, U, USM); Distr. Oxapampa, La Suiza, 10 Dec. 2002, 2200 m, *Vásquez Martínez & Rojas* 27782 (F, HUT, MO, U, USM).

Notes. *Costus rubineus* sp. nov. is a species mostly restricted to relatively high elevations (except for *Foster* 8950A, which was found at 300 m) in the departments of Pasco, San Martín, and Huánuco, Peru. It looks, at first glance, somewhat similar to *C. spiralis* (Jacq.) Roscoe, but it differs from that species by its abaxially oriented flowers and a very short ligule. The living plants look similar to *C. scaber* Ruiz & Pav., but that species differs by a much shorter calyx (3–7 mm vs. 9–15 mm long in *C. rubineus*) and the presence of a row of erect hairs on the adaxially of the lamina (lacking in *C. rubineus*). There are often several flowers at the same time at anthesis, whereas in *C. scaber*, there is usually just one flower at anthesis.

***Costus whiskeycola* Maas & H.Maas, sp. nov.**

urn:lsid:ipni.org:names:77316102-1

Diagnosis. *Costus whiskeycola* sp. nov. (Fig. 20) looks quite similar to *Costus erythrophyllus* Loes., sharing many features of inflorescence and flowers, but it is markedly different in their leaves which lack the distinct plication and which have a dark, olive green adaxial surface.

Type. COLOMBIA, Putumayo: Km 42 of Mocoa-Puerto Asis Road, Quebrada El Whiskey, Finca Santa Marta, Hilltop forest, c. 400 m (“1260 ft”), 5 Nov 1974, *Plowman & Davis* 4396 (holotype COL; isotype PSO).

Description. *Herb* 0.5–0.7 m tall. *Leaves* sheaths 10–18 mm diam; ligule 10–20 mm long, obliquely truncate; petiole 5–15 mm long; sheaths, ligule and petiole glabrous, purple-red to green; lamina ovate-elliptic to narrowly ovate-elliptic, 22–29 × 10–13 cm, dark, olive green adaxially, red-purple to dark purple abaxially, with 5–6 dark green bands corresponding with slightly raised veins above, adaxial and abaxial surfaces both glabrous, base acute, apex acute to shortly acuminate (acumen 3–5 mm long). *Inflorescence* ovoid, ca. 7 × 4.5 cm, terminating a leafy shoot; bracts and appendages of bracts, bracteole, calyx, and ovary glabrous. *Flowers* abaxially oriented; bracts red, coriaceous, broadly ovate, 3–4 × 2–3.5 cm; appendages green, foliaceous, ascending, triangular-ovate, 2.5–5 × 2–3.5 cm, apex acute; bracteole boat-shaped, 24–30 mm long; calyx red, 12–20 mm long, lobes very shallowly triangular, 2–5 mm long; corolla white, 60–75 mm long, glabrous, lobes narrowly elliptic, 45–60 mm long; labellum white, distal edge horizontally spreading, broadly obovate, 60–70 × 50 mm, lateral lobes striped with red, middle lobe reflexed with yellow honey mark, irregularly lobulate, margin crenulate; stamen white, tinged with red, 35–40 × 13–14 mm, not exceeding the labellum, apex 3-dentate, anther 8–10 mm long. *Capsule* not seen.

Distribution. Colombia (Putumayo). Ecuador (Napo), Peru (Loreto) (Fig. 22I).



Figure 20. *Costus whiskeycola* Maas & H.Maas **A** cultivated plant grown from seeds from the *Plowman & Davis* 4396 accession now growing at the Utrecht Botanical Gardens, The Netherlands **B** cultivated plant obtained from the established nursery trade, accession *D.Skinner* R2847 **C** flowers showing spreading labellum with lateral markings and a prominent honey mark **D** bracts with green and red erect appendages **E** comparison of leaf plication between (i) *C. whiskeycola* and (ii) *C. erythrophyllus* **F** comparison of ligule and leaf bases of (i) *C. whiskeycola* and (ii) *C. erythrophyllus* **G** comparison of leaf sections showing thicker mesophyll layer in (i) *C. whiskeycola* v. (ii) *C. erythrophyllus*. Leaf images in **C–D** taken from plants that were pressed and accessioned as *D.Skinner* R3026 (*C. whiskeycola* sp. nov.) and *D.Skinner* R2948 (*C. erythrophyllus*). Photo **A** by Lubbert Westra, photos **B–F** by Dave Skinner.

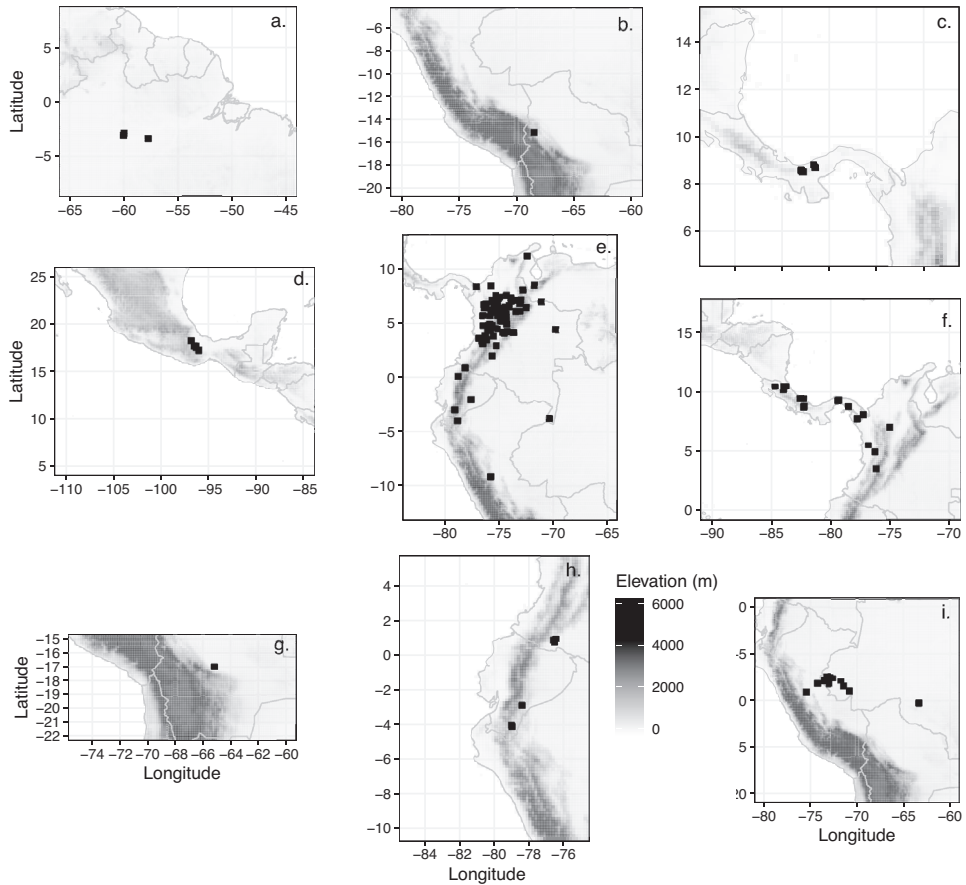


Figure 21. Distribution maps for new species of Costaceae: *Chamaecostus* – *Costus douglasdalnyi*
A *Chamaecostus manausensis* Maas & H.Maas **B** *Costus alfredoi* Maas & H.Maas **C** *Costus alleniiopsis* Maas & D.Skinner **D** *Costus alticolus* Maas & H.Maas **E** *Costus antioquiensis* Maas & H.Maas **F** *Costus callosus* Maas & H.Maas **G** *Costus cochabambae* Maas & H.Maas **H** *Costus convexus* Maas & D.Skinner **I** *Costus douglasdalnyi* Maas & H.Maas.

Habitat and ecology. In tropical wet forests at an elevation of 250–400 m. Flowering period is uncertain.

Etymology. This species has been named after its type locality El Whiskey (Putumayo, Colombia), with “cola” the Latin for “living in”.

Paratypes. COLOMBIA. Putumayo: El Chapo, 250 m, *Skinner R 3463*. **Cultivated Material** Greenhouse of “Sandwijck”, near Utrecht, the Netherlands, 3 Aug 1978, *Maas 74-618* (AAU, COL, K, NY, QCA, P, U161001, U161002, US), from the type collection *Plowman & Davis 4396*; cultivated in USBRG as 1994-680, *Skinner R 2257* (UC); cultivated in Jesse Durko Nursery, *Skinner R 2847*; cultivated by Bob Campos, *Skinner R 2948*; Smith College Botanical Garden, *Skinner R 3026* (UC).

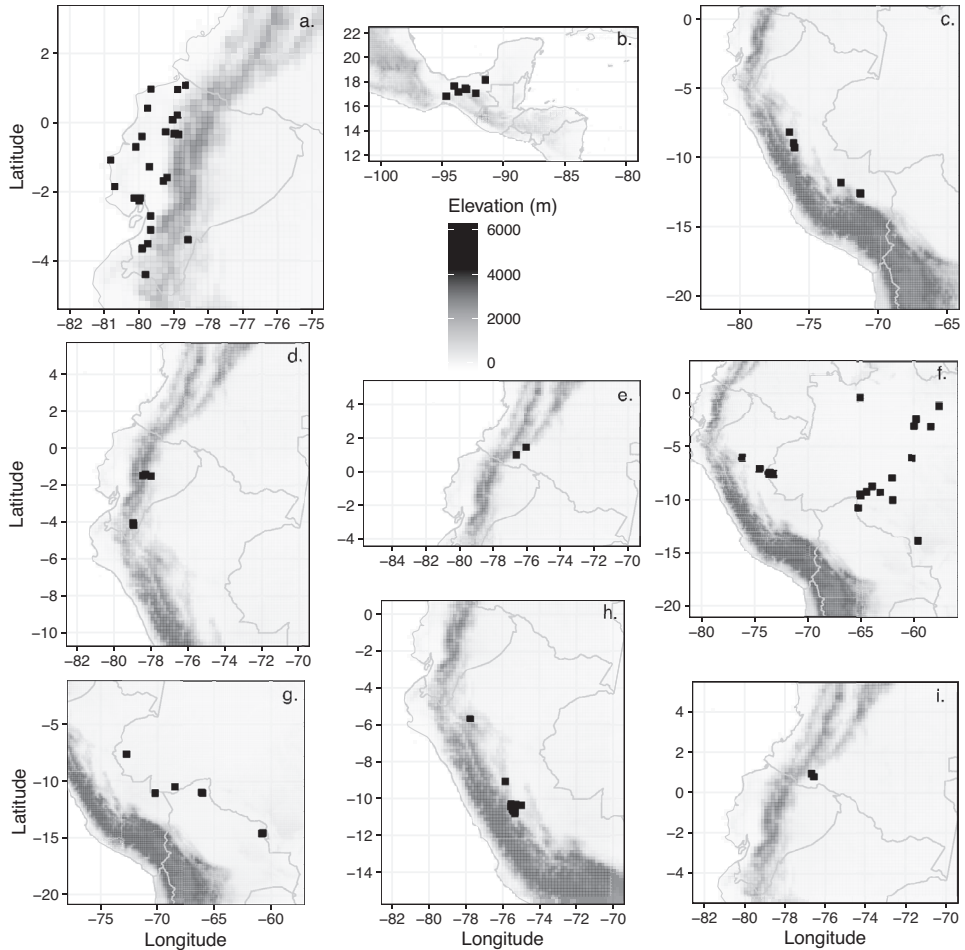


Figure 22. Distribution maps for new species of Costaceae. *Costus gibbosus* – *Costus whiskeycola*
A *Costus gibbosus* D.Skinner & Maas **B** *Costus mollissimus* Maas & H.Maas **C** *Costus obscurus* D.Skinner
 & Maas **D** *Costus oreophilus* Maas & D.Skinner **E** *Costus pitalito* C.D.Specht & H.Maas **F** *Costus prancei*
 Maas & H.Maas **G** *Costus pseudospiralis* Maas & H.Maas **H** *Costus rubineus* D.Skinner & Maas **I** *Costus*
whiskeycola Maas & H.Maas.

Notes. This new species has long been widely cultivated worldwide, originally grown from seeds collected by Tim Plowman when preparing *Plowman & Davis* 4396 at the type locality. Until a late stage in our revision, we united this species with *C. erythrophyllus* Loes. Although the flowers and inflorescence look very much like those of that species, the leaves are quite different in their colour and the almost absence of distinct plication. They also have a thicker mesophyll layer and are waxy in living plants. This species can also be distinguished from *C. erythrophyllus* by the length and shape of the ligules, which are truncate to obliquely truncate instead of being deeply lobed.

In April 2022, Dave Skinner visited the Santa Cruz private reserve of Project Amazonas near Iquitos, Loreto, Peru and found large populations of plants in primary forest that are identical vegetatively and have flowers that match those of *Costus whiskeycola*. However, the bracts lack leafy appendages. It is yet to be determined whether or not these plants are of this same species.

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Appendix I

Identification list

Collections are identified by first collector and number only. The abbreviations behind the collector number refer to the following taxa:

Ch man = *Chamaecostus manausensis* Maas & H.Maas

Co alf = *Costus alfredoi* Maas & H.Maas

Co all = *Costus alleniopsis* Maas & H.Maas

Co alt = *Costus alticolus* Maas & H.Maas

Co ant = *Costus antioquiensis* Maas & H.Maas

Co cal = *Costus callosus* Maas & H.Maas

Co coc = *Costus cochabambae* Maas & H.Maas

Co con = *Costus convexus* Maas & D.Skinner

Co dou = *Costus douglasdalnyi* Maas & H.Maas

Co gib = *Costus gibbosus* D.Skinner & Maas

Co mol = *Costus mollissimus* Maas & H.Maas

Co obs = *Costus obscurus* D.Skinner & Maas

Co ore = *Costus oreophilus* Maas & D.Skinner

Co pit = *Costus pitalito* C.D.Specht & H.Maas

Co pra = *Costus prancei* Maas & H.Maas

Co pse = *Costus pseudospiralis* Maas & H.Maas

Co rub = *Costus rubineus* D.Skinner & Maas

Co whi = *Costus whiskeycola* Maas & H.Maas

Asplund 7833: Co ore; 15412: Co gib; 15895: Co gib; 16421: Co gib; 16494: Co gib; 16763: Co gib; 18160: Co gib; 20068: Co ore – Assunção 72: Co pra;

Balcázar 49: Co ant – Beck 7320: Co coc – Beltrán Cuartas 78: Co ant – Betancur B. 5970: Co ant; 7500: Co ant; 16507: Co ant; 16651: Co ant – Bilby 33: Co pra – Boeke 1009: Co ant – Bohlin 1226: Co gib – Boyle 2556: Co alt – Breedlove 26463: Co mol;

Calderón 2986: Co pra – Camp E 2395: Co ant – Campbell 8951: Co pra – Carreira 315: Co pra – Castro 81: Co pra – Castillo 642: Co rub – Cavalcante 540: Co pra; Cid 5437: Co pra – Cordero-P. 91: Co ant – Cornejo 425: Co gib; 5792: Co gib – Croat 62674: Co dou; 83948A: Co gib; 85008: Co dou; 92034: Co ant – Cuatrecasas 8396: Co ant; 13714: Co ant;

Daly 8606: Co dou – Davidson 8770: Co cal; 9395: Co rub – Dodson 913: Co ore; 1441: Co ore; 5347: Co gib; 13039: Co gib – Dressler 4190: Co cal – Duque-Jaramillo 2251: Co ant; 3912-A: Co ant;

Ehrich 5c: Co pra;

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- Galeano 2036: Co ant – García-Barriga 18240: Co ant – Gentry 52203: Co pra;
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Lücking s.n.: Co cal;
Maas 596: Co ant; 1548: Co cal; 1652: Co all; 1783: Co ant; 2915: Co gib; 4795:
Co gib; 8751: Co pse; 8999: Co dou; 10488: Co ant; 10631: Co ant; 10635: Co
gib; 10688: Co cal; 10707: Co con; 10733: Co pit; P 12972: Co dou; P 13213:
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Aspidistra daibuensis var. *longkiauiensis*, a new variety of *Aspidistra* (Asparagaceae) from Taiwan, identified through morphological and genetic analyses

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Abstract

Aspidistra Ker Gawl. is one of the the most diverse and fastest-growing genera of angiosperm. Most *Aspidistra* species have been discovered in a limited area or a single site through morphological comparison. Because of the lack of population studies, morphological variation within species and the boundaries of some species remain unclear. In recent years, combining genetic and morphological markers has become a powerful approach for species delimitation. In this study, we performed population sampling and integrated morphometrics and microsatellite genetic diversity analyses to determine the species diversity of *Aspidistra* in Taiwan. We identified three species, namely *Aspidistra attenuata* Hayata; *A. daibuensis* Hayata var. *daibuensis*; *A. mushaensis* Hayata var. *mushaensis*; and reduced *A. longiconnectiva* C.T.Lu, K.C.Chuang & J.C.Wang to the variety level, and described a new variety, *A. daibuensis* Hayata var. *longkiauiensis*. The description, diagnosis, distribution, and photographs of this new variety as well as a key to the known Taiwanese *Aspidistra* are provided.

Keywords

Aspidistra, microsatellite, morphometric analysis, new variety, Taiwan

Introduction

The genus *Aspidistra* Ker Gawl. (Asparagaceae) is native to eastern and southeastern Asia, particularly China and Vietnam (Tillich 2014), and *Aspidistra* species typically grow under forest canopies and shrubs in high-rainfall areas. Plants of *Aspidistra* are characterized by a perennial herbaceous habit, conspicuous rhizomes, solitary or two to four tufts' leaves, a variety of fruits and a highly diversified flower structure (Li et al. 2000; Lin et al. 2010). *Aspidistra* is one of the fastest growing genera in Angiosperm (Tillich and Averyanov 2018). In the past 15 years, the number of *Aspidistra* has considerably increased from approximately 93 taxa (Tillich 2008) to more than 200 species (Kalyuzhny et al. 2022). So far, new species of this genus have been discovered continuously. However, phylogenetic analysis of this genus has not yet been complete performed.

In Taiwan, Hayata (1912, 1920) described three species, namely *Aspidistra attenuata* Hayata, *A. daibuensis* Hayata, and *A. mushaensis* Hayata. However, the boundaries of some of these species are inconsistent. For example, Ying (2000) described that these three species should be combined into *A. elatior* Blume and regarded them as a variety, namely *A. elatior* var. *attenuata* (Hayata) Ying. Subsequently, Yang et al. (2001) demonstrated that the previously described three species differed from *A. elatior*, and that *A. mushaensis* is a synonym of *A. attenuata* Hayata. Wang (2004) and Chao (2016) have accepted the species concept of Hayata and reported that *A. daibuensis* is distributed in south and east Taiwan. However, Lu et al. (2020) reported that *A. daibuensis* is restricted to only south Taiwan and did not consider the population in east Taiwan. Wang (2004) suggested that *A. mushaensis* is distributed in not only central Taiwan but also south Taiwan. However, Lu et al. (2020) disagreed with this suggestion. A recent study suggested that the genus *Aspidistra* contains four species: *A. attenuata* Hayata; *A. daibuensis* Hayata; *A. longiconnectiva* C.T.Lu, K.C.Chuang, & J.C.Wang; and *A. mushaensis* Hayata (Lu et al. 2020).

Taxonomists have recognized nearly all *Aspidistra* species through the morphological comparison. However, this approach of species delimitation can cause some identification problems because of the intraspecific morphological variation or small morphological differences between closely related species (Pratt and Clark 2001; Whittall et al. 2004). Advances in many fields, such as molecular genetics, have helped taxonomists determine species boundaries and identify cryptic species (Whittall et al. 2004; Ellis et al. 2006; Bickford et al. 2007).

Population genetic methods, such as the algorithm developed by Pritchard et al. (2000) that was implemented in Structure software, can be used to identify well-differentiated genetic clusters and thus detect gene flow barriers. These methods can be employed to detect distinct species even if they are not yet reciprocally monophyletic at many genes due to incomplete lineage sorting (Duminil et al. 2015). In addition, by determining associations based on morphological data and considering the spatial distribution of detected genetic groups, we can investigate whether these genetic groups correspond to distinct species. Recently, many studies, such as those

on *Ancistrocladus* Wall. (Turini et al. 2014), *Asteropyrum* J. R. Drumm. et Hutch. (Cheng et al. 2021), *Greenwayodendron* Verdc. (Lissambou et al. 2019), *Polygonum* L. (Mosaferi et al. 2016), and *Santiria* Blume (Ikabanga et al. 2017), have reported that the combination of morphological and population genetic data can be used to delineate species complexes.

In this study, we integrated morphological and genetic data to evaluate species delimitation within the genus *Aspidistra* in Taiwan. In addition, we conducted population genetic and morphometric analyses to confirm the previous classification hypotheses and to determine the number of species in the genus *Aspidistra* in Taiwan. We further performed genetic differentiation analysis for groups to determine whether they are the conspecific populations or distinct species.

Materials and methods

Recognition of *a priori* species groups

To classify collected samples into distinct morphological groups, we initially used a subjective approach in accordance with a previous taxonomic study (i.e., Lu et al. 2020). According to studies conducted by Wang (2004) and Chao (2016), the population in east Taiwan that was not considered in the study by Lu et al. (2020) was regarded as *A. daibuensis*. We defined distinct morphological groups as *a priori* groups and used them in this study.

We determined whether *a priori* groups correspond to distinct taxa based on the concept of iterative taxonomy (Yeates et al. 2011), and species hypotheses were iteratively assessed using classical morphometrics and microsatellite genetic data. First, we performed morphometric analyses, including multivariate and univariate analyses, to evaluate quantitative morphological characters. Subsequently, we inferred genetic groups from microsatellite markers by using Structure software and explored genetic diversity and differentiation between genetic groups. Finally, we compared the results of the two datasets and concluded species delimitation.

Plant material sampling

For morphological comparison, living materials of *Aspidistra* were collected from Taiwan (Table 1, Fig. 1). A portion of these samples was converted into herbarium vouchers and deposited at TNU (The herbarium of National Taiwan Normal University, Taipei, Taiwan). For genotyping, the leaf material of these samples was dried using silica gel to preserve DNA and was stored at the Department of Life Sciences, National Taiwan Normal University, Taipei, Taiwan (Table 1, Fig. 1). In addition, herbarium specimens obtained from many herbaria (HAST, PPI, TAI, TAIF, and TNU; the acronyms are based on Index Herbariorum (<https://sweetgum.nybg.org/science/ih/>)) were examined directly, or digital plant specimens were evaluated online.

Table 1. Information on taxa, *a priori* groups, and locations at which samples were collected for morphological and genetic analyses.

Taxa	<i>A priori</i> group	Collected locations	Samples for morphometric analyses	Samples for DNA analyses	Abbrev.	Coordinates	Altitude (m)
<i>Aspidistra mushaensis</i>	AM	Shaolai Trail, Taichung City	10	9	TS	24°12'21.59"N, 121°0'21.6"E	800
		Defulan Trail, Taichung City	12	12	TD	24°10'51.6"N, 120°58'33.59"E	600–700
<i>A. longiconnectiva</i>	AL	Huisun Experimental Forest Station, Nantou County	2	6	NHS	24°05'48.81"N, 121°02'13.99"E	600–701
<i>A. attenuata</i>	AA	Dongpu, Nantou County	11	11	N	23°33'51.81"N, 120°55'31.32"E	1500–1600
		Fengshan, Chiayi County	14	12	C	23°35'54.44"N, 120°44'45.7"E	1100–1300
		Dinghu, Chiayi County	0	8	H	23°29'27.16"N, 120°43'22.51"E	1600–1700
		Kantoushan Trail, Tainan City	2	4	T	24°10'39.97"N, 120°58'35.97"E	600–700
		Liuyishan Trail, Kaohsiung City	3	3	K	23°5'33.97"N, 120°34'26.97"E	600–800
		Peitawushan Trial, Pingtung County	16	16	P	22°36'52.88"N, 120°42'6.2"E	1200–1500
		Shouka, Pingtung County	3	3	PK	22°14'34.79"N, 120°48'50.39"E	300–400
<i>A. daibuensis</i>	ADS	Shuangliou, Pingtung County	11	10	PS	22°13'4.79"N, 120°47'38.39"E	200–300
		Jinlun, Taitung County	0	5	DJL	22°31'7.02"N, 120°54'40.10"E	100–300
		Lijia Forest Road, Taitung County	0	7	DLJ	22°48'57.80"N, 121°1'27.89"E	1000–1200
		Walami Trail, Hualien County	7	8	HW	23°19'40.21"N, 121°13'40.21"E	600–700
	ADE	Dulan Mountain Trail, Taitung County	6	6	DL	22°10'37.79"N, 121°10'37.79"E	700–900
		Wenshan Hot Spring, Hualien County	0	3	HWS	24°12'6.14"N, 121°29'26.61"E	600–700
		Lidao, Taitung County	0	5	NHL	23°8'30.60"N, 121°6'1.08"E	500–600
			97	128			

Identification of morphological groups

In the first step, we selected 128 fresh specimens representing different *a priori* groups, including available flowering specimens (N = 97; Table 1, Fig. 1). We used 5 leaf characters and 16 floral characters for 97 samples (Tables 2, 3, Fig. 2). Raw data were normalized before analysis. Subsequently, we used a clustering method (clustering analysis [CA]) and an ordination method (discriminant analysis [DA]) to project and visualize trends for morphological variability across our samples, including leaf and floral characters. Finally, we determined whether the mean of quantitative traits significantly differed between morphological groups by using one-way analysis of variance (ANOVA). For characters determined to be significant, we again tested each pair of morphological groups through Tukey’s pairwise test and used letters to identify groups that differed significantly. All statistical analyses were performed using PAST statistical software v.4 (Hammer et al. 2001).

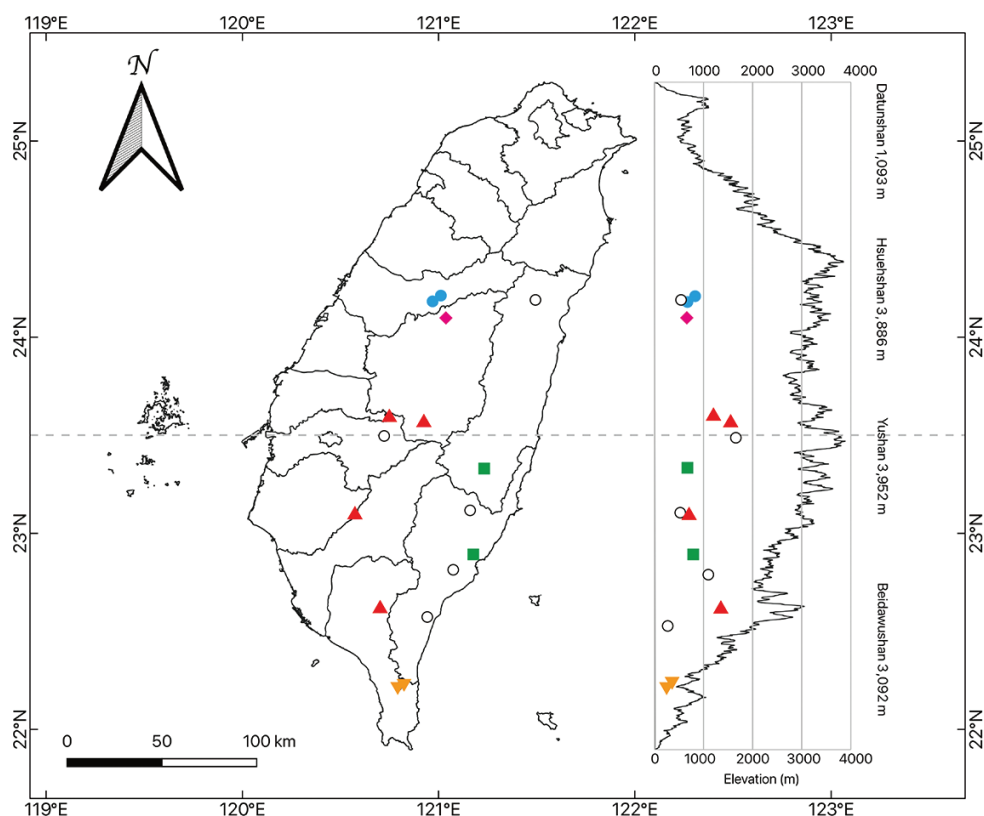


Figure 1. Sample collection location of the five *a priori* *Aspidistra* groups on the basis of morphological and geographical criteria. Color symbols: morphological and genetic data; white spots: only genetic data. Red triangle: AA, yellow inverted triangle: ADS, green square: ADE, fill diamond: AL, blue circle: AM.

DNA extraction and genotyping

DNA was extracted from 15 to 25 mg of leaf material (stored in silica gel) for 128 samples by using the Viogene Plant Genomic DNA Extraction Miniprep System (Viogene-Biotek, Taiwan). On the basis of the study conducted by Lu et al. (2020), five populations without morphological data, namely H, HWS, NHL, DJL, and DLJ, were temporally classified into different *a priori* groups (Table 1). Nine microsatellite markers were amplified using the method of Huang et al. (2014). Genotyping was performed in a 48-capillary sequencer (Labnet MultiGene Gradient, Labnet International Inc., USA) by using 1.5 μ L of DNA, 1000 μ L of HiDi formamide (Thermo Fisher Scientific, Taiwan), and 8.3 μ L of GeneScan-500 LIZ Size Standard (Applied Biosystems, Warrington, U.K.). To identify alleles (sizes of amplified PCR products), the resulting chromatograms were interpreted using Peak Scanner software v.1.0 (Applied Biosystems, Foster City, CA).

Table 2. The abbreviation and meaning of morphological characters measured in this study.

Abbreviation		Meaning
Leaf		
LL		Leaf length
BL/BWL	The ratio of blade length to the length from the blade base to the most width part of the blade	
BL/BW	The ratio of blade length to the most width of the blade	
LL/BL	The ratio of leaf length to blade length	
LL/PL	The ratio of leaf length to petiole length	
Flower		
LT		Lobe thickness
SCL	The curve length of the stigma surface	
LBST	The distance from the lobe base to the stigma apex	
SDA		Stigma angle
SL		Stigma height
SW		Stigma width
LBW		Lobe base width
LBL		Lobe length
PTL2/PTBW	The ratio of the perianth tube length to the width of the perianth tube base	
PTW1/PTBW	The ratio of the perianth tube width on the stamen-attached position to the width of the perianth tube base	
PTW2/PTBW	The ratio of the perianth tube width on the stigma apex to the width of the perianth tube base	
PTW3/PTBW	The ratio of the perianth tube width on the lobe base to the width of the perianth tube base	
PTL1/PH	The ratio of the perianth tube length (exclude lobe) to pistil height	
PTL1/SH	The ratio of the perianth tube length (exclude lobe) to the height of the stamen attached on the perianth tube (stamen height)	
PTL1/LBL	The ratio of the perianth tube length (exclude lobe) to lobe length	
PTL1/PTL2	The ratio of the perianth tube length (exclude lobe) to the perianth tube length	
PH/SH	The ratio of pistil height to the height of the stamen attached on the perianth tube (stamen height)	

Table 3. Leaf and floral quantitative characters of *Aspidistra* in Taiwan measured for the samples of the five *a priori* groups.

Characters		AA (N = 46) (mean ± stand. dev.)	ADE (N = 13) (mean ± stand. dev.)	ADS (N = 14) (mean ± stand. dev.)	AL (N = 2) (mean ± stand. dev.)	AM (N = 22) (mean ± stand. dev.)
Leaf	LL	0.745 ± 0.725 a	−0.100 ± 0.805 b	−1.048 ± 0.447 c	−1.742 c	−0.642 ± 0.574 bc
	BL/BWL	−0.585 ± 0.724 b	0.397 ± 0.879 a	0.644 ± 0.979 a	1.184 a	0.566 ± 0.901 a
	BL/BW	0.587 ± 0.428 a	0.017 ± 0.771 b	−1.881 ± 0.650 c	−0.587 b	−0.096 ± 0.614 b
	LL/BL	−0.182 ± 0.810 bc	0.393 ± 0.959 ab	−0.421 ± 0.659 bc	1.613 c	0.641 ± 1.194 a
	LL/PL	0.151 ± 1.019 b	−0.436 ± 0.484 bc	0.168 ± 0.882 bc	2.935 a	−0.496 ± 0.697 c
Flower	LT	0.237 ± 0.833 b	1.318 ± 0.935 a	−0.905 ± 0.561 c	−1.104 ± 0.386 bc	−0.600 ± 0.471 c
	SCL	0.770 ± 0.743 a	0.143 ± 0.385 b	−1.269 ± 0.365 c	−0.986 ± 0.114 bc	−0.815 ± 0.345 c
	LBST	0.569 ± 1.009 a	−0.056 ± 0.676 ab	−0.948 ± 0.377 cd	−2.148 ± 0.473 d	−0.215 ± 0.295 bc
	SDA	−0.898 ± 0.386 c	1.242 ± 0.296 a	0.552 ± 0.899 b	0.875 ± 1.428 ab	0.625 ± 0.447 b
	SL	0.842 ± 0.753 a	−0.749 ± 0.397 b	−0.727 ± 0.303 b	−0.364 ± 0.068 ab	−0.837 ± 0.533 b
	SW	−0.540 ± 0.936 c	1.470 ± 0.664 a	−0.287 ± 0.515 bc	0.127 ± 0.008 abc	0.369 ± 0.308 b
	LBW	0.184 ± 0.883 a	0.444 ± 1.004 a	−1.279 ± 0.679 b	1.670 ± 0.999 a	0.023 ± 0.720 a
	PTL2/PTBW	0.337 ± 0.989 a	0.484 ± 0.810 a	−0.132 ± 0.390 ab	−2.489 ± 0.313 c	−0.600 ± 0.769 b
	PTW1/PTBW	0.512 ± 1.066 a	−1.051 ± 0.377 b	−0.190 ± 0.877 ab	−0.402 ± 0.276 ab	−0.333 ± 0.461 b
	PTW2/PTBW	−0.173 ± 1.228	−0.283 ± 0.550	0.361 ± 0.889	0.244 ± 0.238	0.297 ± 0.650
	PTW3/PTBW	−0.234 ± 1.124	0.233 ± 0.831	0.057 ± 1.104	1.164 ± 0.156	0.228 ± 0.590
	PTL1/PH	−0.152 ± 0.739 bc	0.415 ± 0.996 ab	−0.531 ± 0.635 c	−2.376 ± 0.566 d	0.776 ± 0.951 a
	PTL1/SH	−0.364 ± 0.686 c	0.324 ± 0.667 ab	1.001 ± 1.232 a	−2.153 ± 0.040 d	−0.102 ± 0.559 bc
	PTL1/LBL	0.001 ± 0.863 b	−0.599 ± 0.786 bc	−0.128 ± 0.821 abc	−0.184 ± 0.177 c	0.693 ± 1.058 a
	PTL1/PTL2	0.504 ± 0.958 a	−0.602 ± 0.594 b	−0.622 ± 0.729 b	−1.322 ± 0.456 b	−0.020 ± 0.704 ab
	PH/SH	−0.211 ± 0.581 b	−0.091 ± 0.689 b	1.014 ± 1.038 a	0.016 ± 0.630 ab	−0.544 ± 0.477 b

The letters after the numbers were used to identify groups with significantly differences.

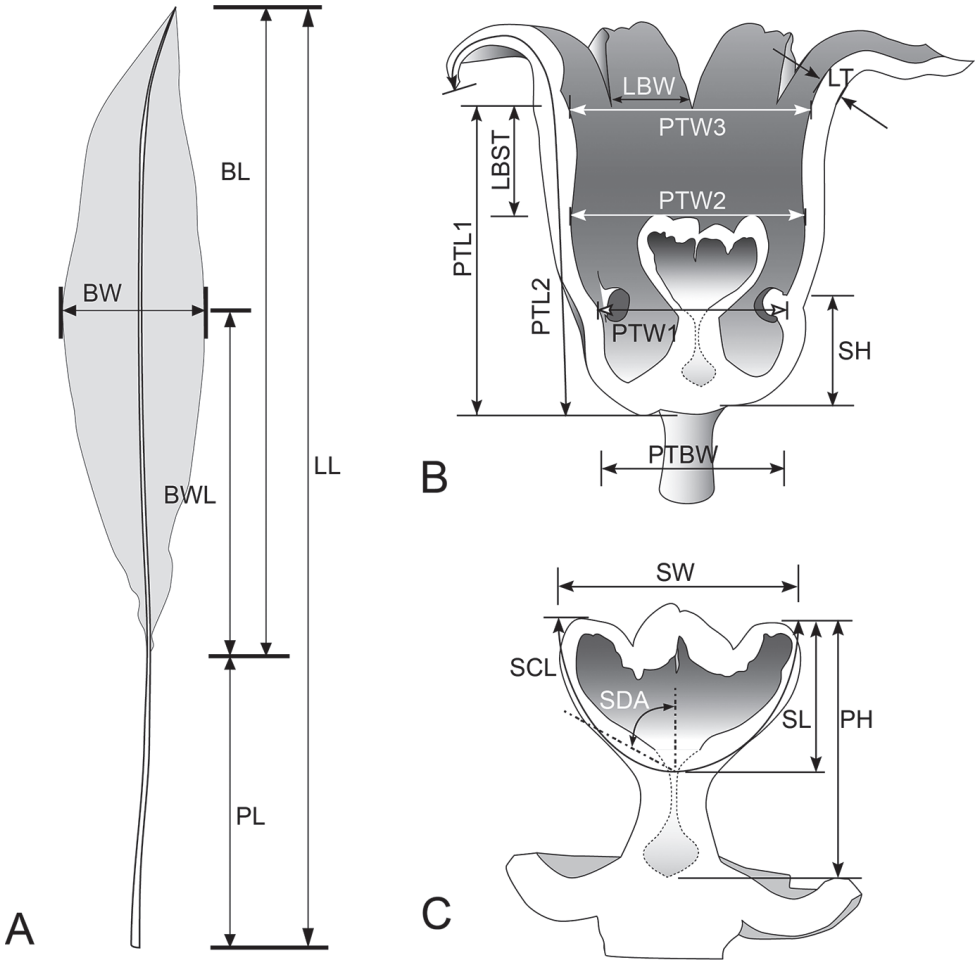


Figure 2. Morphological characters measured in this study **A** leaf characters **B, C** flower characters.

Identification of genetic groups

Genetic clusters were identified using the Bayesian clustering algorithm implemented in Structure v.2.3.4 (Pritchard et al. 2000) without *a priori* grouping. Ten independent runs were performed for each value of K ranging from 1 to 10 under a model assuming admixture and correlated allele frequencies (Falush et al. 2007). Each run comprised a burn-in period of 100,000 replications, followed by a run length of 1,000,000 Markov Chain Monte Carlo iterations. The results of replicated runs for each value of K from 1 to 10 were combined using Structure Harvester v.0.9.94 (Earl and von Holdt 2012). The optimal value of K was determined by calculating log-likelihood values and by using the ΔK method developed by Evanno et al. (2005). To visualize cluster assignments, the outputs of replicated runs were combined using CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007).

Genetic diversity and genetic differentiation between genetic groups

We used Arlequin v3.5.2 (Excoffier and Lischer 2010) to analyze molecular variance (AMOVA) and estimate the genetic diversity of each genetic group on the basis of the number of polymorphic loci as well as observed and expected heterozygosities (H_O and H_E) and the inbreeding coefficient (F). Differences in genetic diversity between genetic groups were characterized by computing the pairwise F_{ST} .

Results

Definition of *a priori* groups

We identified five *a priori* groups on the basis of their morphological and geographical data. The names of these *a priori* groups were derived from one of the four recognized taxon names after matching with one (Table 1). (1) A total of 46 samples obtained from the higher mountain altitude of central to southern Taiwan were called “AA” because they corresponded to the species *A. attenuata*. (2) A total of 22 samples from central Taiwan (Defulan Trail and Shaolai Trail, Taichung City) were called “AM” because they corresponded to the species *A. mushaensis*. (3) A total of 14 samples from the Hengchun Peninsula were called “ADS” because they corresponded to the species *A. daibuensis sensu* Lu et al. (2020). (4) A total of 13 samples, which had distinctly thick perianth lobes, corresponded to the taxon *A. daibuensis sensu* Wang (2004) and Chao (2016) and were called “ADE.” (5) Finally, 2 samples from Huisun Experimental Forest Station, Nantou County, which had elongated stamen connectives, corresponded to the taxon *A. longiconnectiva* and were called “AL.”

Morphological differentiation between *a priori* groups

Fig. 3A presents the results of the CA based on the Ward’s method that was performed to evaluate the 5 leaf characters and 16 floral characters for the 97 specimens. The morphological clustering groups almost fitted *a priori* groups, except for some mismatched samples. In the CA dendrogram, the samples were first divided into two groups: the first group consisted of samples from the AA group, and the second group consisted of samples from the ADE, ADS, AL, and AM groups. The second group was divided into four subgroups, which corresponded to the four *a priori* groups. However, except for the AL group, the other three groups included some mismatched samples, such as two ADE samples, and two ADS samples were assigned to the AM group (Fig. 3A). Axes 1, 2, and 3 of DA together accounted for 94.96% of the total variance. Axis 1 (59.81% of the relative contribution) was mainly determined on the basis of the ratio of the pistil height to the stamen height (PH/SH) and the distance from the perianth tube lobe base to the stigma apex (LBST). Axis 2 (20.9% of the

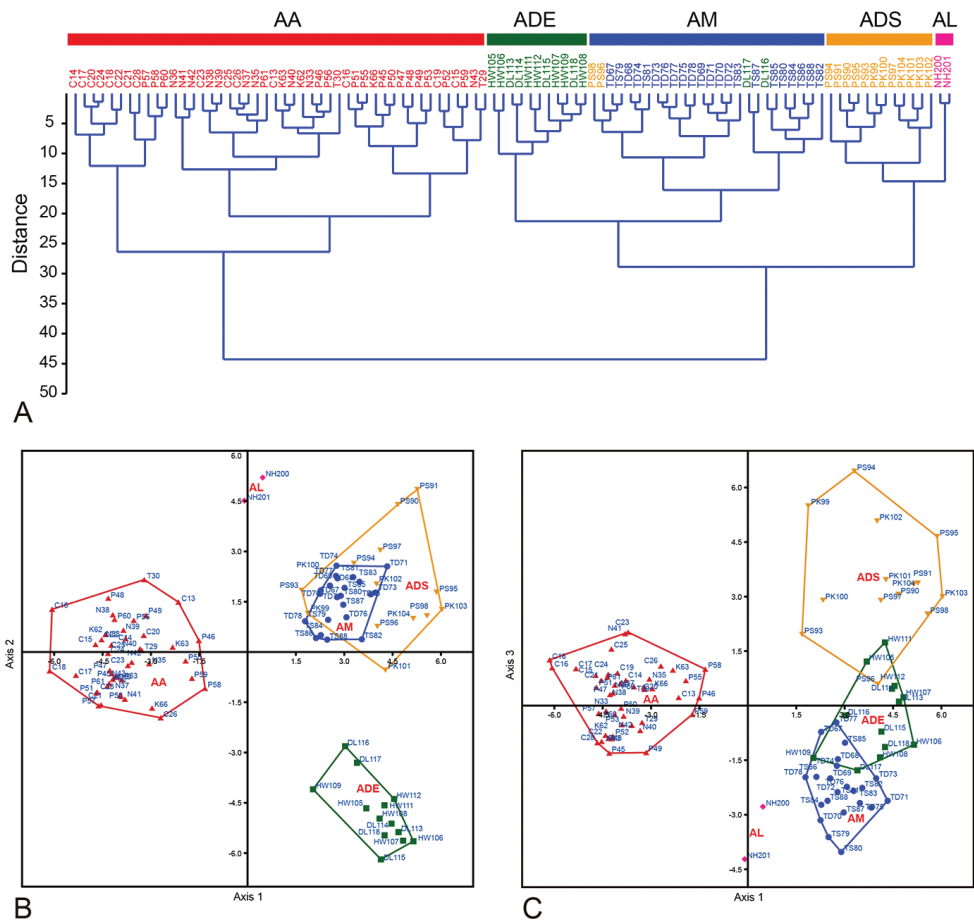


Figure 3. Results of morphometric analyses **A** dendrogram of clustering analysis **B, C** scatterplot of discriminant analysis **B** scatterplot by Axis 1× Axis 2 **C** scatterplot by Axis 1× Axis 3.

relative contribution) was mainly determined on the basis of the distance from the perianth tube lobe base to the stigma apex (LBST), the ratio of the perianth tube length to the width of the perianth tube base (PTL2/PTBW), and the curve length of the stigma surface (SCL). Axis 3 (14.25% of relative contribution) was determined on the basis of the ratio of the blade length to the blade width (BL/BW), PH/SH, and the ratio of the leaf length to the blade length (LL/BL). In DA scatterplots, the AA and AL groups were unambiguously separated along axis 1, whereas the other groups were less pronounced (Fig. 3B, C). The ADE group was separated from the AM and ADS groups along axis 2, but the AM and ADS groups overlapped in the axis 1–axis 2 scatterplot (Fig. 3B). In the axis 1–axis 3 scatterplot, the ADS group could be distinguished from the AM group along axis 3, whereas the ADE group was located between these two groups (Fig. 3C).

A comparison of paired means by using one-way ANOVA revealed that the quantitative variables significantly differed among the five *a priori* groups, except for PTW2/PTBW and PTW3/PTBW (Table 3). The results of Tukey's pairwise test for each pair of morphological groups were as follows. The AA group significantly differed from the ADE, ADS, AL, and AM groups in terms of three of the five leaf characters (LL, BL/BWL, and BL/BW) and the stigma disk angle (SDA). The AL group significantly differed from all the other groups in terms of floral characters (PTL2/PTBW and ratio of the perianth tube length [excluding the lobe] to the pistil height [PTL1/PH], and PTL1/SH). The leaf and floral characters differed among the ADE, ADS, and AM groups. In terms of leaf characters, leaf length (LL) significantly differed between the ADE group and the other two groups, BL/BW significantly differed between the ADS group and the other two groups, and the ratio of the leaf length to the petiole length (LL/PL) significantly differed between the AM and the other two groups. In terms of floral characters, the ADE had significantly thicker perianth lobes. In addition, the SCL, SDA, and stigma width significantly differed between the ADE group and the other two groups. Compared with the other two groups, the ADS group had a significantly smaller lobe base width, a smaller PTL1/PH ratio, and a PH/SH ratio.

Identification of genetic groups

The results of Bayesian structuring analysis performed using nine nuclear microsatellites in Structure software (Pritchard et al. 2000) revealed that the likelihood of data substantially increased with the number of imposed clusters K until K reached a value of 2 and a plateau was reached for a larger K value (Fig. 4A). Application of the delta K method developed by Evanno et al. (2005) demonstrated that the K value of 2 was the most likely number of clusters (Fig. 4B). We found that genetic cluster 1 matched the AA and ADS groups, and that genetic cluster 2 matched the AM, ADE, and AL groups. However, when investigating clustering solutions obtained at higher K values, a good match between the genetic clusters and *a priori* groups was observed at the K value of 4 (Fig. 4C). Cluster 1 matched the AA group, cluster 2 matched the AM and AL groups, cluster 3 matched the ADS group, and cluster 4 matched the ADE group. Therefore, we used the K value of 4 to analyze differences in genetic diversity between different genetic groups.

Furthermore, the genetic clusters indicated that the five populations without morphological data, namely H, HWS, NHL, DJL and DLJ, were temporally classified into different *a priori* groups according to Lu et al. (2020) have slightly different results. The DJL population was formerly classified to the ADS group, but now was allocated to the ADE group.

Genetic diversity and genetic differentiation between genetic groups

The genetic diversity of the genetic groups was substantial: the AL and AM groups displayed the highest diversity ($H_E = 0.4403$), whereas the ADE group exhibited the

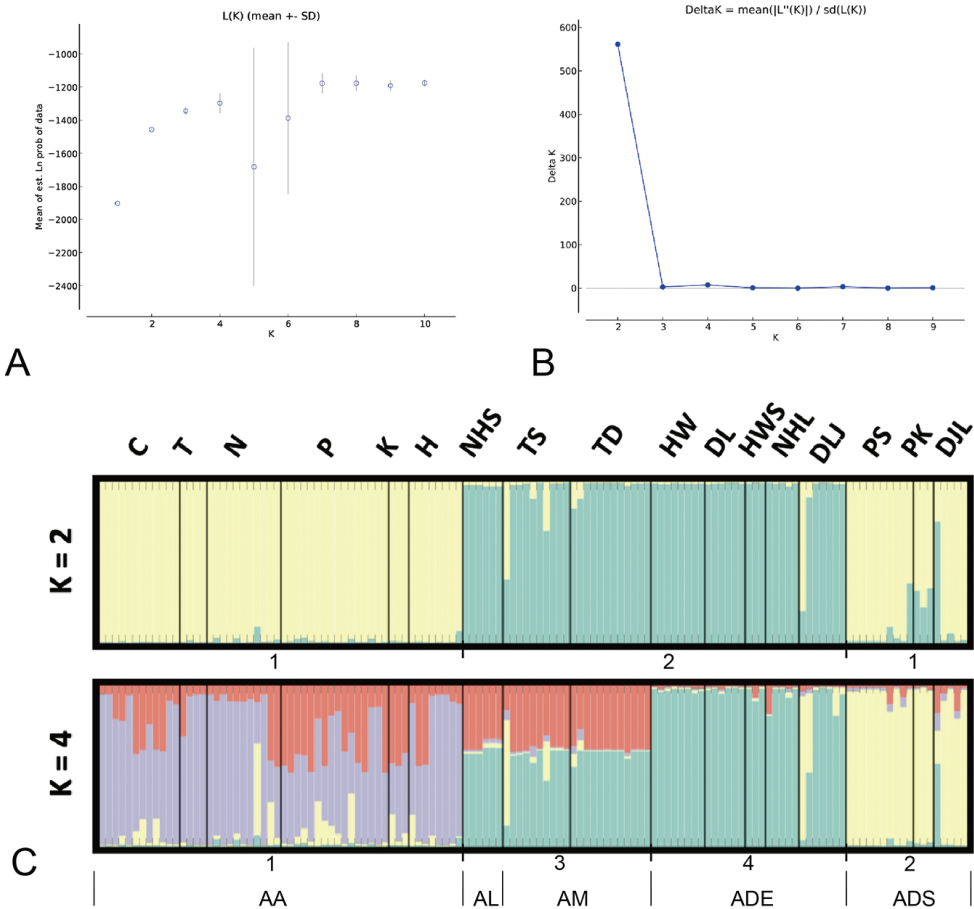


Figure 4. Results of genetic grouping **A**, **B** variation in the likelihood of data, $L(K)$ (**A**) and of Delta K (**B**) as a function of the number of genetic groups (K) identified in 128 *Aspidistra* samples by using the software STRUCTURE **C** histogram of the genetic assignment of 128 *Aspidistra* individuals at $K = 2$ and $K = 4$. Individuals were ordered along the horizontal axis in accordance with their θ group. Vertical bars indicate how the genome of each individual is partitioned into four clusters.

lowest diversity ($H_E = 0.2842$). The AA and ADS groups displayed intermediate diversity (H_E ranged from 0.3428 to 0.3951; Table 4A). The H_O of each genetic group was smaller than its H_E .

The inbreeding coefficient is a key parameter for understanding the number of matings between related individuals in a population. If no heterozygotes are present in a population, the inbreeding coefficient is 1.0. When the heterozygote frequency is equal to the Hardy–Weinberg expected value, the inbreeding coefficient is 0. Our results revealed that the inbreeding coefficients of the four genetic groups were significantly larger than 0, ranging from 0.1442 to 0.3705. This finding indicated that the degree of inbreeding varied within each group.

Pairwise F_{ST} is considered a satisfactory index to explain genetic differences among populations. Index values of 0–0.05, 0.05–0.15, 0.15–0.25, and >0.25 indicate nearly no, moderate, high, and significant genetic differences between populations, respectively (Wright 1978; Hartl and Clark 1997; Balloux and Lugon-Moulin 2002). Our results revealed that the pairwise F_{ST} between the genetic groups ranged from 0.2450 to 0.4939 (Table 4B). The pairwise F_{ST} of five of the six group pairs was >0.25. This result indicated the presence of significant genetic differences among the groups.

We determined differences in genetic diversity among the five *a priori* groups. The results were similar to the aforementioned results (Table 5). Except for the AL group, the H_O of the other four groups were all smaller than the H_E , and the inbreeding coefficient values were all greater than 0, ranging from 0.1442 to 0.3705. The pairwise F_{ST} of almost all the pairs was >0.25.

Table 4. Genetic diversity (A) and differentiation (B) parameters for the four genetic groups of the genus *Aspidistra* in Taiwan detected using nine microsatellite markers.

A. Genetic diversity parameters					
	Sample size	No. of polymorphic loci	H_O	H_E	F
AA	54	8	0.2757 (±0.20422)	0.3428 (±0.24237)	0.1972*
ADE	29	7	0.1801 (±0.31215)	0.2842 (±0.25198)	0.3705*
ADS	18	7	0.3395 (±0.28252)	0.3951 (±0.27706)	0.1442*
AL+AM	28	9	0.3175 (±0.22335)	0.4403 (±0.22806)	0.2826*
B. Genetic differentiation parameters (pairwise F_{ST})					
	AA	ADE	ADS	AL+AM	
AA	0				
ADE	0.4939*	0			
ADS	0.2450*	0.3774*	0		
AL+AM	0.4105*	0.2710*	0.3761*	0	

* $p < 0.05$; N_A : mean number of alleles observed per locus; H_E : expected heterozygosity (unbiased estimator); H_O : observed heterozygosity; F: inbreeding coefficient. * $p < 0.01$.

Table 5. Genetic diversity (A) and differentiation (B) parameters for the five *a priori* groups of the genus *Aspidistra* in Taiwan detected using nine microsatellite markers.

A. Genetic diversity parameters					
	Sample size	No. of polymorphic loci	H_O	H_E	F
AA	54	8	0.2757 (±0.20422)	0.34281 (±0.24237)	0.1972*
ADE	29	7	0.1801 (±0.31215)	0.2842 (±0.25198)	0.3705*
ADS	18	7	0.3395 (±0.28252)	0.3951 (±0.27706)	0.1442*
AL	6	5	0.3333 (±0.43301)	0.3030 (±0.29545)	-0.1111
AM	22	9	0.3131 (±0.27125)	0.4091 (±0.18091)	0.2388*
B. Genetic differentiation parameters (pairwise F_{ST})					
	AA	ADE	ADS	AL	AM
AA	0				
ADE	0.49393*	0			
ADS	0.24497*	0.37740*	0		
AL	0.45724*	0.41715*	0.44917*	0	
AM	0.44411*	0.30664*	0.40586*	0.28430*	0

* $p < 0.05$; H_E : expected heterozygosity (unbiased estimator); H_O : observed heterozygosity; F: inbreeding coefficient. * $p < 0.01$.

Discussion

Comparison with morphological and *a priori* groups

The CA and DA of leaf and floral characters revealed that the five *a priori* groups were well-supported by morphological groups (Fig. 3). Both the CA dendrograms and DA scatterplots demonstrated that the AA group differed from the AL group. The AA group corresponded to *A. attenuata*, and the AL group corresponded to *A. longiconnectiva*. With its longer leaves and concave stigma, *A. attenuata* exhibits clear morphological differentiation. Several authors have recognized all these characters to differentiate species (Hayata 1912; Liu and Ying 1978; Yang et al. 2001; Wang 2004; Chao 2016; Lu et al. 2020). *A. longiconnectiva* is characterized by deep perianth lobes, relatively broad and shallow perianth tubes, and elongated stamen connectives (Lu et al. 2020). The ADE, ADS, and AM groups corresponded to the eastern population of *A. daibuensis*, the southern and southeastern populations of *A. daibuensis*, and *A. mushaensis*, respectively. However, some mismatched samples were noted within these three groups. These mismatched samples may be attributed to the ambiguous morphological characters between the two species. This result is consistent with those of previous taxonomic studies. Wang (2004) and Chao (2016) have considered that the southern and southeastern populations of *A. daibuensis* correspond to *A. mushaensis*, and that the eastern population of *A. daibuensis* corresponds to *A. daibuensis*. However, Lu et al. (2020) believed that *A. mushaensis* exists only in central Taiwan, and that the southern and southeastern populations of *A. daibuensis* correspond to *A. daibuensis* and not *A. mushaensis*. Whether the ADS group corresponds to *A. daibuensis* or *A. mushaensis* remains unclear. However, our morphometric analyses revealed that the ADS group differed from the AM and ADE groups in terms of a wider blade width, a smaller lobe base width, more stigma present near the perianth opening, and the stamen attached on the perianth tube base or near the base. Thus, we suggest that the ADS group should not be classified as the AM or ADE group.

Comparison with genetic groups and *a priori* groups

The results of Bayesian structural analysis revealed that a K value of 2 was the most favorable for genetic clustering. Genetic cluster 1 consisted of the AA and ADS group, and genetic cluster 2 consisted of the ADE, AL, and AM groups. However, this genetic division was inconsistent with the morphological division based on CA, wherein in the CA dendrogram, the samples were divided into the AA group and the other groups (ADE, ADS, AL, and AM). Although two clusters were obtained in our dataset based on ΔK , we determined that a K value of 4 led to more favorable matching with the *a priori* groups. Demographic, environmental, and historical processes are multifaceted and complex and have led to different organization levels in the genetic structure of species. Therefore, Meirmans (2015) suggested that different K values may reflect different demographic processes, and that a biologically interpretable pattern obtained from a suboptimal K value is better than a completely unrealistic pattern obtained from the

optimal K value. Thus, we used the K value of 4 to determine the relationship between genetic and morphological groups. Although genetic clusters almost supported our morphological groups, AL clustered with AM to form a genetic group. This result can be attributable to the small sample size and incomplete lineage sorting. First, the AL group corresponded to *A. longiconnectiva*, which is a rare species in Taiwan, and we only sampled six individuals from a locality. The Structure software usually fails to identify genetic groups represented by few individuals, even when one group is well-represented (Porrás-Hurtado et al. 2013; Wang 2017). Second, AL and AM are morphologically similar (Lu et al. 2020) and are sympatrically distributed. We believe that AL and AM are closely related and may have diverged recently. Genetic data also indicated that they were closely related. Thus, the lineage sorting of these two species is incomplete.

Genetic diversity and genetic differentiation between genetic groups

The inbreeding coefficient indicates the effect of inbreeding on homozygosity by quantifying the deviation in observed genotypic frequencies from those expected under the Hardy–Weinberg equilibrium (Charlesworth 2003). Inbreeding, null alleles, and undetected genetic structure increase observed homozygosity and the inbreeding coefficient, whereas outbreeding, mutation, and inbreeding depression tend to reduce the inbreeding coefficient (Stoeckel et al. 2006; Waples 2018). Our results revealed that the inbreeding coefficients of all the genetic clusters were significantly greater than 0. Therefore, each genetic group had a high possibility of inbreeding. Inbreeding promotes population fragmentation and provides a selective advantage at the population level, resulting in structured populations, which contribute to speciation (Joly 2010; Olsen et al. 2020).

In our genetic differentiation analysis, we observed significantly high pairwise F_{ST} values, ranging from 0.2450 to 0.4939. Among the pairwise F_{ST} values of the six genetic group pairs, the AA with ADE pair and the AA with AL+AM pair exhibited high genetic differentiation (0.4939 and 0.4105, respectively), whereas the pairwise F_{ST} values of the AA with ADS pair was slightly smaller (0.2450) but still indicated high genetic differentiation (Wright 1978; Hartl and Clark 1997; Balloux and Lugon-Moulin 2002). The remaining genetic group pairs, namely ADE with ADS, ADE with AL+AM, and ADS with AL+AM, had significantly high pairwise F_{ST} values (0.3774, 0.2710, and 0.3761, respectively). We believe that these three groups have high genetic differentiation. This result also supports our morphometric analysis finding that ADE, ADS, and AM are distinct entities.

Integration of morphometric and genetic data

We performed an iterative analysis of morphometric and microsatellite markers to define, assess, and delineate species within the *Aspidistra* genus in Taiwan. The integration of the two analyses was almost congruent for the five *a priori* groups. In addition, the population genetic data indicated that frequent inbreeding and high genetic differentiation may shape the species diversity of *Aspidistra* in Taiwan. Therefore, we suggest that the five *a priori* groups should be regarded as five different taxa. Considering the morphological

similarities between these five taxa, their geographical distribution as well as the preliminary ranking rule providing by Tillich and Averyanov (2018), we proposed the following taxonomic treatments to update the taxonomy of *Aspidistra* in Taiwan: AA corresponds to the species *A. attenuata*, which is distributed in the mountain range at higher altitudes in the central and southern Taiwan; AM corresponded to the species *A. mushaensis*, which is only distributed in central Taiwan; AL corresponded to the species *A. longiconnectiva*, because it is genetically similar to *A. mushaensis*, we reduced it to the variety level; ADE corresponded to the species *A. daibuensis*, which has restricted distribution in southeastern Taiwan; ADS morphologically resembles ADE, but they differ in their genetic data. Therefore, we considered regarding it as a new variety of *A. daibuensis*.

Conclusion

Our study demonstrated that combining morphological and population genetic data is effective for the discovery of new *Aspidistra* taxa. By using this approach, we confirmed five *Aspidistra* taxa in Taiwan, namely *A. attenuata* Hayata; *A. daibuensis* Hayata var. *daibuensis*; *A. mushaensis* var. *longiconnectiva* (C.T.Lu, K.C.Chuang & J.C.Wang) C.T.Lu & J.C.Wang; *A. mushaensis* Hayata var. *mushaensis*; and a new variety, *A. daibuensis* Hayata var. *longkiauiensis* C.T.Lu, M.J.Yang & J.C.Wang var. nov. Combining morphometric and genetic studies can help us discover species diversity in this genus. The complete description of the new variety is provided in the taxonomic treatment below.

Taxonomic treatment

***Aspidistra daibuensis* Hayata var. *longkiauiensis* C.T.Lu, M.J.Yang & J.C.Wang, var. nov.**

urn:lsid:ipni.org:names:77316103-1

Figs 5A–G, 6

Chinese name: 瑯嶠蜘蛛抱蛋

Diagnosis. This new variety is similar to *A. daibuensis* Hayata var. *daibuensis* but can be distinguished by its shorter leaf length (72.24 ± 11.68 cm vs. 95.46 ± 21.04 cm), smaller ratio of the leaf blade length to width (3.73 ± 1.43 vs. 8.05 ± 1.73), thinner perianth lobe (1.28 ± 0.38 mm vs. 2.79 ± 0.64 mm), longer curve length of the stigma surface (10.98 ± 1.38 mm vs. 16.23 ± 1.45 mm), and larger ratio of the pistil height to stamen height (2.49 ± 0.59 vs. 2.03 ± 0.78). It also resembles *A. mushaensis* Hayata var. *mushaensis* but differs by its smaller ratio of the leaf blade length to width (3.73 ± 1.43 vs. 7.66 ± 1.35), narrower perianth lobe base (5.12 ± 1.12 mm vs. 7.27 ± 1.19 mm), and larger ratio of the pistil height to the stamen height (2.49 ± 0.59 vs. 1.59 ± 0.26).

Type. TAIWAN. Pingtung County, Shuangliou national forest recreation area, Banyan trail, elev. 200–300 m, 12 Jun 2020, *M.J.Yang s.n.* (holotype: TAIF; isotype: TNU).

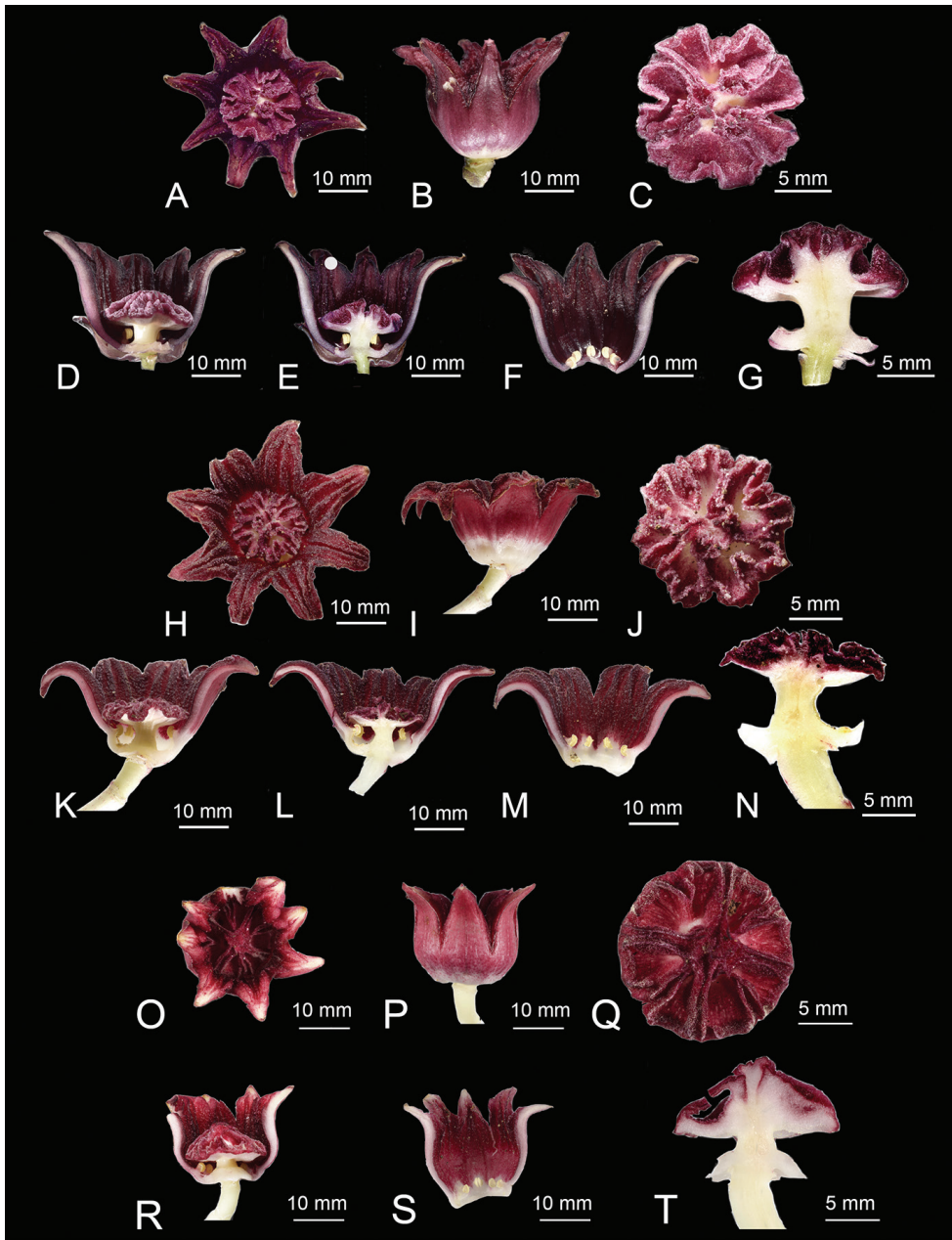


Figure 5. Floral comparison of *Aspidistra daibuensis* var. *longkiauensis* C.T.Lu, M.J.Yang & J.C.Wang; *A. mushaensis* Hayata var. *mushaensis*, and *A. daibuensis* Hayata var. *daibuensis* **A–G** *A. daibuensis* var. *longkiauensis* **H–N** *A. mushaensis* var. *mushaensis* **O–T** *A. daibuensis* var. *daibuensis* **A, H, O** front view of the flower **B, I, P** lateral view of the flower **C, J, Q** stigma surface **D, K, R** half dissection of the perianth tube, showing the pistil and stamens **E, L** half dissection of the perianth tube and stigma **F, M, S** half dissection of the perianth tube, showing the stamen **G, N, T** half dissection of the stigma.

Description. Perennial evergreen herb. Rhizome creeping, 1–1.2 cm in diameter. Internode 0.2–0.4 cm. Cataphylls 3 to 4. Leaves: coriaceous, solitary or rare 2 tufts leaves, blade dark green occasionally with yellow spots; leaf margin with white serrulate, parallel venation; petioles 9.6–36.6 cm long; blade oblanceolate, blade oblique, 40.5–61.8 cm long, 4.6–8.6 cm wide, acute at apex. Flower solitary, bisexual, scape 0.3–4.0 cm long, 3–6 bracts, bract ovate; perianth urceolate to campanulate, fleshy, perianth tube purple, basal white, 13.5–20.5 mm long, 8.5–15.5 mm wide; perianth lobe 7–9, sometimes yellow at apex, ovate triangular, 5–10 mm long, base 3.2–7.4 mm wide, 0.7–2.0 mm thickness, lobe center with two keels, lobe margin with two keels conspicuous. Stamens 7–9 as many as the perianth lobe, inserted at the perianth tube base or near base; anther oblong, 1.3–3.0 mm long; anther connective absent; filament short, 1–2 mm. Pistil 5–8 mm; stigma disk-like, 4 to 5 lobes, conical and lobe marginal concave, stigma nearly covering the perianth tube, 7.6–12.8 mm wide; style 0.5–1.5 mm long; ovary 1.2–1.7 mm with 4–5 locules. Fruit unknown.

Etymology. The specific epithet “*longkia*” means that the geographical distribution of this species is mainly distributed in Hengchun Peninsula, Pingtung County, Taiwan. The area was known as “Longkia” in early records.

Phenology. Flowering from February to June.

Distribution and habitat. *Aspidistra daibuensis* var. *longkiaensis* is geographically confined to the Hengchun Peninsula, the most southern part of Taiwan and the southeastern part of Taiwan. The population typically grows on slopes under the canopies of primary or secondary forests. It is distributed at an elevation of 200–500 m.

Conservation status. *Aspidistra daibuensis* var. *longkiaensis* is known from three populations in Hengchun Peninsula, Pingtung County, Taiwan. Based on the specimen records, the area of occupancy (AOO) is ca. 15 km² by GeoCAT (Bachman et al. 2011). Following the criteria of IUCN (2019), this species is assessed as endangered (EN B2ab(ii)).

Notes. This new variety is regarded as *A. daibuensis* (Chang and Hsu 1974) or *A. mushaensis* (Wang 2004; Chao 2016). We determined that this species does not have thick perianth lobes as *A. daibuensis* var. *daibuensis* (Hayata 1912). In addition, *A. daibuensis* var. *longkiaensis* differs from *A. mushaensis* var. *mushaensis* in terms of the ratio of the blade length to the blade width, perianth lobe base width, the distance from the lobe base to the stigma apex, and the ratio of the pistil length to the stamen height (Table 6). The stamen of this new species is inserted on the perianth tube base or near the base instead of the low part of the perianth tube, as observed in the previous former two species. We considered it can be distinguished from *A. daibuensis* var. *daibuensis* and *A. mushaensis* var. *mushaensis*. However, it is morphologically more similar to *A. daibuensis*, so we considered regard it as a variety of *A. daibuensis*.

Additional specimen examined. TAIWAN. Pingtung County: Kaoshifo, alt. 400 m, 14 Jun 1993, fl., T.-T. Chen et al. 1393 (TAIF!); Mt. Kaoshifo, 27 Feb 2016, fl., P.F. Lu 29262 (TAIF!); Mt. Kaoshifo, alt. 300–400 m, 16 April 2020, fl., C.T. Lu 2662 (TNU!); Shoucha, alt. 470–500 m, 17 Feb 2017, fr., S.W. Chung 12866 (TAIF!).

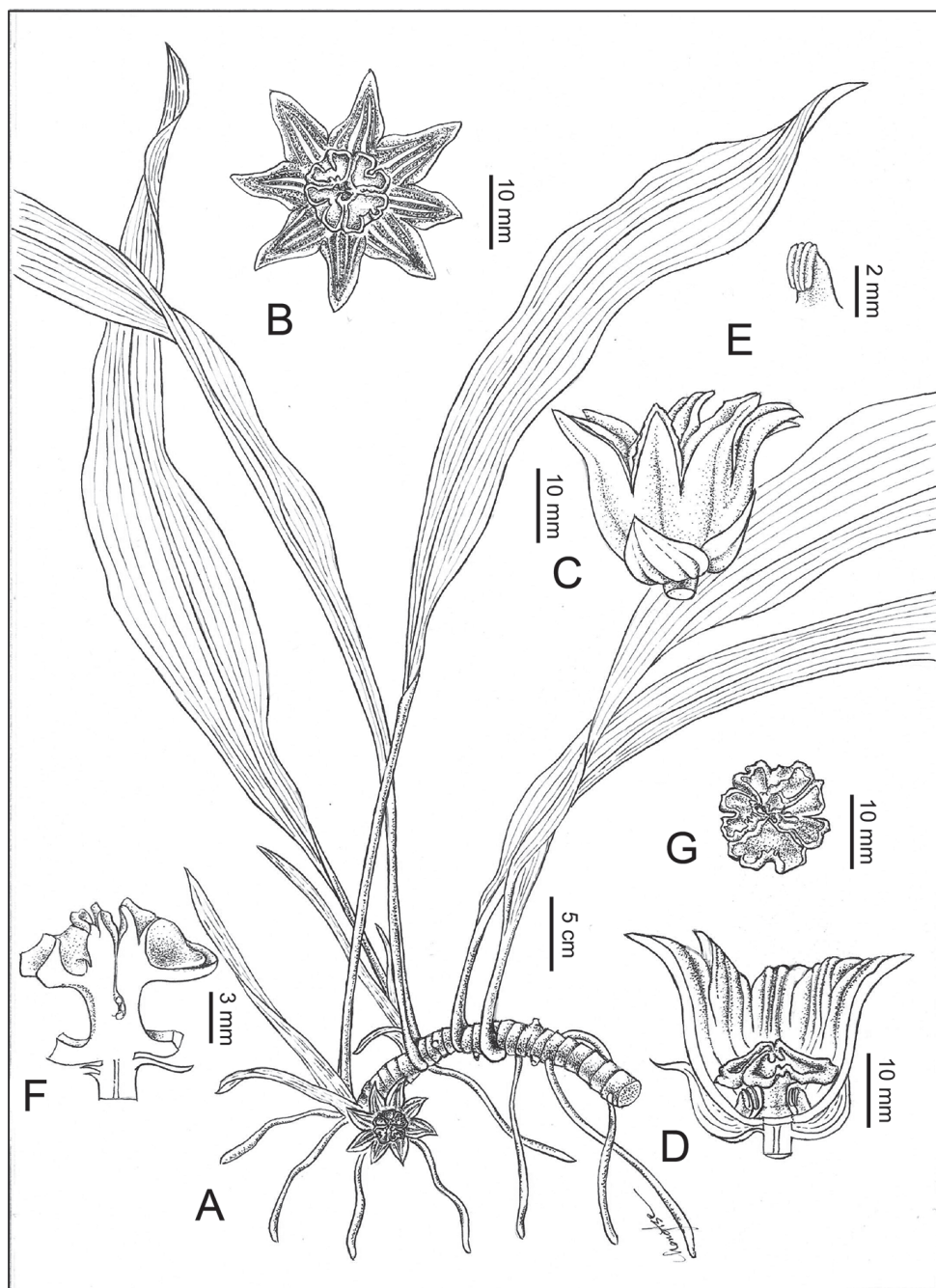


Figure 6. Illustration of *Aspidistra daibuensis* var. *longkiauensis* C.T.Lu, M.J.Yang & J.C.Wang **A** habit **B** flower (front view) **C** flower (lateral view) **D** half dissection of perianth tube, showing pistil and stamens **E** stamen **F** half dissection of pistil.

Table 6. Morphological comparison of *Aspidistra daibuensis* var. *longkiauiensis* with *A. daibuensis* var. *daibuensis*, *A. mushaensis* var. *longiconnectiva* and *A. mushaensis* var. *mushaensis*. *. Mean \pm S.D. (min. value and max. value).

Taxa characters	<i>A. daibuensis</i> var. <i>longkiauensis</i>	<i>A. daibuensis</i> var. <i>daibuensis</i>	<i>A. mushaensis</i> var. <i>longiconnectiva</i>	<i>A. mushaensis</i> var. <i>mushaensis</i>
Leaves				
Leaf length (cm)	72.24 ± 11.68 (54.1–86.5)*	95.46 ± 21.04 (70.2–141.1)	54.1	82.87 ± 14.99 (60.1–106.1)
Leaf length/petiole length	3.40 ± 0.90 (2.4–4.0)	2.83 ± 0.51 (1.9–3.6)	1.19	2.72 ± 0.71 (1.7–3.6)
Blade length/blade width	3.73 ± 1.43 (1.4–6.4)	8.05 ± 1.73 (5.1–10.1)	6.58	7.66 ± 1.35 (6.1–10.1)
Flowers				
Perianth lobe thickness (mm)	1.28 ± 0.38 (0.7–2.0)	2.79 ± 0.64 (1.7–4.0)	1.14 ± 0.27 (1.0–1.3)	1.48 ± 0.34 (1.1–1.9)
Lobe base width (mm)	5.12 ± 1.12 (3.2–7.4)	7.95 ± 1.60 (5.8–9.3)	10.0 ± 1.66 (8.8–11.2)	7.27 ± 1.19 (5.5–8.7)
Curve length of the stigma surface (mm)	10.98 ± 1.38 (9.4–13.4)	16.23 ± 1.45 (13.7–17.5)	12.07 ± 0.43 (11.8–12.4)	12.70 ± 1.30 (10.4–15.7)
Stigma width (mm)	10.33 ± 1.79 (7.6–12.8)	16.43 ± 2.21 (12.7–21.4)	11.76 ± 0.03 (11.7–11.8)	12.59 ± 1.08 (10.2–14.6)
Ratio of the perianth tube length (excluding the lobe) to pistil height	1.71 ± 0.24 (1.3–2.2)	2.02 ± 0.41 (1.3–2.9)	0.91	2.19 ± 0.35 (1.6–3.1)
Ratio of perianth tube length (excluding the lobe) to stamen height	4.20 ± 0.86 (3.4–6.5)	3.88 ± 0.72 (2.7–5.9)	1.99 ± 0.03 (1.9–2.0)	3.43 ± 0.39 (2.7–4.7)
Ratio of perianth tube length to the width of the perianth tube base	1.52 ± 0.13 (1.4–1.8)	1.72 ± 0.27 (1.4–2.2)	0.73 ± 0.11 (0.7–0.8)	1.37 ± 0.26 (1.0–1.9)
Ratio of pistil height to stamen height	2.49 ± 0.59 (1.8–3.8)	2.03 ± 0.78 (1.3–2.5)	1.91 ± 0.35 (1.7–2.2)	1.59 ± 0.26 (1.0–2.1)

Key to the species of *Aspidistra* in Taiwan

- 1 Leaf blade length to leaf blade width > 10 ; stigma surface concave or flat; anthers inserted at one third of the perianth tube.....***A. attenuata***
- Leaf blade length to leaf blade width ≤ 10 ; stigma surface convex; anthers inserted in the low part of the perianth tube or near the perianth tube base.....**2**
- 2 Leaf length to leaf blade length < 1.2 ; the perianth tube length to the width of the perianth tube base < 0.9 ; the perianth tube length (exclude lobe) to the height of the stamen attached on the perianth tube < 2.1***A. mushaensis* var. *longiconnectiva***
- Leaf length to leaf blade length > 1.2 ; the perianth tube length to the width of the perianth tube base > 0.9 ; the perianth tube length (exclude lobe) to the height of the stamen attached on the perianth tube > 2.5**3**
- 3 The perianth tube length (exclude lobe) to pistil height > 1.5***A. mushaensis* var. *mushaensis***
- The perianth tube length (exclude lobe) to pistil height < 1.5**4**
- 4 The curve length of the stigma surface > 13.5 ; lobe thickness up to 4 mm***A. daibuensis* var. *daibuensis***
- The curve length of the stigma surface < 13.5 ; lobe thickness no more than 2 mm.....***A. daibuensis* var. *longkiauwensis***

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Contributions to Ecuadorian butterworts (Lentibulariaceae, *Pinguicula*): two new species and a re-evaluation of *Pinguicula calyptrata*

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Abstract

Comparatively few species of the insectivorous genus *Pinguicula* L. have been recognized in South America so far. In recent years, a number of narrowly endemic taxa from the Andes have been described that simultaneously refined the broad taxonomic concepts of the “historical” species. Here, we describe two striking new species from Southern Ecuador that further condense the circumscription of *Pinguicula calyptrata* Kunth. *Pinguicula jimburensis* **sp. nov.** and *P. ombrophila* **sp. nov.** are clearly beyond the taxonomic scope of the known species and consequently described as new to science. The deviating morphological features of the two new taxa are described and illustrated and the remaining morphological spectrum of *P. calyptrata* in Ecuador is outlined. The two new species add to the exceptional biodiversity in the Amotape-Huancabamba Zone and underline its importance as a biodiversity hotspot in urgent need of protection.

Resumen

Actualmente pocas especies del género insectívoro *Pinguicula* L. se reconocen de forma comparativa en América del Sur. En los últimos años, se han descrito varios taxones micro-endémicos de los Andes que simultáneamente han refinado los amplios conceptos taxonómicos de las especies “históricas”. Aquí, describimos dos nuevas especies sorprendentes del sur del Ecuador que condensan aún más la circunscripción

de *Pinguicula calyptrata* Kunth. *Pinguicula jimburensis* **sp. nov.** and *P. ombrophila* **sp. nov.** están más allá del alcance taxonómico de las especies conocidas y, en consecuencia, descritas como nuevas para la ciencia. Se describen e ilustran los caracteres morfológicos divergentes de los dos nuevos taxones y se discute la variación morfológica restante de *P. calyptrata* en Ecuador. Las dos nuevas especies se suman a la excepcional biodiversidad de la zona Amotape-Huancabamba y se realza su importancia como un punto crítico de biodiversidad que necesita protección urgente.

Keywords

Amotape-Huancabamba, Andes, Cordillera del Cóndor, distribution, Ecuador, endemism, Jimbura, morphological variation, new species, taxonomy

Introduction

Pinguicula L., the second largest genus of the most specious family of carnivorous plants – Lentibulariaceae (Lamiales) – comprises approximately 115 species (Fleischmann 2021). The genus is distributed throughout Eurasia and the Americas, is largely absent from Africa (only Morocco) and not present in Oceania and Antarctica. Roughly half of all taxa occur in Latin America with a clear center of diversity in Mexico, with about 40 endemic species alone. Despite the high diversity in Central America, Mexico and the Caribbean, only a few species are known from South America and restricted to the Andean mountain chain. At the moment, most authors accept seven species here, namely: *P. antarctica* Vahl, *P. calyptrata* Kunth, *P. elongata* Benj., *P. involuta* Ruiz & Pav., *P. jarmilae* Halda & Malina, *P. nahuelbutensis* Gluch and *P. rosmarieae* Casper, Bussmann & T.Henning. All species belong to the monophyletic sect. *Ampullipalatum* Casper within the subgenus *Isoloba*, with the exception of *P. elongata* (sect. *Heterophylliformis* (Casper) Fleischmann & Roccia within subgen. *Themnoceras*).

Significantly, all recently described species are narrow endemics (*P. jarmilae*, *P. nahuelbutensis* & *P. rosmarieae*), whereas the earlier described taxa usually have larger ranges. This already indicates that the relatively low number of taxa known from (Andean) South America might be, at least partially, rather a result of under-collection and a lack of taxonomic studies, than true species poverty.

In Ecuador, the genus is to this date only represented by *Pinguicula calyptrata* Kunth (Taylor 1975; Jørgensen and León-Yáñez 1999). The holotype for this species was collected in the páramo of Saraguro (Loja Province, Ecuador) by Alexander von Humboldt and Aimé Bonpland during their five years expedition to South America (1799–1804). It was published in Karl Sigismund Kunth's "Nova Genera et Species Plantarum" (Humboldt et al. 1818) with a brief and superficial description and without an illustration as was customary at the time. The only taxonomic study that subsequently dealt with this group of plants in Ecuador dates back 48 years (Taylor 1975). His treatment of the Lentibulariaceae for the Flora of Ecuador was, however, mostly based on the scarce herbarium material available at the time and could not deliver a critical review of the genus for Ecuador.

Pinguicula in the Amotape-Huancabamba Zone and adjacent Andean regions

The genus *Pinguicula* is particularly species-rich in Latin America. However, the taxonomic richness south of the genus' center of diversity: Mexico, Central America and the Caribbean, is limited, and most of the few taxa recognized are reported to have large latitudinal distributions. Lately, the continuous botanical exploration of the Andes and especially of the so-called Amotape-Huancabamba Zone (hereafter AHZ), however, shows a more complex picture for the genus in this area than hitherto believed. This phytogeographical zone has been revealed as an area characterized by an exceptional biodiversity and a noticeable accumulation of narrowly endemic taxa (Weigend 2002, 2004). This has been demonstrated for many groups of organisms including numerous angiosperm taxa (e.g. Deanna et al. 2018; Escobar et al. 2018; Acuña-Castillo et al. 2021; Landis et al. 2021; Tejedor and Calatayud 2022; Vieu et al. 2022) and only very recently also for the Lentibulariaceae. With the description of *P. rosmarieae*, the first narrowly endemic *Pinguicula* has been documented for the AHZ (Casper et al. 2020). And the recent description of *Utricularia amotape-huancabambensis* (Henning et al. 2021) revealed the same narrow endemism for the other large genus in the family.

However, despite constantly growing botanical research activities and new collections, taxon delimitation in *Pinguicula* remains problematic. This is particularly true for *Pinguicula calyptrata*, which is distributed from Central Colombia, throughout Ecuador and most of the AHZ until the North Peruvian Departamentos Cajamarca, Amazonas and San Martín (all specimens from Bolivia that we are aware of are misidentified *P. involuta*) and is unsatisfactorily defined in its entirety (Casper et al. 2020). Caspers concept of *Pinguicula*, in both systematics and alpha-taxonomy, was very flower focused (Fleischmann 2021). Species delimitation and the infrageneric classification of the genus in his works largely founded on morphological character complexes of the corolla, traditionally thought to be evolutionary stable. Instead, they were often subject to rapid diversifications and thereby prone to misinterpretations as recent molecular studies repeatedly revealed (Lustofin et al. 2020; Shimai et al. 2021, see Fleischmann 2021 for a detailed discussion). In the course of studying the Andean taxa, the late Siegfried Jost Casper (1929–2021) also based his allocation of new collections/observations on the gross flower morphology and (for the time being) tended to classify any observation from the Northern Andes with more or less spreading rosettes and bluish flowers with a yellow palate as *P. calyptrata* (pers. correspondence). However, similar to the situation reported for a number of Cuban species (Domínguez et al. 2013), gradual morphological variation is found between most of the populations, varying with latitudinal and longitudinal distribution and elevation. Furthermore, on site-conditions such as e.g. moisture, soil type, associated vegetation and presumably the local pollinator fauna – a commonly ignored aspect in the context of speciation in the mosaic-like microhabitats of the Andes – affect genetic exchange between populations and facilitate diversification (Kay and Sargent 2009; Van der Niet et al. 2014). These dynamic and diverse networks of relationships and interactions can have a catalyzing effect on such character complexes that can be subject to rapid alteration and adaptation. In

particular the plasticity of e.g. flower size, shape and color is greater than traditionally assumed and divergent versus convergent developments can be difficult to determine, potentially complicating an unambiguous assignment of taxa (Fleischmann 2021). With the ongoing botanical exploration of the (remaining) remote paramo habitats of Ecuador and Peru (and other Andean countries), the real extent of the morphological spectrum becomes increasingly recognizable and, in the case of *P. calyptrata*, quickly goes beyond the narrow scope of the protologue.

Besides biogeographical data and habitat preferences, the overall bauplan of *Pinguicula* provides only a limited number of characters to take into consideration for taxon delimitation. Basically, the plant body in this genus is reduced to leaves and flowers and morphological characterization boils down to a handful of characters delivered by these two organs. In the light of the aforementioned gradual variability between populations and the known uncertainties in species recognition in Andean South America (e.g. *P. huilensis* – Cuatrecasas, 1945; *P. chilensis* and *P. antarctica* – Gluch 2017; Roccia 2018) it appears appropriate that species delimitation must be subject to significant, quantifiable and consistent morphological differences (see stalk length debate in Gluch 2017 and Roccia 2018) and, in the case of the AHZ, must be considered in relation to omnipresent and often sympatric “typical” *P. calyptrata*. Nevertheless, lumping all taxa of *Pinguicula* from the Northern Andes together under *P. calyptrata* due to a superficial similarity in their flower and rosette morphology would not do the actual diversity any justice and could leave a false impression regarding the actual threat status of potential narrow endemic taxa.

Taking these considerations into account, most Ecuadorian populations of *Pinguicula* fall into the range of *P. calyptrata* and this species in its (original) unsharp definition is present in many suitable Andean habitats sampled, although a more fine-grade analysis of *P. calyptrata* s.l., applying population genetics, is urgently needed and strongly encouraged. However, in two localities, two distinct taxa could be found growing sympatrically. The collections from the Cerro Plateado Biological Reserve show two taxa, that clearly differ in leaf morphology and rosette shape, albeit having similar flowers. Conversely, collections from Yacuri National Park show two taxa, differing considerably in both character complexes: leaves/rosettes and detailed flower morphology.

Despite the disparate grade of morphological dissimilarity observed for the two taxon pairs, the new taxa are both separated at species level to meet the needs of a conservation assessment under the IUCN criteria (IUCN Standards and Petitions Committee 2022) and facilitate potential protective measures. Whether a different taxonomic concept might ultimately be more appropriate to reflect the close relationship among (some) Andean *Pinguicula* species remains open to future studies, ideally within the framework of a more comprehensive approach using both phylogenetic and population genetic methods.

The new taxa are morphologically clearly distinguishable and a consistent spatial segregation is evident. Both new taxa share their habitat with rather typical *P. calyptrata* and are ecologically separated by contrasting habitat requirements, a pattern reported already for *P. rosmarieae* and *P. calyptrata* from North Peru (Casper et al. 2020). Similarly, the taxon pairs found in Ecuador also grow in close proximity to each other without any direct spatial overlap. No morphologically intermediate plants

were found, indicating that the taxa here described are discrete reproductive units without naturally occurring hybrid individuals (unlike recently reported for *Utricularia* – Henning et al. 2021).

Consequently, *P. jimburensis* sp. nov. and *P. ombrophila* sp. nov. are described as species new to science. All aspects of their biology are presented and discussed. Furthermore, the morphological spectrum of *P. calyptrata* throughout the known Ecuadorian populations is outlined and illustrated. A key to the Ecuadorian species is provided and an up-to-date assessment of the taxonomic situation of *Pinguicula* in the Northern Andes is made.

Materials and methods

In the current study, we document two new narrow endemic *Pinguicula* species from southern Ecuador as a result of recent botanical explorations. We conduct detailed morphological analyses for these new species, provide a taxonomic description, present images for all floral and vegetative plant structures, and provide a distribution map. The conservation status of the newly described species and their relationships with other species are also discussed.

Furthermore, the morphological spectrum of *P. calyptrata* throughout the known Ecuadorian populations is outlined and illustrated. The populations of this species have been collected from north to south in all the Ecuadorian Andean provinces from 2100 to 4100 m.

We consulted pertinent literature, examined specimens at ECUAMZ, GUAY, LOJA, QCA and QCNE herbaria (Thiers 2023), and high-resolution images of type material for Neotropical taxa (Tropicos database, <https://www.tropicos.org/> and the JSTOR Global Plants website <http://plants.jstor.org>).

Results

Key to the Ecuadorian species of *Pinguicula*

- 1 leaves (semi) upright, elongated, margins shallowly irregular lobed, flowers without a yellow palate, spur evenly bent (carnassial tooth-like) and with a tapered apex..... *Pinguicula jimburensis* sp. nov.
- rosettes flat on the ground, leaf margins entire, flowers with a distinct yellow palate, spur angled from the tube, often thickened at the end and with a stubby apex..... 2
- 2 terrestrial plant, rosette star-like, leaf blades ovate, margins distinctly curled up, flower scapes (much) longer than rosette diameter..... *Pinguicula calyptrata*
- lithophytic plant, rosette not star-like, leaf blades oblong-obovate-ovate, margins not or only very inconspicuously curled up, flower scapes half as long as rosette diameter *Pinguicula ombrophila* sp. nov.

Taxonomic treatment

Pinguicula jimburensis Á.J.Pérez, Tobar & T.Henning, sp. nov.

urn:lsid:ipni.org:names:77316266-1

Fig. 1

Type. ECUADOR, Loja, Cantón Espíndola, Parroquia Jimbura, Parque Nacional Yacuri, Lagunas Negras de Jimbura, 04°42'46"S, 79°25'50"W, 3400 m, 9 Oct 2022, Á.J. Pérez *et al.* 11891 (holotype QCA (fl, fr, spirit collection) barcode: 245581; isotype LOJA (fl) barcode: 43489).

Diagnosis. *Pinguicula jimburensis* belongs to *Pinguicula* sect. *Ampullipalatum* and is closely allied to the other North Andean species of the section (*P. calyptrata*, *P. ombrophila* and *P. rosmariaeae*). With the latter two it shares the lack of involute leaf margins, but clearly differs from them in its terrestrial habit (vs. litho-/epiphytic) and morphologically by the oblong leaves (vs. widely ovate to rounded). The flowers of *P. jimburensis* lack a distinct yellow palate, a character shared with *P. involuta* and the Peruvian endemic *P. rosmariaeae*, but the evenly bent, tooth-like spur is unique among related species. First and foremost, *P. jimburensis* is characterized by erect leaves that are shallow and irregularly lobed. This character is, despite a terminological similarity with the leaves of only distantly related *P. elongata*, unique among all South American taxa.

Description. Terrestrial, perennial rosette leaved herb with 1 (–2) flowering scapes. Rhizome ~5 mm long, with numerous fibrous roots 1.5–6 cm long. Leaves (4–) 5–7, erect from the ground, ± succulent, red and fussed with green along the medvein, drying dark purple, (15–) 18–52 (–60) mm long × (2–) 3–8 (–9) mm width, the blades oblong, rounded at the tip, slightly attenuated to the base into a enveloping petiole, the margins are irregularly shallowly lobed, upper surface of lamina covered with stalked glandular hairs. Hibernacula (winter buds, dormant buds) absent. Scapes 1–2 (–3), erect, (30–) 40–60 (–85) mm tall, terete, filiform (0.5–1 mm thick), one-flowered, red, scattered with stalked glandular hairs. Flowers small, 10–12 (–15) mm long (including tube-spur-complex). Calyx two-lipped, red, upper surface of sepals scattered with stalked glandular hairs; upper lip divided into three nearly equal-sized oblong lobes, at apex pointed; lower lip up to ¼ divided into two lobes, but appearing to be entire. Corolla two-lipped, purple-whitish with white lobes; upper lip two-lobed, lobes obovate, ~4–6 mm long and ~3–4.2 mm wide, shallowly notched at the apex; lower lip larger and longer than the upper lip, with three obovate-oblong lobes (the median lobe somewhat larger than the two lateral ones), ~4.5–6 mm wide, each distinctly (up to 1/3 of its length) notched. Tube (tube-spur-complex) at the throat funnel-shaped, on both sides broader than the spur, on the back side higher than the spur, proximally cylindrical (nearly as long as wide), on the ventral side merging without any sharp angle into the cylindrical to cone-like stubby, carnassial tooth-like, at apex tapered white to yellow spur, ~6 mm long; the tube-spur-complex externally whitish -blue to purple lengthwise-striped by parallel veins. Palate simple, weakly developed (not clapper-like), inserted immediately behind (~1–2 mm) the corollas' lower-lip middle lobe, blue, set with short-stalked glandular hairs, proximally elongated into a short ventral hair strip;

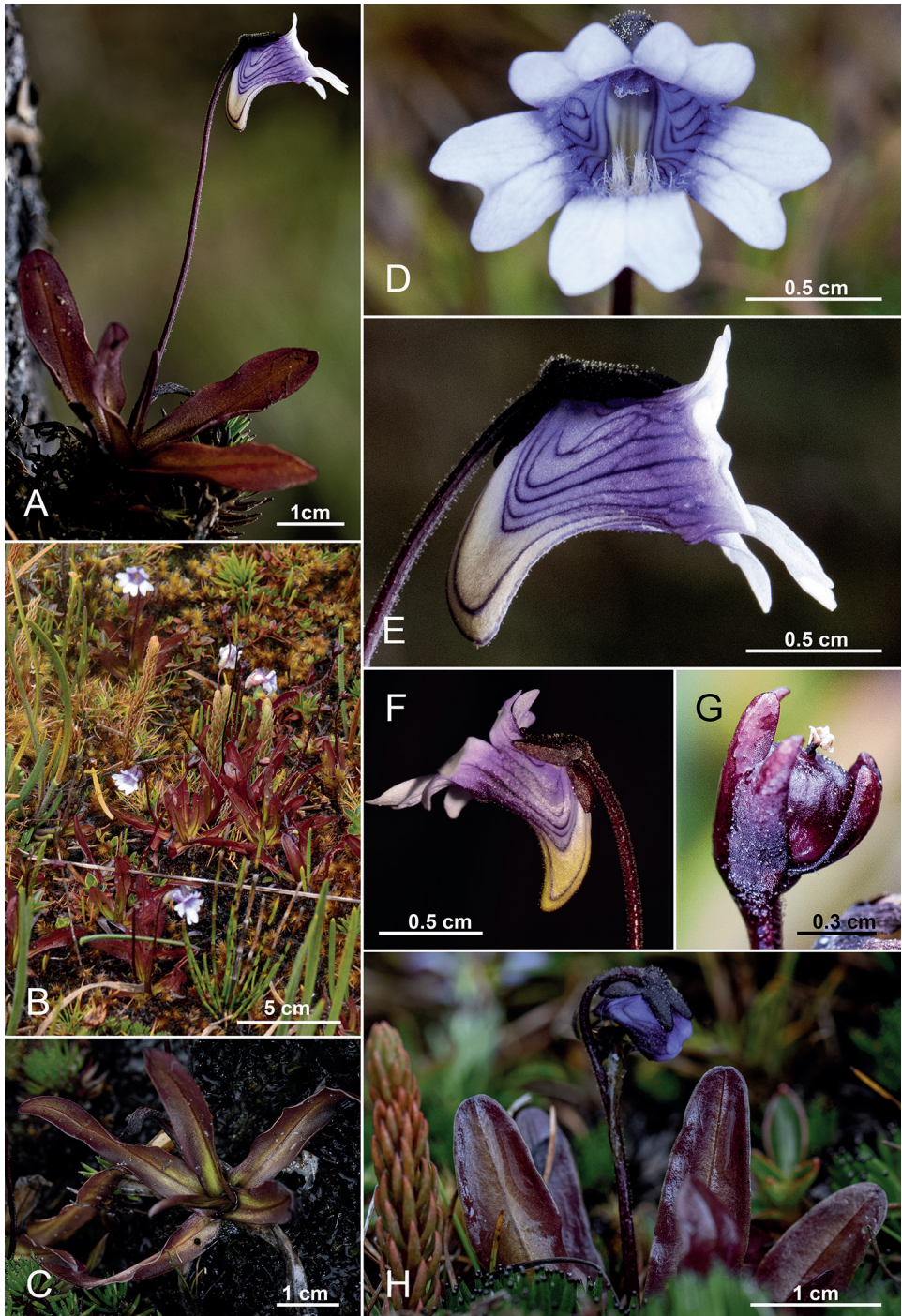


Figure 1. *Pinguicula jimburensis* **A** flowering plant of in lateral view **B** stands of *P. jimburensis* at the Lagunas Negras de Jimbura **C** upper view of the rosette **D** flower, frontal view **E** ditto, lateral view **F** ditto, with yellow spur **G** young capsule **H** plant with developing flower bud. (**A–H** from *Á.J. Pérez et al. 11891*). Photos by Kabir Montesinos.

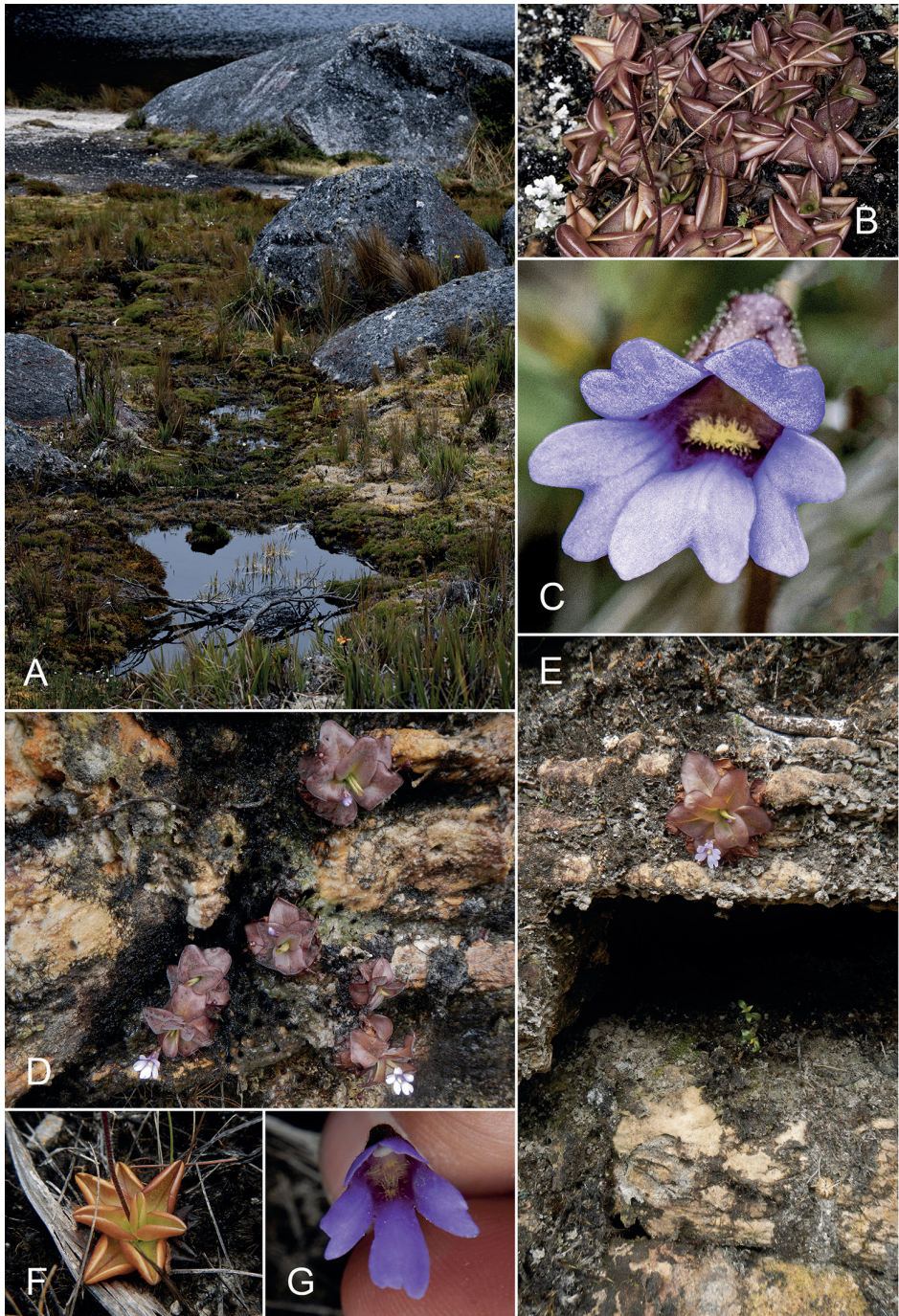


Figure 2. Habitats of the new species and associated *P. calyptrata* **A–C** Lagunas Negras de Jimbura **A** swampy areas between rocks next to the Lagoons where *P. jimburensis* is found **B** neighboring stand of *P. calyptrata* **C** flower of *P. calyptrata*, frontal view **D–G** Reserva Biológica Cerro Plateado **D** small stand of *P. ombrophila* growing on a vertical rock face **E** single plant on top of a rock overhang **F** rosette of sympatric *P. calyptrata* **G** ditto, flower in frontal view. Photos: **A–C** by Kabir Montesinos; **D–G** by Álvaro J. Pérez.

each of the two lateral corolla lobes with short-stalked glandular hairs, stretching proximally along on each side of the inner tube wall. Stamens 2, filaments 1.2–2 mm long, anthers dorsifixed, 1 mm, oval, transverse dehiscing. Ovary 1.2 mm, rounded, slightly covered with short-stalked glandular hairs to glabrescent, style 0.5 mm long, stigma 0.5 mm long, campanulate, glabrous. Capsule 3–4 mm, rounded, slightly covered with short-stalked glandular hairs to glabrescent, splitting in 2 valves. Seed numerous, alveolate, ellipsoid, 0.5–0.8 mm long, yellow. Chromosome number unknown.

Etymology. The specific epithet refers to the type locality, Lagunas Negras de Jimbura, which is part of the Yacuri National Park in the Ecuadorian provinces of Loja and Zamora-Chinchipe.

Distribution, habitat and associated vegetation. Specimens of *P. jimburensis* have so far only been collected around the Lagunas Negras de Jimbura in the Yacuri National Park in the province of Loja. As a result, *P. jimburensis* is endemic and thus far only known growing between the grass and shrubby paramo vegetation around the lagoon complex, especially in swampy areas (Figs 2, 3). According to the Ministerio del Ambiente del Ecuador (2013), this locality lies within a much larger zone dominated by the ecosystem named arbustal siempreverde montano alto del Páramo del sur. Inhabitants of this area call this type of vegetation “paramillo” and the environment is characterized by a constant cloudiness and drizzle with strong winds. The vegetation is dominated by the herbs *Oritrophium* sp., *Paepalanthus lodiculoides* Moldenke, *Chusquea* spp. and *Phlegmariurus* spp., between the shrubby vegetation dominated by *Chuquiraga jussieui* J.F. Gmel., *Monticalia peruviana* (Pers.) C. Jeffrey and *Miconia* spp. Additionally, *Pinguicula calyptrata* has been collected in this locality (Pérez *et al.* 8690, 8755, QCA; Figs 2, 3). The border between Ecuador and Peru is only about 3 km in a straight line from the type locality, hence *P. jimburensis* could also occur in Peru.

Conservation status. Only one population of approximately 50 mature individuals of this species was discovered at the type locality at the Yacuri National Park. The habitat is very close to the shore of the Laguna Negra and exposed to human activities related to spiritual rituals. Additionally, the trail that connects the lagoon complex closely passes by the population of this new species. According to the IUCN Red List criteria (IUCN Standards and Petitions Committee 2022) this species is assessed as Vulnerable (VU, Criterion D2).

Affinities. *P. jimburensis* apparently is a close ally of *P. calyptrata* as are all Ecuadorian and Andean taxa. A similar atypical leaf orientation, with erect, elongated leaves can only be found in *P. elongata* Benj. from Colombia and Venezuela. This strange species is, contrary to traditional placements (Ernst 1961, Casper 1966), not closely related to the other Southern American taxa, but represents an isolated lineage with affinities to Mexican and European clades, as a recent molecular phylogenetic analysis has shown (Shimai *et al.* 2021). Moreover, the leaves of *P. jimburensis* are only slightly elongated and otherwise flat with a distinct abaxial and adaxial leaf lamina and not involute as the almost filiform leaves of *P. elongata*, with the abaxial leaf surface sometimes completely hidden. Furthermore, the leaf margins of *P. jimburensis* are irregularly, shallowly lobed, a unique character among *Pinguicula* from South America so far. With the lack of a yellow palate and the evenly narrowed spur that resembles

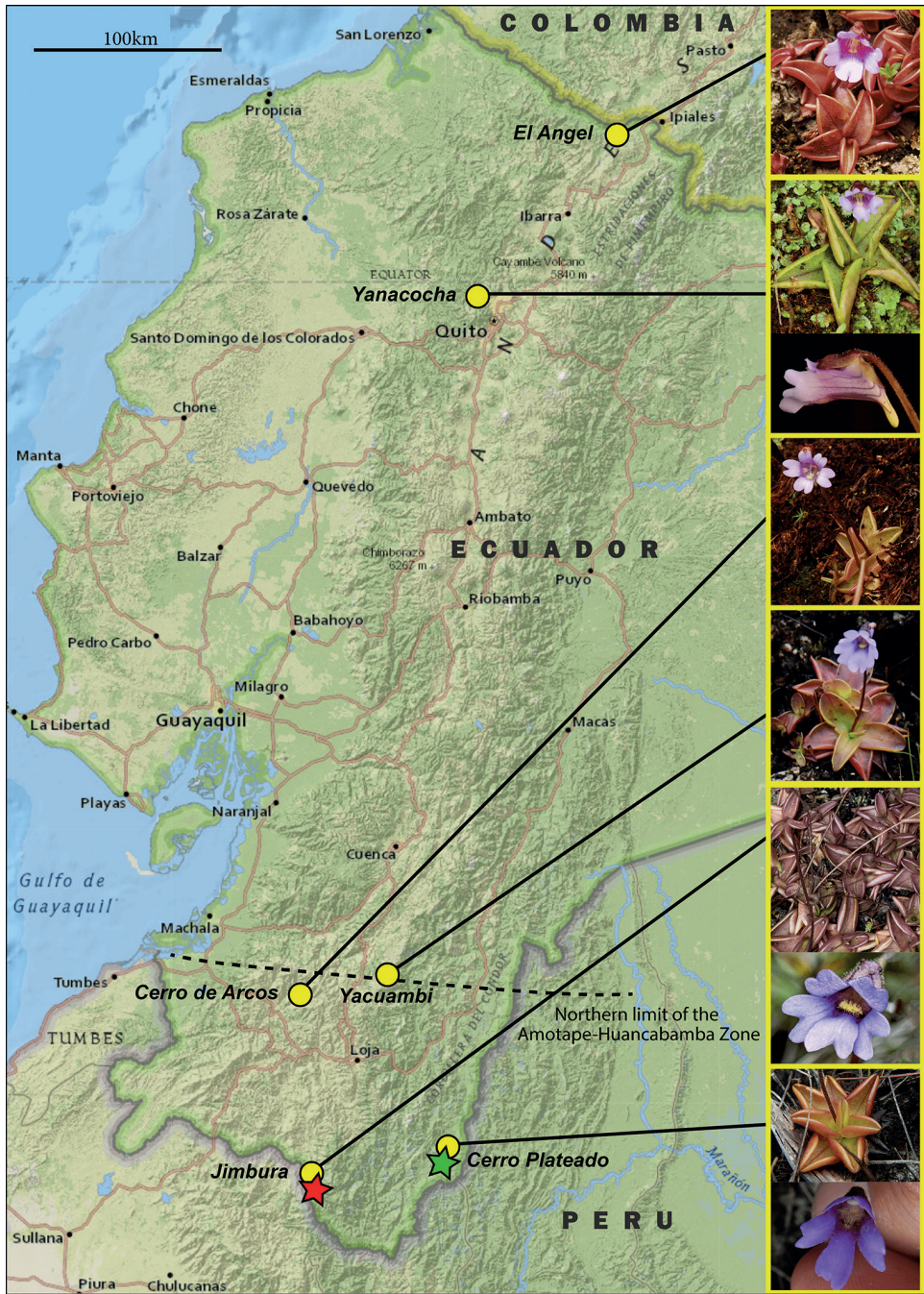


Figure 3. Map showing the distribution of *Pinguicula* spp. in Ecuador and illustrating the morphological spectrum of *P. calypttrata* observed from north to south. The yellow circles mark the respective localities sampled (locality name in *italics*). The two new species (*P. jimburensis* = red star, *P. ombrophila* = green star) are both found at the southern end of Ecuador near the border to Peru and well within the Amotape-Huancabamba Zone (dashed line).

the ripper tooth of a predatory mammal in shape, the flowers appear more similar to those of *P. involuta* than to typical flowers of *P. calyptrata*. The latter is characterized by the presence of a yellow palate and the spur, although very variable, usually has a stubby apex preceded by a narrower or at least straight section that is rather abruptly angled downwards after the throat. Both *P. jimburensis* and *P. involuta* lack a yellow palate and the thorn-shaped, regular curved spurs in both taxa are evenly narrowed towards the apex. However, *P. involuta* has a very different leaf morphology and is overall a much smaller taxon distributed much further south. *P. jimburensis* cannot be confused with any other American *Pinguicula* species based on the exceptional leaf morphology and orientation.

Additional specimens examined (paratypes). ECUADOR. Loja. Cantón Espíndola, Parroquia Jimbura, Parque Nacional Yacuri, Lagunas Negras de Jimbura, 04°42'S, 79°25'W, 3550 m, 10 Sept 2001 (fl), *P. Lozano & R. Bussmann* 7 (LOJA, barcode: 31115); *ibid*, 04°42'46"S, 79°25'50"W, 3100–3200 m, 15 Oct 2018 (fl), *G. Salazar et al.* 10191 (QCNE, barcode: 263036).

Photographic evidence. <https://www.inaturalist.org/observations/138176370>.

***Pinguicula ombrophila* Á.J. Pérez, Tobar & T. Henning, sp. nov.**

urn:lsid:ipni.org:names:77316267-1

Fig. 4

Type. Ecuador. Zamora-Chinchipe, Cantón Nangaritza, Parroquia Nuevo Paraíso, Reserva Biológica Cerro Plateado, -4.6194445, -78.7830556, 2850–2900 m, 27 Sep 2016, Á.J. Pérez, N. Zapata & W. Santillán 10353 (holotype QCA (fl, fr) barcode: 245582).

Diagnosis. *Pinguicula ombrophila* belongs to *Pinguicula* sect. *Ampullipalatum* and is closely allied to the other North Andean species of the section (*P. calyptrata*, *P. jimburensis* and *P. rosmarieae*). It differs from *P. calyptrata* and *P. jimburensis* in its lithophytic (vs. terrestrial) habit and the combination of broad rosettes with flat leaves (unlike *P. jimburensis*) without curled margins (unlike *P. calyptrata*). The flowers of *P. ombrophila* are similar to those of *P. calyptrata* and a distinct yellow palate – absent in *P. rosmarieae* and *P. jimburensis* – is present. It differs from all three other taxa by having very short flower scapes that barely reach leaf-length.

Description. Lithophyte on sandstone rocky walls, perennial rosette leaved herb with 1 (–3) flowering scapes. Rhizome ~12 mm long, with numerous fibrous roots 1.5–6.5 cm long. Leaves (5–) 6–7, flat on the ground, ± succulent (dried translucent-membranous), (15–) 20–30 (–35) mm long, nearly as long as wide, the blades ovate-obovate-oblong in outline, rounded at the tip, attenuated to the base into a sessile petiole, the margins entire, light green along the midvein and purple-brownish throughout the rest, upper surface of lamina covered with stalked glandular hairs. Hibernacula (winter buds, dormant buds) absent. Scapes 1–2 (–3), erect, (20–) 25 (–30) mm tall, terete, filiform (0.5–1 mm thick), one-flowered, purple-brownish, scattered with stalked glandular hairs. Flowers small, ~10–13 (–15) mm long (including

tube-spur-complex). Calyx two-lipped, persistent, purple-brownish, upper surface of sepals scattered with stalked glandular hairs; upper lip deeply divided into three nearly equal-sized oblong lobes, at apex pointed; lower lip deeply divided into two oblong lobes, at apex pointed. Corolla two-lipped, bluish-magenta to bright-violet, scattered with stalked glandular hairs; upper lip two-lobed, lobes obovate, ~5–6 mm long and ~4–6 mm wide, notched at the apex; lower lip larger and longer than the upper lip, with three oblong to obovate-oblong lobes (the median lobe somewhat larger than the two lateral ones), 5–6 mm wide, each distinctly (up to 1/3 of its length) notched. Tube (tube-spur-complex) at the throat funnel-shaped and with dark stripes, proximally getting narrower until the end of the lower calyx lobes and merging relatively abruptly and with a weak angle into the cylindrical stubby, at apex rounded, yellow spur; ~6 mm long; Palate simple, well developed, inserted immediately behind (~1–2 mm) the corollas' lower-lip middle lobe, yellow, set with short-stalked glandular hairs, proximally elongated into two short ventral hair strips; the corollas' inner surface covered with small white hairs, stretching proximally along on each side of the inner tube wall. Stamens 2, filaments 1.5–2 mm long, anthers dorsifixed, 0.5 mm, oval, transverse dehiscent. Ovary 1.2 mm, rounded, glabrous, style 1 mm long, stigma 1 mm long, campanulate, sparsely covered with simple trichomes. Capsule 3–4 mm, rounded, glabrous, splitting in 2 valves. Seed numerous, alveolate, ellipsoid, 0.5–0.8 mm long. Chromosome number unknown.

Etymology. The specific epithet was chosen to point at the particular habitat requirements of the plants. They prefer very wet conditions where they not only receive constant moisture from the surrounding waterlogged paramo-soil, but are also fully exposed to the high amounts of precipitation and fog typical for this area. The name *ombrophila* signifies “rainloving” from the two latin words “ombros” (rain) and “philos” (that loves/is fond of).

Distribution, habitat and associated vegetation. The Cerro Plateado Biological Reserve is one of the governmental protected areas along the Cordillera del Cóndor range in Ecuador, protecting around 26 000 ha of mature forest from 850 to 3100 m in the province of Zamora-Chinchipe. The Cordillera del Cóndor runs 150 km north-south along the border of Ecuador and Peru. This mountain range is isolated from the main Andean chain and is geologically distinct, formed with an intermixture of limestone, quartzitic sandstone, and igneous rock of the Hollin Formation (Gregory-Wodzicki 2000; Neill 2005). The geology of these mountains is similar to the tepuis of the Guyana shield in northwest South America. In fact, a number of angiosperm genera once thought to be endemic to the tepuis of the Guyana shield have also been found along the Cordillera del Cóndor (Berry et al. 1995; Schulenberg and Awbrey 1997).

Pinguicula ombrophila, together with *P. calyptrata* (Neill et al. 17467, ECUAMZ; Pérez et al. 10170, 11711, QCA; Figs 2, 3), were collected during the first botanical expedition to the Cerro Plateado led by Dr. David A. Neill in 2012 and later in 2016 and 2021 by the first author of this paper. Both species were collected at the summit of this Andean tepui, very close to the Peruvian border, that correspond to the highest sandstone plateau of the Cordillera del Cóndor, growing as a lithophyte on exposed quartzitic sandstone rocks with acidic and nutrient poor soils and in an

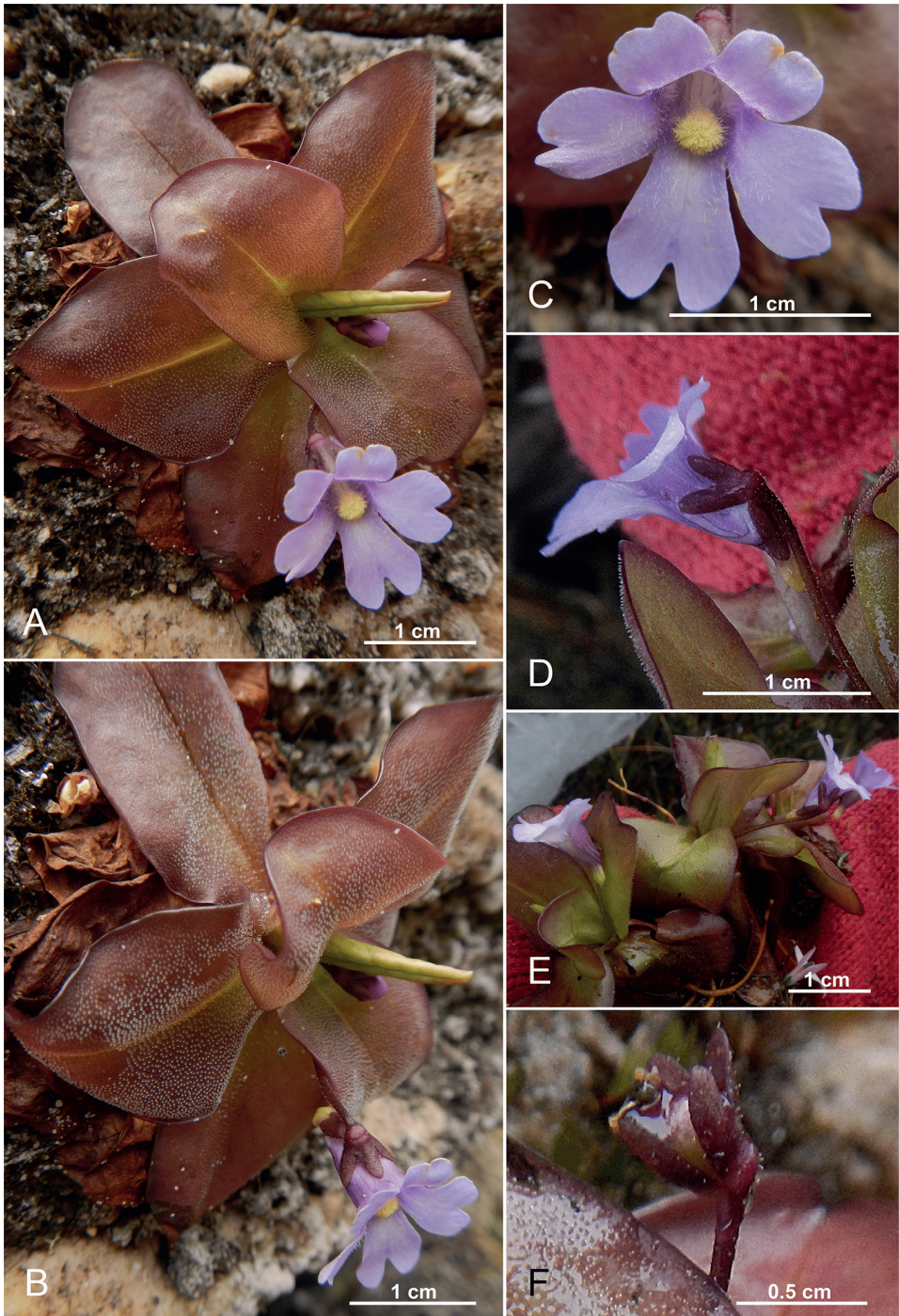


Figure 4. *Pinguicula ombrophila* **A, B** flowering plant in fronto-lateral view in the natural habitat in the Reserva Biológica Cerro Plateado **C** flower, frontal view **D** ditto, lateral view **E** freshly collected specimen, note the angled spurs with the blunt apices **F** young capsule. (**A–F** from *Á.J. Pérez et al.* 10353). Photos by Álvaro J. Pérez.

extremely wet environment (Figs 2, 3). The vegetation is paramo-like, dominated by the grass *Chusquea* cf. *nana* and the bromeliad *Vriesea* sp. along with scattered shrubs of *Clethra concordia* D.A.Neill, H.Beltrán & Quizhpe, *Diplostegium* sp., *Gaultheria lanigera* Hook. and *Valeriana plateadensis* sp. nov. Á.J. Pérez, C. Persson & J.N. Zapata (Persson et al. 2023).

Conservation status. Only one population with ca. 15 mature individuals of this species was discovered at the type locality at the summit of the Cerro Plateado. It is an isolated area and difficult to access; nevertheless, climate change could affect the environmental requirements of this species. According to the IUCN Red List criteria (IUCN Standards and Petitions Committee 2022) this species is assessed as Vulnerable (VU, Criterion D2).

Affinities. *P. ombrophila* is superficially very similar to *P. rosmarieae* with which it shares the wide leaves without involute margins and the resulting large and relatively flat rosettes. Interestingly, both *P. ombrophila* and *P. rosmarieae* are lithophytic (the latter rarely epiphytic) plants that prefer the wettest of all non-submerge locations available in the generally very moist paramo habitats. The rosette morphology likely is an adaptation to that and might be subject to environmental constraints. *P. rosmarieae* shows, however, a different flower morphology with a peculiar box-like corolla-spur complex and the lack of a yellow palate. The flowers of *P. ombrophila* are more similar to *P. calyptata* and the three species are obviously closely related to each other. *P. ombrophila* is furthermore characterized by a very short flower stalk compared to all other north-Andean taxa. The flowers barely exceed the tips of the freshly developing leaves, a character so far only known and typical for, for example, the south-Andean *P. nahuelbutensis* (Gluch 2017).

Additional specimens examined (paratypes). ECUADOR. Zamora-Chinchipe. Cantón Nangaritza, Reserva Biológica Cerro Plateado, Herbaceous páramo-like vegetation on broad, gently sloping summit area of Cerro Plateado, 04°37'10"S, 078°46'59"W, 2915 m, 24 Aug 2012 (fl), *D. Neill et al.* 17465 (ECUAMZ); ibid, colecciones en la cima de la meseta, vegetación paramuna, -4.6194445, -78.7830556, 2900 m, 23 Sep 2016 (fl, fr), *Á.J. Pérez et al.* 10145 (QCA, barcode: 245583); ibid, 8 Aug 2021 (fl, spirit collection), *Á.J. Pérez et al.* 11712 (QCA, barcode: 245580).

Discussion

The results presented in this study show that the assessment of the neotropical biodiversity is far from complete. Even in well-known groups such as the carnivorous plants, new taxa are continuously discovered and described, in particular from remote areas that become accessible in the course of the unlimited urban sprawl. This is both encouraging and worrying at the same time. While there are evidently still pristine habitats left that inhabit an unknown biodiversity, the fact that these ecosystems are now at an accessible distance to human infrastructure puts them under immediate threat of exploitation and destruction. In particular, the eastern slopes and Andean foothills facing Amazonia in the northern Andes border such remaining microhabitats

in the form of isolated paramo sites and cloud forest fragments. Their seclusion in relation to similar neighboring habitats by the rugged terrain led to a mosaic of small scale organismal communities that are often self-contained to a certain extent. In the case of flowering plants, the degree of isolation often is determined by the associated pollen vector, usually the pollinating fauna. While spatial isolation of plant-pollinator communities is considered the main driver of diversification in angiosperms, intra-population competition for resources can also play an important role. In the case of insectivorous *Pinguicula* two potentially limited resources appear to be essential: pollinators and prey. Striving for these resources between neighboring populations can lead to rapid phenotypic diversifications in morphologically plastic parts of the plant body such as leaves and flowers. Furthermore, in co-occurring taxa, these changes can be strongly accentuated towards a differentiation by a phenomenon known as character displacement (Beans 2014). An adaptation for different prey by e.g. the different shape and orientation of the leaves in the sympatric taxon pairs found can only be assumed. However, the differences observed in the flower morphology between populations, both sympatric and throughout the entire distribution range of *P. calypttrata* s.l., point at effective reproductive barriers. The fact that, as far as is known, all species of the section (i.e. *Ampullipalatum*) can be artificially hybridized, but no hybrids have been reported from the wild (Fleischmann 2021) indicates that there are no genetic barriers, but other factors in effect. Plant-pollinator interactions can be very specific and are often controlled by multiple factors, especially in heterogeneous mosaic-like landscapes such as the high Andes. In addition to the morphological adaptations of flowers (e.g., color, size, spur length), traits such as nectar quantity and sugar concentration are often precisely matched to a particular pollinator taxon (Ackermann and Weigend 2006), and even pollen presentation can be the subject of complex and specific plant-pollinator interactions (Henning et al. 2018). Although speculative, such processes likely play a key role in the ongoing diversification of *Pinguicula* and evidently in the phenotypic divergence observed in the Ecuadorian taxa. Studies on the pollination biology of *Pinguicula* are scarce, the specificity of their pollination ecology is largely unknown, and appropriate research is strongly encouraged. However, published pollinator observations as e.g. in the recently described *P. warijia* from Mexico (Zamudio et al. 2023), provide evidence for a single principal pollinator (butterflies) and a respective close interaction.

Morphological variation of *Pinguicula calypttrata* Kunth

Pinguicula calypttrata shows a great morphological variability in all phenological aspects throughout its distributional range and the different populations considerably vary in size, shape and color of all parts of the plant body (Figs 2, 3). Since the protologues of the “historical” taxa are usually based on a single collection and neither provide measurements, nor by any means outline a morphological spectrum, the real taxonomic extent of *Pinguicula* remains hidden in the broad taxonomic concept pursued ever since (Casper and Hellwig 2019; Casper et al. 2020). This is particularly true for *Pinguicula calypttrata* and likely holds true for other South American taxa not yet studied in the

necessary depth. The present description of the two new species should be understood as the continuation of an ongoing taxonomic extension of the genus as anticipated once a thorough molecular study including material from as many populations as possible can be conducted. Such a study has recently shed light on the taxonomy and nomenclature of the *P. vulgaris* aggregate in Europe, which, based on conflicting taxonomic concepts and different methodological approaches, were subject to scientific debates for decades (Majeský et al. 2022). The earlier separations of *P. jarmilae*, *P. nahuelbutensis* and *P. rosmarieae* from their putative sister taxa (*P. involuta*, *P. antarctica* & *P. calypttrata* respectively) were already founded on the evaluation of new collections and their morphological divergence from the broad circumscriptions of the historical taxa. In the case of *P. jarmilae*, which was later and hence invalidly also described as *P. chuquisacensis* by Beck et al. (2008), the latter authors already conducted a molecular study to underscore species delimitation and reveal the relationships among the South American taxa. Their key findings were that, contrary to their initial assumption, not morphologically more similar *P. calypttrata* but *P. involuta* is the closest relative and that the new species showed an unexpectedly high amount of autapomorphies in its sequence data (plastid markers). Furthermore, their preliminary dataset revealed a high genetic divergence among the South American taxa relative to those found in other clades of *Pinguicula*. These findings contrast with the comparatively low number of taxa described from Andean South America to this day. We therefore expect that the number of taxa will grow with the availability of molecular data and a concomitant better understanding of the taxon boundaries and speciation processes in this group of plants. For the time being, we must confine ourselves to describe those taxa as new to science, whose delimitation based on morphology and biogeography alone appears objectively evident.

The newly described species and the still unsatisfactorily documented and understood diversity among Andean *Pinguicula* underline the need to continue with botanical explorations and taxonomic studies and intensify urgently needed conservation efforts. The actual diversity of the Andean flora, in particular that of Ecuador and Northern Peru (AHZ), is still not conclusively determined. The threats to natural ecosystems in general, and those to small scale mountainous habitats in particular, are very worrying. *Pinguicula*, which inhabits very specific ecological niches due to its peculiar habitat requirements, is particularly susceptible to the destructive effects of e.g. mining activities and infrastructure projects (Cross et al. 2020), which penetrate further and further into the remaining remote pristine refugia. To make things worse, climate change increasingly affects local precipitation regimes and temperature profiles, with potentially dramatic consequences for moist habitats such as the paramo wetlands of the high Andes and their unique flora and fauna.

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Taxonomic status and nomenclature of *Cephalotaxus lanceolata* (Cephalotaxaceae)

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Abstract

The nomenclature of *Cephalotaxus lanceolata* is controversial. After a thorough literature investigation, the nomenclatural problems have been resolved. This name was published in W. C. Cheng et al. (1975) and, although ascribed to “K. M. Feng”, there is no suggestion that the descriptive material in the protologue was provided by K. M. Feng. Under Art. 46.5 of the ICN, this name should be attributed to K. M. Feng ex W. C. Cheng et al. but not to K. M. Feng alone. It has been claimed that the name is an illegitimate later homonym of one published by Beissner in 1901, but Beissner never accepted this name in any of his publications and so, under Art. 36.1, he did not validly publish an earlier homonym. *Cephalotaxus lanceolatus* was first validly published in W. C. Cheng et al. (1975).

Keywords

Cephalotaxaceae, *Cephalotaxus lanceolata*, gymnosperms, nomenclature, the Shenzhen Code

The name *Cephalotaxus lanceolata* was described in Cheng et al. (1975: 86) and ascribed to K. M. Feng, but there was no indication in that publication that the diagnosis of the new species was provided by K. M. Feng. Likewise, in Cheng et al. (1978), “Flora Reipublicae Popularis Sinicae”, no author contribution by K. M. Feng was acknowledged. Consequently, under Art. 46.5 of the ICN (Turland et al. 2018), this name should be attributed to the authors of the publication and cited as “K. M. Feng ex W. C. Cheng et al.” or just “W. C. Cheng et al.”. As a result, the attribution of this name to K. M. Feng by Cheng (1983), Farjon (2010) and Lang et al. (2013a, 2013b) is incorrect.

Lang et al. (2013a, 2013b) considered *Cephalotaxus lanceolata* W. C. Cheng et al. to be an illegitimate later homonym because they thought that there was an earlier homonym, *C. lanceolata* Beissner (1901a). Therefore, they proposed the replacement name *C. talonensis* W. C. Cheng & L. K. Fu ex S. G. Lu & X. D. Lang taking up a manuscript name that W. C. Cheng & L. K. Fu and/or K. M. Feng had used on the original specimen. If Lang et al.'s claim was correct, then a replaced name would indeed be required.

I conducted a new investigation on the publications of Beissner (1901a, 1901b, 1909) and found that Beissner did not accept *C. lanceolata* as the name of a species in any of the three publications and so it is not validly published in any of them (Art. 36.1). Beissner (1909) treated *C. lanceolata* as a synonym of "*Cephalotaxus fortunei robusta* hort.", so clearly not accepting the name and the same is the case in the two 1901 publications: Beissner (1901b) wrote: "Nous devons dès lors regarder le *C. lanceolata* hort. comme une forme vigoureuse, multipliée par greffe, de *C. fortunei* Hook." Translated to English as: I must therefore look at *C. lanceolata* hort. as a vigorous, graft-propagated form of *C. fortunei* Hook. Beissner (1901a) wrote: "Damit erledigt sich dann auch der zweite Punkt, dass *C. lanceolata* hört, nicht mit *C. griffithii* gleich ist, sondern als besonders langblättrige, üppige, durch Veredelung fixierte Form zu *C. fortunei* Hook, gehören dürfte." This suggests that *C. lanceolata* hort. is not the same as *C. griffithii*, but should belong to *C. fortunei* Hook. as a particularly long-leaved, luxuriant form fixed by grafting.

As a conclusion, I consider that *C. lanceolata* should be ascribed to K. M. Feng ex W. C. Cheng et al. and that *C. lanceolata* K. M. Feng ex W. C. Cheng et al. is a legitimate name.

The taxonomic status of *C. lanceolata* has been in debate. The name was accepted in Cheng et al. (1978), Cheng (1983), Fu (1984), Huo (1986), Farjon (1998, 2010, 2017) and Fu et al. (1999) as that of a distinct species, but Silba (1990) treated it as a variety: *C. fortunei* var. *lanceolata* (K. M. Feng ex W. C. Cheng et al.) Silba, which is also accepted by Eckenwalder (2009). Tripp (1995) considered *C. lanceolata* as a separate species from *C. griffithii* Hook. f. but Bisht et al. (2021), who thought the name illegitimate, treated it as a synonym of *C. griffithii*. Wang et al. (2022) conducted DNA barcoding research by sampling Chinese materials, and concluded that *C. lanceolata* represented a separate species lineage. Here I follow Tripp (1995), Farjon (1998, 2010, 2017), Fu et al. (1999) and Wang et al. (2022) and accept *C. lanceolata* as a separate species.

Treatment

***Cephalotaxus lanceolata* K. M. Feng ex W. C. Cheng et al., Acta Phytotax. Sin. 13(4): 86 (1975)**

Homotypic synonym. *Cephalotaxus fortunei* var. *lanceolata* (W. C. Cheng et al.) Silba, Phytologia 68(1): 27 (1990); *Cephalotaxus talonensis* W. C. Cheng & K. M. Feng ex S. G. Lu & X. D. Lang, Bull. Bot. Res., Harbin 33(1): 5 (2013), nom. illeg.

Type. CHINA (中国), Yunnan (云南), Gongshan Co. (贡山县), west of Dulong River, alt. 1900 m, in broad-leaved forests nearby the river, 18 Nov. 1959, K. M. Feng (冯国楫) 24347 (holotype: PE00206970, Fig. 1).



Figure 1. Holotype of *Cephalotaxus lanceolata* K. M. Feng ex W. C. Cheng et al. (PE00206970).

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Five new synonyms for *Impatiens procumbens* (Balsaminaceae) in China

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Abstract

In the revision on the genus *Impatiens* L. in China, we found that there were synonyms amongst some species. *Impatiens procumbens* Franch. morphologically resembled *I. reptans* Hook.f., *I. crassiloba* Hook.f., *I. ganpiuana* Hook.f., *I. atherosepala* Hook.f. and *I. rhombifolia* Y.Q.Lu & Y.L.Chen. After a thorough morphological study, based on original literature, type specimens and field surveys, it was found that the above six species of *Impatiens* had no substantial differences in morphological characters and there was continuity in geographical distribution. Therefore, we determined that *I. reptans*, *I. crassiloba*, *I. ganpiuana*, *I. atherosepala* and *I. rhombifolia* are the synonyms of *I. procumbens*. At the same time, we present the color photographs, supplementary descriptions of morphology, and geographical distribution. The lectotype of *I. procumbens* and *I. reptans* are also designated here.

Keywords

Lectotypification, morphology, taxonomy

Introduction

In China, 352 species of *Impatiens* have been recorded, including 273 species endemic to China, which are concentrated throughout the Qinling Mountains, southern Tibet, the Hengduan Mountains, Yunnan–Guizhou–Guangxi karst region, the middle and lower reaches of the Yangtze River and other regions (Yuan et al. 2022). It is well known that *Impatiens* species are notoriously difficult to identify, because of their

abundant character variations and morphological similarities, which makes the boundary between species very blurred (Grey-Wilson 1980; Cong 2007; Tian et al. 2007). From the perspective of research history, there were many factors in the early published species, such as the distance between collectors and researchers, the collection of specimens with the same number in different herbaria, the change of place names etc., all of which have led to taxonomic problems, for example, the same species with different names, the absence of characters, and incorrect records. Additionally, these classification problems have not been resolved so far, which is not conducive to the research of *Impatiens*, but also causes some obstacles in the research for identification of the genus *Impatiens* in China. *I. procumbens* was published by Franchet (1886) on the basis of specimens collected from Dali, Yunnan Province. *I. reptans*, *I. crassiloba*, *I. ganpiuana*, and *I. atherosepala* were described by J. D. Hooker, based on specimens collected from Guizhou Province by E. M. Bodinier and J. P. Cavalerie (Hooker 1908a, b). *I. rhombifolia* was published by Chen and Lu (1990), based on specimens collected from Mount Emei, Sichuan Province. Yu (2008) mentioned that *I. reptans*, *I. ganpiuana*, *I. procumbens* and *I. rhombifolia* were very similar in morphology and may be described repeatedly. Through extensive field investigations and textual research of specimens, it was found that there were no essential differences amongst the above six species. In addition, the guidelines and recommendations of Article 9 of the ICN (Turland et al. 2018) have been followed while designating the lectotype.

Materials and methods

For morphological comparisons, we reviewed the original literature and related records, including the original literature description of each species, information of type specimens, synonyms and geographical distribution. The main sources of original literature are from Tropicos (<http://www.tropicos.org>), IPNI (<http://www.ipni.org>) and other websites. Otherwise, we also critically checked type specimens or high-resolution images of specimens involved in this study in BM, E, K, P, NY, WU, PE, IBSC, IBK, KUN, HGAS, GZAC, GZTM, SWFC and conducted fieldwork of type localities in Sichuan, Guizhou and Yunnan Provinces. We have obtained more than 10 field collections. Herbarium specimens were chosen carefully. We dissected the flowers of the plant in the field or in the habitat itself after collection of the plants. And various morphological characters, such as leaf size and shape, inflorescence type, flower color, etc., were carefully observed, measured and quality photographs were taken.

Results and discussion

After consulting the type specimens (Fig. 1) of *Impatiens procumbens* from Dali, Yunnan, *I. reptans* from Guiyang, Guizhou (Fig. 2) and *I. rhombifolia* from Emei, Sichuan (Fig. 4B), as well as the specimens in domestic and foreign herbaria and relevant

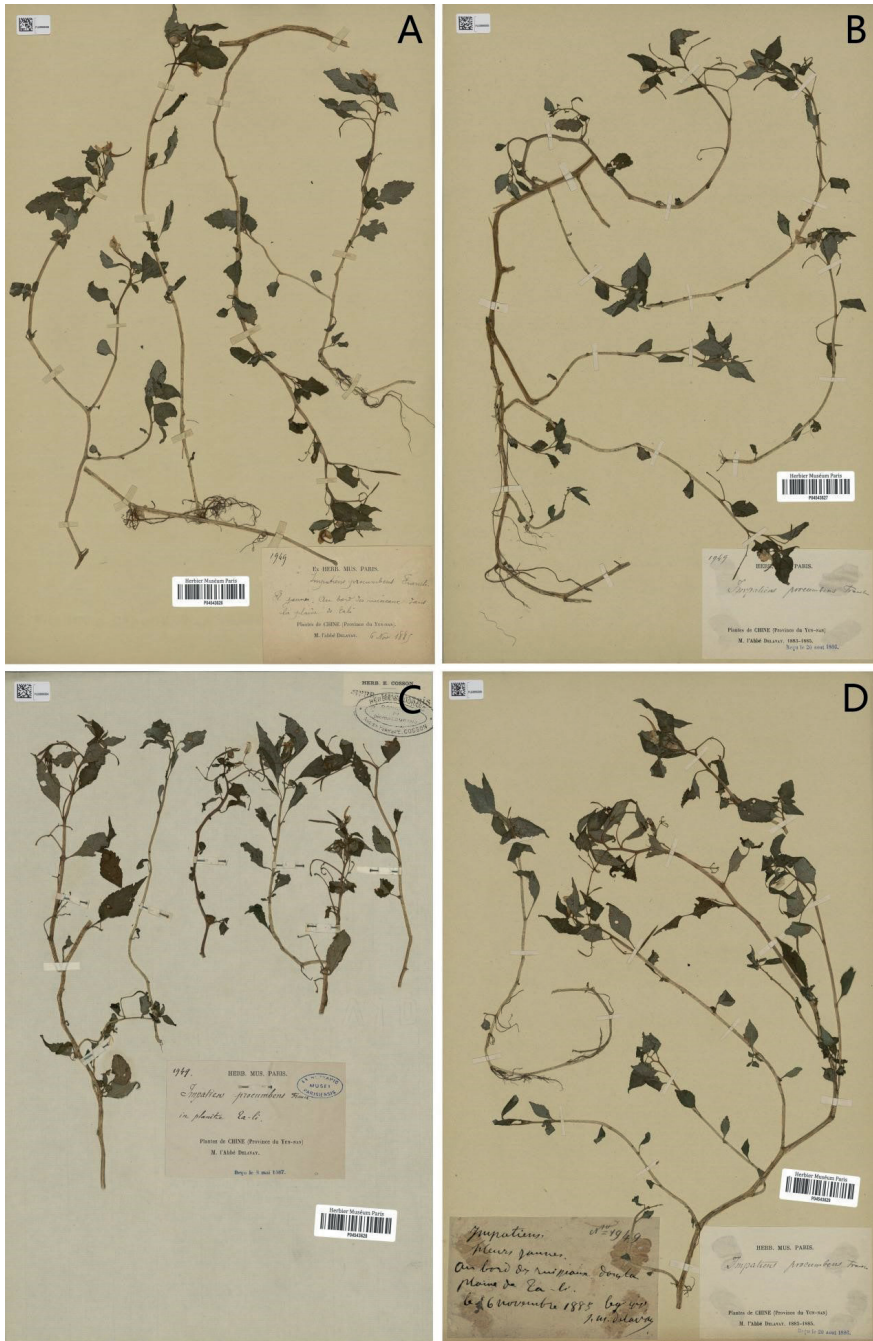


Figure 1. *Impatiens procumbens* **A** lectotype (P04543626) **B–D** isolectotype (P04543629, P04543628, P04543627). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p04543626> (accessed on 1 September 2022). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p04543629> (accessed on 1 September 2022). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p04543628> (accessed on 1 September 2022). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p04543627> (accessed on 1 September 2022).



Figure 2. *Impatiens reptans* **A** lectotype (P00780766) **B** isolectotype (E00313595). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00780766> (accessed on 8 April 2021). Source from <https://data.rbge.org.uk/herb/E00313595> (accessed on 8 April 2021).

literature records, it was found that there were no obvious differences in morphology amongst them (Table 1).

In the process of textual research on the type specimen of *Impatiens reptans* (Figs 2, 5A), we found that J. D. Hooker did not designate a holotype in keeping with the practice of the time, the specimen P00780766 (Fig. 2A) agrees better with the information given in the literature and contains the pencil drawing of flower parts and the dissected flower parts pasted on the sheet. Therefore, it is designated here as the lectotype. We also found that the labelling information of the isolectotype (E00313595) was marked “An idem au no. 1782?”. At the same time, the vernacular name “*Impatiens ganpiuana* Hf Vav” (Fig. 2B) was found on the label at the lower right corner. These two uncertainties point to the need for further textual research of *I. ganpiuana*. Meanwhile, the collection information marked on the type specimens of *I. crassiloba* (Fig. 4A) and *I. ganpiuana* (Fig. 4C) were the same, namely *E. M. Bodinier* 1782, the collection time of the latter being 9 August, 1897. Although there was no collection time on the specimen of the former, it could be inferred that the collection time may be from the same period according to the marked information that the specimens were received on 26

Table 1. Comparison of morphological characters of *Impatiens procumbens*, *I. reptans*, *I. crassiloba*, *I. ganpiuana*, *I. atherosepala* and *I. rhombifolia* (data from protologue and type specimen).

Characters	<i>I. procumbens</i>	<i>I. reptans</i>	<i>I. crassiloba</i>	<i>I. ganpiuana</i>	<i>I. atherosepala</i>	<i>I. rhombifolia</i>
Stem	prostrate or procumbent	prostrate	prostrate	prostrate	no record	prostrate
Leaf	ovate or ovate-lanceolate, margin serrulate, 2–3 cm long, 1–1.5 cm wide, petiole 5–10 mm long	ovate or ovate-elliptic, margin crenate-serrate or serrate, 5–7 cm long, 2.5–3.5 cm wide, petiole 1–2 cm long	ovate, narrowly ovate or ovate-lanceolate, margin coarsely serrate, 2–7 cm long, 1–3 cm wide, petiole 5–20 mm long	elliptic, narrowly elliptic or lanceolate, margin coarsely serrate, 3–6 cm long, 1.5–3.5 cm wide, petiole 1–2 cm long	lanceolate, margin spinescent-serrate, 4–6 cm long, 1.5–2 cm wide, petiole 1–2 cm long	rhombic or subrhombic, margin serrate, 2–5 cm long, 1.2–1.6 cm wide, petiole 5–10 mm long
Lateral veins	5–6	6–7	6–8	4–6	6–8	5–6
Flower	1–2-flowered, 1.5–2 cm deep	2–3-flowered, ca. 2.5 cm deep	1–3-flowered, ca. 2.5 cm deep	1- or 2-flowered, ca. 2 cm deep	1-flowered, ca. 1.1 cm deep	2-flowered, ca. 1.5 cm wide
Lateral sepal	obliquely ovate, subfalcate, 3-veined, apex long cuspidate, 3–4 mm long	falcate-ovate, 3-veined, midvein apically stoutly mucronulate, 3–5 mm long	lanceolate, small, 3–5-veined, apex cuspidate, 3–5 mm long	ovate, abaxial midvein narrowly carinate, 3–4 mm long	ovate, aristate, arista ca. as long as sepals, 3-veined, ca. 5 mm long	yellow-green, ovate-orbicular, small, apex acute, ca. 5 mm long
Dorsal petal	orbicular, abaxial midvein thickened, narrowly carinate, 8–10 mm in diam.	orbicular, abaxial midvein fine, narrowly carinate, ca. 15 mm in diam.	orbicular, abaxial midvein carinate, ca. 10 mm in diam.	elliptic, abaxial midvein rostellate, carinate, ca. 10 mm long, ca. 8 mm wide	orbicular, abaxial midvein cristate, ca. 8 mm in diam.	orbicular, large, abaxial midvein thickened, ca. 15 mm long, ca. 13 mm wide
Lateral united petals	ca. 17 mm long, basal lobes subtrigonous, distal lobes obovate-oblong	ca. 22 mm long, basal lobes orbicular, small; distal lobes dolabriform	ca. 16 mm long, basal lobes orbicular, small; distal lobes large, apex 2-lobed	ca. 18 mm long, basal lobes broadly ovate; distal lobes dolabriform	ca. 12 mm long, basal lobes orbicular; distal lobes dolabriform, longer	ca. 18 mm long, basal lobes red spotted, orbicular, distal lobes dolabriform
Lower sepal	navicular, narrowed into an incurved spur, ca. 20 mm long	subnavicular, narrowed into an incurved spur, acute or divaricate, ca. 20 mm long	navicular, narrowed into a curved, long spur, ca. 18 mm long	funnelform, narrowed into an incurved spur, ca. 10 mm long	navicular, abruptly contracted into an incurved spur, ca. 15 mm long	navicular, narrowed into an erect spur; spur ca. 18 mm long
Anther	acute	acute	obtuse	obtuse	acute	acute

April 1898. Compared with the character description of the protologue, the differences mainly lay in the shape of the lower sepal and anther (Table 1), but by examining type specimens (Figs 4C, 5B), the shape of the lower sepal of *I. ganpiuana* should be cymbiform, so there was no obvious difference, except for the different anther.

At the same time, we found that *Impatiens atherosepala* is relatively close to the above plants of genus *Impatiens*, the type locality actually being Pingfa, Guiding County, which the “Flora of China” (Chen et al. 2007) misquoted as Pingba. From the type specimen (Figs 4D, 5D), it can be seen that the typical features are leaves lanceolate, margin with spinescent-serrate, 1-flowered inflorescence and lateral sepals ovate, with long aristate. The population we found in Pingfa, Guiding County (Fig. 6A–D) conformed to the description of these characters. Through extensive field investigations (Figs 6, 7), it was found that the leaf shape of genus *Impatiens* varied greatly and as a

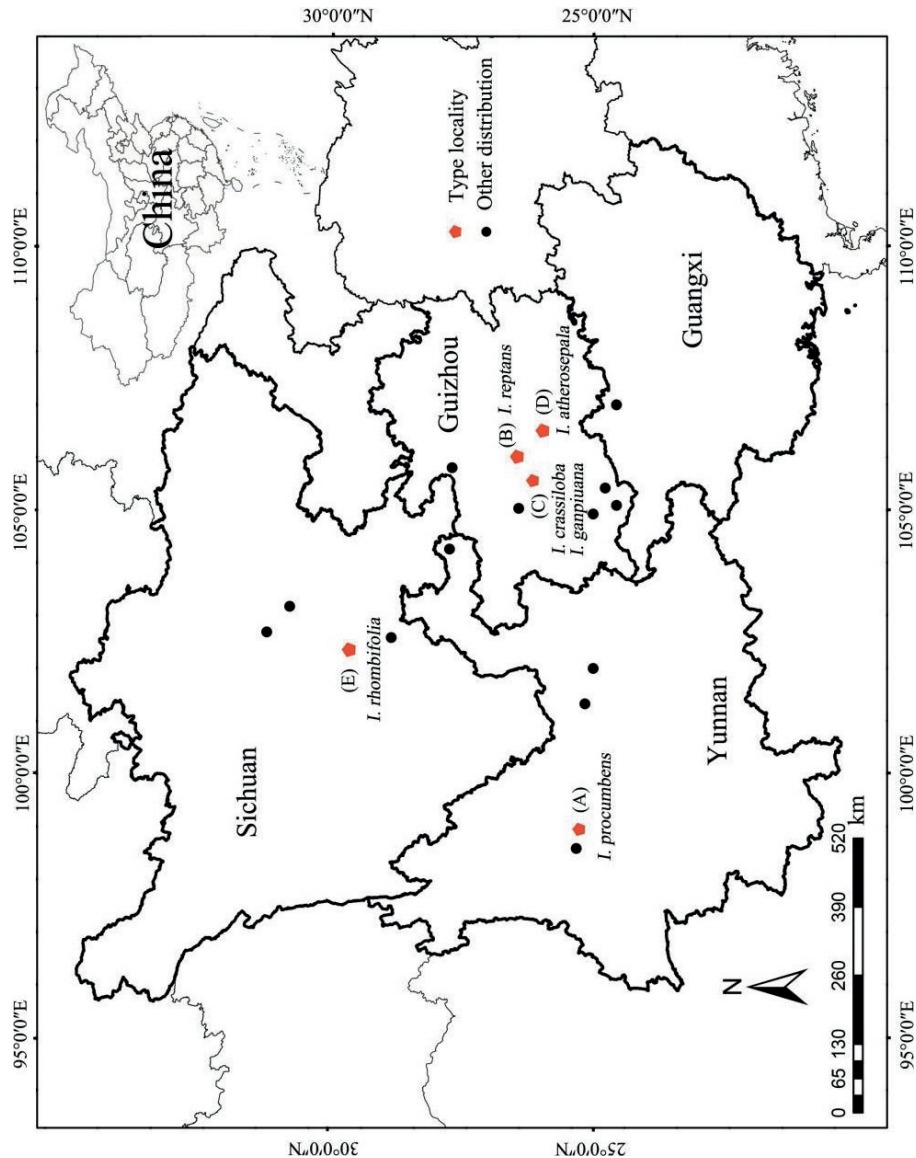


Figure 3. Geographical distributions **A** Dali City: the type locality of *Impatiens procumbens* **B** Guiyang City: the type locality of *I. reptans* **C** ganpin: the type locality of *I. crassiloba* and *I. ganpiuana* **D** pinfa: the type locality of *I. atherosepala* **E** mount Emei: the type locality of *I. rhombifolia*.

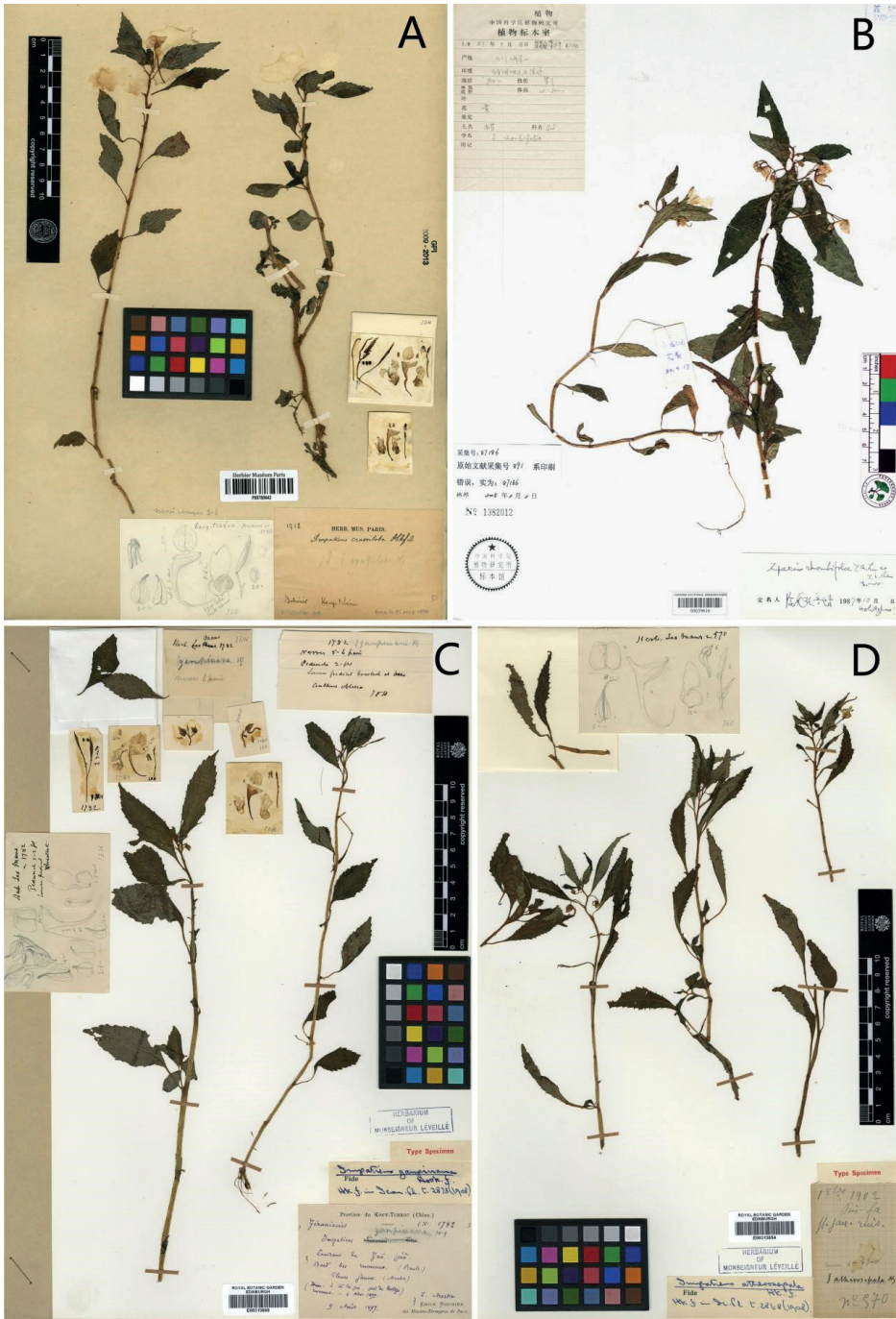


Figure 4. Type specimens **A** *Impatiens crassiloba* (E. M. Bodinier 1782; P00780642) **B** *I. rhombifolia* (Lu YingQing 87186; PE00039616) **C** *I. ganpiuana* (E. M. Bodinier 1782; E00313669) **D** *I. atherosepala* (E. M. Bodinier 1782; E00313654). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00780642> (accessed on 12 July 2021). Source from <https://data.rbge.org.uk/herb/E00313669> (accessed on 20 March 2021). Source from <https://data.rbge.org.uk/herb/E00313654> (accessed on 20 March 2021).

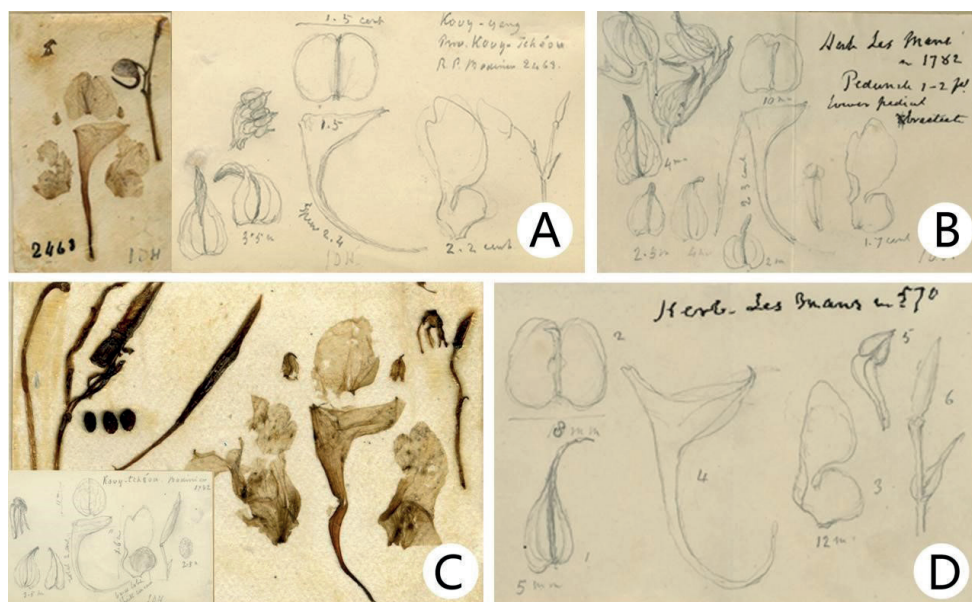


Figure 5. Flower dissected and drawing **A** *Impatiens reptans* (on the part of E00313595 and P00780766) **B** *I. ganpiuana* (on the part of E00313669) **C** *I. crassiloba* (on the part of P00780642) **D** *I. atherosepala* (on the part of E00313654). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00780766> (accessed on 8 April 2021). Source from <https://data.rbge.org.uk/herb/E00313595> (accessed on 8 April 2021). Source from <https://data.rbge.org.uk/herb/E00313669> (accessed on 20 March 2021). Source from <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00780642> (accessed on 12 July 2021). Source from <https://data.rbge.org.uk/herb/E00313654> (accessed on 20 March 2021).

taxonomic character, it was not reliable. Meanwhile, the lateral sepals of *I. procumbens*, *I. reptans*, *I. crassiloba*, *I. ganpiuana*, *I. atherosepala* and *I. rhombifolia* had a tendency to extend and grow.

In addition, it can be seen from the type localities and other distribution points that the geographical distribution of these species is continuous and there is no obvious geographical isolation (Fig. 3).

It can be seen that there is no obvious difference in the morphology of the above six species and the geographical distribution is widespread. Since *Impatiens procumbens* is the earliest name published, we concluded that *I. reptans*, *I. crassiloba*, *I. ganpiuana*, *I. atherosepala* and *I. rhombifolia* are the synonyms of *I. procumbens*. Franchet (1886) did not specify the type specimen when publishing the *I. procumbens*. Four specimens (collection no. 1949, collected by M. l'Abb é Delavy) made of flowering plants collected on 16 November 1885 are now in P (P04543629, P04543628, P04543627, P04543626), of which specimen P04543626 (Fig. 1A) is more consistent with the information described in the protologue, as well as containing complete plant organs and collection information. Therefore, P04543626 is designated as the lectotype here and P04543627, P04543628 and P04543629 are the isoelectotypes.

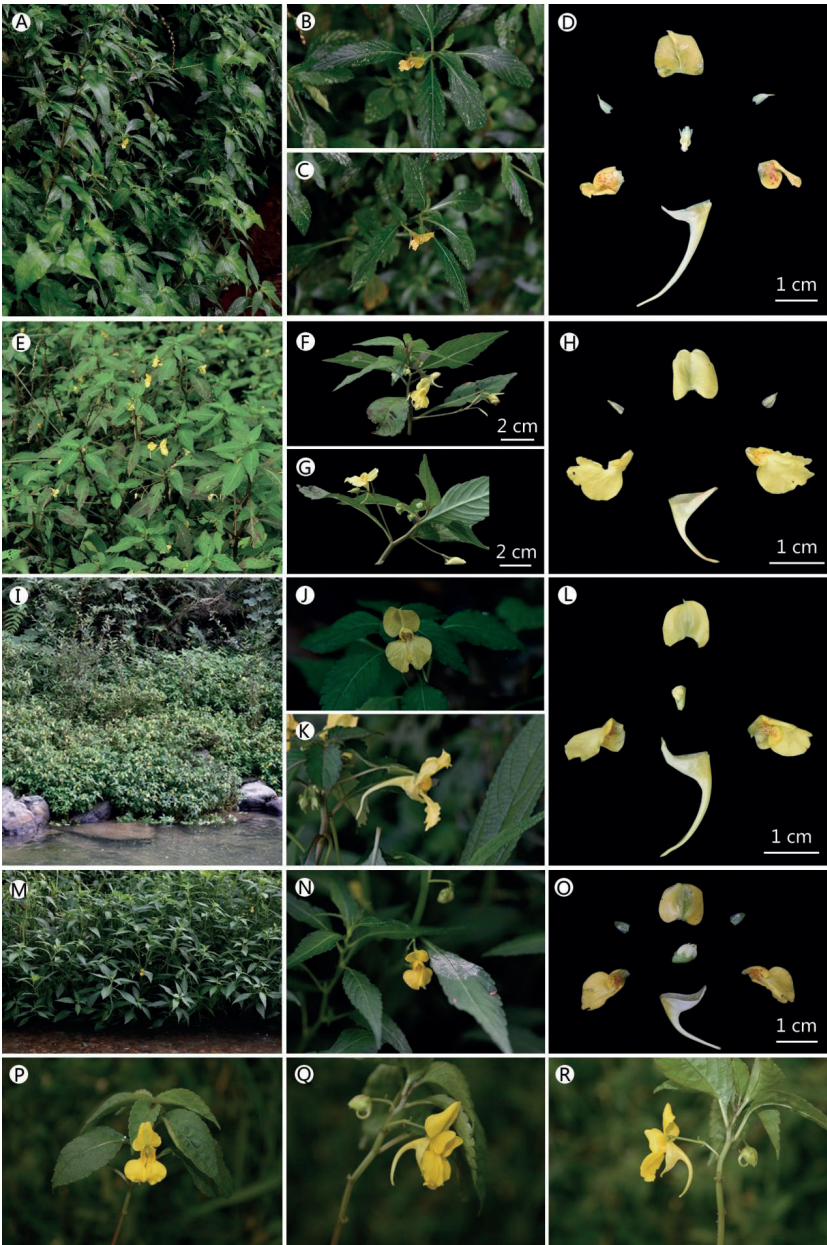


Figure 6. **A–D** population: Guiding County (type locality of *Impatiens atherosepala*: Pinfa) **A** habit **B** flower in face view **C** flower in lateral view **D** flower dissected **E–H** population: Mount Emei (type locality of *I. rhombifolia*) **E** habit **F** flower in face view **G** flower in lateral view **H** flower dissected **I–L** population: Kaiyang County (type locality of *I. reptans*: Guiyang) **I** habit **J** flower in face view **K** flower in lateral view **L** flower dissected **M–O** population: Guanshanhu District (near type locality of *I. crassiloba* and *I. ganpiuana*: ganpin) **M** habit **N** flower in face view **O** flower dissected **P–R** population: Dali City (type locality of *I. procumbens*) **P** flower in face view **Q, R** flower in lateral view. Photographs (**Q, R**) was taken in Dali City by QIN-WEN LIN, and other photographs by XIN-XIANG BAI.

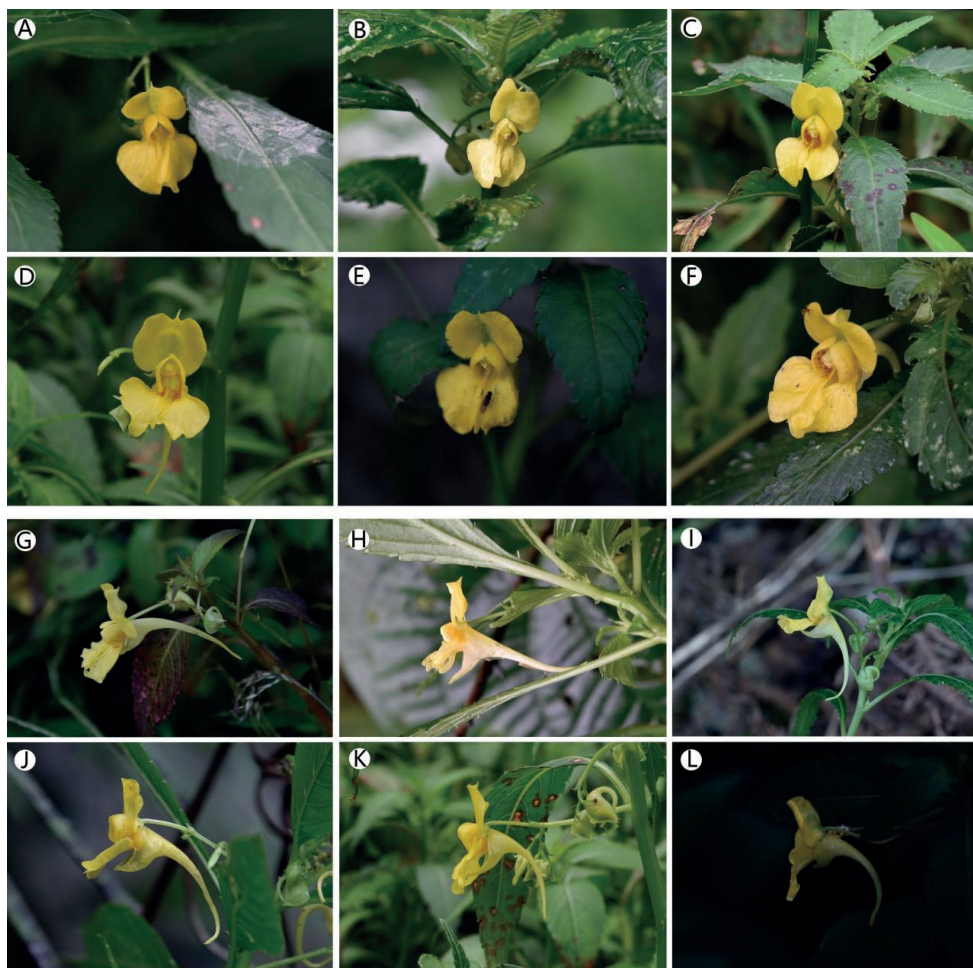


Figure 7. Floral variations (A–F) flower in face view A Guanshanhu District B Suiyang County C Huishui County D Zhenning County E Zhijin County F Guiding County G–L flower in lateral view G Danzhai County H, I Guiding County J Huishui County K Zhenning County L Zhijin County. Photographs by XIN-XIANG BAI.

Taxonomic treatment

Impatiens procumbens Franch. in Bull. Soc. Bot. France 33: 447. 1886.

Fig. 8

=*Impatiens rhombifolia* Y. Q. Lu & Y. L. Chen syn. nov. Type:–CHINA. Sichuan: Mt. Omei, 18 September 1987, *Yingqing Lu* 87186 (holotype: PE [PE00039616]).

=*Impatiens reptans* Hook. f., syn. nov. Type:–CHINA. Guizhou: Guiyang, 11 September 1898, *E. M. Bodinier* 2463 (lectotype, designated here: P [P00780766]; isolectotype: E [E00313595]).

=*Impatiens crassiloba* Hook. f., syn. nov. Type:—CHINA. Guizhou: Ganpin, without date, *E. M. Bodinier* 1782 (holotype: P [P00780642]).

=*Impatiens ganpiuana* Hook. f., syn. nov. Type:—CHINA. Guizhou: Ganpin, 09 August 1897, *E. M. Bodinier* & *L. Martin* 2463 (holotype: E [E00313669]).

=*Impatiens atherosepala* Hook. f., syn. nov. Type:—CHINA. Guizhou: Pinfa, 01 October 1902, *J. P. Cavalerie* & *J. Pierre* 570 (holotype: E [E00313654]).

Type. CHINA. Yunnan: Dali City, 6 November 1885, *M. l'Abbé Delavay* 1949 (lectotype, designated here: P [P04543626]; isolectotype: P [P04543629, P04543628, P04543627]).

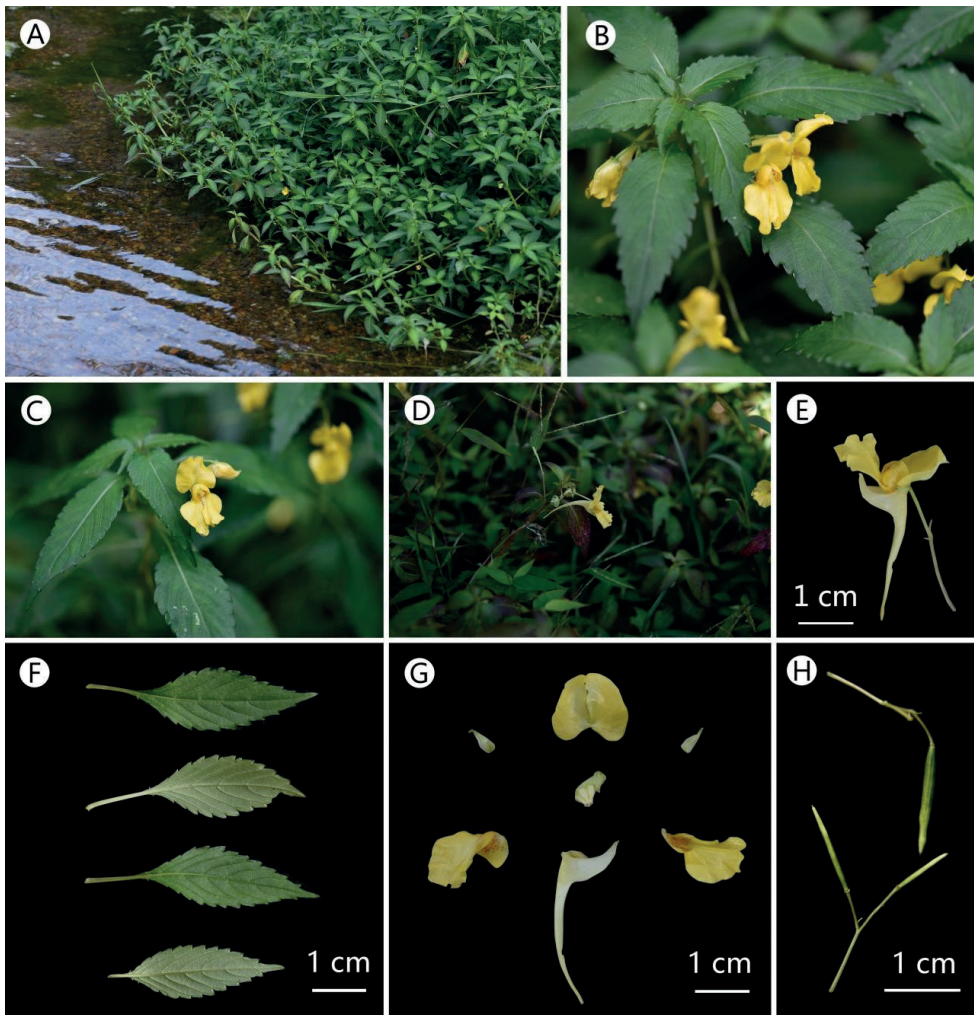


Figure 8. *Impatiens procumbens* **A** habit **B** plant **C** flower in face view **D, E** flower in lateral view **F** seed **G** leaf **H** flower dissected. Photographs by XIN-XIANG BAI.

Revised description. Annual herbs, glabrous, 30–65 cm tall. Stem long prostrate basally, lower nodes with adventitious roots, green. Leaves alternate, lamina 4–7 cm long, 1–4 cm wide, lanceolate to ovate or ovate-elliptic, apex acuminate, margin crenate-serrate or spinescent-serrate, apex acuminate, base cuneate, blade dark green adaxially, pale green abaxially, herbaceous, lateral veins 6–9 pairs. Inflorescences axillary, racemose, 18–3 mm long, 1–3-flowered, bracteate base or above middle; bract 1, small, persistent, ovate-lanceolate, 3–4 mm long, 1–1.5 mm wide, herbaceous, green. Flower yellow, 2–3 cm deep, 1.5–2.5 cm wide. Lateral sepals 2, obliquely ovate, 4–6 mm long, 2–3 mm wide, abaxial mid-vein slightly thickened, green, herbaceous, 3-veined. Lower sepal cymbiform, 2–3.5 cm deep (including spur), mouth vertical, apex slightly acute, narrowed into a long spur, 1.5–2 cm long. Dorsal sepal orbicular, ca. 1 cm in diam., base cordate, apex retuse, abaxial midvein fine, narrowly carinate. Lateral united petals 2-lobed, not clawed, basal lobes small, orbicular, ca. 3 mm long, ca. 2 mm wide, with red spots; distal lobes dolabriform, ca. 12 mm long, ca. 6 mm wide, with dorsal inflexed auricle and notch in subapical portion on dorsal side. Filaments short, anther acute. Ovary erect, 5-carpellate. Capsule linear, 1.5–3 cm long, seeds 3–6, ca. 3 mm long.

Distribution and habitat. Yunnan; Sichuan; Guizhou; Guangxi. The species grows on damp ground near hills and streams in the understorey at an elevation of 800–1500 m.

Phenology. Flowering and fruiting occur from July to November.

Additional specimens. **CHINA.** **Guizhou:** Guiyang City, *Yuanxin Xiong* 86009 (PE); Zhenfeng County, *Tsiang* 4512 (PE); Wangmo County, *Gongfan Wang* 1-0142 (PE); Cengheng County, *Fengcai Wang* (PE); Renhuai City, *Lijiao Lou* MT571 (ZY); Zhijin Country, *Fang Cheng* 522425150910005LY (GZTM). **Guangxi:** Nandan County, *Shengxiang Yu* 3709 (PE). **Hunan:** *Daike Tian et al.*, LS-2677 (CSH). **Yunnan:** Dali City, *Qiwu Wang* 63390 (PE); Luquan County, *Yongjie Guo* 10CS1920 (KUN); Yangbi County, *Renchang Qin* 25280 (KUN, PE); Weixin County, *Spice Plant Investigation Team* 870552 & 870553 (KUN); Songming County, *Y.P. Chang* 0130 (PE). **Sichuan:** Chengdu City, *C.Y. Wang* 7469 & 7446 (PE); *Wenpei Fang* 13140 & 13701 (KUN); Emeishan City, *Jihua Xiong et al.*, 33487 & 33574 (IBK, PE); *Shengxiang Yu* 3274 (PE); *Hsiung et al.*, 33574 (IBSC); *Xiaojie Li LiXJ* 175 (KUN); *Zhongwu Yao* 3361 (PE); *Caiqi Li* 3998 (PE); *Sichuan Plants, Western Academy of Sciences* 891 (PE); *Gensheng Zhou* 81205 (PE); *Zhengyi Wu* 6439 (KUN); *T.N. Liou & C. Wang* 797 (PE); *Cehong Li* 96-39 (PE); *Chenglie Zhou* 7267 (PE); Mabian Country, *Dejun Yu* 4176 & 4175 (PE); *Deyuan Hong et al.*, s.n. (PE); Dujiangyan City, *Wenpei Fang* 6046 (PE).

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