

A novelty in *Ceratozamia* (Zamiaceae, Cycadales) from the Sierra Madre del Sur, Mexico: biogeographic and morphological patterns, DNA barcoding and phenology

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Abstract

Ceratozamia is a genus of cycads occurring in eastern Mexico and Central America. In this study, we describe a new species from the Pacific region of Mexico in Guerrero state. This locality represents the most northwestern Mexico distribution for the genus. We focus the comparison of this species with the most geographically proximate and phenotypically relevant lineages for this taxon. We followed an integrative taxonomy approach to evaluate the classification of these species, including geographic location, morphology, DNA barcoding and phenology as primary sources of systematic data. Within the morphological dataset, reproductive structures are described in detail and new characters are proposed for microsporophylls. The comparative morphology of these structures facilitated the elucidation of differences in forms and species for identification. The two chosen DNA barcoding markers – namely, the chloroplast genome coding region *matK* and the nuclear ribosomal internal transcribed spacer (ITS) region – had low divergence, allowing only 61% of species identification, suggesting slow molecular evolutionary rates. Besides employing these three basic sources of evidence, we introduced phenology as additional information for species circumscription. In addition, this work includes a brief review of the genus at the species-level. This is therefore the most recent review for *Ceratozamia* across its full geographic range (latitudinal and elevational). Overall, this work further contributes to a comprehensive framework for systematic studies in Mexican cycads.

Keywords

cloud forest, cycads, Guerrero, integrative taxonomy

Introduction

The Sierra Madre del Sur (SMS) is a biogeographic province assigned to the Mexican Transition Zone, which includes nearctic and neotropical ecosystems (Marshall and Liebherr 2000). This Mexican province runs northwest-southwest parallel to the Pacific Ocean coast throughout 1,200 km. It has great diversity of species, due to its high climatic and topographical heterogeneity (Morrone 2005; Santiago-Alvarado et al. 2016). Its rugged orography has been influenced by ancient geological events. In parallel, regional climatic cycles are relevant to explain its ecological heterogeneity, including overlaps between the Neotropical and Nearctic biotas, both of which house high species richness and endemics (Morrone 2010). The SMS has been divided into three subprovinces, in which the eastern Sierra Madre del Sur subprovince is the most heterogeneous in structural composition. This subprovince includes two districts: the Guerrero and Oaxaca highlands (Morrone 2017).

One of the least represented habitats in the SMS, and perhaps the least studied, is the SMS cloud forest. This type of vegetation is distributed unequally between 600 and 1,800 meters of elevation, and has a very disjointed and fragmented range caused by different climatic cycles (Luna-Vega et al. 1999). Moreover, the cloud forest is the most diverse habitat in Mexico per unit area and is found in small fragmented zones mainly in the Guerrero and Oaxaca highland districts (Rzedowski 1996; Challenger 1998).

The distribution of *Ceratozamia* Brongn. (Cycadales) is restricted to areas with high humidity in the main mountain systems of Mexico and Central America. The genus occurs in a spectrum of habitats: evergreen tropical forest, oak-pine forest, and cloud forest in the Sierra Madre Oriental (SMO), Sierra Madre de Chiapas (SMCh), SMS proper, and mountains of eastern Central America. The greatest diversity of this genus is found in the SMO. In the SMS, *Ceratozamia* has only been reported in Oaxaca (Vovides et al. 2004; Contreras-Medina 2016), but the north central portion of the SMS also may have suitable habitats for the genus. This is particularly so in Guerrero, which has small patches of cloud forest and is part of this biogeographic province (Luna-Vega et al. 1999).

Ceratozamia is easily diagnosed by the presence of two horns at the distal end of the sporophylls (Norstog and Nicholls 1997). This genus is one of the most complex morphological assemblages within the family Zamiaceae, because some of its species show considerable intrapopulation variation and most characters commonly used to diagnose species in other cycad genera are polymorphic (Martínez-Domínguez et al. 2017). Taxonomic and molecular systematic studies have contributed to clarify species identification and to provide taxonomic revisions with keys at the regional level (e.g. Pérez-Farrera et al. 2009; Martínez-Domínguez et al. 2016, 2017, 2018a). Gener-

ally, quantitative morphological characters provide limited information among closely related species (Martínez-Domínguez et al. 2017). However, reproductive characters have been poorly studied at the species level, both morphologically and in terms of phenological cycles. Detailed studies and descriptions of organs in the reproductive structures – mainly micro- and megasporophylls – could yield potentially useful diagnostic characters in the genus (Martínez-Domínguez et al. 2018a,b).

Recently, during a review of collections of *Ceratozamia* deposited at the FCME herbarium, our research group found a specimen collected in 1984 from the state of Guerrero. However, this material lacked reproductive structures indispensable for unequivocal identification. Because the previously known biogeographic pattern of this genus was restricted to southeastern and central Mexico, this discovery was a novelty, as the corresponding coordinates would represent the northernmost locality of *Ceratozamia* for the Mexican Pacific. Given that similar specimens from the same geographic point had not been collected again, we explored the corresponding area in search of this underdescribed population. After conducting extensive fieldwork in Guerrero to collect fresh material and monitor the attendant reproductive process, we compared the new specimens with all known species in the genus with an initial focus on similar species using geographic and morphological criteria. Formally, we adopted an integrative taxonomic approach (*sensu* De Salle et al. 2005; Goldstein and DeSalle 2010) that involves a set of inferential rules for corroboration or refutation of species hypotheses based on multiple sources of evidence ('taxonomic circle' *sensu* DeSalle et al. 2005). Elsewhere, we have successfully used this approach for species-level identification in *Ceratozamia*, explicitly considering intraspecific morphological variation (Martínez-Domínguez et al. 2017). To identify this unidentified herbarium sheet and additional specimens from two Guerrero populations, which ultimately turned out to match the new taxon herein described, we use geographic location information, both qualitative and quantitative morphological characters, character-based DNA barcoding and phenological data. Finally, we have explored how this taxonomic discovery might alter our understanding of biogeographic and evolutionary patterns in *Ceratozamia*.

Materials and methods

Specimen collection and morphological character coding

Twenty-one specimens were collected for the newly described taxon from two localities in Guerrero, Mexico. Leaf tissue was collected from all individuals for DNA sequencing and preserved in silica-gel. In total, we sampled 8 to 10 specimens approximately per population for *Ceratozamia robusta* Miq., and *C. subroseophylla* Mart.-Domínguez & Nic.-Mor., three and two populations, respectively (Appendix I). These specimens were collected during 2014 and 2017 by our research group, which have been deposited at the CIB and MEXU. In these collections, we have included the type locality and associated populations that correspond to the distribution range. Species selection was

further based upon morphological similarities with the initial *Ceratozamia* specimen from Guerrero. For this species, a set of 40 continuous, quantitative characters were coded along with another series of 39 discrete, qualitative characters. Additionally, we examined all herbarium specimens for the 30 species recognized in the genus, which are deposited at CIB, ENCB, FCME, MEXU, US, NY, and XAL. From the herbarium specimens for all species, we evaluated a set of 25 continuous and 23 discrete characters.

Biogeographic information for specimens

Herbarium specimens of *Ceratozamia* were reviewed and their geographic coordinates were used to compile a database. This information was verified in the geographic information system ArcMap GIS v.10.2. Ambiguous and/or doubtful geographic data were omitted; in cases where precise locality data were available, we georeferenced each locality with Google Earth Pro (2020) (<http://earth.google.es/>). To determine elevation for all registered samples, points of occurrence were superimposed on the ‘digital elevation model’ available from CONABIO (Guevara and Arroyo-Cruz 2016). The vegetation type for the populations of Guerrero taxon was characterized and classified following the biogeographical provinces of cloud forest according to CONABIO (2008).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from five individuals for each population collected in Guerrero using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Additionally, DNA was extracted from a single individual for *Ceratozamia chamberlainii* Mart.-Domínguez, Nic.-Mor. & D.W. Stev., *C. delucana* Vázq.Torres, A. Moretti & Carvajal-Hern., *C. mexicana* Brongn., and *C. totonacorum* Mart.-Domínguez & Nic.-Mor. Samples from these taxa were not included in previous works (cf. Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017; Medina-Villarreal et al. 2019). For amplification of the nuclear ribosomal internal transcribed spacer (ITS) region, primers 5a fwd (5'CCTTATCATTTAGAGGAAGGAG3') and 4 rev (5'TCCTCCGCTTATTGATATGC3') were used, whereas primers fwd (5'ATACCCCATTTTATTCATCC3') and rev (5'GTACTTTTATGTTTACGAGC3') were used for the maturase K (*matK*) chloroplast genome locus. The latter region was amplified in *Ceratozamia subroseophylla* (an ITS region sequence was previously available) and included in the molecular matrix. Choice of markers followed previous publications where they performed best in terms of number of diagnostic sites (Martínez-Domínguez et al. 2016, 2017). Amplification products were visualized in 1% ethidium bromide-stained, agarose gels. Bands of the expected molecular weight were purified with a QIAquick PCR Purification Kit (QIAGEN) and sent to Laboratorio de Secuenciación Genómica de la Biodiversidad y

la Salud (LANABIO; Instituto de Biología, UNAM) for automated DNA sequencing. All DNA sequence data were deposited in GenBank.

Character-based DNA barcoding

Electropherograms were edited and assembled using the program Sequencher v.4.8 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences were aligned in BioEdit v.7.0.9 using the 'multiple alignment' option in Clustal X (Thompson et al. 1997; Hall 1999). We manually checked and edited these alignments with MacClade v.4.03 (Maddison and Maddison 2001). A matrix of these sequences, plus a subset of sequences for *Ceratozamia* including previously published ITS and *matK* sequences currently available in GenBank, was then saved in Nexus format for further use (Suppl. material 1: File S1). All markers were assembled with SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The resulting dataset corresponding to all species currently and correctly ascribed to the genus in Mexico (Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017) was subject to character-based DNA barcoding in CAOS (Character Attribute Organization System; Sarkar et al. 2008). Following the recommendations in the CAOS manual, a guide tree was prepared using the molecular matrix of *Ceratozamia* DNA sequences. The total length of the concatenated ITS and *matK* data matrix was 1813 nucleotide sites, including gaps. Cladistic analyses on this matrix in TNT v.1.5 (Goloboff et al. 2008) generated nine shortest, equally parsimonious trees. Because relationships in the corresponding strict consensus tree were not completely resolved (Suppl. material 2: Fig. S1), this tree was manually edited to avoid polytomies, preserving the arrangement of resolved clades in all trees. The topology of the resulting guide tree was saved in the Nexus format for further CAOS analyses. Identification of DNA diagnostics was carried out with the *P-gnome* program. Only simple pure ('sPu' *sensu* Sarkar et al. 2008) characters (i.e. attributes) with confidence value of 1.00 were selected.

Reproductive phenology data

The populations registered for the Guerrero taxon, as well as two populations for both *Ceratozamia robusta* and *C. subroseophylla*, were monitored for phenological observations of ovulate strobili (Appendix I). In addition, herbarium specimens of known wild collections in CIB, MEXU, and XAL were examined for complementary information in this regard (Suppl. material 3: File S2). All observations were categorized into one of the following three states, which were determined according to Martínez-Domínguez et al. (2018b): (i) receptivity (R); (ii) late ovulate (LO); and (iii) degraded (D). Determining phenological patterns for other species in the genus was not possible due to the lack of reproductive structures in herbaria.

Results

Biogeographic patterns

The genus *Ceratozamia* has a continuous but restricted distribution along two of the major mountain ranges in Mexico – namely, the Sierra Madre Oriental (SMO) and the Sierra Madre del Sur (SMS), Belize, and some lowlands in Los Tuxtlas (Veracruz, Mexico) and Honduras (Fig. 1). Elevational ranges for most species are broad, usually occurring between 800 to 1,400 meters. However, some species are distributed at the extremes of this general pattern. *Ceratozamia miqueliana* H.Wendl., can occur at elevations as low as 19 meters, and *C. zaragozae* Medellín-Leal at elevations up to 2,030 meters (Fig. 2). The species with the greatest elevation range are *C. fuscoviridis* W. Bull, *C. miqueliana*, *C. robusta*, and *C. zoquorum* Pérez-Farr., Vovides & Iglesias. In turn, the two species with the narrowest range of elevational variation are *C. hildae* G.P.Landry & M.C.Wilson and *C. euryphyllidia* Vázq.Torres, Sabato & D.W.Stev. The new populations registered from Guerrero are the northernmost for the genus on the western ranges of the coastal Pacific Ocean region; this location corresponds to the northern end of the SMS province, from 1,100 to 1,400 meters of elevation (Fig. 1).

The *Ceratozamia* taxon herein described from Guerrero is found in relictual cloud forest and the transition zone between cloud forest and oak forest on rocky limestone slopes. In contrast, *C. robusta* is found in evergreen tropical forest and oak forest, whereas *C. subroseophylla* only inhabits evergreen tropical forest. These two related *Ceratozamia* species occur on clay soils with isolated rocks of volcanic origin, whereas the Guerrero taxon occurs on karstic rocks. In the context of classifications of biogeographical provinces of cloud forest, the Guerrero taxon occurs in southern coastal mountain range and the Putla subregion.

Comparisons of quantitative and qualitative morphological characters

In terms of vegetative morphology, three general groups of species within *Ceratozamia* can be distinguished. These groups include plants with (i) very wide leaflets, between (2.5) 4.5–17.6 cm, oblong to oblanceolate and obovate, (ii) wide leaflets of 2.3–4.6 cm, lanceolate to linear, and (iii) narrow leaflets, 0.8–2.2 cm wide, linear to lanceolate. The first group includes the species *C. miqueliana*, *C. zoquorum*, *C. latifolia* Miq., *C. huastecorum* Avendaño, Vovides & Cast.-Campos, *C. euryphyllidia*, *C. decumbens* Vovides, Avendaño, Pérez-Farr. & Gonz.-Astorga, *C. hildae*, *C. morettii* Vázq.Torres & Vovides, *C. santillanii* Pérez-Farr. & Vovides, *C. hondurensis* J.L.Haynes, Whitelock, Schutzman & R.S.Adams, *C. chamberlainii*, and *C. totonacorum*. Plants belonging to this group of species also have hypogeous stems, few leaves, and small ovulate strobili, with the exception of *C. miqueliana*, *C. chamberlainii*, and *C. totonacorum*, which have ovulate strobili up to 30 cm long and epigeous stems. The second group has epigeous stems, many leaves, and cylindrical and long ovulate strobili; this group contains

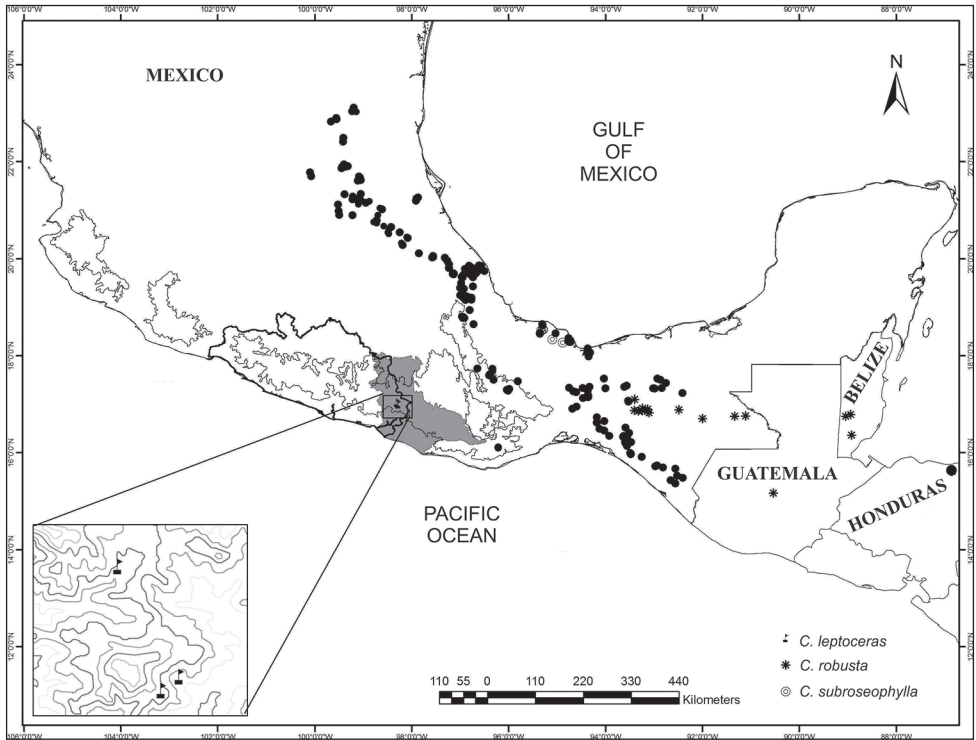


Figure 1. Distribution map of the genus *Ceratozamia* (black solid circles). Distributions for species morphologically similar to *Ceratozamia leptoceras* are represented with an asterisk and a double circle. Guerrero state and Putla subregion of cloud forest are shown with outline and shaded area, respectively. Inset: points corresponding to the Guerrero mountain range where *Ceratozamia* was collected.

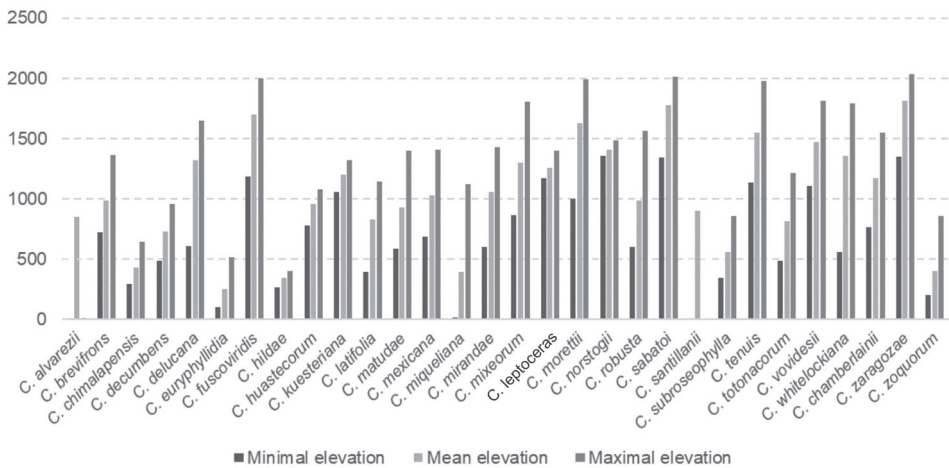


Figure 2. Elevational distribution of *Ceratozamia*. Intervals of altitudinal distribution of *Ceratozamia* species are shown.

C. mexicana, *C. subroseophylla*, *C. robusta*, *C. whitelockiana* Chemnick & T.J.Greg., *C. mixeorum* Chemnick, T.J.Greg. & Salas-Mor., *C. delucana*, and *C. brevifrons* Miq. The third group has epigeous and semi-epigeous stems and greater variation in relation to the size of ovulate strobili; it includes *C. zaragozae*, *C. norstogii* D.W.Stev., *C. alvarezii* Pérez-Farr. Vovides & Iglesias, *C. mirandae* Vovides, Pérez-Farr. & Iglesias, *C. vovidesii* Pérez-Farr. & Iglesias, *C. matudae* Lundell, *C. tenuis* (Dyer) D. W. Stev. & Vovides, *C. sabatoi* Vovides, Vázq.Torres, Schutzman & Iglesias, *C. chimalapensis* Pérez-Farr. & Vovides, *C. fuscoviridis*, and *C. kuesteriana* Regel. In preparation for the integrative taxonomy analysis, morphological comparisons revealed that the taxon from Guerrero has epigeous stems, median leaflets of 1.9–2.8 cm width, and long ovulate strobili. These data strongly suggest affinity of the Guerrero taxon to the second group.

In this context, the Guerrero taxon has a close morphological similarity to *Ceratozamia subroseophylla* and *C. robusta*; however, it has differences when compared to all species within the second group (see the taxonomic key below, for more details). Further morphological comparisons between these three entities reveal that only a few vegetative morphological characters provide support for species delimitation. The detailed morphological differences between *C. subroseophylla*, *C. robusta* and the Guerrero taxon are listed in Tables 1, 2. In this regard, the leaves of the Guerrero taxon show great morphometric affinity with *C. robusta* and *C. subroseophylla* with a leaf length of ranging from 1.20 to 2.80 meters. In contrast, the leaf position of the new taxon is descending, whereas in the other two species it is erect (Table 1). Additionally, the Guerrero taxon bears linear and membranaceous leaflets in contrast to *C. robusta* and *C. subroseophylla*, which have lanceolate and papyraceous to subcoriaceous leaflets

Table 1. Comparison of diagnostic qualitative morphological characters between *Ceratozamia leptoceras* and morphologically similar species.

Characters	Species		
	<i>C. leptoceras</i>	<i>C. robusta</i>	<i>C. subroseophylla</i>
Leaf color at emergence	<i>Green with copperish-green petiole</i>	Dark brown	Yellowish-brown
Leaf position	<i>Descending</i>	Ascending	Ascending
Prickles on petiole	<i>Thin</i>	Robust	Robust
Leaflet shape	<i>Linear</i>	Lanceolate	Lanceolate
Leaflet texture	<i>Membranaceous</i>	Papyraceous	Papyraceous
Leaflet base color	Green (brown only in articulation)	Green (yellow in juvenile leaves)	Green (brown in juvenile leaves)
Ovulate strobilus color	<i>Copperish-green with greyish-black pubescence</i>	Dark green with dark trichomes	Rosaceous-green with brown trichomes
Ovulate strobilus apex	<i>Acute</i>	Acuminate	Mucronate
Megasporophyll horns shape	<i>Straight</i>	Straight	Straight
Megasporophyll distal face form	<i>Prominent</i>	Prominent	Prominent
Microsporophylls horns direction	<i>Straight</i>	Recurved	Recurved
Microsporophylls horns shape	<i>Thin</i>	Robust	Robust
Infertile portion of microsporophylls	<i>Linear</i>	Rounded	Rounded
Fertile portion of microsporophylls	<i>Deeply lobate</i>	Deeply lobate	Lobate

Autapomorphic character states are shown in italics.

Table 2. Comparison of diagnostic quantitative morphological characters between *Ceratozamia leptoceras* and morphologically related species; values are given in centimeters. The reproductive structures were measurements at maturity.

Characters	Species		
	<i>C. leptoceras</i>	<i>C. robusta</i>	<i>C. subroseophylla</i>
Pairs of leaflets*	22–61	13–58	15–40
Distance between median leaflets	1.8–2.8	2–3.9	0.9–3.9
Length of median leaflets	28–43.5	30.5–44.5	23.5–44.5
Width of median leaflets	1.9–2.8	3.1–3.9	2.4–4
Length of ovulate strobilus	23.5–28	26–40	15.5–23.5
Diameter of ovulate strobili	9.5–11	11.5–14.5	7–10
Length of ovulate strobili peduncle	11–16	5–6.2	9.8–17.5
Number of orthostichies*	8–9	9–12	9–11
Number of megasporophylls per column*	7–9	17–20	11–13
Length of megasporophyll horns	<i>0.60–0.81</i>	0.38–0.50	0.41–0.62
Length of pollen strobili	<i>42–45</i>	60–70	15–30
Diameter of pollen strobili	6.0–7.8	7–8.5	6.2–8
Length of pollen strobili peduncle	13–19	14–17	10–15
Width of microsporophylls	1.09–1.35	1.14–1.80	1.01–1.24
Length of microsporophylls	2.21–2.55	2.33–3.0	1.47–2.80
Length infertile portion of microsporophylls	<i>0.83–0.96</i>	0.45–0.65	0.49–0.59
Distance between microsporophyll horns	0.44–0.56	0.55–0.75	0.55–0.42
Length of microsporophyll horns	<i>0.1–0.23</i>	0.26–0.40	0.27–0.38

Diagnostic character states are in italics. *Meristic characters.

(Fig. 3). Furthermore, the shape of prickles on the petiole are robust in *C. subroseophylla* and *C. robusta*, whereas in the Guerrero taxon the prickles are thin.

The primary diagnostic morphological characters lie in the reproductive structures. However, quantitative characters traditionally used for species distinction overlap in this case, both in length and diameter of ovulate strobili and peduncle length of ovulate strobili (Table 2). The most relevant and diagnostic characters are, instead, the number of megasporophylls per orthostichy and the length of horns in the megasporophylls (Table 2). Moreover, the pollen strobilus in the Guerrero taxon has several differences when compared to species in the second group, especially in terms of microsporophyll morphology: in the Guerrero taxon, the horns of microsporophylls are straight, thin, the infertile portion is linear, and the fertile portion is deeply lobate (Fig. 4).

Character-based DNA barcoding

Sequences of *matK* and ITS allowed the molecular identification of 19 out of 31 *Ceratozamia* species (Table 3). The ITS region alone granted 45% identification success of recognized species, which increased to 61% after the inclusion of *matK*. Surprisingly, molecular diagnosability determined through character-based DNA barcoding in CAOS indicated that the taxon from Guerrero has two autapomorphies within *matK* [i.e. ‘simple pure’ (sPu) characteristic attributes *sensu* Sarkar et al. 2008] in all

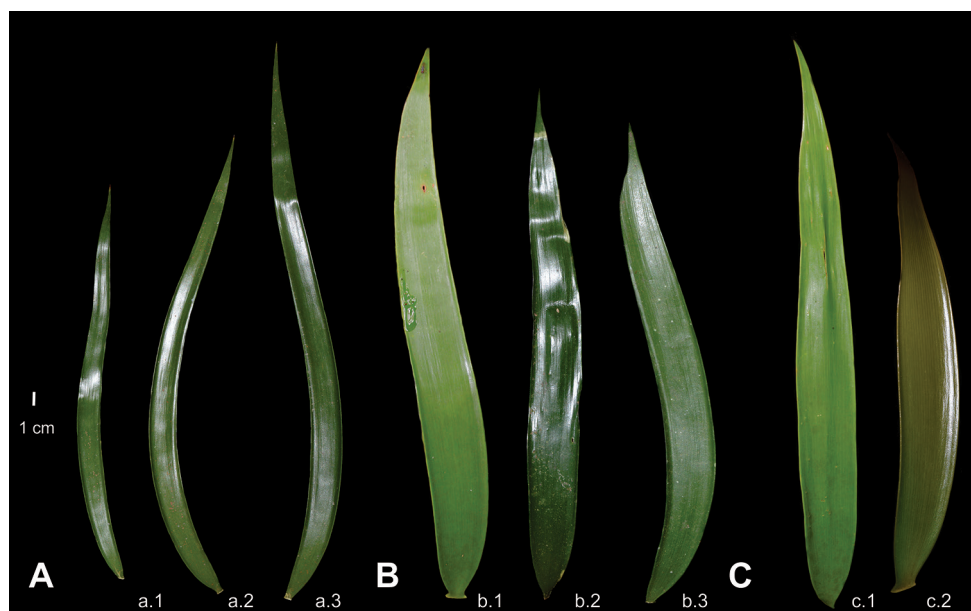


Figure 3. Leaflet variation at the population level **A** *Ceratozamia leptoceras*, a.1, a.2 San Pedro Cuitlapan, a.3 Riverbank “Chipili” **B** *C. robusta*, b.1 Cañón del Sumidero, b.2, b.3 Cuchumbak **C** *C. subroseophylla*, c.1 Sinapan, c.2 “El Vigía”. All leaflets were collected from middle and right side of leaf with exception of two first leaflets for *C. leptoceras* (left).

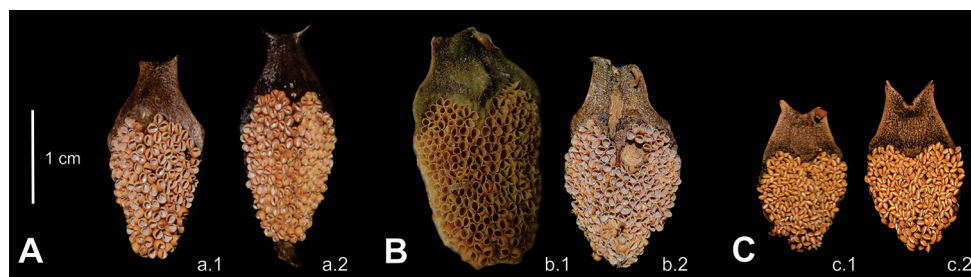


Figure 4. Abaxial view of microsporophylls **A** *Ceratozamia leptoceras*, a.1, a.2 San Pedro Cuitlapan **B** *C. robusta*, b.1 Cuchumbak, b.2 Cañón del Sumidero **C** *C. subroseophylla*, c.1 “El Vigía”, c.2 Sinapan. For more detail of differences in character states see Table 1.

five sequenced individuals (G: 1592; C: 1807). No interindividual or intrapopulation polymorphic sites were detected.

Among the species with morphological affinity to the Guerrero taxon, *Ceratozamia robusta* and *C. subroseophylla* were diagnosable with ITS and ITS+*matK*, respectively. Notably, *C. subroseophylla* has a 15 nucleotide deletion in region 569–583 of the

Table 3. Species identification using the candidate combination of loci for character-based DNA barcoding in *Ceratozamia*. The dash indicates absence of diagnostic sites.

Species	ITS region	matK	Total DNA diagnostic sites
<i>C. alvarezii</i>	1	–	1
<i>C. brevifrons</i>	–	–	–
<i>C. chamberlainii</i>	–	1	1
<i>C. chimalapensis</i>	–	–	–
<i>C. decumbens</i>	–	–	–
<i>C. delucana</i>	–	–	–
<i>C. euryphyllidia</i>	1	–	1
<i>C. fuscoviridis</i>	–	–	–
<i>C. hildae</i>	–	–	–
<i>C. hondurensis*</i>	–	–	–
<i>C. huastecorum</i>	–	–	–
<i>C. kuesteriana</i>	6	3	9
<i>C. latifolia</i>	1	–	1
<i>C. leptoceras</i>	–	2	2
<i>C. matudae</i>	4	2	6
<i>C. mexicana*</i>	1	–	1
<i>C. miqueliana</i>	–	–	–
<i>C. mirandae</i>	–	2	2
<i>C. mixeorum</i>	3	–	3
<i>C. moretii</i>	–	3	3
<i>C. norstogii</i>	–	–	–
<i>C. robusta</i>	1	–	1
<i>C. sabatoi</i>	1	1	2
<i>C. santillanii</i>	2	–	2
<i>C. subroseophylla</i>	1	1	2
<i>C. tenuis</i>	2	–	2
<i>C. totonacorum</i>	–	–	–
<i>C. vovidesii</i>	–	3	3
<i>C. whitelockiana</i>	–	–	–
<i>C. zaragozae</i>	1	–	1
<i>C. zoquorum</i>	8	–	8

Gray squares indicate the presence of at least one DNA diagnostic site for the corresponding locus. For species with an asterisk, only ITS sequences are available.

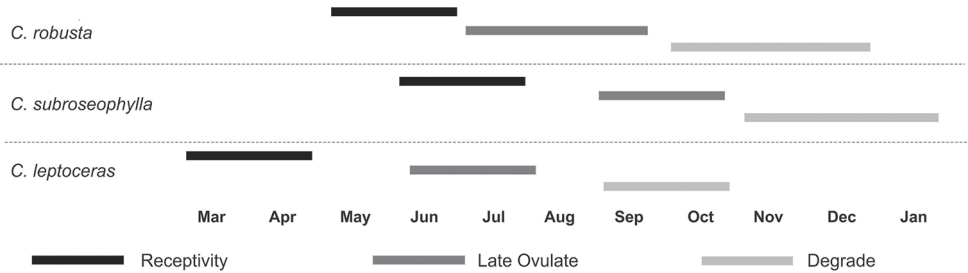


Figure 5. Phenological patterns of ovulate strobili in *Ceratozamia leptoceras* and morphologically similar species.

aligned matrix; this type of deletion is not present in any other *Ceratozamia* species (Table 3; Suppl. material 4: Table S1) and, thus, represents an autapomorphy. Our samples from Guerrero are more distant genetically from other phenotypically similar species such as *C. mexicana*, *C. mixeorum*, *C. delucana*, *C. whitelockiana* and *C. brevifrons*. The latter three species did not have any diagnostic sites; however, a closer visual inspection of groups generated by CAOS showed divergences with the taxon from Guerrero and greater similarities with *C. miqueliana* and *C. morettii*.

Ceratozamia kuesteriana had the greatest number of diagnostic sites (nine in total). This species was followed by *C. matudae* and *C. zoquorum*, with six and five diagnostic characters, respectively. The remaining species of the genus had low values of DNA diagnostics; the number of diagnostic sites by species ranges from one to three DNA diagnostics, and for nine species diagnostic sites were consistently absent (Table 3). In the case of *C. chamberlainii* only a gap in *matK* was recovered as a potential diagnostic site and it was included as a diagnostic character due to its location in a coding region.

Ovuliferous reproductive phenology

Overall, the Guerrero taxon has a phenological reproductive pattern that differs from its most morphologically related species (Fig. 5). The phenological cycle of *Ceratozamia robusta* and *C. subroseophylla* is mutually more similar; however, in the first species, the R phase occurs from May to June, whereas in *C. subroseophylla* this phase occurs from June to July. OT and D phases for these species have different offsets (Fig. 5). In contrast, the R phase in the Guerrero taxon occurs from March to April, while the D phase runs from September to October.

Integrative taxonomic inference of a new *Ceratozamia* species

According to the inferential rules of the ‘taxonomic circle’ in our integrative taxonomy approach, the specimens collected from Guerrero were marked with a ‘red flag’ – i.e. as a hypothetical species demanding test. This hypothesis was corroborated after detection of morphological diagnostic characters, DNA diagnostics and phenological differences, and after the establishment of the particular geographic locality of the collected specimens. Formally, these specimens are recognized here as belonging to a new species based on the presence of (i) distinct morphological qualitative characters, particularly in reproductive structures; (ii) exclusive DNA diagnostic sites in *matK*; (3) distinctions in the phenological pattern in comparison to similar species; and (4) a separate geographical range, which suggests allopatric geographic isolation (i.e. barriers to gene flow). In summary, the new *Ceratozamia* species from Guerrero is diagnosable according to all tested criteria.

Taxonomic treatment

***Ceratozamia leptoceras* Mart.-Domínguez, Nic-Mor., D.W. Stev. & Lorea-Hern., sp. nov.**

urn:lsid:ipni.org:names: 77211166-1

Figures 6–8

Type. MEXICO. Guerrero: Tlacoachistlahuaca, 3 Km NW of San Pedro Cuitlapan, 1,400 m, 26 Jun. 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1867 ♀ (holotype CIB; isotypes MEXU, NY).

Ceratozamia leptoceras is most similar to *C. robusta*, but can be distinguished by its linear membranaceous leaflets and petioles with thin prickles. In addition, *C. leptoceras* is easily distinguished from its congeners by having obconic microsporophylls with a long, linear infertile portion (0.83–0.96 cm), and two thin horns; ovulate strobilus with abundant pubescence at base of megasporophylls, 8–9 orthostichies, and 7–9 sporophylls per orthostichy.

Additional specimens examined (paratypes). MEXICO. **Guerrero:** Cochoapa El Grande, 4 km to W-NW of San Pedro by a logging road, 1,170 m, 4 Feb 1984, *F. Lorea-Hernández* 2928 (FCME); Tlacoachistlahuaca, San Pedro Cuitlapan, riverbank “Chipili”, 1,200 m, 29 May 2019, *L. Martínez-Domínguez et al.* 1756 (CIB), 1757 (CIB, MEXU), 1758 (XAL), 1759 (CIB, MEXU); *F. Nicolalde-Morejón et al.* 3173 (XAL), 3174 (FCME), 3175 (CIB); 3 km NW of San Pedro Cuitlapan, 1,400 m, 26 June 2019, *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1860, 1861 (MEXU), 1862–1866 (CIB).

Description. *Stem* epigeous, erect to decumbent, 30–150 cm in length, 11–35 cm in diameter, covered with leaf bases. *Cataphylls* persistent, reddish-brown, densely brownish tomentose abaxially at emergence, pubescent at maturity, triangular, apex acuminate, 9–11 × 2.5–3 cm at base. *Leaves* 7–50, descending, 93.5–281 cm, green at emergence with sparse reddish-brown pubescence, glabrous at maturity. *Petiole* terete, linear, 45–85 cm, armed with long (0.48–0.68 cm) and thin prickles, copperish-green in mature leaves. *Rachis* terete, linear, 75–196 cm, armed with long and thin prickles, green in mature leaves. *Leaflets* 22–61 pairs, linear, abaxially curved, not basally falcate, membranaceous, flat, opposite to subopposite, plane, green, adaxial and abaxial surfaces glabrous, acuminate and symmetric apex, attenuate at base, with conspicuous and green veins; median leaflets 28–43.5 × 1.9–2.8 cm, 1.8–2.8 cm between leaflets; articulations generally copperish-green. *Pollen strobilus* generally solitary (rarely 2), cylindrical, erect, 40–45 cm in length, 6.0–7.8 cm in diameter, brownish-yellow at emergence, yellowish-green with brownish trichomes at maturity; peduncle tomentose, reddish-brown to brown, 13–19 cm in length, 1.5–2.0 cm in diameter; microsporophylls 2.1–2.45 × 1.09–1.30 cm, obconic, non-recurved distal face, fertile portion deeply lobate, infertile portion 0.83–0.96 cm, linear, horns 0.1–0.23 cm, straight, thin, 0.44–0.56 cm between horns, 180–230 sporangia on abaxial side. *Ovulate strobilus* solitary, cylindrical, erect,

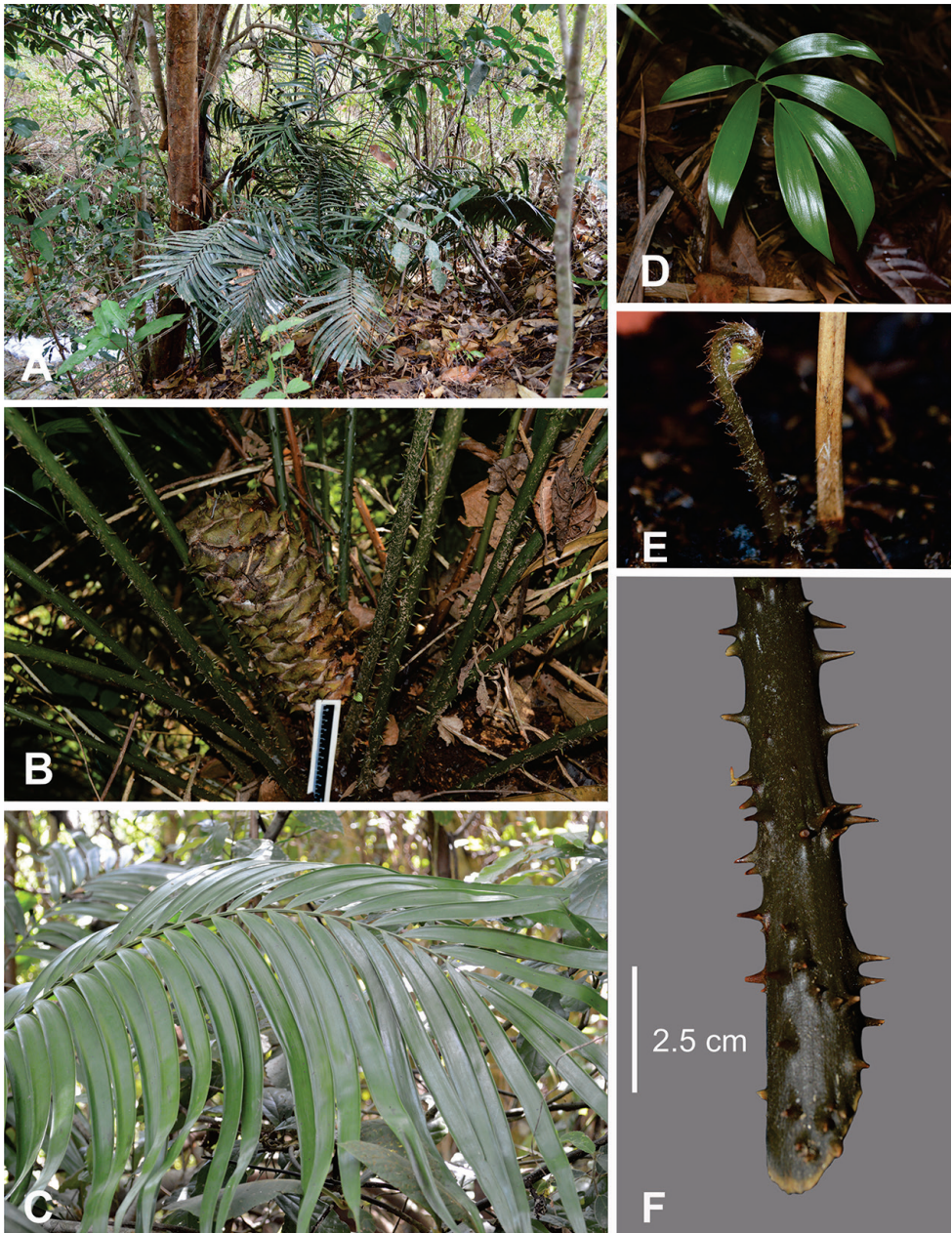


Figure 6. *Ceratozamia leptoceras* **A** adult plant in habit **B** ovulate strobilus **C** detail of leaflets **D** seedling **E** ptyxis **F** prickles on petiole.

23.5–28 cm in length, 9.5–11 cm in diameter, brownish-green with greyish-black trichomes at emergence, copperish-green with greyish-black pubescence at maturity, acute apex; peduncle tomentose, brown, 11–16 cm in length, 1.5–2.0 cm in diameter; megasporophylls 56–81, 8–9 orthostichies (column), 7–9 sporophylls per column, 4.9–5.6 ×

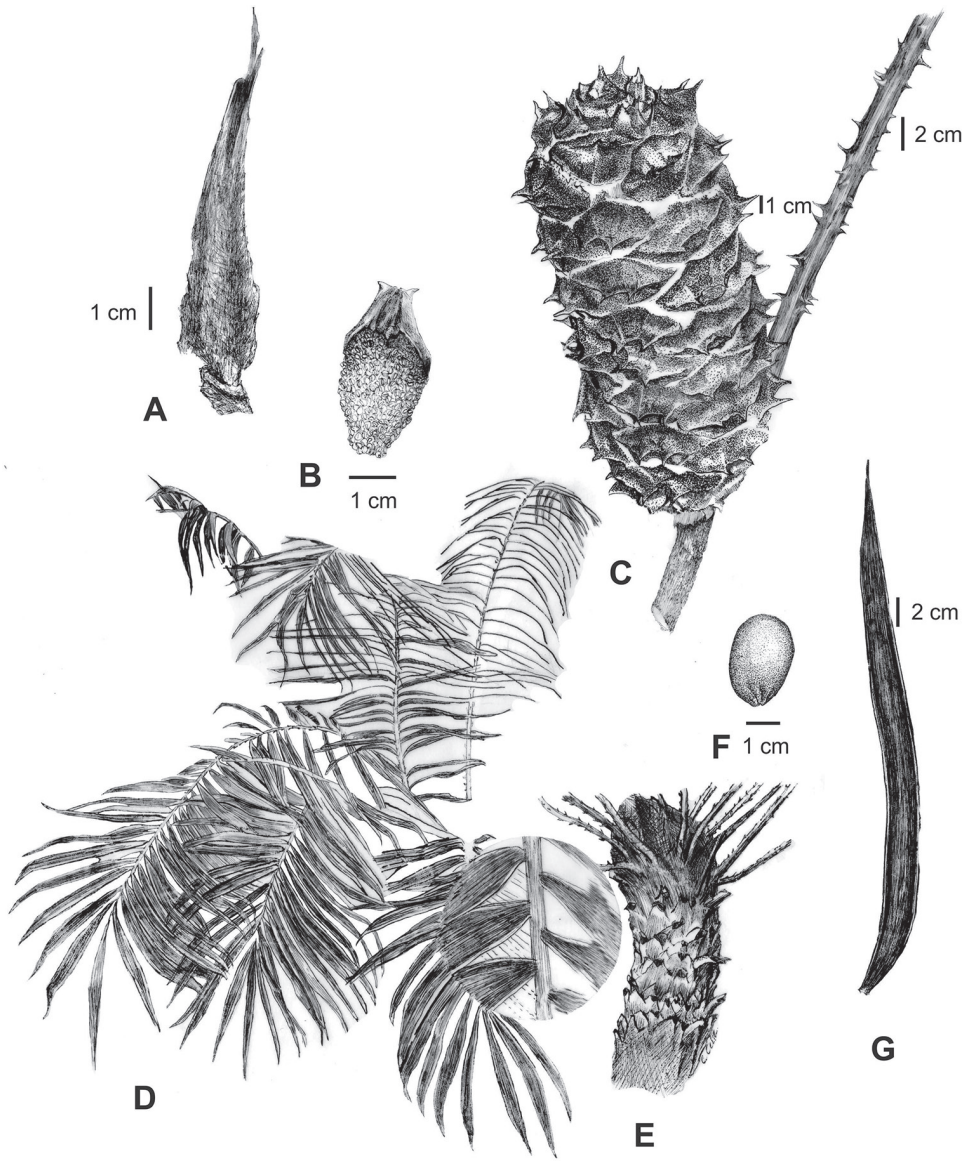


Figure 7. Illustration of *Ceratozamia leptoceras* **A** cataphyll **B** microsporophyll **C** ovulate strobilus **D** leaves and detail of leaflets **E** stem **F** seed **G** leaflet. This illustration is based on L. Martínez-Domínguez & F. Nicolalde-Morejón 1867, with exception microsporophyll, which is based on L. Martínez-Domínguez et al. 1757.

2.2–2.6 cm, prominent distal face, horns 0.63–0.81 cm, straight, 0.95–1.35 cm between horns, straight angle between horns. *Seeds* ovoid, 2.43–2.71 cm in length, 1.4–1.8 cm in diameter, sarcotesta whitish-pink when immature, light brown at maturity.

Etymology. The specific epithet alludes to microsporophylls horns shape, which are short and thin. This name comes from the Greek “lepto”, which means thin or fine, and “ceras” in reference to horns.

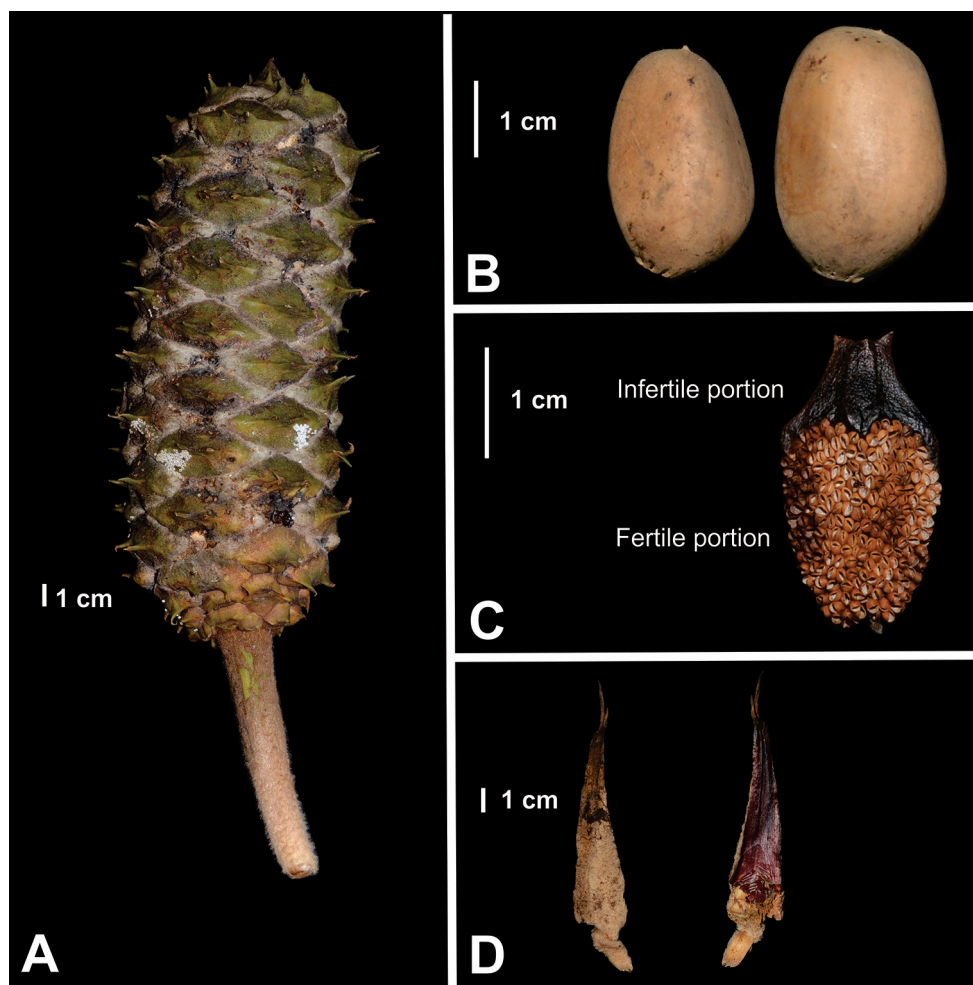


Figure 8. *Ceratozamia leptoceras* **A** detail of ovulate strobilus and megasporophylls **B** seeds **C** abaxial view of microsporophylls **D** cataphylls **A, B, D** are based on *L. Martínez-Domínguez & F. Nicolalde-Morejón* 1867; **C** is based on *L. Martínez-Domínguez et al.* 1757.

Distribution and habitat. Only known from Guerrero, Mexico, on the karstic rocks within the elevation range of 1,170–1,400 m of the Sierra Madre del Sur subprovince of the Guerreran district (Morrone 2017) (Fig. 1). This species occurs in cloud forest. The climate type is (A) C (w2)-semi-warm temperate subhumid with summer rains, and the annual precipitation is from 2, 000 to 2, 500 mm (García 2004).

Phenology. The leaves are produced in groups of 9 to 15 and mature almost simultaneously. Ovulate strobili mature from June to July; seeds mature from August to September. Pollen strobili mature from January to May.

Common names. The common local name for this species by the “Mixteco” ethnic group is *Shalukaá*.

Taxonomic keys to *Ceratozamia leptoceras* and morphologically related species

Key for vegetative plants

- 1 Leaflets coriaceous.....2
- Leaflets membranaceous to papyraceous.....3
- 2 Leaflets keeled; petiole with long (0.3–0.6 cm) prickles robust and abundant (> 50).....*C. brevifrons*
- Leaflets flat; petiole with short (0.1–0.2 cm) prickles thin and sparse (< 40) 4
- 4 Leaves ascending; new leaves yellowish-green at emergence. Cataphylls pubescent to tomentose*C. delucana*
- Leaves descending; new leaves light-green at emergence. Cataphylls pubescent to scarcely pubescent*C. mexicana*
- 3 Leaves descending; petiole unarmed to armed with thin prickles.....5
- Leaves ascending; petioles armed with robust prickles8
- 5 Leaflets linear, membranaceous. New leaves green with copperish-green petiole and rachis..... *C. leptoceras*
- Leaflets lanceolate, papyraceous. New leaves green6
- 6 Petiole unarmed to armed with scarce prickles (< 15)..... *C. whitelockiana*
- Petiole armed with abundant prickles (> 20)7
- 7 Leaflets with adaxial side glabrous; leaflets in median portion inserted at acute angle along rachis.....*C. mixeorum*
- Leaflets glaucous; leaflets in median portion inserted at right angle along rachis*C. delucana*
- 8 New leaves dark brown at emergence. Leaflets in median and apical portion abaxially curved *C. robusta*
- New leaves yellowish brown at emergence. Leaflets abaxially curved in median and apical portion mostly planar.....*C. subroseophylla*

Key for microsporangiate plants

- 1 Microsporophylls discoid, infertile portion orbicular.....*C. delucana*
- Microsporophylls obconic, infertile portion rounded to linear2
- 2 Microsporophylls with straight horns.....3
- Microsporophylls with recurved horns4
- 4 Pollen strobili 60–70 cm long; microsporophylls with infertile portion deeply lobate..... *C. robusta*
- Pollen strobili < 50 cm long; microsporophylls with infertile portion lobate6
- 6 Peduncle of pollen strobili > 3.5 cm, tomentose reddish-brown to light brown .7
- Peduncle of pollen strobili <3 cm, glabrous to scarcely pubescent..... *C. whitelockiana*
- 7 Pollen strobili green with black trichomes, peduncle 3.5–5 cm long *C. mexicana*
- Pollen strobili light green with brown-reddish trichomes, peduncle 10–15 cm long*C. subroseophylla*

- 3 Microsporophylls with infertile portion linear 5
- Microsporophylls with infertile portion rounded *C. mixeorum*
- 5 Microsporophylls with infertile portion of 0.4–0.61 cm long, horns 0.24–0.33 cm long *C. brevifrons*
- Microsporophylls with infertile portion of 0.83–0.96 cm long, horns 0.1–0.23 cm long *C. leptoceras*

Discussion

In this synthesis of *Ceratozamia*, we constructed a database for the genus based with geographic, morphological, and molecular data through the use of comparative and character-based DNA barcoding methods. In addition, we investigated the potential value of phenological reproductive patterns for species delimitation; this type of ecological information has been scarcely studied in cycads (Stevenson 1981; Clugston et al. 2016) or in integrative taxonomy studies of gymnosperms. Considering the different male plants in a population, the phenology of pollen strobili shows a broader cycle at the population level; the lifespan of a pollen strobilus is shorter than for ovulate strobili, and an open pollen phase is prolonged in different individual plants (Martínez-Domínguez et al. 2018b). In contrast, duration of ovulate strobili exhibits high specificity in the receptivity phase, which suggests that these data are taxonomically informative. Our findings on the length of the lifespan of reproductive structures and phenological phases coincide with our previous observations in *C. tenuis* (Martínez-Domínguez et al. 2018b). *Ceratozamia leptoceras* has a different phenological pattern for ovulate strobili compared to its morphologically similar congeners, which perhaps represents a reproductive barrier in the field (Fig. 5). Unfortunately, these data are not available for all *Ceratozamia* species.

Despite the species diversity of *Ceratozamia* and its restricted geographic distribution, a formal infrageneric classification for the genus has not been proposed. This is mainly because previously reconstructed phylogenetic relationships display remarkable differences in the number of clades and the members of the clades (cf. González and Vovides 2002, 2012; Medina-Villarreal et al. 2019). This situation has led to an incorrect identification of specimens that unfortunately has limited the inference of evolutionary relationships, with contradictory results inexplicably unrecognized (cf. Medina-Villarreal and González-Astorga 2016; Medina-Villarreal et al. 2019). The groups within the genus are based on similarities in geographic distribution and morphology (Vovides et al. 2004); however, *Ceratozamia* species complexes defined by these authors are more influenced by geography. In this work, we follow the proposal of Stevenson et al. (1986) to address morphology in this genus. Our morphological description for all *Ceratozamia* species recognized tree groups based on the affinity of morphological characters, both vegetative and reproductive, which facilitates comparisons between species.

Under integrative taxonomy criteria (*sensu* De Salle et al. 2005), we have proposed a new *Ceratozamia* species from the Sierra Madre del Sur. This biogeographic province

is a salient area in terms of gymnosperm diversity in the country (Contreras-Medina 2016). This new record for the genus in Guerrero represents an expansion of the distribution pattern for the genus on the Pacific seaboard, which opens new questions on the influence of mountain systems on the diversity of the Mexican flora. Our survey shows that the vast majority of *Ceratozamia* species grow at relatively high elevations (Fig. 2); the genus has standard elevation gradient patterns and does not exhibit occurrences at higher elevations or lower elevations. Therefore, it appears that *Ceratozamia* is poorly adapted at lower and very high elevations (Fig. 2). Species with the lowest elevation distributions occur in southern Mexico and Central America, and those at higher elevations occur in the SMO, Highlands of Chiapas, and SMS. This atypical distribution pattern seems to be favored by mountain regions, mainly in areas with cloud forest.

Historically, taxonomic research in the genus *Ceratozamia* has been characterized by difficulties in species identification (Miquel 1868). Recently, new species have been described (e.g. Martínez-Domínguez et al. 2018a) but only some of them have been evaluated. Particularly, in relation to DNA datasets, only 19 species could be diagnosed with ITS+*matK*. Other loci already tested (*psbK/I*, *rpoC1*, *rbcL* and *atpF/H*) have not provided enough variation for improved resolution (see Nicolalde-Morejón et al. 2011; Martínez-Domínguez et al. 2017). As an approach to molecular species identification, character-based DNA barcoding is susceptible to the addition of new sequences (Nicolalde-Morejón et al. 2010); therefore, further sequencing of new loci (e.g. single-copy nuclear genes; Salas-Leiva et al. 2014) could contribute new molecular diagnostic characters and improved resources for automated species identification in *Ceratozamia* and other cycad genera.

In terms of morphological taxonomic evidence, vegetative characters have been widely used in the genus to identify species (Vovides et al. 2004; Whitelock 2004; Pérez-Farrera et al. 2009). However, these characters are polymorphic in some species, which hinders the construction of dichotomous keys (Martínez-Domínguez et al. 2017). The classification of strictly allopatric taxa, as is the case in some *Ceratozamia* species, will always remain arbitrary to some degree so that the evaluation of new characters is a pressing task. In this work, we introduced previously unreported reproductive characters such as the internal parts of the strobilus, which have not been described before in detail for the genus *Ceratozamia* (Fig. 4; Table 1). Comparisons of pollen strobili and the microsporophylls among different species indicated strong morphological divergence limited to certain groups of related species (Fig. 4). For this reason, we only present a key for the species group that includes the newly described taxon. Thus, it is necessary to explore these reproductive characters for the rest of the species in the genus. Keys based on vegetative features still preserve their relevance due to the dioic condition of these gymnosperms, and the lack of reproductive structures in materials deposited at the consulted herbaria (Moretti et al. 1989). However, we consider that, in view of the complexity of vegetative morphological variation, keys based on reproductive characters could function as excellent support tools in the systematics of cycad genera such as *Ceratozamia*.

Species delimitation in *Ceratozamia* exhibits a high degree of complexity (Whitelock 2004). The morphological and molecular evidence available for the genus at pre-

sent does not allow the diagnosability of all species. For example, *C. hildae* is a morphologically distinctive species, but lacks molecular diagnostics; in contrast, *C. vovidesii* and *C. mirandae* are species that share many morphological affinities, but variation at the molecular level facilitates their taxonomic differentiation (Table 3). This taxonomic-systematic scenario points to the need to explore new molecular markers and the evaluation of variation between populations with morphological, molecular, and phenological evidence for some closely related species. In particular, there are some populations in Oaxaca and Chiapas that still await closer inspection. In the present work, 31 species have been recognized for the genus (Stevenson et al. 1986; Nicolalde-Morejón et al. 2014; Vovides et al. 2016; Pérez-Farrera et al. 2017; Martínez-Domínguez et al. 2017, 2018a), with *C. becerrae* and *C. microstrobila* treated as synonyms of *C. zoquorum* and *C. latifolia*, respectively (Stevenson et al. 1986; Martínez-Domínguez et al. 2017, 2018a).

Conclusions and future directions

This study adds to recent research that suggests the significant role of topography in SMS as a speciation driver in shaping its high species diversity (Santiago-Alvarado et al. 2016). The geographical barriers and ecological changes in this biogeographic province could have allowed the intermingling of different species; these processes could have favored the development of a number of centers of endemism (Morrone 2010; Santiago-Alvarado et al. 2016).

Our integrative taxonomic assessment provided support for the recognition of a new species, *Ceratozamia leptoceras*. However, the taxonomic complexity of the genus indicates the need of further systematic revisions using multiple sources of evidence, particularly in some groups of species with problematic boundaries. In addition, we have demonstrated the value of investigating ovulate and pollen strobili – particularly, microsporophylls – for the construction of refined morphological matrices for *Ceratozamia*; and finally, that the construction of dichotomous keys with vegetative characters should consider variation at the population level.

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Appendix I

Localities of sampled populations for morphologically similar *Ceratozamia* species to Guerrero taxon.

Species	State	Municipality	Population	Elevation (m)
<i>C. robusta</i>	Chiapas	Berriozábal	Cuchumbak	1,129
<i>C. robusta</i>	Chiapas	Tuxtla Gutiérrez	Cañón del Sumidero	1,263
<i>C. robusta</i> *	Chiapas	San Fernando	Cuauhtémoc	1,200
<i>C. subroseophylla</i>	Veracruz	Santiago Tuxtla	Hill El Vigía	474
<i>C. subroseophylla</i>	Veracruz	Santiago Tuxtla	Sinapan	425
Guerrero taxon	Guerrero	Tlacoachistlahuaca	Near Riverbank “Chipili”	1,200
Guerrero taxon	Guerrero	Tlacoachistlahuaca	Near San Pedro Cuitlapan	1,400

* Population not monitored in field for reproductive phenology data.

Supplementary material I

File S1. GenBank accession numbers of sequences used in the analyses for ITS and matK, respectively. Sequences were generated by this study are in bold.

Authors: Lili Martínez-Domínguez, Fernando Nicolalde-Morejón

Data type: GenBank accession numbers of sequences used in the analyses

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Link: <https://doi.org/10.3897/phytokeys.156.53502.suppl1>

Supplementary material 2

Fig. S1. Strict consensus tree of nine equally parsimonious trees

Authors: Lili Martínez-Domínguez, Fernando Nicolalde-Morejón

Data type: Strict consensus tree

Explanation note: Heuristic searches were conducted with 1000 random-addition, tree bisection-reconnection (TBR) branch swapping, and collapse zero-length branches off. All characters were treated as equally weighted and unordered.

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Supplementary material 3

File S2

Authors: Lili Martínez-Domínguez, Fernando Nicolalde-Morejón

Explanation note: Herbarium specimens consulted for phenology data.

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Supplementary material 4

Table S1

Authors: Lili Martínez-Domínguez, Fernando Nicolalde-Morejón

Data type: DNA diagnostic sites

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Link: <https://doi.org/10.3897/phytokeys.156.53502.suppl4>

Comparative wood anatomy of Korean *Viburnum* L. (Adoxaceae) and its taxonomic implication

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Abstract

Comparative wood anatomy of *Viburnum* was carried out to understand the differences in wood features amongst the species which might be useful for taxonomic discrimination in the genus. Altogether, nine taxa belonging to five clades were investigated using a sliding microtome and light microscopy. The growth rings are well represented and earlywood and latewood are distinguishable in cross-section. Some of the important wood features include angular, oval and rounded vessels with scalariform perforation plates, opposite to scalariform inter-vessel pitting, rounded pits with slit-like apertures, thick-walled xylem tracheids with simple, rounded bordered pits, diffuse axial parenchyma, uni- and multiseriate rays, 2–4 cells wide. In general, there is a remarkable uniformity in the qualitative wood features in *Viburnum* species, although quantitative measurement showed some disparities. The most significant quantitative wood variables which might be useful for taxonomic groupings of the species comprise a frequency of vessels and rays, the diameter of the vessels and tracheids in the radial and tangential planes and height and width of rays in the tangential plane.

Keywords

Viburnum, wood anatomy, light microscopy, taxonomy

Introduction

Investigation of wood anatomical features and their correlation with one another, as well as with different habitats and environmental parameters, has been a long practice (Frost 1930, 1931; Carlquist 1975, 2001; Bissing 1982; Metcalfe and Chalk 1983; Baas 1986; Baas and Schweingruber 1987). Plant taxonomists understand that wood

anatomical features can provide useful information to unravel the phylogenetic relationships amongst the genera of angiosperms and gymnosperm (Oskolski 1994, 1995; Baas et al. 2000; Oskolski and Lowry 2000; Visscher and Jagels 2003; Lens et al. 2007; Esteban and de Palacios 2009). Nevertheless, due to the insufficient knowledge of the microscopic structure of wood in many taxonomic groups, the studies incorporating wood features in phylogenetic analysis are limited (e.g. Baas et al. 1988; Zhang 1992; Gasson 1994, 1996; Noshiro and Baas 1998; Klaassen 1999; Olson 2002; Malécot et al. 2004; Lens et al. 2007).

The genus *Viburnum* L. (Adoxaceae) consists of about 175 to 230 species of shrubs and small trees distributed in the temperate region of the Northern Hemisphere to the subtropical region of Asia and Latin America (Malécot and McNeill 2002; Donoghue et al. 2004; Moura et al. 2015). The genus was initially classified under the family Caprifoliaceae by Linnaeus (1753) which was later given its own family Viburnaceae, including *Sambucus*, by Rafinesque (1820). However, contemporary molecular phylogenetic analysis (e.g. Backlund and Donoghue 1996; Backlund and Bremer 1997; Kim et al. 1999; Olmstead et al. 2000; Bell et al. 2001; Donoghue et al. 2001) suggested that *Viburnum* and *Sambucus* are more closely related to *Adoxa*. As a result, the Angiosperm Phylogeny Group (APG) system recognises both these genera under the family Adoxaceae (APG 2009, 2016). A proposal to conserve the name Viburnaceae was made by Reveal (2008) and accepted by the General Committee (Wilson 2016) and approved by the Nomenclature Section of the Shenzhen International Botanical Congress. As such, currently the name Viburnaceae, is conserved and has priority over the name Adoxaceae. However, because a super-conservation proposal for the name Adoxaceae has still to be assessed by the relevant committees, ICN Rec. 14A.1 recommends following existing usage, as we have done here in using Adoxaceae as the correct name of the family.

In recent decades, *Viburnum* has been extensively studied and much progress has been made in the understanding of its phylogeny (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005; Clement and Donoghue 2011, 2012; Clement et al. 2014). Despite the uniform flower and fruit morphology, *Viburnum* species are well known for their striking variation in several morphological features, like presence or absence of naked buds, sterile flowers around the margin of the inflorescence, endocarp shape, inflorescence form and leaf morphology, based on which the genus has been subdivided into ten sections (Hara 1983) or in 12 (Winkworth and Donoghue 2005) to 16 clades (Clement et al. 2014).

In Korea, ten taxa of *Viburnum* belonging to five clades have been described (Hong and Im 2003; Kim 2007). Based on the DNA analysis, Choi et al. (2018) distinguished six out of ten Korean species at the species level. Moreover, Choi and Oh (2019) found that Korean *Viburnum* is easily distinguishable, based on their morphological features, like the character of bud, leaf, extra-floral nectary, inflorescence, corolla, fruit and stone. Wood anatomy of *Viburnum* species is scattered in literature and most wood anatomical studies include a limited number of species from restricted geographical areas (Gundersen 1910; IAWA Committee 1989; Ogata 1988; Eom and Chung 1996; Lens et al. 2016). In this study, we present the wood

anatomy of nine out of ten Korean *Viburnum* (except *V. koreanum*). The principal objectives of this study are: (i) to provide an overview of wood anatomical variation within *Viburnum* species, (ii) to identify the systematic significance of wood features in *Viburnum* and (iii) to relate the wood anatomical data to DNA and other morphological features within the *Viburnum*.

Materials and Methods

Light microscopy

Mature branches were collected from the natural populations. Names of the studied taxa, voucher number and collection sites are presented in Table 1. Collected wood materials were preserved in 50% ethyl alcohol before sectioning. Preserved wood samples were cut into approximately 2 cm-long circular blocks. For microtome sectioning, the samples were prepared depending on the size of wood; cubic pieces were cut (transverse) or split (radial and tangential) from circular blocks. Three such pieces – representing three planes: transverse, radial and tangential – of each sample were prepared. The blocks were preserved in softener solution (glycerine 10 parts/10% aerosol OT 3 parts/distilled water 87 parts) until sectioning and were sectioned according to the standard technique for light microscopy.

After preparing permanent slides, microscopic observations and wood-feature analyses were carried out under an AXIO Imager A1 light microscope (Carl Zeiss, Germany). We studied several quantitative and qualitative features of the wood structure and pertinent features of them are summarised in Table 2. Observations and measurements of quantitative features of vessels, tracheids and rays were made with a Hirox 3D microscope (Hirox, Japan). Mean values for each feature were calculated from the

Table 1. Name of taxa and collection information. KH, Herbarium Korea National Arboretum.

Taxa	Clade	Voucher no. (KH)	Collection site
<i>Viburnum dilatatum</i> Thunb.	Succotinus	Paik, 160607-0012	Korean National Arboretum, Gyeonggi-do
<i>Viburnum erosum</i> Thunb.	Succotinus	Paik, 160509-001	Uijeongbu Dobongsan, Gyeonggi-do
<i>Viburnum japonicum</i> (Thunb.) C.K. Spreng.	Succotinus	Lee, 160519-010	Dogsilsan, Sinan-gun, Gagae-do
<i>Viburnum wrightii</i> Miq.	Succotinus	Paik, 160614-0001	Odaesan, Gangneung, Gangwon-do
<i>Viburnum burejaeticum</i> Regel & Herd.	Euviburnum	Paik, 160502-001	Pyeongchang-gun, Gangwon- do
<i>Viburnum carlesii</i> Hemsl.	Euviburnum	Paik, 160420-001	Samcheok-si, Gangwon-do
<i>Viburnum odoratissimum</i> var. <i>awabuki</i> (K. Koch) Zabel ex Rümpler	Solenotinus	Lee 160616-014	Seogwipo-si, Sioreum, Jeju-do
<i>Viburnum furcatum</i> Blume ex Maxim.	Pseudotinus	Lee, 160429-007	Seogwipo-si, 1100 Hill, Jeju-do
<i>Viburnum opulus</i> for. <i>hydrangeoides</i> (Nakai) Hara	Opulus	SGUB, 160528-001	Cheorwon-gun, Soisan, Gangwon-do

Table 2. Distinguishing wood features in *Viburnum* species included in this study.

Growth rings	Distinct
Wood	Diffuse-porous
Vessels	Solitary
Vessel outline	Angular/oval/rounded
Perforation plates	Scalariform
Scalariform perforation plate bars	20–40
Inter-vessel pits	opposite, scalariform
Helical thickenings in vessel elements	Absent or indistinctly present
Vessel frequency	40–220
Septate fibers	Absent
Fiber pits	Common on radial and tangential walls
Helical thickenings in fiber cells	Present
Axial parenchyma	Diffuse
Ray width	1–4 cells
Ray in radial section	Procumbent, upright, square marginal
Ray frequency	10–58

measurements taken from the same species, but different samples and standard deviations were also calculated. Photographs of the best sections with characteristic features were taken using a digital camera system attached to the light microscope.

Statistical analysis

The biometric data were analysed statistically. For each wood feature, one-factor analysis of variance (ANOVA) was used to examine differences in means amongst the included species. Pearson’s correlation coefficients were used to estimate the relationship amongst the vessel number (VN), vessel diameter in tangential (VDT) and radial (VDR) planes, vessel wall thickness (VW), fibre diameter in radial (TDR) and tangential (TDT) planes, fibre wall thickness (TW), bordered pit (BP), ray number (RN), ray length (RL) and ray wall thickness (RW). All of the statistical analyses were carried out using the SPSS statistical programme (IBM SPSS Statistics for Windows Version 20.0., IBM Corp., Armonk, USA). Principal component analysis (PCA) was also performed to verify whether wood features allowed the grouping of the species by using the statistical programme R (RStudio, Inc., USA).

Results

Altogether, nine taxa belonging to five clades of *Viburnum* from Korea were investigated in this study. The qualitative wood features and quantitative wood variables of all included species are presented in Tables 2, 3. Figures 1–6 show the detailed wood features in the cross, radial and tangential sections. All the taxa included have well-defined growth rings with the gradual transition from early to latewood (Figs 1A–D). In all species, the quantity of latewood is very small and the fibres are

Table 3. Wood variables in different *Viburnum* species (mean and standard deviation). *Abbreviations:* VN, Number of vessels; VDR, Vessel diameter in radial plane; VDT, Vessel diameter in tangential plane; VW, Vessel wall thickness; TDR, Tracheid diameter in radial plane; TDT, Tracheid diameter in tangential plane; TW, Tracheid wall thickness; BP, Bordered pit; RN, Number of rays; RH, Ray height; RW, Ray thickness.

Taxa	VN (mm ²)	VDR (µm)	VDT (µm)	VW (µm)	TDR (µm)	TDT (µm)	TW (µm)	BP (µm)	RN (mm ²)	RL (µm)	RW (µm)
<i>V. ordoratisissimum</i> var. <i>auabuki</i>	164.06±18.67	23.97±4.57	18.33±3.24	2.43±0.45	8.04±1.65	5.96±1.12	2.65±0.48	6.51±0.72	15.89±2.05	669.6±144	39.14±6.33
<i>V. dilatatum</i>	97.83±9.43	28.27±5.53	26.61±4.8	2.77±0.36	6.88±1.45	7.81±1.99	3.55±0.41	6.69±0.76	20.74±2.38	656.11±114.86	34.87±9
<i>V. erosum</i>	83.14±10.6	27.98±6.58	25.06±6.38	2.81±0.41	5.65±1.36	6.58±1.67	3.69±0.63	5.53±0.66	19.2±3.23	629.86±81.21	43.11±9.43
<i>V. japonicum</i>	53.77±8.64	31.99±5.53	20.46±3.34	3.27±0.47	8.22±2.27	7.89±1.52	3.12±0.64	4.31±0.51	20.77±2.95	680.89±135.11	23.86±4.34
<i>V. burejaticum</i>	195.03±10.06	24.41±7.52	18.55±3.51	2.01±0.29	6.06±1.89	4.7±1.24	2.22±0.36	5.08±1	47.77±5.54	294.53±107.08	9.31±2.53
<i>V. carlesii</i>	185.5±17.23	27.05±5.13	20.08±3.64	2.21±0.36	6.79±1.83	6.67±2.19	2.11±0.31	6.64±0.83	44.09±3.97	346.53±94.94	19.93±4.68
<i>V. furcatum</i>	112.51±9.78	18.23±3.54	19.14±3.95	2.26±0.37	5.63±1.33	5.15±1.08	2.34±0.39	5.72±0.68	41.23±2.92	479.2±94.38	29.26±4.24
<i>V. opulus</i> f. <i>hydrangoides</i>	152.06±6.82	35.39±6.81	31.7±6.62	2.61±0.34	8.5±2.43	9.54±3	2.89±0.44	4.59±0.79	36.4±2.93	557.6±104	19.55±4.24
<i>V. wrightii</i>	70.17±9.28	26.42±4.16	16.73±3.34	2.33±0.41	7.49±1.76	6.67±1.58	2.97±0.56	4.52±0.74	14.74±2.13	750±170.24	32.53±6.38
ANOVA	F = 683.64 P<0.001	F = 26.42 P<0.001	F = 42.51 P<0.001	F = 34.87 P<0.001	F = 12.9 P<0.001	F = 24.83 P<0.001	F = 47.55 P<0.001	F = 55.44 P<0.001	F = 564.1 P<0.001	F = 63.1 P<0.001	F = 109.01 P<0.001

tangentially elongated with the narrow lumen. The wood is diffuse porous. The vessels are exclusively solitary, narrow, mostly angular, oval and round in cross-section (Figs 1A–D; 2A–D). The widest vessels in radial and tangential diameter are in *V. opulus* f. *hydrangeoides* (ranges from 18.8–47.1 μm) and (19.4–44.5 μm), respectively, whereas the narrowest vessels in radial diameter are in *V. furcatum* (11.7–27 μm) and tangential diameter in *V. wrightii* (11.1–25.3 μm). The number of vessels is highest in *V. burejaeticum* (frequency ranges 170–220 mm^{-2}) followed by *V. carlesii* (frequency ranges 146–212 mm^{-2}) and the lowest number of vessels is recorded in *V. japonicum* (frequency ranges 40–78 mm^{-2}) followed by *V. wrightii* (frequency range 56–96 mm^{-2}). The vessel wall thickness ranges from 1.6–2.6 μm in *V. burejaeticum* to 2.3–4.2 μm in *V. japonicum*.

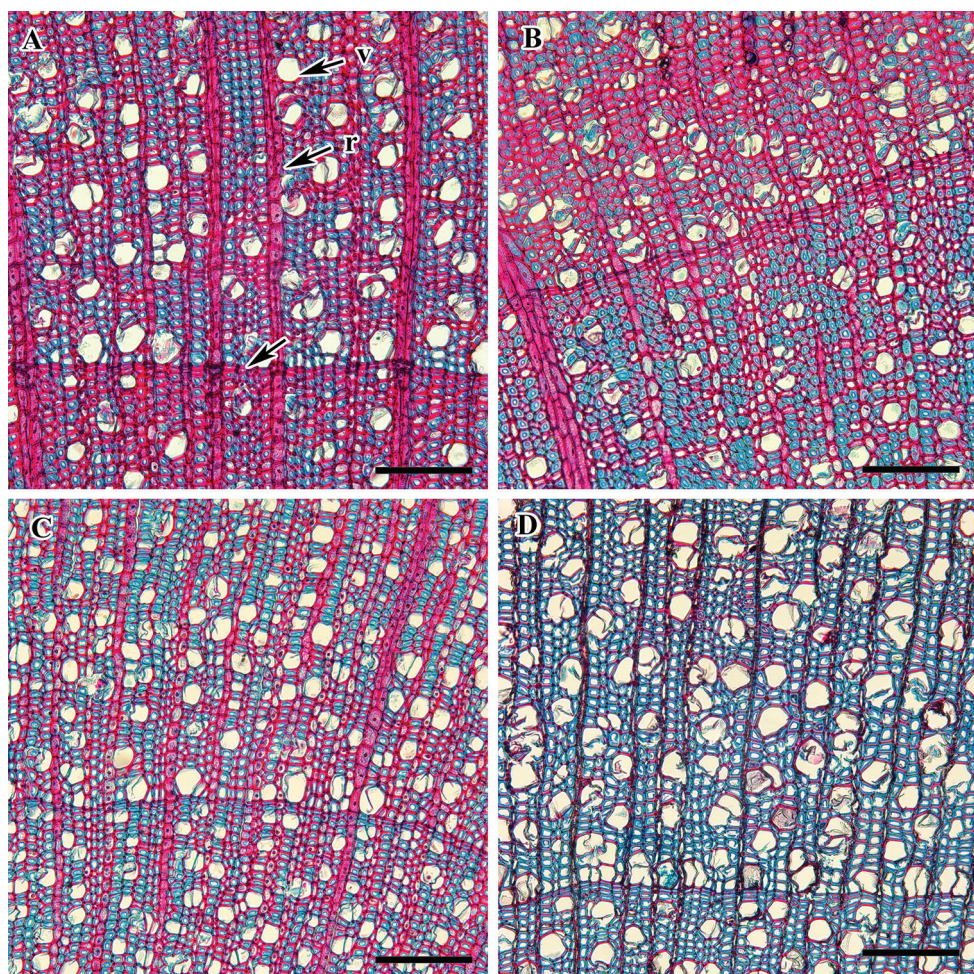


Figure 1. Cross section of *Viburnum* wood showing growth ring, vessels, tracheids, and rays. **A** *V. dilatatum* (arrow indicates growth ring) **B** *V. erosum* **C** *V. carlesii* **D** *V. opulus* f. *hydrangeoides*. Abbreviations: r, ray; v, vessel. Scale bars: 0.1 mm.

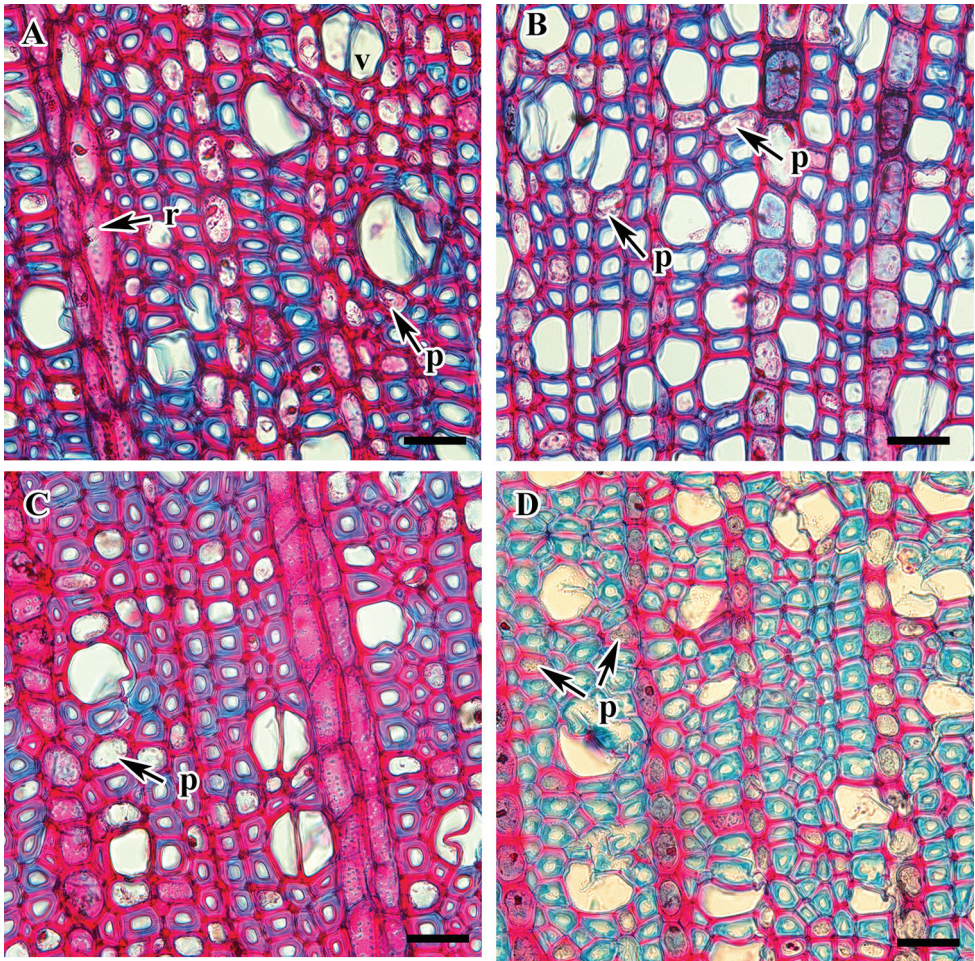


Figure 2. Cross section of *Viburnum* species showing axial parenchyma (magnified). **A** *V. japonicum* **B** *V. ordoratisissimum* var. *awabuki* **C** *V. wrightii* **D** *V. furcatum*. Abbreviations: p, parenchyma cell; r, ray. Scale bars: 20 μm .

Perforation plates are scalariform with 20–40 bars and vessel tails are gradual in all species (Fig. 3A–D). Inter-vessel pitting is opposite or scalariform, pits are rounded or oval, with an elliptical or slit-like aperture (Fig. 3B, C). Smooth or faintly helical thickening occurs on the vessel walls. Vessel-ray and vessel axial parenchyma pits are distinctly bordered and are similar to the inter-vessel pits. Tracheids are thick walled (wall thickness ranges 1.6–2.7 μm in *V. carlesii* to 2.4–5 μm in *V. erosum*), with helical wall thickenings and narrow lumen. Radial and tangential tracheid diameter ranges (radial: 4–9.1 μm in *V. furcatum* to 4.6–12.7 μm in *V. opulus* f. *hydrangeoides*; tangential: 2.9–8.4 μm in *V. burejaeticum* to 5–14.7 μm in *V. opulus* f. *hydrangeoides*). The bordered pits are circular with a slit-like aperture and occur in both radial and tangential walls. The diameter of bordered pits ranges from 3.5–5.5 μm in *V. japonicum* to 5.3–8.7 μm in *V. dilatatum*.

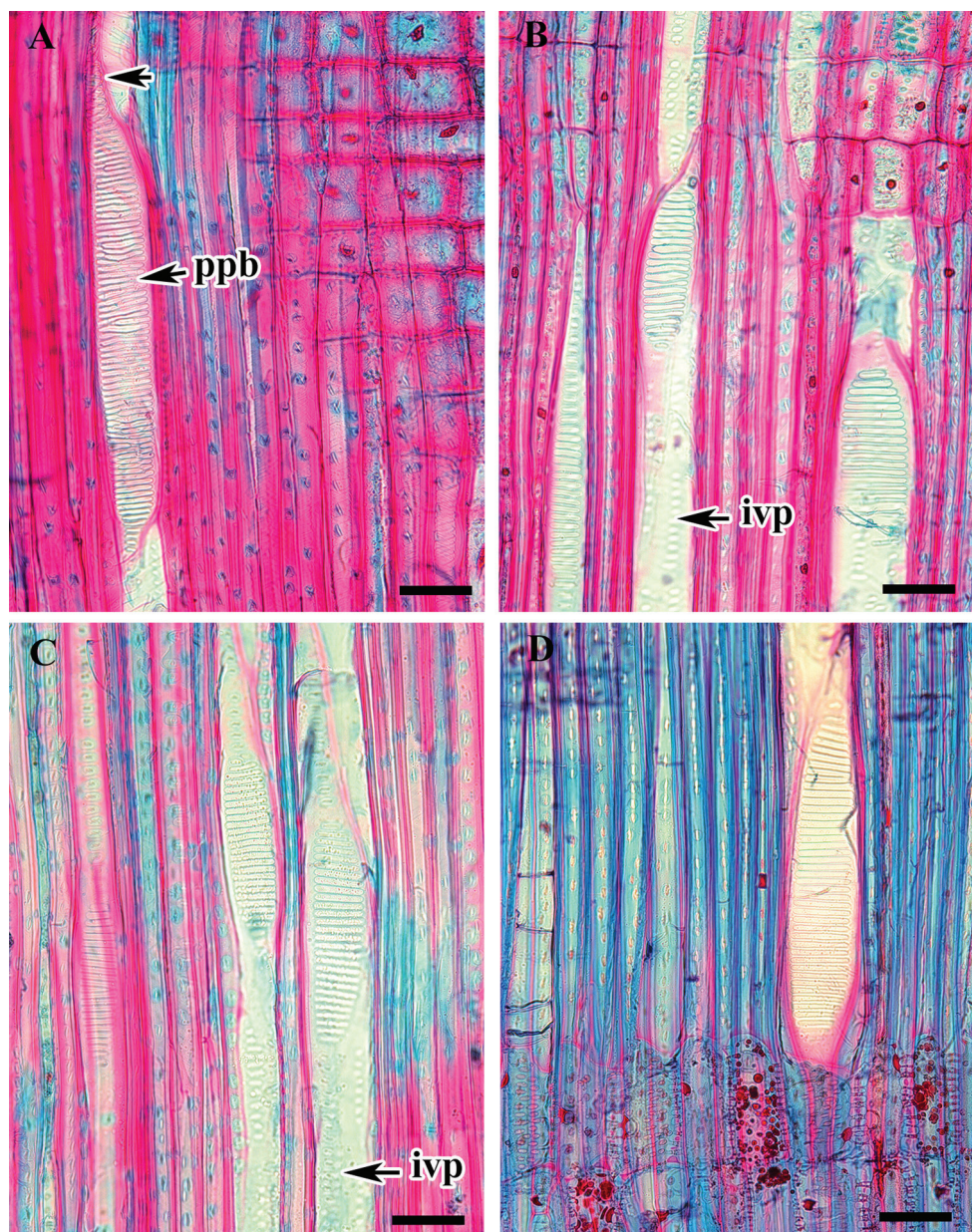


Figure 3. Radial longitudinal section (RLS) of *Viburnum* wood showing inter-vessel pits and scalariform perforation plates. **A** *V. wrightii* (arrow indicates gradual vessel tail) **B** *V. carlesii* **C** *V. burejaeticum* **D** *V. opulus* f. *hydrangeoides*. Abbreviations: ppb, bars on perforation plate; ivp, inter-vessel pits. Scale bars: 20 μ m.

The rays are mostly uniseriate rarely biseriate (*V. burejaeticum* and *V. japonicum*), uni- and biseriate (*V. carlesii*, *V. furcatum*, *V. opulus* f. *hydrangeoides* and *V. wrightii*), multiseriate, 1–3 seriate (*V. odoratissimum* var. *awabuki*, *V. dilatatum*) or 1–4 seriate (*V. erosum*) (Fig. 4A–D). Three types of cellular composition are found in ray cell:

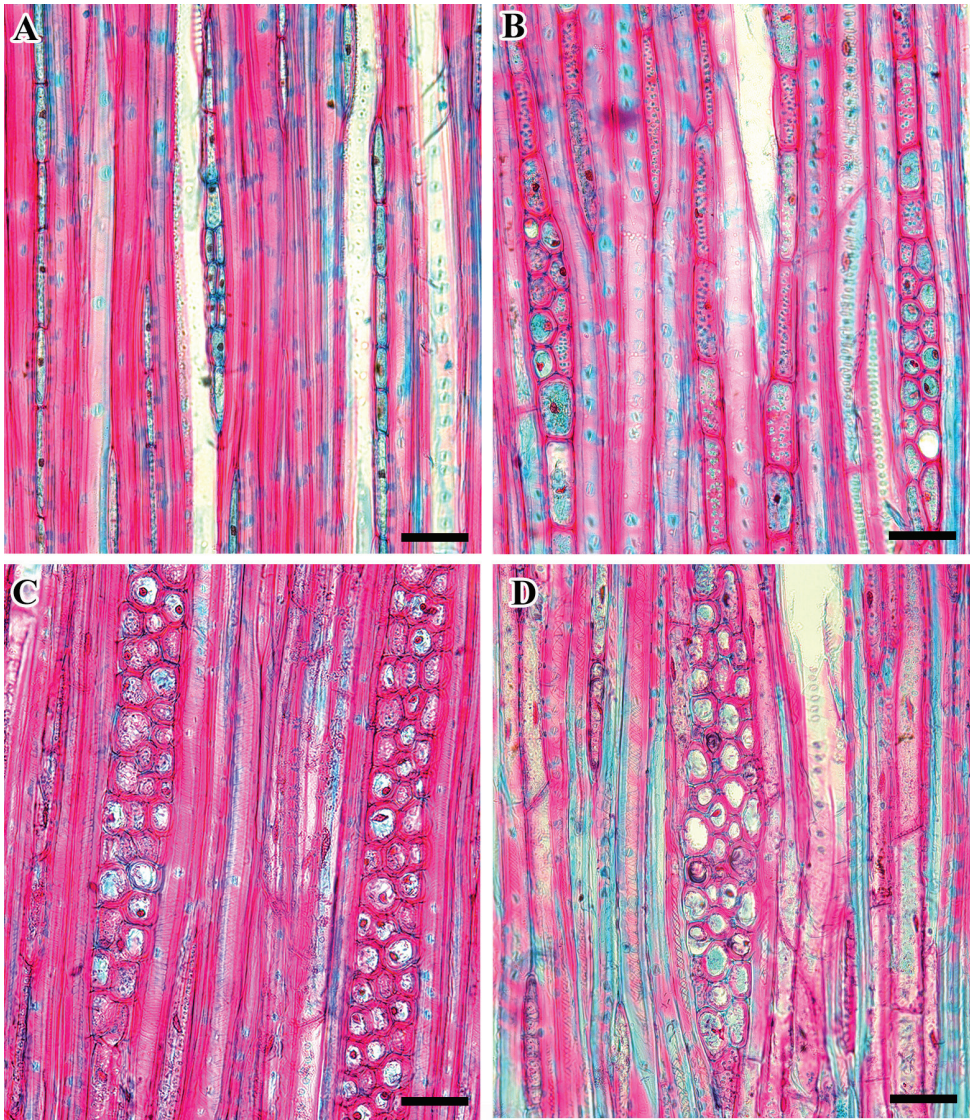


Figure 4. Tangential longitudinal section (TLS) of *Viburnum* wood showing different types of cells in the ray. **A** *V. burejaeticum* **B** *V. carlesii* **C** *V. dilatatum* **D** *V. erosum*. Scale bars: 20 μ m.

body cells procumbent with mostly 2–4 rows of upright and/or square marginal cells (*V. opulus* f. *hydrangeoides*, *V. erosum*), body cells procumbent with more than 4 rows of upright and/or square marginal cells (*V. odoratissimum* var. *awabuki*, *V. carlesii*), body cells square with 2–4 rows of upright marginal cells (*V. furcatum*, *V. burejaeticum*, *V. dilatatum*, *V. wrightii*, *V. japonicum*) (Fig. 5A–D). The number of rays per square mm is highest in *V. burejaeticum* (frequency ranges 38–58) followed by *V. carlesii* (ranges 36–51) and lowest in *V. wrightii* (ranges 10–19) followed by *V. odoratissimum* var. *awabuki* (ranges 12–21). The ray height in the tangential section is less

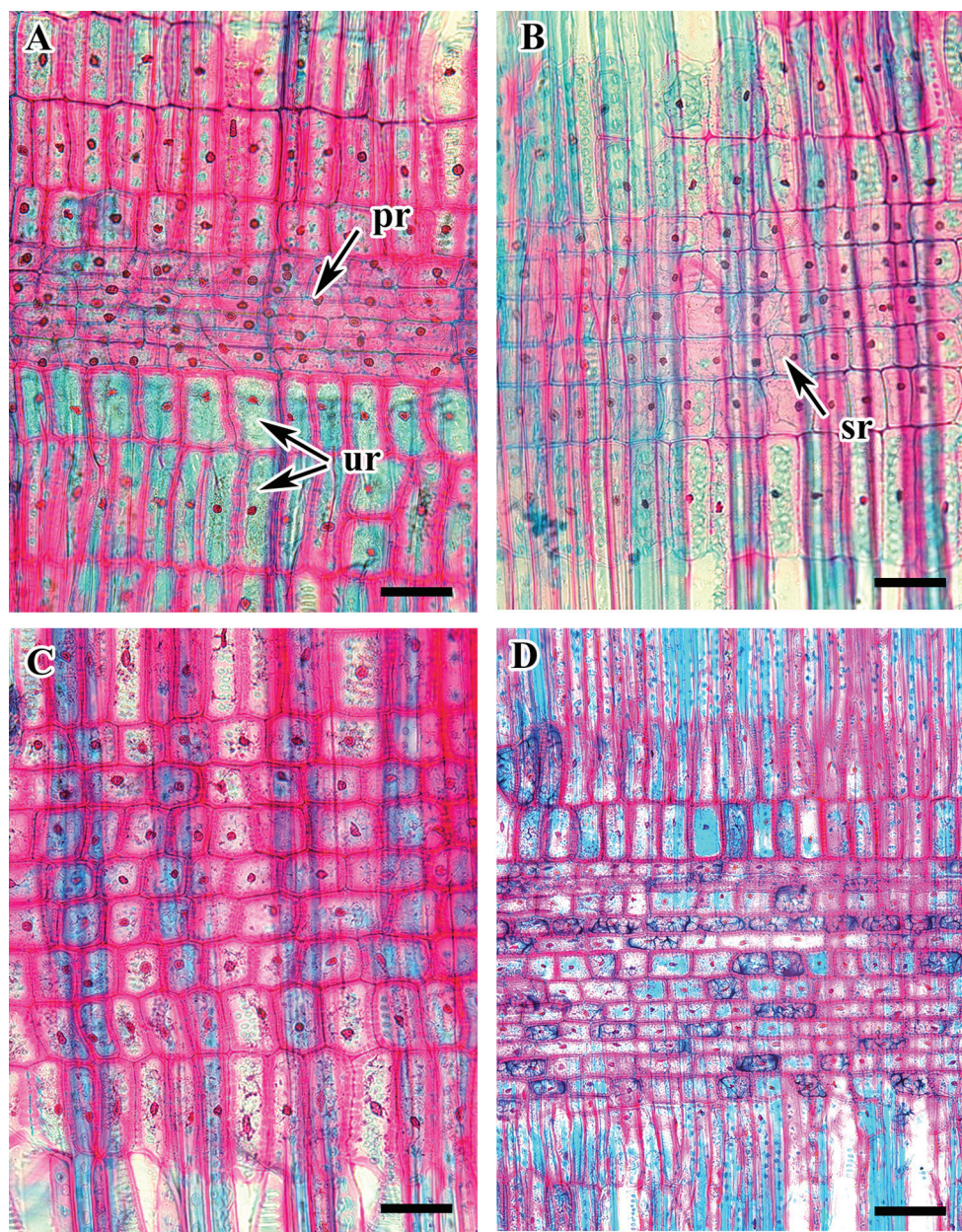


Figure 5. Radial longitudinal section (RLS) of *Viburnum* wood showing ray and parenchyma. **A** *V. erosum* **B** *V. carlesii* **C** *V. burejaeticum* **D** *V. furcatum*. Abbreviations: pr, procambium ray cells; sr, square ray cells; ur, upright ray cells. v, vessel. Scale bars: 20 μm .

than 1 mm for all species. The tallest rays are in *V. wrightii* (ranges 428.3–978.3 μm) followed by *V. ordoratisimum* var. *awabuki* (ranges 402.2–912.4 μm), whereas the shortest rays are in *V. burejaeticum* (106–559.3 μm) followed by *V. carlesii* (ranges 134.4–541.9 μm).

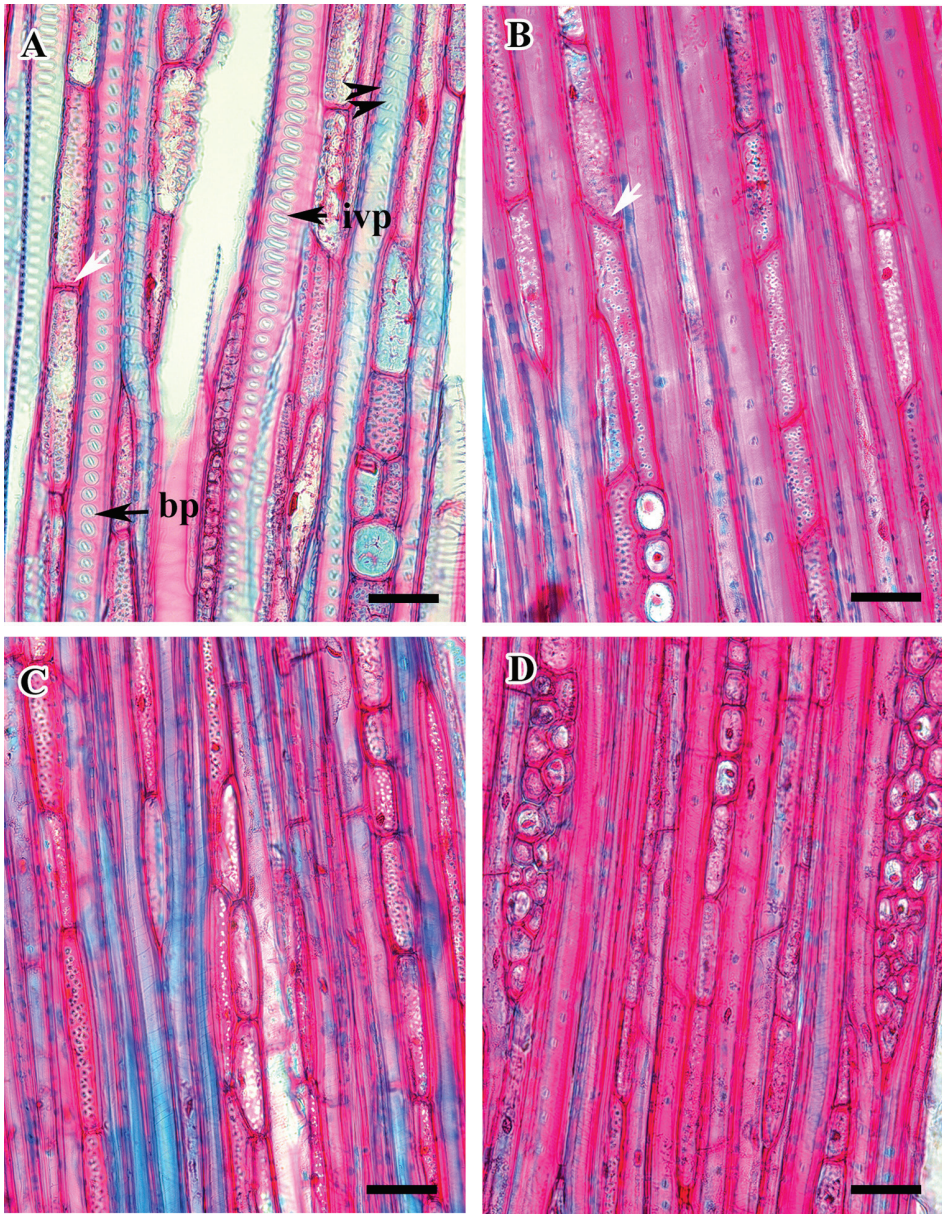


Figure 6. Tangential longitudinal section (TLS) of *Viburnum* wood showing ray and parenchyma. **A** *V. wrightii* (double arrow head indicate wall thickenings in tracheids, white arrow indicates simple cross wall in parenchyma cells) **B** *V. odoratissimum* var. *awabuki* (white arrow indicates, oblique cross wall in parenchyma cell) **C** *V. japonicum* **D** *V. dilatatum*. Abbreviations: bp, bordered pits; ivp, inter-vessel pits. Scale bars: 20 μm .

Axial parenchyma is diffuse with scanty paratracheal parenchyma in solitary strands adjacent to the vessel elements (Fig. 2A–D). Axial parenchyma consists of tubular cells with mostly oblique or sometimes horizontal end walls (Fig. 6A–D). The wall is smooth with minute pits.

Table 4. Pearson’s correlation coefficients between different wood features in *Viburnum* species. Numbers in bold indicate significant. *Abbreviations:* VN, Number of vessels; VDR, Vessel diameter in radial plane; VDT, Vessel diameter in tangential plane; VW, Vessel wall thickness; TDR, Tracheid diameter in radial plane; TDT, Tracheid diameter in tangential plane; TW, Tracheid wall thickness; BP, Bordered pit; RN, Number of rays; RH, Ray height; RW, Ray thickness.

	VN	VDR	VDT	VW	FDR	FDT	FW	BP	RN	RH	RW
VN											
VDR	-.109										
VDT	-.037	.467**									
VW	-.496**	.278**	.274**								
FDR	-.042	.167**	.106	.125*							
FDT	-.191**	.276**	.348**	.313**	.251**						
FW	-.538**	.226**	.243**	.356**	.062	.191**					
BP	.284**	-.160**	-.043	-.158**	-.125*	-.167**	-.058				
RN	.650**	-.129*	.006	-.386**	-.169**	-.115*	-.545**	.050			
RH	-.604**	.117*	.042	.345**	.165**	.213**	.428**	-.128*	-.725**		
RW	-.445**	-.068	.055	.253**	-.050	.079	.412**	.208**	-.656**	.556**	

**Sig. at 0.01 level *Sig. at 0.05 level.

Statistical analysis

One-factor ANOVA was performed on 11 quantitative traits and the differences between species were found to be highly significant (Table 3). Pearson’s correlation coefficient also indicated a significant correlation between wood features (Table 4). Figure 7 (A, B) shows the variation in vessel number and ray number per square millimetre. The relationships amongst the species were revealed using PCA analysis (Fig. 8). The first four components explain 92.61% of the total variance of the analysed data. The first axis of the complete sample set explained 48.21% of the total variance and showed strong positive loadings for vessel numbers and ray numbers per square area (VN and RN) and strong negative loadings for vessel and fibre wall thickness (VW and FW) in association with ray height (RH). The second axis explained 23.61% of the total variance and showed strong positive loadings for vessel and tracheid diameter (VDT, VDR, TDT and TDR) and negative loadings for ray wall thickness and bordered pits (RW and BP). Amongst the four, three species (*V. erosum*, *V. dilatatum* and *V. wrightii*) of the clade Succotinus were grouped on the negative side of both axes whereas *V. japonicum* remained on the positive side of axis two and negative side of axis one. Similarly, both species of the clade Euviburnum, included in this study (*V. carlesii* and *V. burejaeticum*), were grouped on the positive side of both axes.

Discussion

Descriptions of wood anatomical features of some *Viburnum* species from different geographical areas are available in literature (Gundersen 1910; Ogata 1988; IAWA Committee 1989; Eom and Chung 1996; Lens et al. 2016). Lens et al. (2016) con-

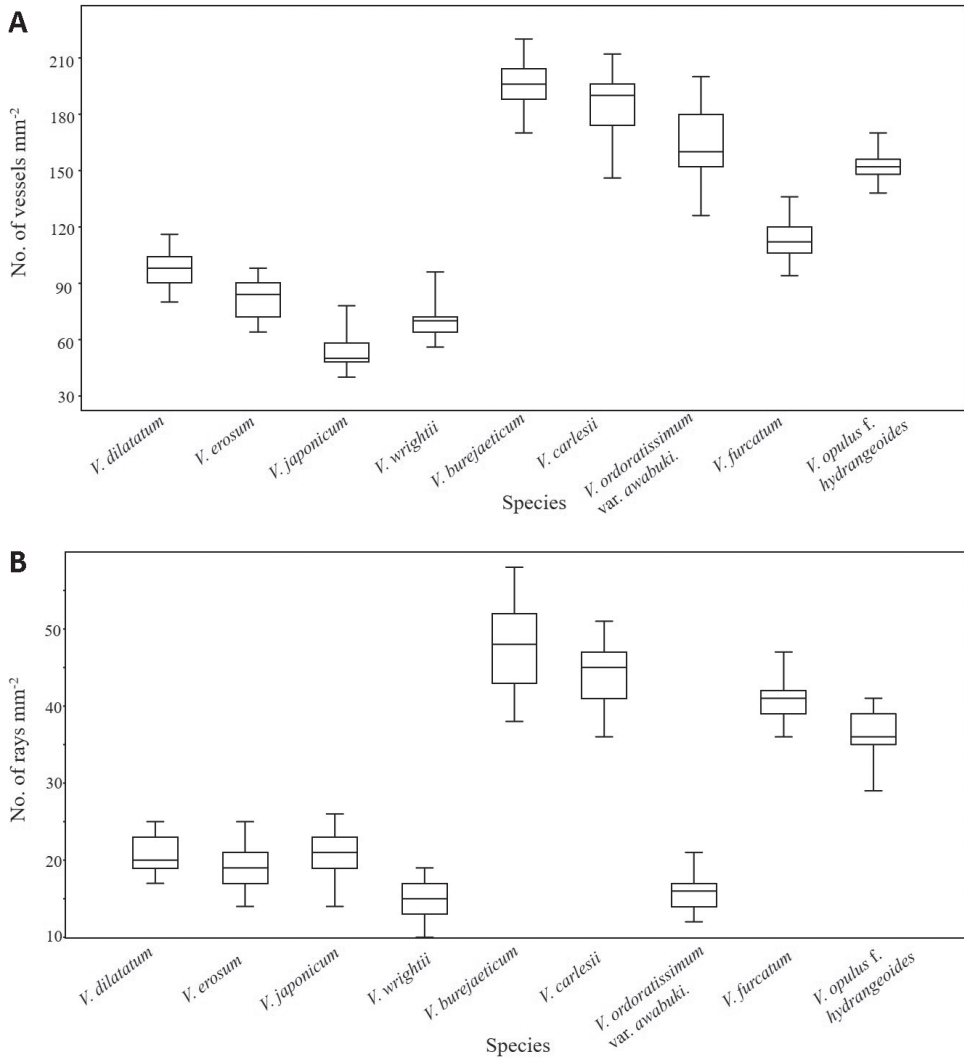


Figure 7. Box plot of ray vessel number and ray numbers per square millimetre in *Viburnum* species.

firmed that the wood anatomy of *Viburnum* perfectly fits within the Dipsacales and also showed some key differences with its close relative *Sambucus*. In his report, the wood anatomy of the Caprifoliaceae of Japan, Ogata (1988) included 13 species of Japanese *Viburnum* along with *Sambucus racemosa* and compared with other members of the Caprifoliaceae (*Abelia*, *Lonicera*, *Weigela* and *Zabelia*). In this early 21st century, phylogenetic relationships, based on molecular data, imply revision of familial limits in Dipsacales and APG (2009, 2016) classification included both *Viburnum* and *Sambucus* in Adoxaceae family, which is sister to Caprifoliaceae. In this study, we compared the wood features of nine out of ten species of *Viburnum* distributed in Korea.

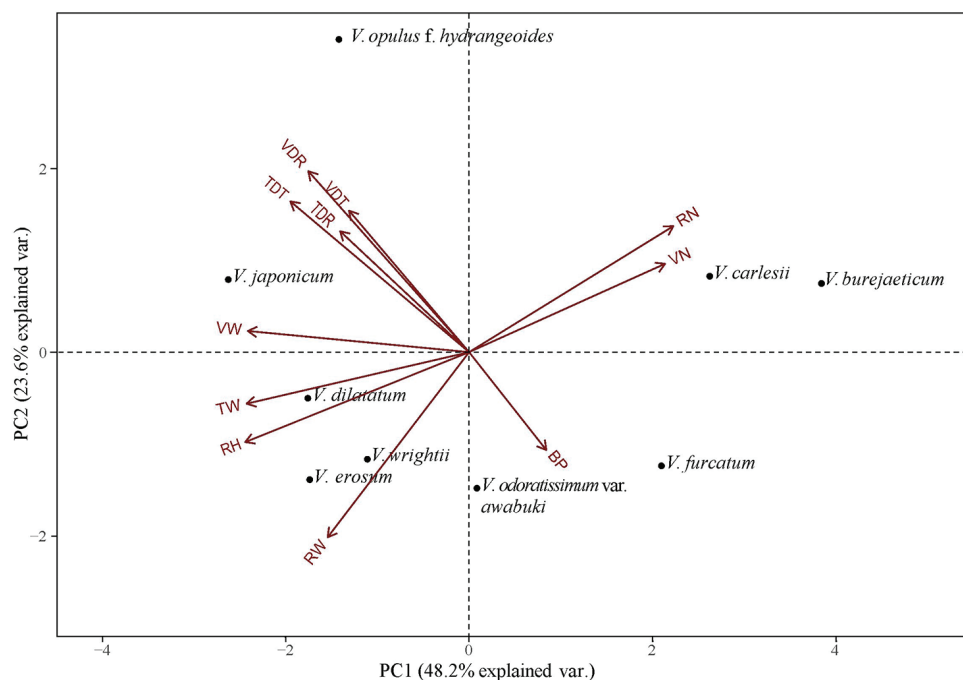


Figure 8. Principal component analysis of 11 different wood variables of *Viburnum* species. VN, Number of vessels; VDR, Vessel diameter in radial plane; VDT, Vessel diameter in tangential plane; VW, Vessel wall thickness; TDR, Tracheid diameter in radial plane; TDT, Tracheid diameter in tangential plane; TW, Tracheid wall thickness; BP, Bordered pit; RN, Number of rays; RH, Ray height; RW, Ray thickness.

Relationship amongst wood variables

Most of the characteristic wood features observed in this study, for instance, distinct growth rings, diffuse porous wood, solitary vessel, scalariform perforation plates, opposite or scalariform inter-vessel pits, wall thickenings in tracheids, diffuse axial parenchyma and heterocellular rays are similar to those reported in previous studies on *Viburnum* (Ogata, 1988; IAWA Committee, 1989; Lens et al. 2016). However, we found variations in quantitative traits, such as vessel density, vessel diameter, ray width, ray height and ray density. When compared, the relatively lower numbers of vessels per square millimetre were counted for some species in our samples particularly, *V. carlesii* (146–212 vessels/mm²) and *V. erosum* (64–98 vessels/mm²) than that of Ogata (1988) who observed 168–294 and 90–117 vessels/mm² for these species, respectively. On the other hand, we recorded slightly higher numbers of vessel/mm² for *V. furcatum* (94–136 vessels/mm²) than Ogata (1988) (58–128 vessels/mm²) and for *V. dilatatum* than Lens et al. (2016) (60–82 vessels/mm²). Our result was more comparable with Lens et al. (2016) for *V. carlesii* (150–185 vessels/mm²) and *V. furcatum* (100–130 vessels/mm²) in terms of vessel frequency, but they did not observe *V. erosum*. However, for the rest of the species, we found fairly similar data to Ogata (1988), excluding

V. ordoratisissimum var. *awabuki*, *V. burejaeticum* and *V. opulus* f. *hydrangeoides* which were not included on his observations.

The ray height (RH) barely exceeds one millimetre in all the taxa included in this study. However, Ogata (1988) recorded rays more than one millimetre (even 4+ mm in *V. furcatum*) in height for all species he studied. Young (2015) also found rays more than one millimetre high in *V. ordoratisissimum* var. *awabuki*. Although the heights of the tallest rays were over one millimetre, the average ray heights recorded by Lens et al. (2016) for *V. dilatatum*, *V. carlesii* and *V. furcatum* are similar to ours. The observations were also similar for ray width. The widest rays were in *V. dilatatum* and *V. erosum* with one- to three- and four- celled wide rays, respectively, whereas the remaining species usually had one- to two- (rarely three- in *V. ordoratisissimum* var. *awabuki*) celled rays.

In general, we found 10–19 (*V. wrightii*) to 38–58 (*V. burejaeticum*) rays per square millimetre. Although we did not count multi- and uni-seriate rays separately, these numbers are higher than those of Lens et al. (2016) as they counted 6–9 multiseriate and 7–9 uni-seriate rays in *V. carlesii* which has the highest number of rays per square millimetre in their observation. On our side, we found 36–51 rays in *V. carlesii* which is the second-highest number in our observations. The result of ANOVA indicated that the variation in the ray number (RN) in the *Viburnum* species is significant ($P < 0.001$) (Table 4). In addition, there was a strong negative significant relationship between ray numbers and ray height (RL) ($r = -0.725$; $P < 0.001$) and ray width (RW) ($r = -0.656$; $P < 0.001$) and a strong positive relationship between ray number and vessel number ($r = -0.065$; $P < 0.001$) (Table 4).

The analysis of biometric data indicated that vessel wall thickness (VW) is positively related to vessel diameter in both planes (VDR and VDT) and negatively related to vessel numbers per square millimetre. It is noteworthy that the vessel number and vessel diameter in both planes are also negatively related, but without statistical significance. This is an obvious and well-known phenomenon related to the water transport system according to Baas (1973) and Carlquist (1975). Wider vessels are more efficient water conductors than narrow ones, but they are more vulnerable against cavitation (Baas et al. 1983; Choat et al. 2005).

Taxonomic significance of wood variables

We included nine taxa belonging to five clades of *Viburnum* for the comparative study. The phylogeny of *Viburnum* is very well studied using DNA of both nuclear and chloroplast regions incorporating some morphological features (Donoghue et al. 2004; Winkworth and Donoghue 2004, 2005; Clement and Donoghue 2011, 2012; Clement et al. 2014). However, we have not found any reports of *Viburnum* wood structures considered as a diagnostic feature for the genus as a whole. According to Lens et al. (2016), scalariform perforations in *Viburnum* is the only wood feature that distinguished this genus from its close relative *Sambucus* which has simple perforations. Although our study did not find any particular wood features that support intraspecific relationship within the genus, biometric data exhibited some groupings which are

almost congruent with current phylogeny of *Viburnum*. In particular, our PCA result revealed groups that corresponded closely to the clades identified by molecular analyses (Clement et al. 2014; Choi et al. 2018).

Viburnum dilatatum, *V. erosum*, *V. wrightii* and *V. japonicum*, members of clade Succotinus, formed a highly-supported clade in the phylogenetic tree inferred from chloroplast and nuclear DNA (Choi et al. 2018). Morphologically, these species are characterised by free bud scales, serrate leaves with pinnate veins and an extra-floral nectary at proximal regions on the abaxial leaf surface (Choi and Oh 2019). Our results partially agree with previous studies, as these species exhibited comparable wood variables, for instance; vessel and ray numbers per square millimetre (Fig. 7A, B), vessel diameter, tracheid wall thickness and ray height and thus form a close group in the PCA plot (Fig. 8). A similar result is obtained for *V. carlesii* and *V. burejaeticum* which belongs to clade Euviburnum. These two species are grouped together on the positive axis of both components and exhibit comparable vessel and ray numbers per area, vessel and tracheid diameter and wall thickness. These two species with stellate trichomes on branchlets, petioles and leaves, naked buds and stones with two dorsal and ventral grooves also form strongly-supported monophyletic groups in a phylogenetic analysis (Choi et al. 2018; Choi and Oh 2019).

In conclusion, despite the limited taxa sampling from a restricted geographical region, the results of this study demonstrated the considerable quantitative variations that exist in the wood features of *Viburnum* species. Although most of the qualitative wood features exhibited uniformity amongst the species, quantitative variables displayed significant relationships with each other and also provided some support for the taxonomic groupings. Further studies considering many more species from different clades will help to clarify the taxonomic problems within the genus. In addition, the methods used in this study can be applied to other taxa, as well as offering valuable basic information about how the wood variables can contribute to taxonomic relationships.

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Rediscovery and taxonomic placement of *Solanum polyphyllum* Phil. (Solanaceae), a narrow endemic from the Chilean Atacama Desert

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Abstract

Although the original description of *Solanum polyphyllum* Phil. was made in 1891, this species was not seen until it was re-discovered 128 years later in 2019 in the Atacama Desert. Fruits and seeds were previously unknown and a complete description is provided here. This species was not treated in the most recent monograph of *Solanum* sect. *Regmandra*, but it should be incorporated in this section due to its glabrous, sessile and entire leaves, which are decurrent onto the stem. Morphologically, *S. polyphyllum* is similar to *S. paposanum*, also of section *Regmandra*, but differs in the entire leaves (against margins with 4–5 acute lobes in *S. paposanum*) and glabrous leaves (moderately pubescent adaxially and velutinous abaxially in *S. paposanum*). The rediscovery of *S. polyphyllum* at a new locality at the same altitudinal belt as the type, re-affirms its restricted distribution and endemism and supports a potential conservation status as an endangered species.

Keywords

Chile, rediscovery, *Regmandra*, *Solanum*, *Solanum polyphyllum*, *Solanum* sect., Tarapacá Region

Introduction

Solanum is a sub-cosmopolitan genus of about 1400 species (Bohs 2005, Weese and Bohs 2007), with the highest diversity in the Andes of South America (Knapp 2002). In Chile, it includes 63 species (44 native and 19 endemic) (Moreira-Muñoz 2011), distributed along an immense latitudinal gradient from arid environments at 17°35'S latitude to hyper-humid southern Chile around 50°S. *Solanum* species are also found in Juan Fernández archipelago, Desventuradas Islands and Easter Island (Rapa Nui). During a recent botanical survey of plant and insect diversity along a transect in the Atacama desert-highlands in 2019, a remarkable specimen of *Solanum* with flowers and fruits was collected.

Rémy (1849) was the first to group and describe Chilean *Solanum* species (18 spp.). Rudolph A. Philippi, the prolific naturalist, described 40 additional species between 1858 and 1895 (e.g. Philippi 1858, 1891, 1895) including *Solanum polyphyllum*. In the second *Flora of Chile*, Reiche (1909) grouped the 50 Chilean *Solanum* species he recognised into six groups. He placed *S. polyphyllum* in his Group II corresponding to section *Pachystemonum* subsection *Dulcamara*, in accordance with a manuscript sent to him by the specialist Miss J. Witasek from Vienna (see note in Reiche 1909, pg. 716).

Reiche indicated that his Group II included herbaceous species with markedly winged stems due to the decurrent leaves. In it he included three species: *S. phyllanthum* Cav. (with lobulate leaves, hispid indumentum, now recognised as *S. montanum* L.), *S. herbabona* Reiche (with velutinous, lobulate leaves) and *S. polyphyllum* Phil. (with entire, glabrous leaves). He also included, in his Group III (prismatic stems, sometimes marked by prominent lines) of section *Pachystemonum* Subsection *Dulcamara*, the herbaceous species with pinnatifid up to bipinnatifid leaves; here he treated 12 species, six of them currently considered members of section *Regmandra* (Bennett 2008).

Bennett (2008) included eleven species in his circumscription of section *Regmandra*, of which nine are native or endemic to Chile, but did not include *S. polyphyllum*. As part of the survey of the flora of Tarapacá, a plant of what appeared to be *Solanum polyphyllum* was found in Compe, on the road to Camiña, in May 2019. That season was relatively wetter than normal, and the vegetation was fully developed and green. These types of greenings or desert blooms associated with the El Niño phenomenon are better known from the southern Atacama, but seem to be a new phenomenon in the northern part of Atacama, in the region of the transition from the driest desert (1900 to 2200 m) to the more humid precordillera and Altiplano. Specific climatic data are sparse, and remote sensing approaches have only recently been undertaken (Chávez et al. 2019). Recollected specimens of the *S. polyphyllum* were compared with the type and the description, and we realised this was a species that had not been collected since 1891. Here we provide a modern description for this rare species, and suggest it belongs in section *Regmandra*. We also provide a key to the Chilean species of section *Regmandra* to assist others in distinguishing *S. polyphyllum* in order that it might be found again.

Taxonomic treatment

Solanum polyphyllum Phil., *Anales Museo Nacional, Botánica* 2: 64, 1891

Figures 1, 2

Type. Chile. Tarapacá Region: prov. Tarapacá, Pachica, 12 Mar 1885, *C. Rahmer s.n.* (lectotype, designated here: SGO [SGO000004586 acc.#55603]; isoelectotypes: B [destroyed, F neg. 2739], CORD [CORD00004258], SGO [SGO000004587 acc.#42779]). Images available via <https://plants.jstor.org/>.

Description. Perennial, robust herbaceous plant up to 100 cm tall. *Stems* thick, glabrescent, internodes with a wing up to 3 mm wide. *Leaves* simple, the blades 3–4 (–8) cm long x 2–4 cm wide, ovate-lanceolate, sessile, base decurrent on the winged petiole, shiny or with few thick hairs, with yellow crystals included in the midrib and stems; upper leaves shorter and thinner. In the buds, there is an oval leaf, with a petiole that has some whitish hairs. *Inflorescence* 7–11 cm long, leaf-opposed, with 12–30 flowers; pedicels filiform 10–12 mm long, with few white hairs 0.5 mm long, which continue in the calyx; calyx 4–5 mm long, with a short tube, $\frac{1}{4}$ – $\frac{1}{3}$ of its length, divided into five almost linear divisions, 1 mm wide. *Corolla* blue, ca. 15 mm in diameter, pentagonal, with five shallow divisions, purple colored at its base forming a star, that is alternated with notorious oblong and yellow-green nectaries, pubescent abaxially mainly towards the apices of the divisions, glabrous adaxially. *Stamens* unequal; anthers differing in size, 4 of 6–9 mm long, 1 shorter of 4–7 mm long, or 3 shorter and 2 longer, tapered, the narrow apex with an elongating pore, the longer anthers curving towards the shorter ones. *Style* curved and longer than the anthers, stigma capitate. *Fruit* 5–6 mm in diameter, a glabrous, globose, shiny green-orange berry. *Seeds* white, ca. 2 mm long, ca. 1.5 mm wide, reniform, with a reticulate surface (Fig. 2).

Distribution. Endemic to the Atacama desert of northern Chile; only known from two localities in the precordillera of Tamarugal province, Tarapacá region.

Ecology and habitat. *Solanum polyphyllum* grows between loose rocks of an alluvial cone on the north side of the Camiña river. It corresponds to a plant of 100 cm high, erect stems, very blue flowers and green-orange berries. The population is composed of only a dozen exemplars. The vegetation of the site is an open bush of low coverage (15%). Other species present on the site are: *Cistanthe amarantoides* (Phil.) Carolin ex Hershkovitz (Montiaceae), *Encelia oblongifolia* DC., *Helogyne apaloidea* Nutt. (both Asteraceae), *Malesherbia tenuifolia* D.Don (Passifloraceae), *Huidobria fruticosa* Phil. (Loasaceae), *Allionia incarnata* L. (Nyctaginaceae) and *Exodeconus integrifolius* (Phil.) Axelius (Solanaceae).

Conservation status. The limited representation in herbaria, as well as the low abundance in the field, makes the *Solanum polyphyllum* a candidate for threatened status. Due to its distribution in the two known locations of Compe and Pachica it is Data Deficient, pending further field work. *Solanum polyphyllum* potentially could be found in Isluga National Park but new surveys are required to corroborate its presence in this protected area.



Figure 1. *Solanum polyphyllum* Phil. (lectotype: C. Rahmer s.n., SGO000004586 acc.#55603).

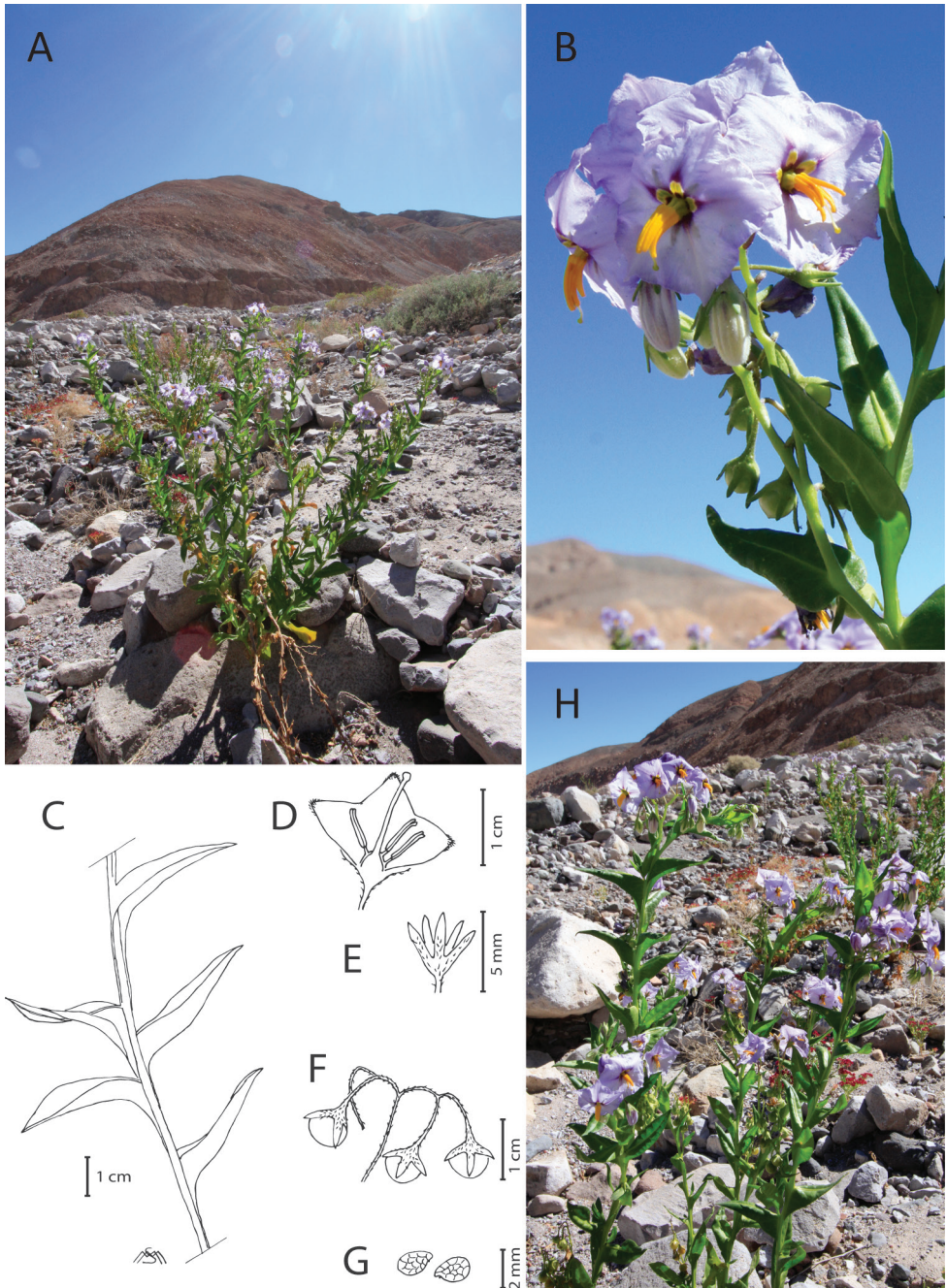


Figure 2. *Solanum polyphyllum* at an alluvial cone at the Tarapacá precordillera **A** habitat **B** flower details **C** leaves **D** flower **E** calyx **F** fruits **G** seeds **H** habit. Photos by A. Moreira-Muñoz, drawings by M. Muñoz-Schick.

Discussion. In its broadly decurrent leaves on the stem, *S. polyphyllum* is similar to *S. paposanum* Phil., which grows both in Perú and in Chile, from 200–3500 m elevation. It differs from *S. paposanum* in its entire (versus margins with 4–5 acute lobes in *S. paposanum*) and glabrous leaves (versus moderately pubescent adaxially and velutinous abaxially in *S. paposanum*).

Solanum polyphyllum was described by Rodulfo Amando Philippi based on specimens collected in Pachica (19°51'58"S, 69°25'56" W, 1622 m alt.) by Carlos Rahmer during a journey to Tarapacá made in 1885. Of this gathering, two duplicates are conserved in SGO. We designate as lectotype SGO000004586 (acc.#55603, Fig. 1), which is very complete and has the handwritten label of R.A. Philippi. The duplicate specimen (SGO000004587, acc.#42779) has a label written by Federico Philippi (son of R.A. Philippi) who also participated in the 1885 expedition.

The rediscovery of this remarkable plant provides an opportunity to promote more intensive fieldwork in the Tarapacá cordillera, at the transition belt between the desert and precordillera vegetation types, where vegetation greening seems to occur more often due to regional climate change. Despite the sparse vegetation that dominates in the Atacama, new botanical discoveries in different plant families are currently happening, as in Asteraceae [*Senecio*] (Calvo and Moreira-Muñoz 2019, 2020), Basellaceae [*Anredera*] (Moreira-Muñoz and Muñoz-Schick 2018), and Solanaceae [*Schizanthus*] (Morales et al. 2020).

Additional specimens examined. Chile. Tarapacá Region, Route A-45 towards Camiña, Compe locality, 19°21'9"S, 69°31'18"W, 1950 m alt., 16 May 2019, A. Moreira 3038 (SGO).

Key to Chilean species of *Solanum* section *Regmandra* (based on Bennett 2008)

- 1 Leaf blades pinnatifid or bipinnatifid 2
- 1a Leaf blades entire, subentire or lobed 3
- 2 Segments of leaves 1 mm wide; corolla 5–7 mm in diameter; Chile: San Ambrosio Island and the coast of northern Chile (Tarapacá, Antofagasta, Atacama Regions) *S. brachyantherum* Phil.
- 2a Segments of leaves 3 mm wide; corolla 11–22 mm in diameter, northern Chile (Antofagasta, Atacama and Coquimbo Regions).... *S. remyanum* Phil.
- 3 Anthers unequal in length..... 4
- 4 Blades with lobed margins, lobes with a smaller secondary lobe arising obliquely at its upper part, both leaf surfaces densely tomentose, Chile (Atacama, Coquimbo and Valparaíso Regions) on dunes and sandy lomas *S. trinominum* J.R.Benn.
- 4a Blades with entire, subentire or shallowly crenate margins 5
- 5 Leaf base cuneate, leaf surface scabrous, Chile (Valparaíso Region) on sandy slopes..... *S. indivisum* Witasek ex J.R. Benn.
- 5a Leaf base decurrent 6

- 6 Leaves with scabrous surface; Chile (southern coast of Coquimbo Region) on sandy slopes ***S. coquimbense* J.R. Benn.**
- 6a Leaves with glabrous surface; Chile (Tarapacá Region, 1600–1950 m)..... ***S. polyphyllum* Phil.**
- 3a Anthers equal in length.....7
- 7 Leaves densely velutinous.....8
- 8 Leaf base decurrent on prominently winged petioles, the wings up to 10 mm wide; coast to high altitudes, 3500 m; Chile (Arica and Parinacota to Coquimbo Regions) and Perú..... ***S. paposanum* Phil.**
- 8a Leaf base truncate, leaves usually petiolate, the petioles without or with a wing up to 1 mm wide; Chile (coastal Atacama Region), ca. 800 m..... ***S. herbabona* Reiche**
- 7a Leaves glabrous to moderately pubescent9
- 9 Inflorescence with 4–10 flowers; leaf margin subentire to deeply lobed, with 2–3 pairs of lobes, the lobe length less than a third of the total leaf width; Chile (coast of Tarapacá) and Perú; on sandy or rocky lomas ***S. montanum* L.**
- 9a Inflorescence with 12–40 flowers; leaf margin with 3–5 pairs of lobes, the lobe length a third to a half of the leaf width; Chile (Arica and Parinacota, Tarapacá, Atacama, Coquimbo, Valparaíso, O’Higgins and Biobío Regions) dunes and coastal slopes ***S. pinnatum* Cav.**

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FlorItaly – the portal to the Flora of Italy

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Abstract

Digital data concerning the flora of Italy are largely fragmented among different resources hosted on different platforms, and often with different data standards, which are neither connected by a common access point, nor by web services, thus constituting a relevant obstacle to data access and usage. Taxonomic incongruences add a further complication. This paper describes “FlorItaly”, an online information system which allows to access and query updated information on the checklist of the flora of Italy, aiming at becoming an aggregator for Italian botanical resources. “FlorItaly” was developed in a collaborative effort by more than 50 taxonomists, with the support of the Italian Botanical Society, and of Project “Dryades” (University of Trieste), to provide a better and reliable organization of botanical knowledge in Italy, as well as a relevant simplification for data retrieval, and a further stimulus towards a more collaborative approach in botanical research.

Keywords

biodiversity data, checklist, floristics, taxonomic standard

Introduction

Taxonomic checklists summarize the hitherto available knowledge of the biodiversity in a given area. They can be simple lists of names, or provide detailed information on each taxon. In well-explored areas, they are the basis for the development of a true flora,

while in poorly known areas they provide a baseline for further investigation. Furthermore, checklists are also a mean for nomenclatural stability, providing a reliable taxonomic backbone. Checklists can potentially be of use for connecting information from different sources, ideally making biodiversity data interoperable through names (or Unique Identifiers – UUIDs – associated with names). While being a fundamental tool for researchers, they can be relevant also for decision-makers, as they provide a baseline for informed decisions. National checklists are especially relevant, since environmental decisions are taken at national level. Being open-ended works, checklists can greatly benefit from a digital “publication”, which allows their updating with new information, continuously, or at regular intervals. Digital, online publication also makes a checklist more visible and accessible to target users (Martellos 2012). Plus, providing open access to information has several positive effects, such as facilitating research, avoiding duplication of efforts, stimulating contributions, etc. (Berendsohn et al. 2018)

The development of online plant data repositories, especially focused on taxonomic information as nomenclatural backbones, is the main aim of several international initiatives. In Europe, the Euro+Med PlantBase (Euro+Med 2006) is a long-lasting effort which aims at achieving an agreed taxonomic core for all families, genera, species, subspecies and, where appropriate, cultivars described from the Euro-Mediterranean region, involving experts from all over Europe. The Plants of the World Online portal (POWO 2020) has a broader aim, since it focuses on enabling users to access information on all seed-bearing plants known worldwide by 2020. It is part of the Science Strategy of Royal Botanic Gardens, Kew, which aims at disseminating Kew’s scientific knowledge of plants and fungi to maximize its impact in science, education, conservation policy and management. Another “global” effort is the World Flora Online (WFO 2020), a project carried on since 2010 in the framework of the Global Strategy for Plant Conservation (GSPC) of the U.N., and supported by the Conference of the Parties to the Convention on Biological Diversity. It aims at becoming a Web-based compendium of the world’s plant species, as a first step towards the development of a consolidated global information service on the world’s flora.

In Italy, efforts for the creation of a network of databases on the flora and vegetation of the country date back from far before the Rio Conference. As discussed by Nimis et al. (1984), Italy was one of the first countries in which a relevant, cooperative effort aimed at creating a distributed network of digital resources for botanical data was initiated. A taxonomic backbone, the first edition of the Flora of Italy (Pignatti 1982) was databased, to serve as a central aggregation core for other databases, especially vegetation data. The authors of that effort already foresaw future steps, such as the integration of digital identification keys in the network. Their vision, however, had not the luck it deserved, and presently Italy still lacks a national network of databases for botanical data. In 2005, the first edition of the checklist of the Italian vascular flora (Conti et al. 2005) was an opportunity for members of the Working Group “Floristics, Systematics, and Evolution” of the Italian Botanical Society to establish an effective network of scientific collaborations (Peruzzi 2018) for updating the list and distributional data up to 2007 (Conti et al. 2007). Other important collaborative efforts followed (Celesti-

Grapow et al. 2009a, b, 2010; Peruzzi et al. 2015, 2019; Brundu et al. 2017; Orsenigo et al. 2018), so that – after 13 years – the time was ripe for an updating of taxonomic, and geographic knowledge on the Italian vascular flora. Thanks to the close cooperation of more than 50 authors, two separate new checklists were published in 2018: one of the native (and doubtfully native) vascular flora (Bartolucci et al. 2018a), the other concerning alien taxa (archaeo- and neophytes) (Galasso et al. 2018a). Thanks to Project “Dryades” of the University of Trieste (Nimis et al. 2003; Nimis and Martellos 2009; Martellos and Nimis 2015), a few months after their publication, nomenclatural, taxonomical and distributional data, integrated with their first updates (Bartolucci et al. 2018b, c, 2019a, b; Galasso et al. 2018b, c, 2019a, b), were organized into an information system on the vascular flora of Italy.

This paper details the result of this effort, “FlorItaly”, which is accessible online at the address <http://dryades.units.it/floritaly>, and is being updated every six months.

Materials and methods

The core of “FlorItaly” is a software written in PHP language, which works on data stored in a MySQL database, running on the servers of the Project “Dryades”, hosted at the Department of Life Sciences, University of Trieste. “FlorItaly” organizes nomenclatural and distribution data from the recent checklists of the Italian native and alien vascular flora and their subsequent updates, which are published every six months, and makes them interoperable with other resources.

Checklist data

The taxonomic circumscription of families follows PPG I (2016) for ferns and fern allies, Christenhusz et al. (2011b) for Gymnosperms, and Angiosperm Phylogeny Group (2016) for Angiosperms, with the exception of Dipsacales (Reveal 2011), Caryophyllales (Hernández-Ledesma et al. 2015) and Boraginales (Luebert et al. 2016). Authors’ citations of plant names were standardized following the Rec. 46A Note 1 of the ICN (McNeill et al. 2012), i.e. according to IPNI (2012). The checklist includes also apomictic taxa belonging to *Alchemilla* and *Rubus* (Rosaceae), *Hieracium*, *Pilosella* and *Taraxacum* (Asteraceae), and the *Ranunculus auricomus*-complex (Ranunculaceae). Taxa at varietal rank and hybrids were not considered. The system organizes 10,898 infra-generic taxa, and 12,887 synonyms, plus 64,001 vernacular names, and an archive of more than 220,000 digital images.

The main data source of “FlorItaly” are the two checklists of the native (Bartolucci et al. 2018a), and alien (Galasso et al. 2018a) vascular flora of Italy, which are updated every six months by a team of researchers of the Working Group “Floristics, Systematics, and Evolution” of the Italian Botanical Society (Bartolucci et al. 2018b, c, 2019a, b; Galasso et al. 2018b, c, 2019a, b). The updates, regularly published on the journal

“Italian Botanist”, are integrated in the online version immediately after their publication. Each new online version is labeled with the year plus an “a” or “b”, depending on the semester of the update.

Distribution data (taken from the checklists, and expressed as presence-absence) are given for each of the 20 administrative regions of Italy (two enclave-countries Republic of San Marino and Vatican City State are not considered). When information on the occurrence of a given subspecies for a region is missing, only the occurrence at species level is reported. For each region, the presence and occurrence status of each taxon is provided by using the following categories: a) occurring, b) doubtfully occurring, c) no longer recorded (reliable historical record), d) extinct or possibly extinct, e) recorded by mistake, f) alien at regional and/or national level (casual, naturalized, invasive, undefined invasion status), g) Italian endemic (status attributed to those taxa occurring only in Italy, or in Italy and Corsica, or in Italy and Malta), h) cryptogenic, i.e. a doubtfully native taxon, whose origin in Italy is unknown, i) taxonomically doubtful, j) data deficient (unknown regional distribution; unknown alien status), k) archaeophyte, and l) neophyte. Occurrence status can also be provided at national level, when relevant, with the following categories: i) confirmed/not confirmed; ii) extinct; iii) doubtful; iv) data deficient; v) erroneously reported for the country; vi) endemic; vii) cryptogenic; viii) esoticity (neo- or archaeophyte). Presence and occurrence status at regional level are depicted in a distribution map (see description of a taxon page below), while those at national level are reported as textual information. A national standardized system was developed by Celesti-Gratow et al. (2009a, b, 2010) to identify taxa alien to Italy. Definitions used in the system were provided by Pyšek et al. (2004):

- **casual:** alien plants that may thrive and even produce offspring occasionally outside cultivation, but that usually disappear, since they are unable to form self-maintaining populations. Hence their persistence relies on repeated introductions;
- **naturalized:** alien plants that occur with self-maintaining populations without direct human intervention;
- **invasive:** alien plants that occur with self-maintaining populations without direct human intervention, and produce fertile offspring which can reach considerable distances from the parent individuals, thus being able to spread over a large area;
- **archaeophytes:** alien plants introduced to Italy before 1492 (approximate date corresponding to the discovery of America);
- **neophytes:** alien plants introduced to Italy after 1492.

Taxa involved in former domestication processes are separated into two categories:

- **culton:** plant distinct from its wild relative(s) and capable to conserve its taxonomic independence in cultivation only; records from the wild are regarded as casual occurrences;
- **feral:** wild plant originated from a culton escaped from domestication, and usually taxonomically distinct from its wild relative; it can either belong to the same taxon of the culton or to a different taxon.

Taxa at varietal rank were not considered; hybrids were considered only for the alien flora.

All of these data are released under a under a CC BY-SA 4.0 license.

Interoperability with other resources

“FlorItaly” makes the checklists data inter-operable with other resources (see below for a comprehensive list) which use the same taxonomy and the same UUIDs of the checklist. This is achieved through:

- web services. “FlorItaly” uses a web service embedded into Wikiplantbase (Bagella et al. 2015; Peruzzi and Bedini 2015; Barberis et al. 2016; Domina et al. 2016) to retrieve information on whether it hosts data for a given infrageneric taxon, and generates a link to the Wikiplantbase taxon page, reporting the number of available records. Queries are sent out encoded in KVP format, and results are retrieved encoded in json format. Web services are also used to access some of the “Dryades” resources, especially image archives. In this case, queries are sent out encoded in KVP format, and results are retrieved in XML format.
- direct querying to external databases, in the case of several “Dryades” resources. Since the developers of all the resources of “Dryades” and “FlorItaly” are the same, it was possible to directly access the other databases by querying through taxon name.
- auto-generated links, made to provide access to resources of Acta Plantarum (2020). A link is dynamically generated by using the UUID of each taxon in the checklist. This provides access to the I.P.F.I. (Index of Plants of the Flora of Italy) taxon page.

Results

“FlorItaly” is accessible online since June 20th, 2018, at the address: <http://dryades.units.it/floritaly>. It has an average of 2,200 page views, and 220 unique visitors per day, and a total of ca. 1,100,000 page loads since its publication on the Internet. Users’ retention rate – calculated for March 2020 – is 61%.

The information system

“FlorItaly” has 3 query interfaces: 1) basic, 2) standard, and 3) advanced. In the second and third interface, users are allowed to combine several parameters in order to perform complex queries. The combination of parameters is transparent to the users, and makes use of the two logical operators OR (when two or more parameters are selected, if either is true, the complex expression is true) and AND (when two or more parameters are selected, all of them must be true for the complex expression to be

true). The two operators are combined in the queries differently in each query interface (see below). All interfaces allow to query by taxon name. The query can be done by inputting an accepted name, a synonym, or part of their names. The query is case-insensitive, and no special characters are allowed (e.g., querying from a string followed by the character “*” will return no results, since the character “*” is read as text, and not as a “jolly” character). Each interface always returns a list of accepted names and/or synonyms, each giving access to a taxon page.

Basic query interface. It allows to quickly access all the information on each taxon, organized in “taxon pages”, which are dynamically generated from the database. When a synonym is typed as a query string, the thesaurus of synonyms is invoked, providing a link to the accepted name. For each query, a list of all synonyms, if present, is provided after the list of the accepted names. The basic interface also allows to filter the query by family (selecting one family from a drop-down menu). Furthermore, it permits to query the Thesaurus of Italian and local names of Project “Dryades”. When an Italian name is used as query string, the system provides a list of vernacular names together with the related scientific name(s). The latter give access to the taxon pages.

Standard query interface. This interface allows simple queries on national and regional floras by including/excluding: i) taxa known from reliable historical records only; ii) extinct taxa; iii) taxa reported by mistake; iv) taxa known from doubtful records only; v) alien taxa. The first four parameters are combined by the logical operator OR (in the same query users can include/exclude more than one of them). The last parameter (alien taxa) is combined with the others by logical operator AND. This interface can also display the results in the form of an image gallery.

Advanced query interface. This interface allows complex queries on the flora of the whole country, or on the floras of different Operational Geographical Units (OGUs), consisting of one or more administrative regions. If no OGU is selected, the system operates on the whole national flora, including extinct taxa, and those reported by mistake. For Italy, or for any other OGU, it is possible to refine the query by using the following seven groups of parameters:

- A) occurrence status: 1) taxa known from reliable historical records only; 2) extinct taxa; 3) taxa reported by mistake; 4) taxa known from doubtful records only; 5) data deficient taxa (those recorded from Italy, but without sufficient knowledge on regional records);
- B) alien status: 6) invasive; 7) naturalized; 8) casual; 9) other alien taxa (currently without invasiveness status); 10) cryptogenic taxa (doubtfully native);
- C) alien, by period of introduction: 11) neophytes (since 1492); 12) archaeophytes (until 1492);
- D) feral/culton status: 13) feral; 14) culton;
- E) 15) Italian endemics;
- F) 16) exclusive endemics (Italian endemics whose distribution is restricted to the selected OGU);
- G) 17) taxonomically doubtful taxa.

Inside each group of parameters, the system uses the logical operator OR, while among the groups the system uses the logical operator AND, as described for the standard query interface (see above).

Taxon pages and external resources

The taxon pages, which are the final outcome of a query, display data from the checklist, and aggregate, or link different external resources (Fig. 1).

At the top of the taxon page there is the accepted name, followed by synonyms, and the presence in the 20 administrative subdivisions of the country. Other information, such as whether the taxon is an archaeo-/neophyte, endemic, etc. are reported immediately below, when present.

This first block is followed by two external resources, a link to a cladogram, with the relative taxonomic information, and an image, which are displayed on the left. On the right of the page, a distribution map is dynamically generated by using the presence in the administrative regions, plus the other data on the taxon (e.g. whether it is an invasive).

The cladograms and the taxonomic information are external resources, deriving from an archive of cladograms from Project “Dryades”, which follows Smith et al. (2006), Schuettpeitz and Pryer (2008), Ran et al. (2010), Christenhusz et al. (2011a, 2011b), and the Angiosperm Phylogeny Group (2016). The image comes from the archive of digital images of vascular plants of Project “Dryades” (see: <http://dryades.units.it/cercapiante/index.php>).

These two sections are followed by a link to the I.P.F.I. database of Acta Plantarum, and a link to the Wikiplantbase Italy project. Each link reports the name of the resource, and its logo, and opens in a new page. Acta Plantarum is one of the most active groups of amateur botanists in Italy, and the resources present in the I.P.F.I. pages include digital images, altitudinal distribution, growth forms, etymology, discussions, etc. The Wikiplantbase initiatives (Bagella et al. 2015; Peruzzi and Bedini 2015; Barberis et al. 2016; Domina et al. 2016) collect and organize geo-referenced occurrence data (Bedini et al. 2016; Peruzzi et al. 2017) for different administrative regions of Italy. Originally, “FlorItaly” linked each of the regional initiatives, but, since they are growing in number, it was decided to switch to a link to the national Wikiplantbase portal, which aggregates data from all regional initiatives. These links are followed by names from the Thesaurus of vernacular names for Italy of Project “Dryades”, largely based on those proposed by Pignatti (1982), and especially by Penzig (1924).

At the bottom of the taxon page there is an image gallery, which currently derives from the archives of Project “Dryades”. However, since “FlorItaly” can be made interoperable with other resources, potentially other archives can be accessed, and integrated in the taxon pages (or linked as external resources). Images are displayed as thumbnails, and can be enlarged by a simple click. Each image is coupled with metadata that specify author, source, license of use, locality, and date of the shot, and any other metadata, when available. When the license is not specified, the author of the

Figure 1. Taxon page for *Poa annua* L. from *FlorItaly* [accessed on 18 June 2020]. The page lists taxon name, synonyms, distribution, also depicted in a distributional map, taxonomic position (in this case in the APG IV scheme), an image, links to external resources, Italian names, and a photo gallery.

image reserves all rights. Approximately 80% of the images of Project “Dryades” are original, and released under a CC BY-SA 4.0 license, which means that anyone can use them, for any purpose, provided that author, source and license are mentioned.

Discussion

Global access to biodiversity information is considered mandatory for research, and decision making since the UNESCO Conference of Rio outputs (Berendsohn and Seltmann 2010; Berendsohn et al. 2010; Berents et al. 2010; Vollmar et al. 2010). Furthermore, the Convention on Biological Diversity includes as its Target 1 the need for “An online flora of all known plants”. Several important actions have been taken in the last 20 years to digitize and mobilize biodiversity data. Large distributed databases (e.g., GBIF, <http://www.gbif.org>, or BioCASE, Güntsch et al. 2007) have been created to organize and share primary biodiversity data, and several approaches to digital identification have been investigated (Dallwitz et al. 2002; Martellos 2010; Nimis and Vignes Lebbe 2010). The digitization of checklists has been also addressed. One of the first efforts to publish online national checklists was the “Index Synonymique de la Flore de France” (Brise and Kerguélen 1994; Kerguélen 1994; <https://www2.dijon.inra.fr/flore-france/>). More recently, thanks to the EDIT platform for cybertaxonomy (Berendsohn 2010), a number of checklists were published online (<https://cybertaxonomy.eu/references>): the second edition of the Flora of Greece, the Flore du Gabon, the Flora of Cyprus, the Flora of Central Africa, etc.

Given the relevance of the two checklists of native and alien vascular flora of Italy (Bartolucci et al. 2018a; Galasso et al. 2018a), the mobilization of their rich content after their publication in paper-printed form was a further step (Peruzzi 2018). Data mobilization is the focus of several efforts in biodiversity informatics, since it became evident that data stored in the form of journal articles need to be extracted, organized in line with appropriate standards, and aggregated into online databases. Some recent examples in this direction are the BIOfid information service (Driller et al. 2018), the PLAZI workflow (Agosti et al. 2019), and the Open Biodiversity Knowledge Management System (OBKMS) initiative (Penev et al. 2018). For the creation of “FlorItaly”, a collaborative effort was initiated between Project “Dryades” of the University of Trieste (Nimis et al. 2003), and the leading authors of the two checklists, in order to make their data accessible online as an actual information system capable of complex queries, and as a “core” for aggregating, and linking further data and resources. In Italy, botanical digital data are currently fragmented in a wealth of different resources, and the role of “FlorItaly” as an aggregator could become very relevant in the next years. As an example, primary biodiversity data for plants are available in the Wikiplantbase repositories, in the Italian Biodiversity Network of the Ministry of Environment (Martellos et al. 2011), and in several online herbaria (e.g., the Virtual Herbarium of the University of Palermo, http://www.ortobotanico.unipa.it/virtual_herbarium.html), often hosted on different platforms, and adopting different standards, thus making

access to data quite complex and fragmented, and creating a relevant obstacle to data retrieval. Taxonomic incongruences add a further complication. The use of a checklist as an aggregator provides a necessary taxonomic backbone to all other resources. All members of the Working Group are committed on agreeing on a common taxonomic backbone (see data and methods), adopted at the national level. The use of “FlorItaly” as an aggregator provides a better and reliable organization of botanical knowledge in the country, as well as a relevant simplification for accessing data by researchers. At the same time, it will provide a further stimulus towards a more collaborative approach in botanical research, allowing quick and solid answers to challenging questions, especially now that global change-related issues require fast and reliable answers from science.

As far as sustainability of the system is concerned, “FlorItaly” will be maintained by the Department of Life Sciences of the University of Trieste, which will assure regular updates of software, and data. Furthermore, a backup instance of “FlorItaly” will be installed in the forthcoming LifeWatch (Basset and Los 2012) Center for Botanic Diversity Data, which will be hosted at the Department of Biological, Geological, and Environmental Sciences, “Alma Mater Studiorum” University, Bologna. As far as data are concerned, sustainability and regular updates are provided by the volunteer, collaborative work of the members of the Working Group “Floristics, Systematics, and Evolution” of the Italian Botanical Society, which come from the academia, or are private citizens, committed in a medium-long term effort.

While originally developed to target an academic audience, “FlorItaly” can be useful for a wider target audience, such as decision makers, and citizens, in the fields of formal education, life-long learning, and citizen science. Other resources (such as primary biodiversity repositories, a *loci classici* database, etc.) are planned to be made interoperable in “FlorItaly”. Furthermore, the system could be potentially connected to digital identification keys. Some keys have been already developed in the framework of Project “Dryades”, by using software FRIDA (Martellos 2010). These keys are particularly suitable to be connected to “FlorItaly”, since FRIDA allows to generate keys to lists of taxa (local floras, or any group of plants, e.g., aquatic plants of N Italy), such as those which result from querying “FlorItaly”. Thus, potentially, any query could produce not only a mere list of taxa, but a digital identification key to those taxa as well. Other planned changes in the system will be introduced in the next technical releases. They will be decided on the basis of users’ feedback, and will focus on improved usability of the interfaces, and on the possibility of changing query parameters in the results page, without the need of performing another query. Furthermore, the possibility to download results data in csv format will be also explored.

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New species of *Anthurium* Schott (Araceae) from the Kōkōi Eujá Natural Traditional Reserve of the Pacific coast, Cauca Department, Colombia

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Abstract

The Kōkōi Eujá Natural Traditional Reserve was created in 2019 to protect the golden poison frog (*Phyllobates terribilis* Myers, Daly & Malkin, 1978) in the Cauca Department of Colombia. As part of the biodiversity inventory of the reserve a new species of *Anthurium* with scandent habit was discovered. The new species is endemic to Colombia and it is more similar to *A. caldodsonii* Croat, *A. boekei* Croat, and *A. scandens* (Aubl.) Engl., but differs by having widely lanceolate leaves with acuminate apex, cuneate base, and acrodromous venation with three pairs of basal veins.

Resumen

La reserva natural tradicional Kōkōi Eujá fue creada en 2019 en el departamento de Cauca, Colombia, para proteger la rana dardo dorado (*Phyllobates terribilis* Myers, Daly & Malkin, 1978). Durante el proceso de realizar el inventario de la biodiversidad de la reserva se descubrió una especie nueva del género *Anthurium*. La especie nueva es endémica de Colombia, y es similar a *A. caldodsonii* Croat, *A. boekei* Croat y *A. scandens* (Aubl.) Engl., de las cuales se diferencia por tener hojas lanceoladas con ápice acuminado y base cuneada, y venación acródroma con tres pares de venas basales.

Keywords

Eperãra Siapidaará, *Phyllobates terribilis*, section *Digitinervium*, section *Tetraspermium*

Palabras clave

Eperãra Siapidaará, *Phyllobates terribilis*, sección *Digitinervium*, sección *Tetraspermium*

Introduction

The genus *Anthurium* Schott has more than 1000 described species (WCSP 2020), but the estimated number is 2000 (Boyce and Croat 2018). Colombia is believed to be the country with the highest *Anthurium* diversity, and the Pacific slope of the Andes is the most diverse region in the country (Croat et al. 2010). This region overlaps with one of the world biodiversity hotspots, the Tumbes-Choco-Magdalena region (Myers et al. 2000), which remains poorly known, especially in the departments of Valle del Cauca, Cauca and Nariño, to the south of Colombia. The Pacific slope of the Andes region is not only important because of its biological diversity but also because of its cultural diversity. Several indigenous communities still survive in this part of Colombia, including the Eperára Siapidará, which are known for the traditional use of the frog *Phyllobates terribilis* Myers, Daly & Malkin, 1978, the golden poison frog (Kókói in Sia language) as a source of poison used for hunting (Myers et al. 1978). However, the survival of these communities and the forests they inhabit is being threatened by factors like illegal mining, illegal timber extraction, and drug trafficking.

By initiative of four communities of the Eperára Siapidará people belonging to the indigenous Calle Santa Rosa Reservation of the municipality of Timbiquí, in the Cauca Department, a new protected area was declared in order to protect the habitat of the golden poison frog. The new reserve was named the Kókói Eujá Natural Traditional Reserve and was included in the National Protected Areas System of Colombia (Paz et al. 2019). The process was led by the Ecohabitats Foundation and the Corporación Autónoma Regional del Cauca, with the support from the Rainforest Trust. The Calle Santa Rosa Indigenous Reservation is located between the municipalities of Timbiquí and López de Micay, and it covers about 21,320 ha. It is currently made up of four communities (La Sierpe, Calle Santa Rosa, Las Peñas and Unión Málaga) (Quiro 2017) with a population of 1,027 people in 234 families distributed in 115 homes. Its economy is based on fishing, and some crops, including bananas, cassava and coconut, in addition to the sugar cane cultivation to produce viche (an alcoholic beverage), which is sold in the municipal capital (Paz et al. 2019). The new reserve comprises about 11,641 ha corresponding to 56 percent of the total area of the reservation, the most representative land coverage is primary forest (11,412 ha), followed by secondary vegetation (144.9 ha), clean pastures (68.3 ha) and crops (15.9 ha).

Methods

During 2018 the first author carried out field work off the Pacific coast in Timbiquí and López de Micay, in the Cauca Department of Colombia, to generate a baseline for the creation of the Kókói Eujá Natural Traditional Reserve. A new species of the genus *Anthurium* was found, and is described and illustrated here following Croat and Bunting (1979). All measurements are based on dried specimens.

Taxonomic treatment

Anthurium siapidaarae Zuluaga & Sánchez-Taborda, sp. nov.

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Figs 1–3

Type. COLOMBIA. Cauca: municipio de Lopez de Micay, resguardo indígena Calle Santa Rosa, camino entre la orilla de la quebrada Bibango, afluente del río Saija, 02°57.467'N, 77°32.967'W y el bosque primario en la parte alta de la colina 02°58.056'N, 77°32.878'W, 16–65 m de altura, 10 September 2018, Jhon Alexander Sánchez-Taborda, Luis Alfonso Ortega, Carlos Robinson Quiro, José Tovar & Jainer Mejía 3141 (Holotype CUVCI!).

Diagnosis. *A. siapidaarae* differs from *A. caldodsonii* Croat, *A. boekei* Croat and *A. scandens* (Aubl.) Engl. by having widely lanceolate leaves with acuminate apex, cuneate base, and acrodromous venation with three pairs of basal veins, one of them 0.3–1 mm from the margin (versus leaves acuminate apex, cuneate base, and acrodromous venation with two pairs of basal veins).

Description. Epiphytic climbing herb; roots loose and spreading, slender, brown; stems cylindrical, green, drying green-brown; internodes 3–4.5 cm long, (2.7–) 3.7–4.4 mm diam when dry, weakly glossy, green, drying grayish-green; cataphylls 4–5.6 cm long, acuminate at apex, brown and glossy, drying light brown mate, persistent as fibers at the upper nodes, with only a few fibers remaining at basal nodes. **Leaves** scattered along stem; petioles ribbed canaliculate adaxially, (3.2–) 4.2–5.6 cm long, 0.2–0.3 mm diam, olive green, drying grayish-green; sheath 3–4 mm long, 2–4 mm width at midpoint, occupying less than ¼ the length of the petiole; geniculum 3–6 mm long, 3 mm diam, green, darker than the petiole and drying dark-brown; blades coriaceous, 4.6–7 cm long, 2.8–4.4 cm wide, 1.2–1.4 times longer than wide, widely lanceolate, acuminate at apex, cuneate at base, adaxial surface glossy dark-green, abaxial surface glossy light-green, black punctations present on both sides, margins slightly revolute; midrib impress and slightly paler above, prominent and dark green below; basal veins 2 per side when the plant is young, 3 per side when adult, one of them, the marginal collective vein, 0.3–1 mm from the margin; primary lateral veins 8–9 per side when the plant is young, 13 when adult, arising at an angle of 35–40° degree, concolorous and impress above, prominent and discolorous below. **Inflorescence** pendant; peduncle curved, cylindrical, slightly striate longitudinally, 8.5 cm long, 0.3 cm diam, 1.7 times longer than the petiole, reddish, weakly glossy, drying brown; spathe reddish and glossy, coming out at a 70° angle to the spadix, 1.7 cm long, 0.5 cm wide, lanceolate, with acuminate apex and decurrent base; stipe cylindrical ca. 2 mm long, 3 mm diam, green yellow, drying grayish-green; spadix light yellow, erect, cylindrical, 2.4 cm long, 0.4–0.5 cm diam, 1.4 times longer than spathe; **flowers** 5–6 in the principal spiral, 9–10 in the secondary spiral, outline rhombic in frontal view, ca 1.5 mm diam; tepals yellow to reddish, weakly glossy, drying dark-brown, 0.4 mm long, 0.7 mm wide when

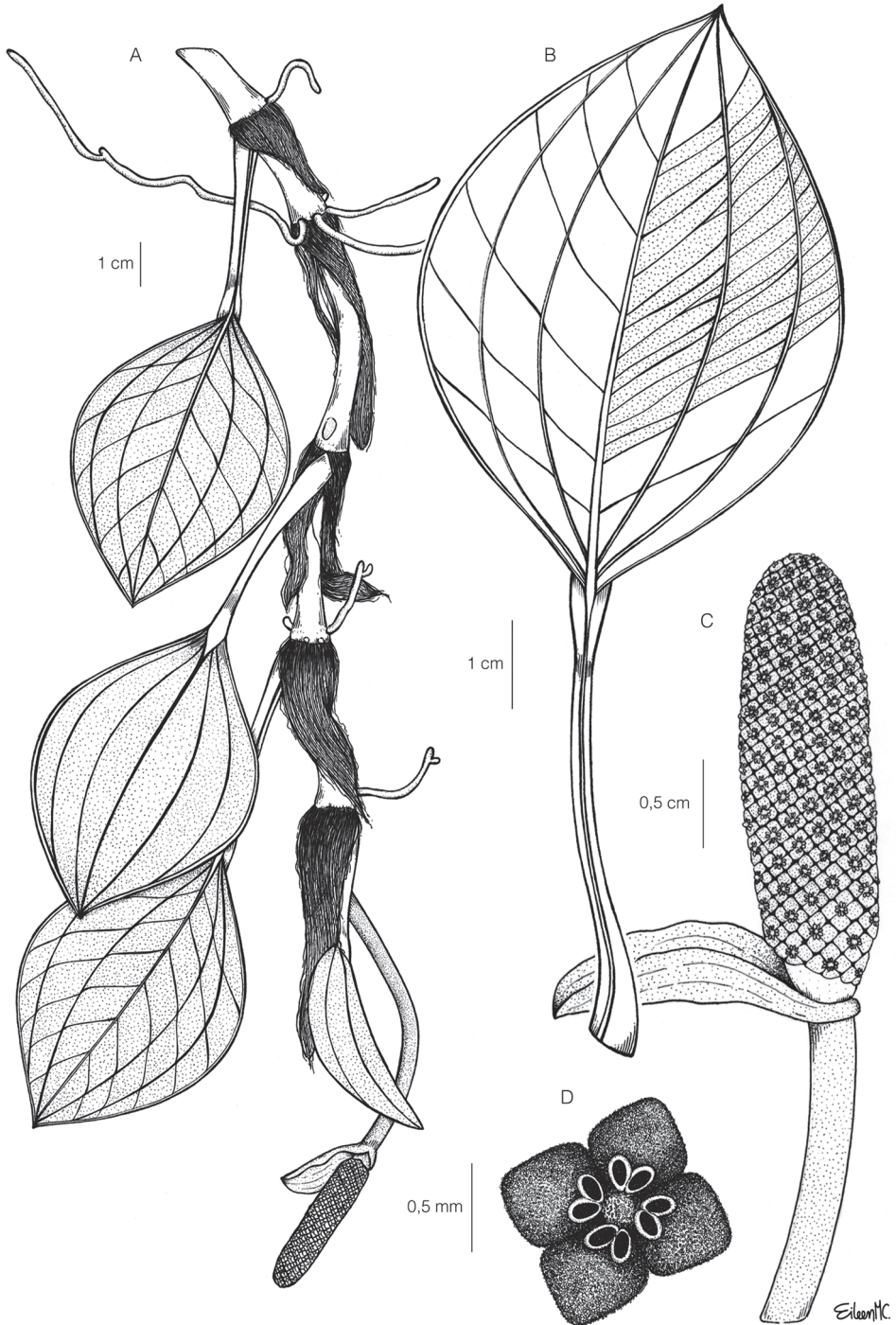


Figure 1. Illustration of *Anthurium siapidaarae* Zuluaga & Sánchez-Taborda, sp. nov. **A** habit and adult plant **B** leaf detail, adaxial view **C** inflorescence with spathe and peduncle **D** flower, frontal view. Illustration by Eillen Muñoz, based on the type collection J.A. Sánchez-Taborda 3141.

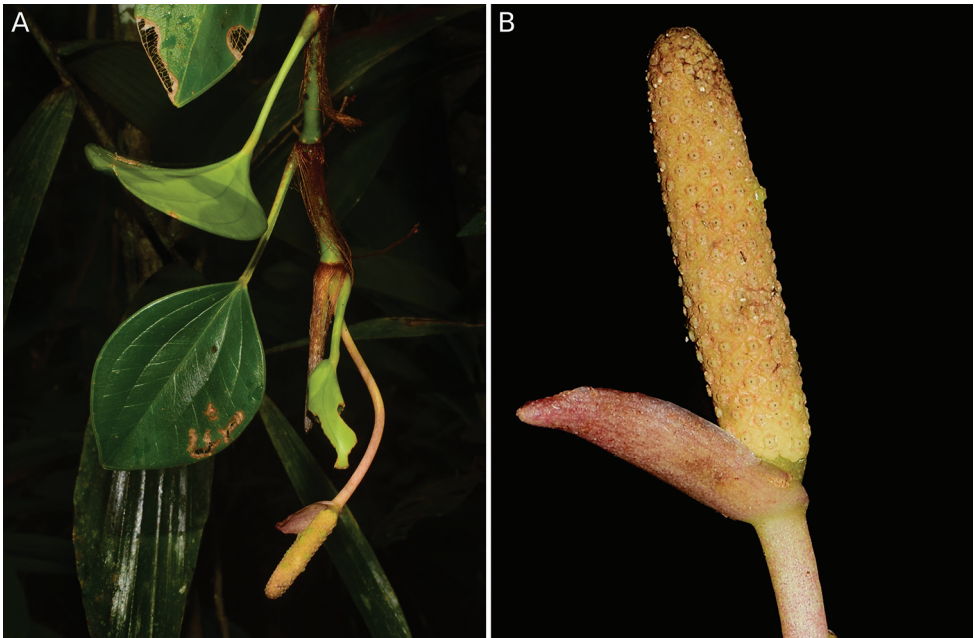


Figure 2. *Anthurium siapidaarae* Zuluaga & Sánchez-Taborda, sp. nov. **A** habit of living plant showing leaves and cataphylls **B** inflorescence. (Photographs by Jhon A. Sánchez-Taborda).

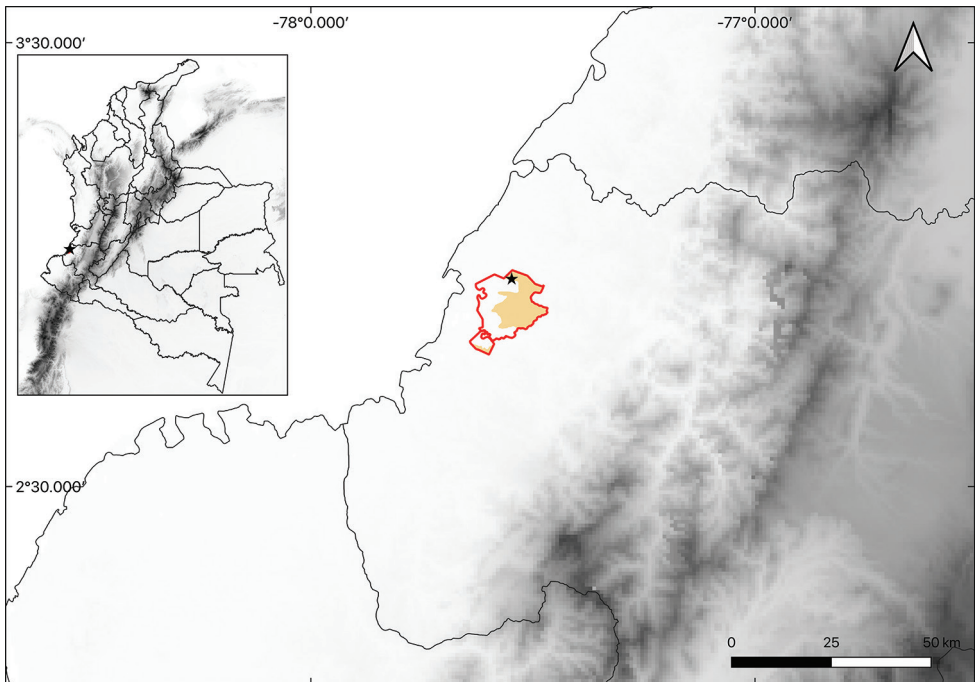


Figure 3. Distribution of *Anthurium siapidaarae* Zuluaga & Sánchez-Taborda, sp. nov. Boundaries of the Calle Santa Rosa Indigenous Reservation are shown in red, the area of the Kókoi Eujá Natural Traditional Reserve in yellow. The star shows the locality where the species was collected.

dry; pistil ca 1.3 mm diam, stigma capitate ca 0.4 mm diam with trichomes; stamens slightly exserted, filament short, anthers 0.3 mm wide; **fruits** not seen.

Distribution and ecology. *Anthurium siapidaarae* is endemic to Colombia, only known from the type locality in the municipality of López de Micay, Cauca. This species inhabits the Tropical Rain Forest between 30 and 100 m above sea level. *A. siapidaarae* is locally scarce, growing as an epiphyte inside dense forest with a closed canopy that exceeds 35 m in height, dominated by species of the genera *Protium* Burm. f., *Pouteria* Aubl., *Ficus* L., *Otoba* (A. DC.) H. Karst., *Ocotea* Aubl. and *Inga* Mill.

Phenology. *Anthurium siapidaarae* was found flowering in September.

Etymology. *Anthurium siapidaarae* is named after the indigenous community inhabiting the Calle Santa Rosa Indigenous Reservation. They belong to the Eperára Siapidaará people who live in the departments of Valle del Cauca, Cauca and Nariño in southwest Colombia. The word Siapidaara makes reference to the language Sia Pedecé spoken by these indigenous people.

Preliminary conservation status. *Anthurium siapidaarae* is only known from one locality, where it is not abundant. Its populations are under protection thanks to the Kókoi Eujá Natural Traditional Reserve, which has an area of 11641 ha (ca 115 km²). Despite being under protection, there are several pressures affecting the conservation of these forests, especially the increase of illegal crops surrounding the reservation and deforestation, which had a rate of 7.8 ha per year between 2001–2018 within the protected area (Paz et al. 2019). Under the IUCN criteria (IUCN 2017) we consider this species should be listed as Vulnerable.

Notes. *Anthurium siapidaarae* could belong to sections *Tetraspermium* (Schott) Engl. Or *Digitinervium* Sodiro. The main character of section *Tetraspermium* is the presence of four seeds per fruit, so we cannot be completely sure of this placement due to the absence of fruits in the samples. There are also some similarities with species from section *Digitinervium*, mainly the thick leaves with glandular punctations, and three pairs of acrodromous veins. It is most similar to *A. caldodsonii*, *A. boekei*, endemic from Ecuador and *A. scandens*, a widespread species. All four species share the characters of section *Tetraspermium*, having scandent habit, long internodes, persistent fibrous cataphylls at least in the terminal nodes, and small leaves with a glandular-punctate lower surface. *A. siapidaarae* differs from the other species by having acrodromous venation with three pairs of basal veins, one of which, the collective marginal vein, is 0.3–1 mm from the margin (vs acrodromous venation with two pairs of basal veins in all other three species). It also differs from *A. caldodsonii* and *A. boekei*, only known from Ecuador, by having lanceolate leaves less than 1.4 times longer than wide, with acuminate apex and cuneate base (versus ovate to ovate-elliptical leaves, with truncate base and apex), and from *A. scandens*, by having a peduncle 8.5 cm long (vs. 1.5–6.5 cm long).

Additional specimens examined (paratypes). COLOMBIA. Cauca: municipio de López de Micay, resguardo indígena Calle Santa Rosa, camino entre la orilla de la quebrada Bibango, afluente del río Saija, 02°57.467'N, 77°32.967'W y el bosque primario en la parte alta de la colina 02°58.056'N, 77°32.878'W, 16–65 m de altura. 11 September 2018, Jhon Alexander Sánchez-Taborda, José Tovar & Jainer Mejía 3245 (CUVC).

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Conifer wood assemblage dominated by Podocarpaceae, early Eocene of Laguna del Hunco, central Argentinean Patagonia

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Abstract

During the early Eocene, Patagonia had highly diverse floras that are primarily known from compression and pollen fossils. Fossil wood studies from this epoch are scarce in the region and largely absent from the Laguna del Hunco flora, which has a highly diverse and excellently preserved compression assemblage. A collection of 26 conifer woods from the Laguna del Hunco fossil-lake beds (early Eocene, ca. 52 Ma) from central-western Patagonia was studied, of which 12 could be identified to genus. The dominant species is *Phyllocladoxylon antarcticum*, which has affinity with early-diverging Podocarpaceae such as *Phyllocladus* and *Prumnopitys*. A single specimen of *Protophyllocladoxylon francisiae* probably represents an extinct group of Podocarpaceae. In addition, two taxonomic units of cf. *Cupressinoxylon* with putative affinity to Podocarpaceae were found. Diverse Podocarpaceae taxa consistent with the affinities of these woods were previously reported from vegetative and reproductive macrofossils as well as pollen grains from the same source unit. Some of the woods have galleries filled with frass. Distinct growth ring boundaries indicate seasonality, inferred to represent seasonal light availability. Growth ring widths suggest that the woods came from mature trees, whereas the widths and types of some rings denote near-uniform temperature and water availability conditions.

Keywords

fossil forests, Huitrera Formation, Paleogene, Podocarpaceae, South America, wood anatomy

Introduction

The early Eocene Earth had warm climates worldwide (e.g., Zachos et al. 2001). In Patagonia, the southernmost region of South America, early Eocene ecosystems had highly diverse floras from mesothermal rainforest environments (Wilf et al. 2003, 2005, 2009; Barreda and Palazzesi 2007).

The volcanic-lacustrine strata of the Tufolitas Laguna del Hunco of the Eocene Huitrera Formation exposed at Laguna del Hunco in northwestern Chubut, central Patagonia, have long been known for their diverse and superbly preserved plant fossils (e.g., Berry 1925). According to previous paleobotanical studies, an extremely diverse mesothermal flora dominated by angiosperms, coupled with a significant presence of ferns and conifers, was present in the area (e.g., Wilf et al. 2003, 2005; Barreda et al. 2020). Among the conifers, Podocarpaceae (Wilf 2012, 2020; Wilf et al. 2017; Andruchow-Colombo et al. 2019), Cupressaceae (Wilf et al. 2009), and Araucariaceae (Wilf et al. 2014; Barreda et al. 2020; Rossetto-Harris et al. 2020) are well-represented in the flora. The modern biogeographic affinities of the Laguna del Hunco flora are diverse, and especially large concentrations of survivor genera are found in the tropical West Pacific region (e.g., Wilf et al. 2013).

Although fossil leaves, reproductive structures, and pollen are well documented, a comprehensive study of the woods from Laguna del Hunco is lacking. Petersen (1946) reported fossil trunks exposed in the upper portion of the fossil lake beds, but so far, the only described silicified specimen is a stem of the osmundaceous fern *Todea* from the southern exposures of the Tufolitas (Bippus et al. 2019; Bomfleur and Escapa 2019). In general, studies of Patagonian Eocene woods are scarce. From Argentinean Patagonia, Brea et al. (2009) described a conifer collected from the Koluel-Kaike Formation, and recently Pujana and Ruiz (2019) described an assemblage from the Río Turbio Formation with woods of Araucariaceae, several Podocarpaceae, Proteaceae, Cunoniaceae, and Nothofagaceae. From the Ligorio Márquez Formation in central-south Chilean Patagonia, Terada et al. (2006a) described a few woods of Araucariaceae, Podocarpaceae, and Cunoniaceae, and Terada et al. (2006b) described another small assemblage with Araucariaceae, Podocarpaceae, and Nothofagaceae from the Loreto Formation in southern Chilean Patagonia.

During a recent field season, we collected a large sample of fossil woods from the Tufolitas Laguna del Hunco, including both angiosperms and conifers. In this contribution, we treat the conifer woods from Laguna del Hunco for the first time. This work comprises the largest study to date of the Laguna del Hunco flora from permineralized wood specimens, otherwise known almost entirely from compression and palynomorph remains.

Materials and methods

Fossil-wood samples were collected 3–5 December 2016 from 10 localities in the exposures of the Tufolitas Laguna del Hunco, Huitrera Formation, at Laguna del

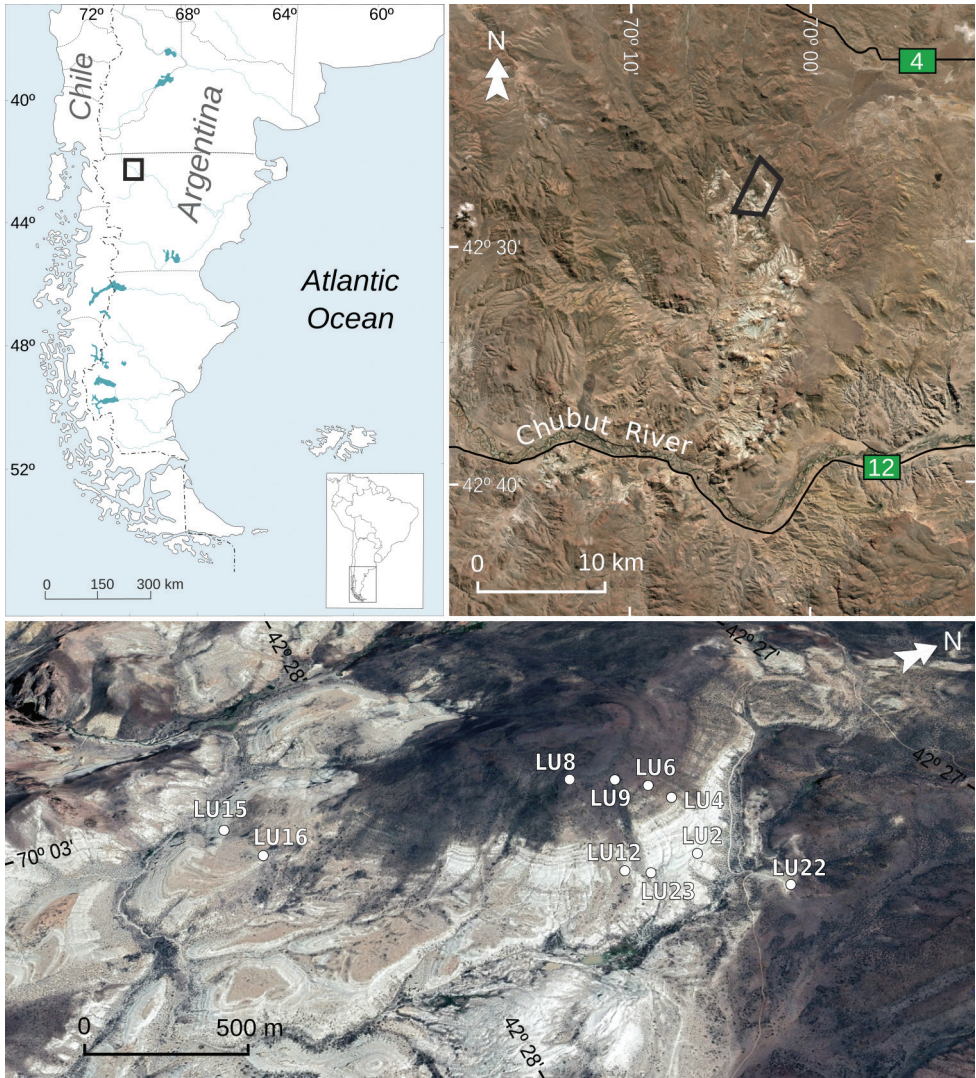


Figure 1. Location map and satellite images (Instituto Geográfico Nacional de la República Argentina, upper, and Google, CNES / Airbus, below) showing the Laguna del Hunco section and sampling locations. Scale in the satellite image below (tilted) varies across the map.

Hunco (Fig. 1; Table 1). The Tufolitas Laguna del Hunco are tuffaceous caldera-lake sediments that belong to the volcanoclastic-pyroclastic complex of the middle Chubut River (Aragón and Mazzoni 1997). The age of the Tufolitas Laguna del Hunco at Laguna del Hunco is constrained to the early Eocene (Ypresian) by the combination of a 52.22 ± 0.22 Ma ^{40}Ar - ^{39}Ar age analyzed from sanidines in a tuff from the middle of the 170 m section, additional ^{40}Ar - ^{39}Ar ages from the lake beds as well as associated units, and paleomagnetic data (Wilf et al. 2003, 2005; Wilf 2012; Gosses et al. 2020). The studied fossil woods were found on strata throughout the local Tufolitas section

Table 1. Geographical coordinates of the localities where the fossils were collected.

Sampling locality	Geographical coordinates (DDM)	n° of conifer woods studied (identified to genus)
LU2	42°27.53'S, 70°02.26'W	1 (1)
LU4	42°27.51'S, 70°02.43'W	2 (1)
LU6	42°27.54'S; 70°02.48'W	4 (2)
LU8	42°27.68'S; 70°02.56'W	1 (1)
LU9	42°27.60'S; 70°02.52'W	2 (0)
LU12	42°27.67'S; 70°02.28'W	1 (0)
LU15	42°28.28'S; 70°02.92'W	1 (1)
LU16	42°28.23'S; 70°02.76'W	6 (1)
LU22	42°27.42'S; 70°02.09'W	7 (4)
LU23	42°27.63'S; 70°02.25'W	1 (1)

of Wilf et al. (2003; Fig. 1), including the upper third of the section that contains few compression fossils, and even the uppermost lake beds below the hill-capping *Andesitas Huanache* (per Aragón and Mazzoni 1997). Most specimens were found exposed on slopes, clean of attached sediment and with abraded surfaces, indicating that they were reworked downslope to an unknown extent from various possible source levels within the Tufolitas.

The 26 conifer fossil woods studied here (Table 2) are part of a larger collection of 87 wood samples. All studied specimens are decorticated and consist of permineralized (mostly by silica) secondary xylem, and their preservation is quite variable; only 12 of the 26 specimens could be assigned to generic level. Of the remaining specimens, 56 are identified as angiosperms (under separate study) and five, due to very poor preservation, could not be determined to any taxonomic group.

All specimens are housed at the Museo Paleontológico Egidio Feruglio, Trelew, Chubut Province, Argentina, repository acronym MPEF-Pb (Table 2). Thin sections of each sample bear the specimen number followed by a lower case series letter. We prepared thin sections (transverse, TS; tangential longitudinal, TLS; radial longitudinal, RLS) following standard techniques and studied them using light microscopy. Small fragments (radial views) of the samples were gold-coated and observed with scanning electron microscopy (SEM, Philips XL30 located in the Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina). Microscopic images were taken with a Leica DM500 microscope with a Leica DFC420 camera. Images were processed with GIMP 2.8.22 software, and measurements from the images were taken with IMAGEJ 1.52 software.

We followed the terminology of the IAWA Softwood Committee (2004) and the Si and Cp indices of Pujana et al. (2016) for describing wood anatomy. These two indices quantify the intertracheary pitting; e.g., Si = 1.00 indicates that all the intertracheary pits are uniseriate, S > 1.00 indicates that there are two- or more-seriate pits, Cp = 0% that pits are non-contiguous, and Cp = 100% that all pits are contiguous (Pujana et al. 2016). We also followed the definitions of Philippe and Bamford (2008) for classifying intertracheary pitting into the categories abietinean, mixed, and araucarian. In abietinean intertracheary pitting, most (ca. > 90%) of the pits in the radial walls are non-contiguous, are rounded in outline, and when in rows are opposite. In araucarian

pitting, most (ca. > 90%) of the pits are contiguous and commonly alternate and angular in outline. Mixed pitting is when the pitting arrangement is between araucarian and abietinean. When possible, at least 15 measurements or observations of each character were made per specimen. Measurements are expressed as the mean followed by the range and mean standard deviation (SD) in parentheses. Measurements reported from species with more than one specimen were taken from all samples. Taxonomic determination was implemented using the criteria of Philippe and Bamford (2008) for delimiting conifer fossil-genera, while Bengtson (1988) was followed for open nomenclature names.

For growth ring classification, we followed the scheme of Creber and Chaloner (1984). Minimum estimated diameters (MED) of the trunks/stems were measured directly on the sample or roughly calculated based on the curvature of the growth rings; when they had virtually straight growth ring boundaries, a 50 cm diameter was assigned.

Systematic Paleontology

Genus *Protophyllocladoxylon* Kräusel

Protophyllocladoxylon francisiae Pujana, Santillana & Marensi

Figure 2A–F

Studied material. MPEF-Pb 10694.

Locality. LU2 at Laguna del Hunco (Fig. 1, Table 1), Chubut Province, Argentina.

Stratigraphic provenance. Tufolitas Laguna del Hunco, Huitrera Formation (Ypresian, early Eocene).

Description. Growth ring boundaries are distinct (Fig. 2A, B), latewood with 1–3 rows of tracheids (Fig. 2B). Tracheids are roundish to polygonal as seen in transverse section (Fig. 2B). Intertracheary pitting in radial walls is mixed, uni- to biseriate, predominantly uniseriate ($Si = 1.25$), contiguous ($Cp = 88.1\%$), and mostly alternate, rarely opposite, when biseriate (Fig. 2C, D). Intertracheary pits are hexagonal to rounded in outline; 19.2 (13.8 – 24.6 , $SD = 1.9$) μm in vertical diameter (Fig. 2C, D). Tracheid tangential diameter is 44.5 (30.3 – 61.2 , $SD = 7.0$) μm . Cross-fields have 1–4, mean 1.9, pits per cross-field (Fig. 2E, F). Cross-field pits are circular with simple borders (rarely with narrow borders); 14.8 (11.8 – 18.4 , $SD = 1.8$) μm in vertical diameter (Figs 2E, F, 6A). Horizontal walls of ray parenchyma cells are smooth (Fig. 2E). Wall alteration (not helical thickening) of the secondary walls of tracheids is observed (Fig. 2G). Average ray height is medium, 5.6 (1 – 13 , $SD = 3.2$) cells high, rays are exclusively uniseriate (Fig. 2H, I) and with a frequency of 3.5 (2 – 5 , $SD = 0.9$) rays per mm.

Remarks. This specimen is characterized by its distinct growth ring boundaries, uni- to biseriate mixed intertracheary radial pitting, cross-fields usually with one or two mostly simple pits, relatively wide tracheids, uniseriate rays, and absence of resin-plugs and axial parenchyma. These characters indicate that this wood belongs to the fossil-genus *Protophyllocladoxylon*, because of the mixed radial pitting, simple large pits in the

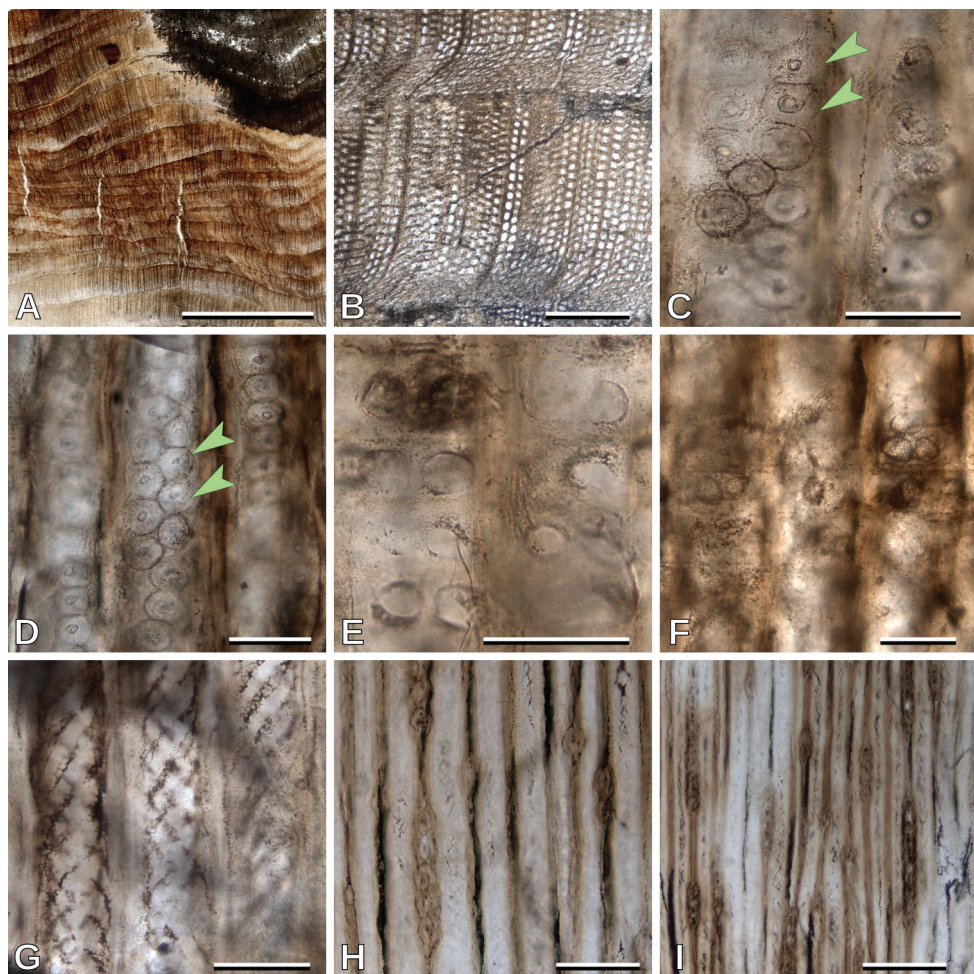


Figure 2. *Protophyllocladoxylon francisiae*, MPEF-Pb 10694: **A** Growth rings of type D (transverse section, TS) **B** detail of a growth ring of type D boundary (TS) **C** opposite (arrowheads) intertracheary radial pits (radial longitudinal section, RLS) **D** alternate (arrowheads) intertracheary radial pits (RLS) **E** and **F** cross-fields (RLS) **G** wall alteration of the secondary walls of tracheids (tangential longitudinal section, TLS) **H** uniseriate rays (TLS) **I** uniseriate rays (TLS). Scale bars: 5 mm (**A**); 500 μ m (**B**); 50 μ m (**C**, **D**, **E**, **F**, **G**); 100 μ m (**H**); 200 μ m (**I**).

cross-fields, uniseriate rays, and smooth ray cell walls (Philippe and Bamford 2008). Conservation of the name *Protophyllocladoxylon* was recently proposed by Zijlstra and Philippe (2020). Among the more than 20 species of the genus, *P. francisiae* is distinguished by its distinct growth ring boundaries, uni- to biseriate and mixed radial pitting, and absence of axial parenchyma and resin plugs (Zhang et al. 2010; Pujana et al. 2014).

Protophyllocladoxylon francisiae was first described by Pujana et al. (2014) from material collected from the Eocene La Meseta Formation, Seymour/Marambio Island, Antarctica, and it was later reported from the Paleocene Cross Valley and Sobral formations

that crop out on the same island (Pujana et al. 2015; Mirabelli et al. 2018). It is also present in the Eocene-Oligocene Río Turbio Formation, Santa Cruz Province, southern Patagonia (Pujana and Ruiz 2019). Interestingly, as is the case at Laguna del Hunco, this species is always a minor component of its floras and never dominates the assemblages.

The fossil-genus *Protophyllocladoxylon* is quite controversial. Vajda et al. (2016) suggested that *Protophyllocladoxylon* represents various unrelated botanical groups, principally because of its long temporal range from the Paleozoic to the Cenozoic (Zhang et al. 2010; see also Andruchow-Colombo et al. 2019). Pujana and Ruiz (2019) suggested that *P. francisiae*, in particular, could represent an extinct member of the Podocarpaceae because it has the general wood anatomy of the family but does not conform to any of the extant genera.

Genus *Phyllocladoxylon* Gothan

Phyllocladoxylon antarcticum Gothan

Figure 3A–L

Studied material. MPEF-Pb 10707, 10710, 10747, 10765, 10767, 10773 and 10776.

Localities. LU6, LU15 and LU22 at Laguna del Hunco (Fig. 1, Table 1), Chubut Province, Argentina.

Stratigraphic provenance. Tufolitas Laguna del Hunco, Huitrera Formation (Ypresian, early Eocene).

Description. Growth ring boundaries are distinct (Fig. 3A, B), latewood with ca. 3–10 rows of tracheids (Fig. 3B). Tracheids are roundish to polygonal as seen in transverse section (Fig. 3B, C). Intertracheary pitting in radial walls is abietinean, mostly uniseriate, rarely biseriate ($Si = 1.03$), mostly non contiguous ($Cp = 11.9\%$), and opposite when biseriate (Fig. 3D–F). Intertracheary pits are rounded in outline; 18.3 (12.5 – 26.4 , $SD = 1.9$) μm in vertical diameter (Fig. 3D–F). Tracheid tangential diameter is 33.2 (16.3 – 56.6 , $SD = 4.7$) μm . Cross-fields have mostly 1, very rarely 2, mean 1.04 , pits per cross-field (Fig. 3G–I). Cross-field pits are ellipsoidal with simple borders (rarely with narrow borders) and sometimes pointed; 13.0 (7.8 – 17.6 , $SD = 1.6$) μm in vertical diameter (Fig. 3G–I, 6C). Wall alteration (not helical thickening) of the secondary walls of tracheids is observed (Fig. 3J). Horizontal walls of ray parenchyma cells are smooth (Fig. 3G, H). Average ray height is medium, 8.2 (1 – 34 , $SD = 5.0$) cells high, rays are exclusively uniseriate (Fig. 3K, L) and with a frequency of 6.5 (3 – 11 , $SD = 0.2$) rays per mm.

Remarks. Abietinean intertracheary radial pitting and cross-fields with usually one large simple pit (Philippe and Bamford 2008) are the observed key characters, allowing confident placement of these Patagonian woods within *Phyllocladoxylon*. Distinct growth ring boundaries, absence of axial parenchyma and resin plugs, and predominantly uniseriate radial pitting are characteristics of the species *Phyllocladoxylon antarcticum* (Gothan 1908; Pujana et al. 2014).

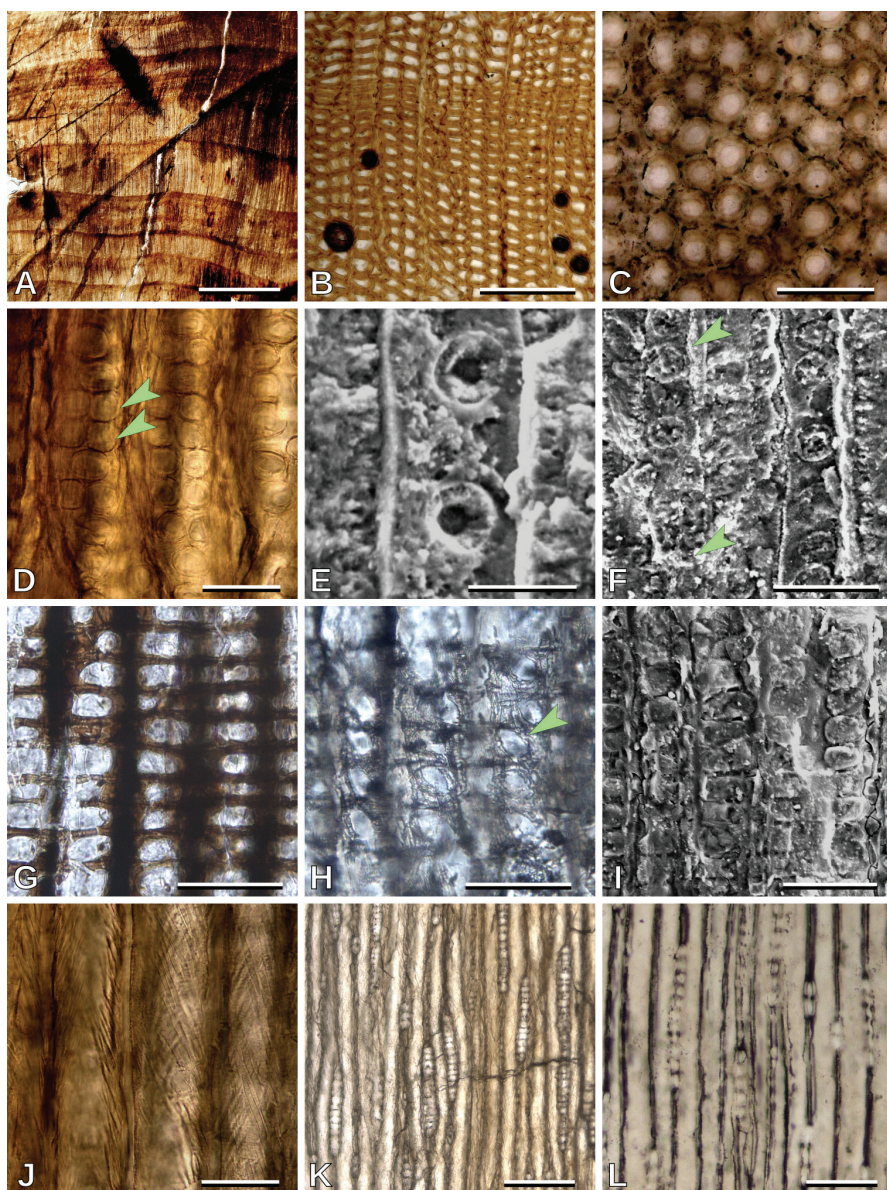


Figure 3. *Phyllocladoxylon antarcticum*: **A** Growth rings of type D (TS), MPEF-Pb 10747 **B** detail of a growth ring of type D boundary (TS), MPEF-Pb 10776 **C** detail of roundish tracheids (TS), MPEF-Pb 10765 **D** opposite contiguous biseriate intertracheary radial pits (arrowheads) (RLS), MPEF-Pb 10767 **E** uniseriate non contiguous intertracheary radial pits (scanning electron microscope, SEM), MPEF-Pb 10776 **F** uniseriate contiguous (arrowheads) and non contiguous intertracheary radial pits (SEM), MPEF-Pb 10776 **G** cross-fields with large simple pits (RLS), MPEF-Pb 10707 **H** cross-fields with large pointed and narrow-bordered pits (RLS), MPEF-Pb 10765 **I** cross-fields with large simple pits (SEM), MPEF-Pb 10710 **J** wall alteration of the secondary walls of tracheids (RLS), MPEF-Pb 10767 **K** uniseriate rays (TLS), MPEF-Pb 10767 **L** uniseriate rays (TLS), MPEF-Pb 10747. Scale bars: 5 mm (**A**); 200 µm (**B**, **K**); 100 µm (**C**, **L**); 50 µm (**D**, **F**, **G**, **H**, **I**, **J**); 20 µm (**E**).

Specimen MPEF-Pb 10767 frequently has biseriate opposite pits (Fig. 3D), and wider (in tangential section) tracheids, similar to *Protophylladoxylon*. However, most of its pits are non-contiguous (Cp= 23.1%), the growth rings are wider, and it has one pit per cross-field, all of which are features of the species *Phyllocladoxylon antarcticum*. Two other specimens, MPEF-Pb 10733 and 10778, are not very well preserved and are assigned to cf. *P. antarcticum* because two of the main characters (intertracheary radial pitting and cross-fields) are poorly preserved and therefore barely discernible (Table 2).

Phyllocladoxylon antarcticum is the most common species in our sample of conifer woods from Laguna del Hunco. In Patagonia, it was previously recorded in the Cretaceous (Nishida et al. 1990), Eocene (Pujana and Ruiz 2019), and in sediments of unknown ages (Kräusel 1924). On the Antarctic Peninsula, the fossil-species is the dominant component of the Eocene of Seymour/Marambio Island wood flora (Torres et al. 1994; Pujana et al. 2014) and a common component of wood floras from the Late Cretaceous of James Ross Island (Pujana et al. 2017), the Paleocene of Seymour/Marambio Island (Pujana et al. 2015; Mirabelli et al. 2018), and the Eocene of the Fildes Peninsula of King George/25 de Mayo Island (Torres and Lemoigne 1988; Oh et al. 2020).

Torres and Lemoigne (1988) suggested a possible relationship of *P. antarcticum* with the extant genera *Phyllocladus* Rich., *Dacrydium* Sol. ex G.Forst., *Microcachrys* Hook. *Prumnopitys* Phil., and *Podocarpus* Labill. Pujana et al. (2014) suggested affinities with several basal extant Podocarpaceae: the prumnopityoid clade (including *Phyllocladus* and *Prumnopitys*), *Microstrobos* Garden and LAS Johnson, and *Microcachrys* (Knopf et al. 2012); all of those taxa share with the fossils similar wood anatomy, abietinean radial pitting, and, predominantly, one large simple pit per cross-field (Pujana et al. 2014).

Recently, a compressed branch bearing phylloclades from Laguna del Hunco was assigned to the newly described fossil-genus *Huncocladus* Andruchow-Colombo et al., a stem relative of *Phyllocladus* (Andruchow-Colombo et al. 2019), and pollen having affinity with *Microcachrys* (Barreda et al. 2020) was also reported from Laguna del Hunco. These fossils could be related to *Phyllocladoxylon antarcticum*, although more evidence is necessary to confirm this hypothesis. *Prumnopitys andina* (Poepp. ex Endl.) de Laub., the only extant species of its genus from Patagonia, and *Phyllocladoxylon antarcticum* share similar wood anatomy (Pujana et al. 2017), and it is possible that the fossil-species could be related to the extant *P. andina*.

Genus *Cupressinoxylon* Göppert

cf. *Cupressinoxylon* sp. 1

Figure 4A–I

Studied material. MPEF-Pb 10733.

Locality. LU8 at Laguna del Hunco (Fig. 1, Table 1), Chubut Province, Argentina.

Stratigraphic provenance. Tufolitas Laguna del Hunco, Huitrera Formation (Ypresian, early Eocene).

Description. Growth ring boundaries are distinct (Fig. 4A, B), with a gradual transition from earlywood to latewood (Fig. 2B). Tracheids are roundish to polygonal as seen in transverse section (Fig. 4C). Intertracheary pitting in radial walls is abietinean, predominantly uniseriate (Si = 1.01), very rarely biseriate, non-contiguous (Cp = 7.2%), and opposite when biseriate (Fig. 4D). Intertracheary pits are rounded in outline; 13.9 (10.4–15.8, SD = 1.1) μm in vertical diameter (Fig. 4D). Tracheid tangential diameter is 24.2 (18.8–32.4, SD = 3.3) μm . Axial parenchyma is present, scarce, and apparently with a tendency to be tangentially zonate (Fig. 4C, G, I). Cross-fields have 1–2, mostly one, mean 1.2, pits per cross-field (Fig. 4D–G). Cross-field pits are

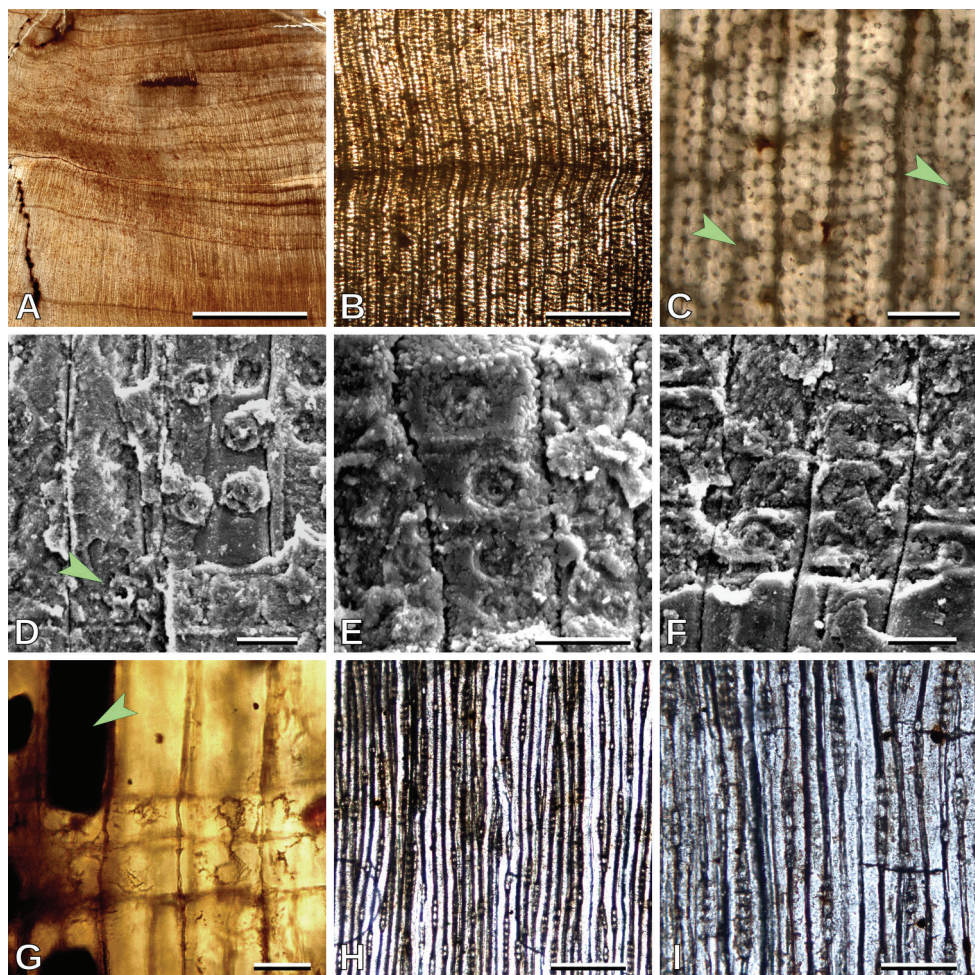


Figure 4. cf. *Cupressinoxylon* sp. 1, MPEF-Pb 10733: **A** Growth rings (TS) **B** detail of a growth ring boundary (TS) **C** Detail of roundish tracheids and axial parenchyma (arrowheads) (TS) **D** uniseriate non contiguous intertracheary radial pits and cross-field pit (arrowhead) (SEM) **E** and **F** cross-fields with bordered pits (SEM) **G** axial parenchyma (arrowhead) and cross-fields with bordered pits (RLS) **H** uniseriate rays (TLS) **I** uniseriate rays (TLS). Scale bars: 5 mm (**A**); 500 μm (**B**); 100 μm (**C**, **I**); 20 μm (**D**, **E**, **F**, **G**); 200 μm (**H**).

circular and bordered, apparently the border is usually wider than the aperture, and the aperture is rounded; 7.4 (5.2–9.6, SD = 1.2) μm in vertical diameter (Figs 4D–G, 6B). Horizontal walls of ray parenchyma cells are smooth (Fig. 4E–G). Average ray height is medium, 4.0 (1–11, SD = 1.9) cells high, rays are exclusively uniseriate (Fig. 4H–I) and with a frequency of 6.7 (4–8, SD = 1.2) rays per mm.

Remarks. Abietinean intertracheary radial pitting and apparently cupressoid pits in the cross-fields (bordered pits with the border wider than the aperture) indicate that this specimen belongs to the genus *Cupressinoxylon*, following Philippe and Bamford (2008). *Cupressinoxylon* includes wood with affinity to Cupressaceae and Podocarpaceae, with cupressoid cross-field pits (Pujana et al. 2014).

Cross-field pit border width is a key character of *Cupressinoxylon*. The poor preservation of this specimen prevents clear observation of the cross-field pits and also of the axial parenchyma walls; consequently, this specimen cannot be assigned with confidence to this fossil-genus and it is left as cf. *Cupressinoxylon*. Philippe and Bamford (2008) suggested that specimens in which the pit border is thinner than the aperture can also assigned to *Podocarpoxylon* Gothan.

The particular specimen studied here seems to be more similar to Podocarpaceae than to Cupressaceae because of the number of pits per cross-field. One, rarely two, pits per cross-field is common in the Podocarpaceae, whereas it is rarely observed in Cupressaceae (Tainter 1968; Greguss 1972; Meylan and Butterfield 1978; Roig 1992; Vidaurre Echeverría et al. 1987; Woltz et al. 1998; Vásquez Correa et al. 2010). Nevertheless, at this point we are not able to determine with certainty its affinity.

cf. *Cupressinoxylon* sp. 2

Figure 5A–I

Studied material. MPEF-Pb 10778.

Locality. LU23 at Laguna del Hunco (Fig. 1, Table 1), Chubut Province, Argentina.

Stratigraphic provenance. Tufolitas Laguna del Hunco, Huitrera Formation (Ypresian, early Eocene).

Description. Growth ring boundaries are distinct (Fig. 5A, B), with a gradual transition from earlywood to latewood (Fig. 5B). Tracheids are mostly polygonal as seen in transverse section (Fig. 5B). Intertracheary pitting in radial walls is abietinean, uni- to biseriate, predominantly uniseriate (Si = 1.06), non-contiguous (Cp = 6.3%), and opposite when biseriate (Fig. 5C–E). Intertracheary pits are mostly rounded in outline; 18.4 (14.9–23.9, SD = 2.2) μm in vertical diameter (Fig. 5C–E). Tracheid tangential diameter is 32.7 (18.7–46.1, SD = 7.1) μm . Cross-fields have 1–2, mostly one, mean 1.1, pits per cross-field (Fig. 5F–H). Cross-field pits are circular and bordered, apparently the border is usually wider than the aperture, and the aperture is rounded; 11.7 (7.7–13.7, SD = 1.8) μm in vertical diameter (Fig. 4F–H, 6D). Horizontal walls of ray parenchyma cells are smooth (Fig. 4F–H). Average ray height is medium, 5.9 (2–15, SD = 3.1) cells high, rays are exclusively uniseriate (Fig. 5I) and with a frequency of 6.6 (4–9, SD = 1.4) rays per mm.

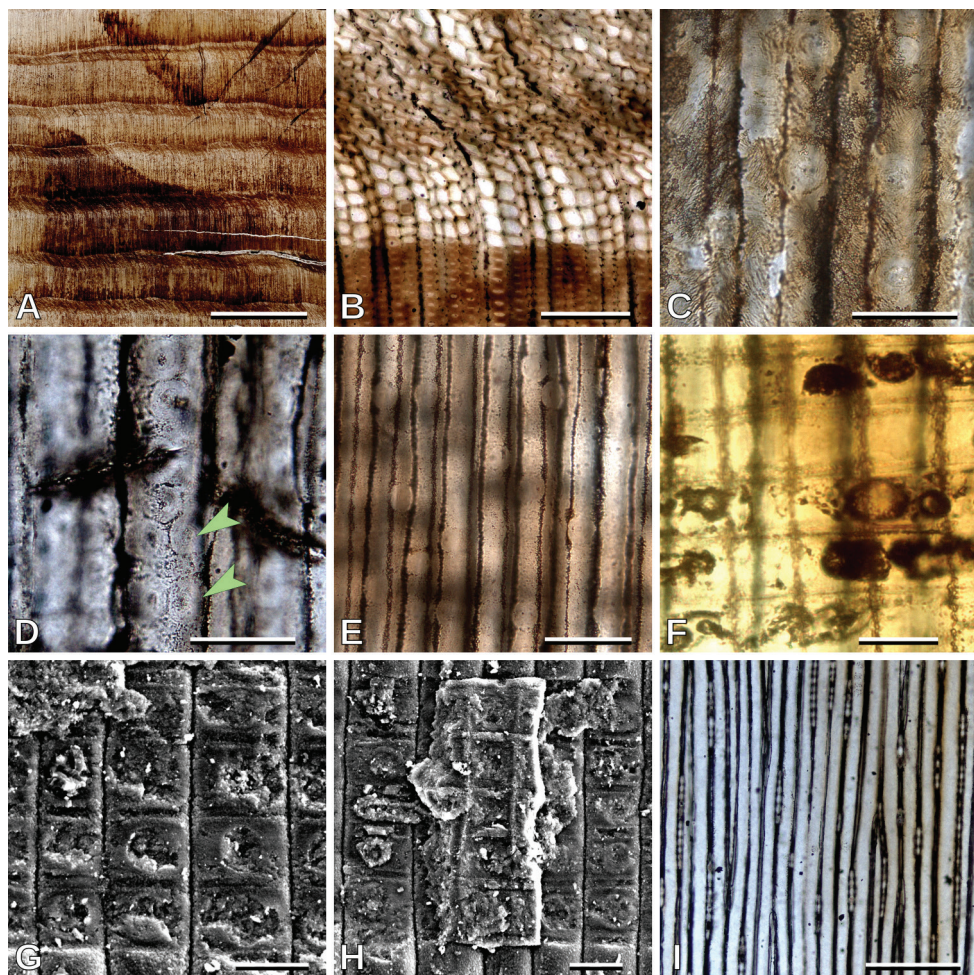


Figure 5. cf. *Cupressinoxylon* sp. 2, MPEF-Pb 10778: **A** Growth rings of type B (TS) **B** detail of a growth ring of type B boundary (TS) **C** uniseriate non contiguous intertracheary radial pits **D** uni- and biseriate intertracheary radial pits, opposite when biseriate (arrowheads) **E** uniseriate non contiguous intertracheary radial pits **F** cross-fields with bordered pits (RLS) **G** and **H** cross-fields with bordered pits (SEM) **I** uniseriate rays (TLS). Scale bars: 5 mm (**A**); 200 µm (**B**, **I**); 50 µm (**C**, **D**, **E**); 20 µm (**F**, **G**, **H**).

Remarks. This specimen shares with cf. *Cupressinoxylon* sp. 1 the abietinean intertracheary radial pitting and bordered cross-field pits, and it also conforms to *Cupressinoxylon* according to Philippe and Bamford (2008). Unfortunately, it is also poorly preserved and could not be assigned with confidence to *Cupressinoxylon*. It differs from cf. *Cupressinoxylon* sp. 1 because sp. 2 has larger pits and lacks axial parenchyma. It also seems to be more similar to the Podocarpaceae than to Cupressaceae because they share the number of pits per cross-field (one, rarely two). In addition, in this specimen the mean diameter of the cross-field pits exceeds 10 µm, a feature present in

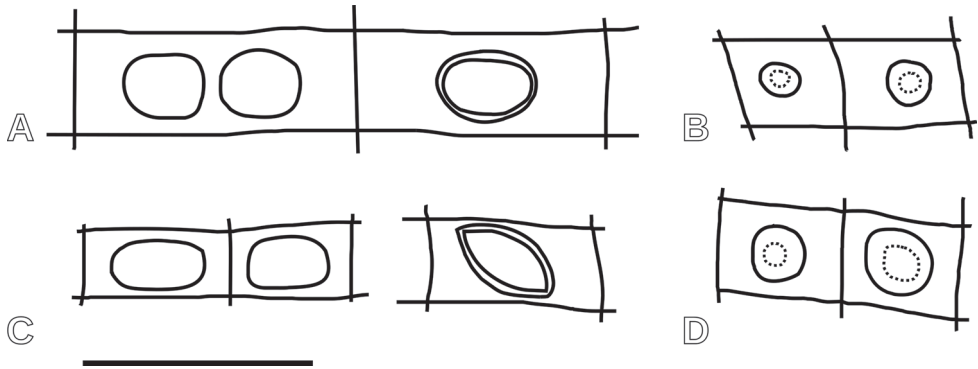


Figure 6. Schematic drawing of the cross-fields: **A** *Protophylladoxylon francisiae* **B** cf. *Cupressinoxylon* sp. 1 **C** *Phyllocladon antarcticum* **D** cf. *Cupressinoxylon* sp. 2. Scale bar: 50 μ m.

South American species of *Prumnopitys* (Woltz et al. 1998; Vásquez Correa et al. 2010) and in other Podocarpaceae genera (Greguss 1955) but mostly absent in Cupressaceae, which usually have smaller pits (Greguss 1955, 1972; Roig 1992).

Growth rings

Due to preservation, complete growth rings were only observed in a few specimens. Nonetheless, all the samples have distinct, well-defined growth ring boundaries (e.g., Figs 2A, 3A, 4A, 5A). Growth ring widths were measured where possible (Table 3). Growth rings are of types B and D (Table 3) of Creber and Chaloner (1984). The majority are type D, with abrupt transition from earlywood to latewood (Figs 2A, B, 3A), and only one specimen is type B, with a gradual transition from earlywood to latewood (Fig. 5A, B; Table 3). The mean ring width can reach 2.8 mm (Table 3). The minimum estimated age of the trees was grossly calculated to be 54 to 110 years, based on the curvature of the rings and the mean ring width (Table 3).

Galleries

Two samples of indeterminate conifers (Fig. 7; Table 2) have frass-filled galleries inside. Specimen MPEF-Pb 10736 has a gallery ca. 1.2 mm in diameter, horizontally oriented and parallel to the growth rings, filled with apparently spherical, sometimes slightly ellipsoidal, coprolites of ca. 200–300 μ m in diameter (Fig. 7A). Unfortunately, this sample is not well preserved, and the content may have been modified during the fossilization process. On the other hand, sample MPEF-Pb 10725 has a gallery of ca. 1.3 mm diameter that abruptly narrows and bifurcates (Fig. 7B), also horizontally oriented and parallel to the rays. The fill of this gallery has better preservation, and

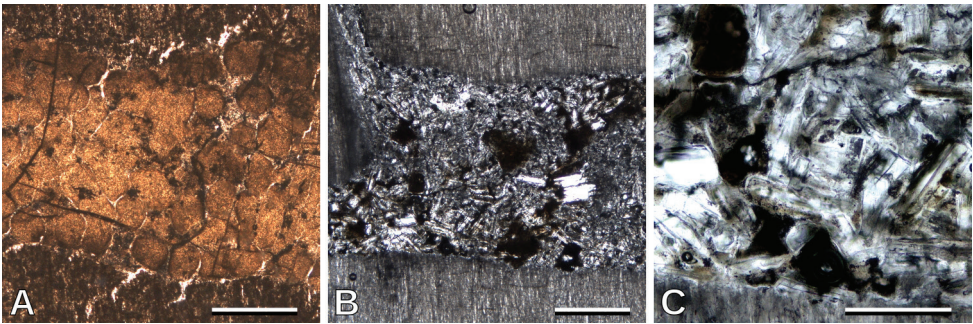


Figure 7. Galleries in two indeterminate conifer woods: **A** gallery filled with apparently spherical coprolites (TS), MPEF-Pb 10736 **B** gallery filled with compact frass (RLS), MPEF-Pb 10725 **C** detail of the frass (RLS), MPEF-Pb 10725. Scale bars: 500 µm (**A**, **B**); 200 µm (**C**).

Table 2. Wood anatomy of studied conifer samples. Locality (LU); Seriation index (Si); Contiguity percentage (Cp) [%]; Mean vertical diameter of radial pits (VDRP) [µm]; Mean tracheid tangential diameter (TTD) [µm]; Mean pits per cross-field (PxCF); Mean vertical diameter of cross-field pits (VDCP) [µm]; Mean ray height (RH) [cells]; Mean rays per mm (R×M). * indicates fewer than 15 measurements.

MPEF-Pb	Taxonomic unit	LU	Si	Cp	VDRP	TTD	PxCF	VDCP	RH	R×M
10694	<i>Protophyllocladoxylon francisiae</i>	2	1.25	88.1	19.2	44.5	1.97	14.8	5.6	3.5
10697	cf. <i>Phyllocladoxylon</i>	4	1.00*	5.6*	15.6	?	1.00*	11.4*	?	?
10700	Indeterminate conifer	4	1.00*	7.1*	14.6	?	?	?	?	?
10707	<i>Phyllocladoxylon antarcticum</i>	6	1.00	12.5	19.3	33.1	1.07	10.8	10.1	8.2
10710	<i>Phyllocladoxylon antarcticum</i>	6	1.03	8.5	20.9	29.5	1.11	12.0	7.9	8.4
10724	Indeterminate conifer	6	?	?	?	?	?	?	?	?
10725	Indeterminate conifer	6	?	?	?	?	?	?	?	?
10733	cf. <i>Cupressinoxylon</i> sp. 1	8	1.01	7.2	13.9	24.2	1.15	7.4	4.0	6.7
10736	Indeterminate conifer	9	?	?	?	?	?	?	?	?
10739	Indeterminate conifer	9	?	?	?	?	?	?	?	?
10742	Indeterminate conifer	12	?	?	?	?	?	?	?	?
10747	<i>Phyllocladoxylon antarcticum</i>	15	1.00	9.6	17.5	32.1	1.03	14.0	5.9	5.7
10748	Indeterminate conifer	16	?	?	?	?	1.00*	?	?	?
10749	cf. <i>Phyllocladoxylon</i>	16	1.00*	9.5*	16.8*	?	1.00*	13.7*	9.5	9.6*
10750	Indeterminate conifer	16	1.00*	12.5*	?	?	?	?	?	?
10751	Indeterminate conifer	16	?	?	?	?	1.00*	?	?	?
10753	Indeterminate conifer	16	?	?	?	?	?	?	?	?
10754	Indeterminate conifer	16	?	?	?	30.7	?	?	5.8	5.8*
10765	<i>Phyllocladoxylon antarcticum</i>	22	1.00	13.4	16.4	31.7	1.00	13.6	5.6	4.0
10766	Indeterminate conifer	22	?	?	?	?	?	?	?	?
10767	<i>Phyllocladoxylon antarcticum</i>	22	1.17	23.1	19.1	42.1	1.00	14.6	11.6	5.1
10771	Indeterminate conifer	22	?	?	?	?	?	?	?	?
10773	<i>Phyllocladoxylon antarcticum</i>	22	1.00*	5.3*	17.1*	31.2	1.00	12.9	6.2	8.1*
10775	Indeterminate conifer	22	1.00*	16.3*	15.1	?	1.00*	?	?	?
10776	<i>Phyllocladoxylon antarcticum</i>	22	1.00	10.6	17.5	32.5	1.06	13.2	9.9	6.3
10778	cf. <i>Cupressinoxylon</i> sp. 2	23	1.06	6.3	18.4	32.7	1.09	11.7	5.9	6.6

the content (frass) is clearly seen. The frass is compact, powdery, and made up of fragments of tracheids of ca. 100–300 µm in length, sometimes solitary and sometimes still united to adjacent tracheids (Fig. 7C).

Table 3. Growth ring analysis of selected samples. Growth ring type (GRT); Mean width (MW) [μm]; Minimum and maximum growth ring width (Min.-Max.) [μm]; Standard deviation (SD) [μm]; Number of rings counted (n); Minimum estimated diameter (MED) [cm]; Minimum age (MA) [years], $\text{MA} = (\text{MED} \times 10000 / \text{MW}) / 2$.

MPEF-Pb	Taxonomic unit	GRT	MW	Min.-Max.	SD	n	MED	MA
10694	<i>Protophyllocladoxylon francisiae</i>	D	681	262–1314	327	21	15	110
10707	<i>Phyllocladoxylon antarcticum</i>	D	816	300–1324	307	19	12	73
10747	<i>Phyllocladoxylon antarcticum</i>	D	1400	371–3056	678	15	15	54
10778	cf. <i>Cupressinoxylon</i> sp. 2	B	2786	2258–3742	517	7	50	90

Discussion

The conifers represent about 30% of the total collected wood assemblage, indicating an important presence of this clade within the forest canopy. Even though not all the conifer woods could be identified, two taxa belong undoubtedly to Podocarpaceae, *Protophyllocladoxylon francisiae* and *Phyllocladoxylon antarcticum*, while the other two, cf. *Cupressinoxylon* sp. 1 and cf. *Cupressinoxylon* sp. 2, are putative Podocarpaceae. Consequently, we found a significant species richness (four types of woods) from only twelve samples that could be identified to genus.

The family Podocarpaceae was apparently the dominant conifer component within the Laguna del Hunco wood flora, with the caveat of small sample size. The family is also abundant and strikingly diverse at generic level in the intensively collected compression flora (>7,000 specimens), including remains of *Acropyle* Pilg., *Dacrycarpus* (Endl.) de Laub., *Podocarpus*, and *Retrophyllosum* CN Page as well as the extinct phyllocladoid genus *Huncocladus* (Wilf 2012, 2020; Wilf et al. 2005, 2017; Andrucho-Colombo et al. 2019). Moreover, the pollen grains of the Podocarpaceae are the most abundant among all gymnosperms so far recorded at Laguna del Hunco; they are represented by seven species within five fossil-genera (*Gameroites* Archangelsky, *Dacrycarpites* Cookson and Pike, *Lygistipollenites* Harris, *Microcachrydites* Cookson, and *Podocarpidites* Cookson; Barreda et al. 2020).

Nevertheless, Araucariaceae, which are not yet known in the wood flora, are the most abundant conifer compression fossils at Laguna del Hunco, where *Agathis* Salisb. (formerly “*Zamia*”) and *Araucaria* Juss. compressions are each more common than any podocarp genus (Wilf et al. 2005, 2014; Rossetto-Harris et al. 2020). *Papuacedrus prechilensis* (Berry) Wilf et al. (Cupressaceae) is also well-represented in the compression flora (Wilf et al. 2005, 2009) but does not correspond exactly to any of the wood fossils because the living genus usually has one to five pits per cross-field (Greguss 1972).

This discrepancy in the family proportional abundances between the woods and compression macrofossils could result from many factors, most likely including the number of fossils studied, local variations of the source flora in time and space, and well-known differences in the taphonomic pathways for wood vs. other plant parts (e.g., Behrensmeyer et al. 2000). For example, many wood specimens were found in the upper part of the section at Laguna del Hunco (Fig. 1), and thus they must be younger than and possibly represent a slightly different source composition from

the great majority of the compression samples, which are much more abundant in the middle part of the section (e.g., Wilf et al. 2003). The upper part of the section is more tuffaceous than the middle, probably reflecting rapid volcanic fill during the late phases of the caldera lake and a more frequently disturbed paleoenvironment (e.g., Aragón and Mazzoni 1997). We were only able to identify with certainty 12 specimens, and it is probable that with a larger wood sample, the occurrence of other conifer families from the compression flora, such as Araucariaceae and Cupressaceae, could be confirmed in the future. Nevertheless, the dominant status of the Podocarpaceae in fossil wood assemblages is a pattern observed for Patagonian and Antarctic woods during the Eocene (Pujana et al. 2014; Pujana and Ruiz 2017, 2019), whereas Cupressaceae and Araucariaceae, if present, were usually uncommon in all the wood floras of this epoch.

Podocarpaceae are today distributed mainly in the Southern Hemisphere and Malesia and are almost entirely restricted to rainforest or wet montane environments (de Laubenfels 1969; Hill and Brodribb 1999; Brodribb 2011; Cernusak et al. 2011). The family is ancient, with potential fossils from the Middle Triassic of Antarctica (e.g., Axsmith et al. 1998), and its fossil record from the Mesozoic through most of the Cenozoic is restricted to Southern Hemisphere land masses (Hill and Brodribb 1999). Interestingly, Podocarpaceae are often the most abundant gymnosperm group in living angiosperm-dominated rainforests (Brodribb 2011), as is the case for the Laguna del Hunco wood flora.

Growth ring boundaries of all samples are usually distinct (although some boundaries are not very well-defined), which indicates seasonality. The growth rings (type D; e.g., Figs 2A, B, 3A) are associated with the retardation of cambial activity, while the presence of type B (e.g., Fig. 5A, B) indicates a long growing season with an adequate water supply (Creber and Chaloner 1984). However, some Podocarpaceae with type D growth rings have wood that is not significantly affected by environmental factors, and can only be used in analyses of ring widths (Creber and Chaloner 1984). These types (B and D) of growth rings are consistent with the light regime at the paleolatitude of the sediments (about 47°S) and with previous paleotemperature and paleoprecipitation estimates based on leaf physiognomy and inferred drought-intolerance of many of the conifer taxa. The Laguna del Hunco compression assemblage, especially the conifer fraction, indicates no significant rainfall seasonality and very mild temperature seasonality (Wilf et al. 2003, 2005, 2009; Wilf 2012; Merkhofer et al. 2015).

The estimated minimum ages based on growth ring widths suggests that the specimens were mature trees at the time of deposition. We infer that the remains of *Protophyllocladoxylon francisiae* came from a tree older than 100 years (Table 3). In one sample, the type of growth ring (type B according to Creber and Chaloner 1984) and width of the growth rings reveal a significant and uniform growth of more than 5 mm in diameter annually (mean ring width 2.8 mm, widest ring of 3.7 mm, Table 3) which is similar to those of Podocarpaceae growing in wet Patagonian forests today (e.g., Rozas et al. 2016). Interestingly, the plant would have grown more than the global mean ring width of ca. 1.1 mm of extant conifers (Falcon-Lang 2005).

Galleries found in the woods were apparently produced by arthropod borers. They are filled with coprolites and particulate frass. This type of fill is produced by many types of arthropods (e.g., Platypodidae beetles, Tarno et al. 2011). The frass found is insufficient for its identification, mainly because the literature on wood debris produced by modern arthropods is scarce (Nuorteva & Kinnunen 2008) and mostly focused on northern hemisphere taxa (e.g., Hay 1968; Solomon 1977; Tarno et al. 2011). However, arthropod galleries are often found in Patagonian fossil woods (e.g., Genise 1995; Pujana et al. 2020), and mite coprolites were found in the permineralized *Todea* stem from southern exposures of the Tufolitas Laguna del Hunco (Bippus et al. 2019).

Conclusions

Herein, we report the first taxonomic study of conifer fossil woods from the highly fossiliferous Laguna del Hunco exposures. The proportion of conifers in this fossil wood assemblage (ca. 30%) indicates a significant presence of this group within the paleoflora. We document the family Podocarpaceae with confidence as the dominant component of the Laguna del Hunco wood paleoflora. The family is represented by two fossil species, *Protophyllocladoxylon francisiae* and *Phyllocladoxylon antarcticum*. Additionally, two species assigned to the genus *Cupressinoxylon* (cf. *Cupressinoxylon* sp.1 and sp.2) are probably representatives of the family as well. Although sample size is small compared with the compression flora, these data strongly indicate that Podocarpaceae were important components of the Laguna del Hunco flora.

Podocarpaceae dominance in the fossil woods is consistent with diverse, abundant podocarpaceous macrofossil compressions and pollen grains previously described from the same section, including vegetative and reproductive structures related to several extant podocarp genera. However, in the Laguna del Hunco compressions, Araucariaceae are the dominant conifers, Cupressaceae are also well represented, and both families are found in the palynoflora. Neither family is yet known from the wood flora, presumably a result of lower sample size available for the woods by two orders of magnitude (10s of wood fossils vs. 1000s of compression fossils) or unknown taphonomic factors.

Growth rings indicate seasonality, probably because of the seasonal light regime at paleolatitude ca. 47°S, and mature tree development. The galleries found in two woods indicate arthropod interactions.

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Primula sunhangii (Primulaceae): a new species from Hubei, Central China

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Abstract

This report provides a description of *Primula sunhangii* from the Shennongjia Forestry District, Hubei Province in Central China, which is categorized as a new species of the primrose family. *Primula sunhangii* is morphologically similar to *P. involucrata* Wall. ex Duby in terms of its simple umbel, efarinose, and prolonged bracts. However, *P. sunhangii* is distinguished by its glabrous sepal, short petiole (compare with blade) and cylindrical calyx and capsule. Molecular phylogenetic analysis based on nuclear and cpDNA genes demonstrates that *P. sunhangii* and *P. involucrata* are closely related. Combining genetic and morphological data, the recognition of *P. sunhangii* as a unique new species is supported.

Keywords

morphological and molecular data, new species, *Primula*, Shennongjia

* The authors contributed equally to this study.

Introduction

The well-known horticultural genus *Primula* is the largest in the Primulaceae. There are approximately 500 *Primula* species worldwide, with the majority distributed in the North Temperate Zone and a small number of outlying species located in the mountainous regions of Africa (e.g. Ethiopia), tropical Asia (e.g. Java and Sumatra), and South America (Richards 2002). In general, *Primula* is distinguished from other genera by being multi-scapose with a long corolla and heterostylous flowers. There are approximately 300 native *Primula* species in China, for which the preferred habitat is the relatively warm and humid regions of the Himalayas and Hengduan mountainous regions, in particular the Yunnan and Sichuan provinces of Southwest China that represent *Primula* biodiversity hotspots (Hu and Kelso 1996).

Currently, many *Primula* species are poorly characterized, with a number of descriptions based on a single sample, which potentially results in undocumented details of characteristics such as pin or thrum morphology, fruiting, and ecology (Hu and Kelso 1996). Taxonomic study of *Primula* in China remains insufficient and requires continued efforts, as demonstrated by the large number of reports describing new species, including *P. pengzhouensis* C. M. Hu, G. Hao & Y. Xu. (Xu et al. 2017), *P. anthemifolia* G. Hao, C. M. Hu & Yuan Xu (Xu et al. 2015), and *P. mianyangensis* G. Hao & C. M. Hu (Wu et al. 2013) collected from Sichuan; *P. hubeiensis* X. W. Li (Li et al. 2017) collected from Hubei; and *P. undulifolia* G. Hao, C. M. Hu & Y. Xu (Xu et al. 2016) collected from Hunan.

The Shennongjia Forestry District, located in western Hubei, Central China, adjacent to eastern Sichuan, is well known for its rich biodiversity, in particular the rare and endangered golden monkey. The Shennongjia mountain range is a large geographical area (ca. 3,250 km²) known as the roof of central China, which represents the second geographical step of China in a west-east direction and is characterized by the majority of its mountain peaks being over 3,000 m. The flora of this area is mainly influenced by the monsoon from the Pacific Ocean, which differs from the weather systems that affect Yunnan or western Sichuan in Southwest China. Moreover, its environment is moist from March to November, which is maintained by the local atmosphere, influenced by both the Yangtze and Hanjiang rivers that surround the Shennongjia area. (Deng et al. 2018)

In 2011, during several field expeditions in Shennongjia, an unusual *Primula* population was discovered, comprised of plants similar to *P. involucrata*, belonging to Sect. *Aleuritia* Duby, with comparable glabrous and efarinose leaves, denticulate leaf margins, umbels, cylindrical calyx, funnel-form corolla and bracts base prolonged below into auriculate appendage. However, these individuals were clearly distinguished by their glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape. We thus hypothesized this population represents a new species, which was verified by subsequent morphological and molecular phylogenetic comparisons and thus described as *Primula sunhangii*.

Materials and methods

Morphological analysis

Morphological analysis was performed using recently collected specimens and those sampled in 2011 from Shennongjia. A vernier caliper and a dissecting microscope were used in the measurement. We referred to the keys to sections and species in *Flora Reipublicae Popularis Sinicae* (Hu 1990) and *Flora of China* (Hu and Kelso 1996). We also examined all species in Sect. *Aleuritia*. Specimens are deposited in the herbaria of Kunming Institute of Botany (**KUN**) and Jishou University (**JIU**).

Taxon sampling and outgroup selection

The phylogenetic analysis was mainly based on the recently published framework of *Primula* (Yan et al. 2015), with Sect. *Aleuritia* as the focal group. We performed sequencing of nuclear ITS as well as *rbcL* and *matK* of *P. sunhangii*, and downloaded 84 sequences of 28 species from GenBank. *Omphalogramma delavayi* (Franch.) Franch. was selected as the outgroup. All GenBank accession numbers of studied taxa are listed in Suppl. material 1.

DNA extraction and sequencing

Molecular materials were collected from Shennongjia in 2011. A Tiangen DNA extraction kit was used. PCR was performed according to standard protocols (Kusukawa et al. 1990). The primers used were those described by Yan et al. (2015) (Table 2). DNA sequencing was performed by Tsingke Biological Technology in Kunming.

Phylogenetic Analysis

The raw sequences were manually edited and aligned using BioEdit 7.0.4.1 (Hall 2001). In the ITS, *rbcL*, *matK* and combined (including all three markers above) phylogenetic analysis, maximum likelihood (ML) analysis was conducted using RAxML 8.2.10 (Stamatakis 2014) and run for 1,000 bootstrap iterations under a GTRGAMMA model. Bayesian inference (BI) analysis was carried out using MrBayes 3.2.6 (Ronquist et al. 2012) and run for 2,000,000 generations under a GTR + I + G substitution model, which was selected using jModelTest2 2.1.6 (Darriba et al. 2012).

Taxonomic treatment

***Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun, sp. nov.**

urn:lsid:ipni.org:names:77211168-1

Type. CHINA, Hubei Province, Shennongjia Forestry District, Hongping, Dashuping. 31°26.67'N, 110°16.01'E Alt. 2877 m. 5 Jun 2011, D. G. Zhang et al. Zdg20110605023 (Holotype **KUN!**) (Fig. 1). – **Isotype:** CHINA, Hubei Province, Shennongjia Forestry District, Hongping, Dashuping. 31°26.67'N, 110°16.01'E Alt. 2877 m. 5 Jun 2011, D. G. Zhang et al. Zdg20110605023 (**JIU!**).

Diagnosis. *Primula sunhangii* is similar to *P. involucrata* in glabrous, efarinose, ovate leaves, and lanceolate bracts base prolonged below into auriculate appendage and its length. But it differs from *P. involucrata* in the following characters: glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape (at most 3 times the leaves).

Description. Herbs perennial, glabrous, efarinose. Leaves 5–10, all basal in a rosette; petiole green, basal white to pink, 1–5.6 cm long, 0.2–0.4 cm diam.; leaf blade green, ovate or oblong, 0.8–4.8 cm long, 0.5–2 cm wide, papery, base truncate or acute, margin entire or slightly denticulate, apex obtuse to rounded. Scapes 5–19 cm in length; umbels 2–4-flowered; bracts 5, lanceolate, 5–10 mm long, 1–3 mm wide, membranous, base prolonged below into 3–6 mm auriculate appendage. Pedicel 0.5–3.5 cm. Calyx 5–7 mm in length, 3 mm diam., cylindrical, parted to 1/4 to 1/3; lobes lanceolate, apex narrowly acute. Corolla funnel-form, pink or purple; tube 8–10 mm in length, 8–10 mm diam., limb 1–2 cm diam.; lobes obovate, deeply emarginated apex.

Pin flowers: corolla tube ca. 8.5 mm; stamens ca. 3.5 mm above base of corolla tube; the style is not or slightly exerted.

Thrum flowers: corolla tube ca. 1 cm; stamens toward apex of corolla tube; the style is slightly shorter than calyx.

Capsule cylindric, apex irregular dehisces. (Fig. 2)

Distribution and habitat. Fissures of rocks on mountain slopes; ca. 2,800 m. Shennongding (Hongping, Shennongjia, Hubei), Laojunshan (Muyu, Shennongjia, Hubei). (Fig. 3)

Phenology. *Primula sunhangii* flowers from June to July.

Etymology. The specific epithet refers to the Chinese botanist Hang Sun.

Vernacular name: Simplified Chinese: 神农架报春; Chinese Pinyin: Shén-nóngjià Bào-chūn.

Conservation status and IUCN preliminary assessment. The new species has only two known populations described thus far. The populations are both in subareas of Shennongjia, one in Shennongding and another in Laojunshan. Each population has ca. 40 individuals. The habitats are situated in a tourist attraction zone, with an average of 20,000 tourists visiting daily. According to IUCN red list categories and criteria, conservation status of this species should be Critically Endangered (CR) (B2abiii).

Relationship with related species. Based on its glabrous, efarinose, ovate leaves, lanceolate bracts base prolonged below into auriculate appendage, and its

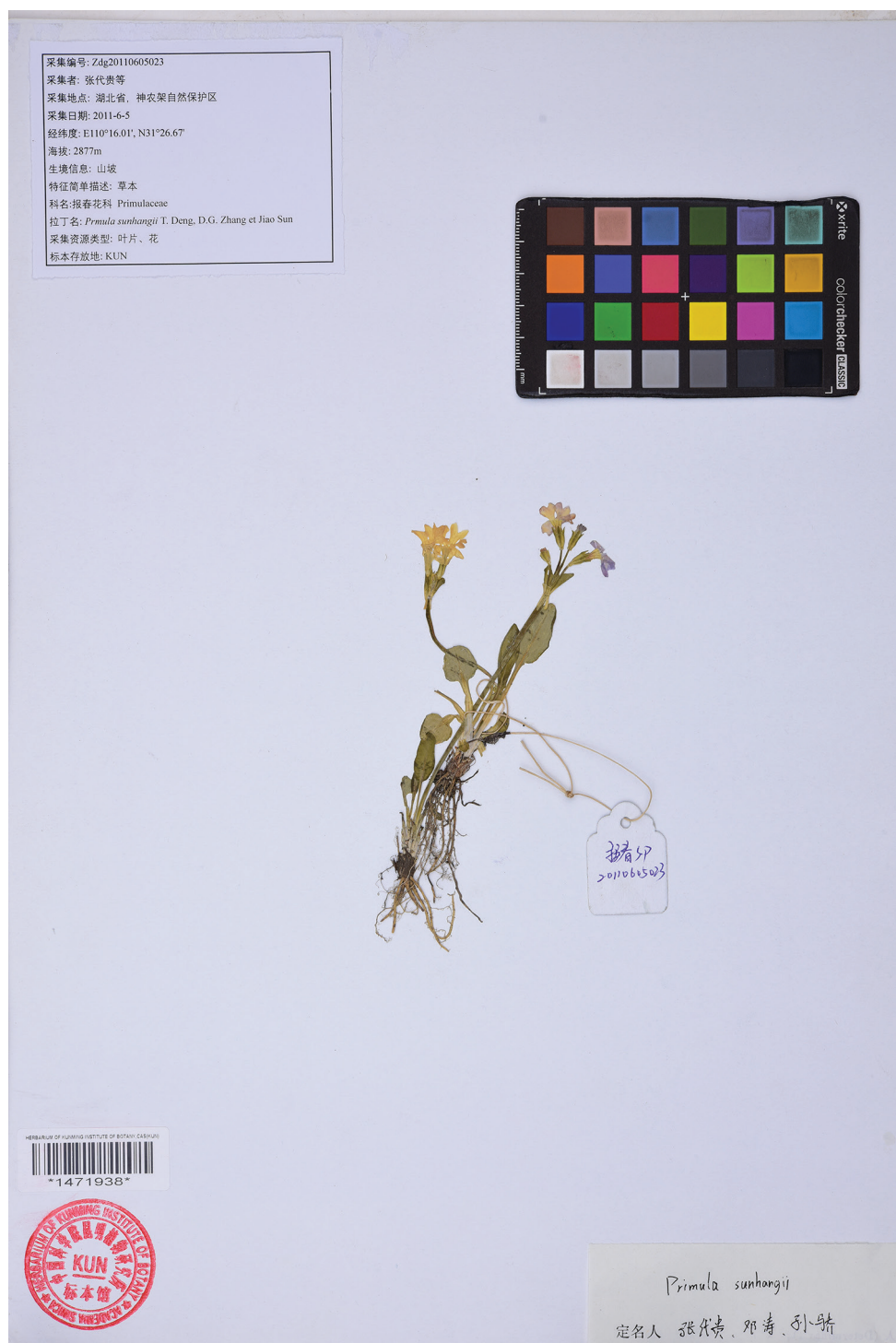


Figure 1. Holotype of *Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun, sp. nov.

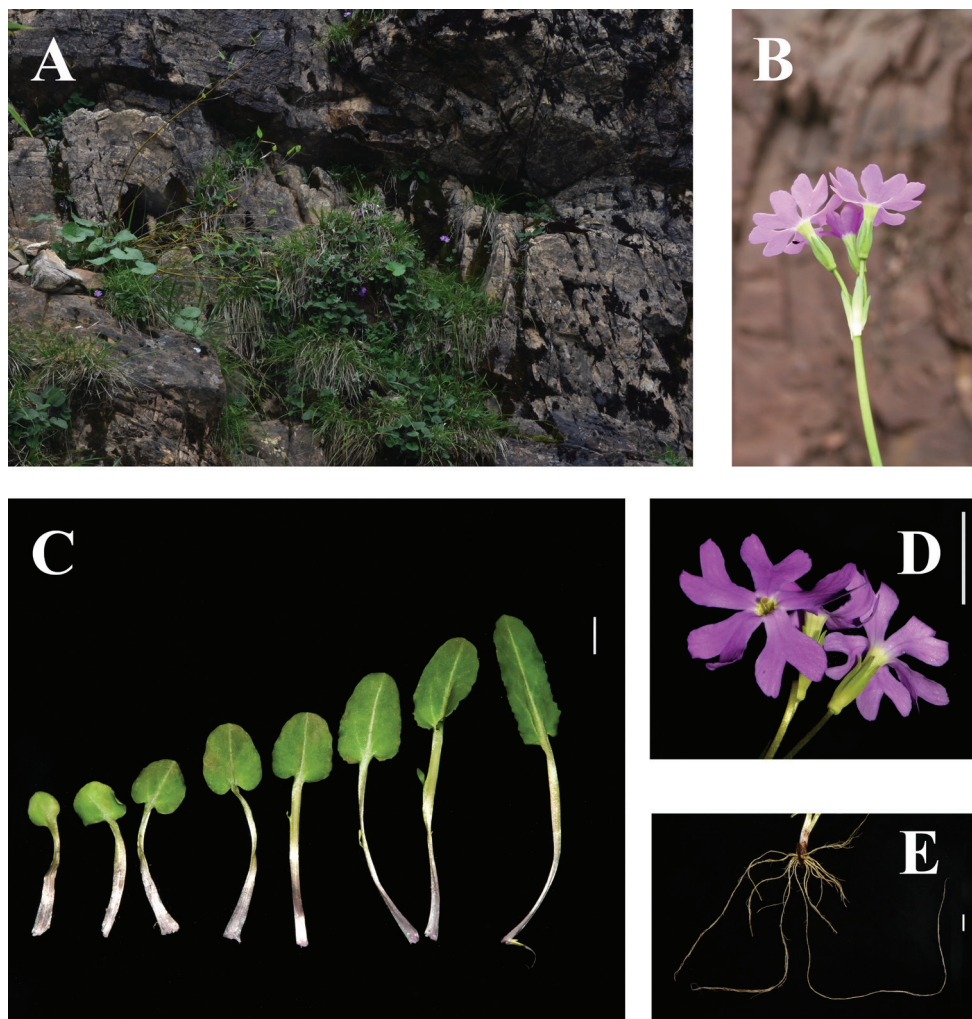


Figure 2. Images of live *Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun, sp. nov. **A** habitat **B** inflorescence **C** leaves **D** flower, front and back side **E** root. Scale bars: 1 cm in (**C**, **D**, **E**). Photographer: Qun Liu.

length, *P. sunhangii* is most morphologically similar to *P. involucrata* (Hu 1990; Hu and Kelso 1996). But *P. sunhangii* differs from *P. involucrata* in glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape (at most 3 times the leaves). (Table 1).

We performed phylogenetic analyses using nuclear ITS as well as *rbcL* and *matK* of *P. sunhangii* and related species. Separate analyses for all three markers get six trees (ML and Bayesian) with similar structures. Combined molecular phylogenetic analyses shows that *P. sunhangii* and *P. involucrata* are sister taxa, with high support (Fig. 4). They have 19 different sites in the 2020 bp nucleotide sequence.

These genetic results and morphological data clearly support that *Primula sunhangii* be recognized as a distinct new species.

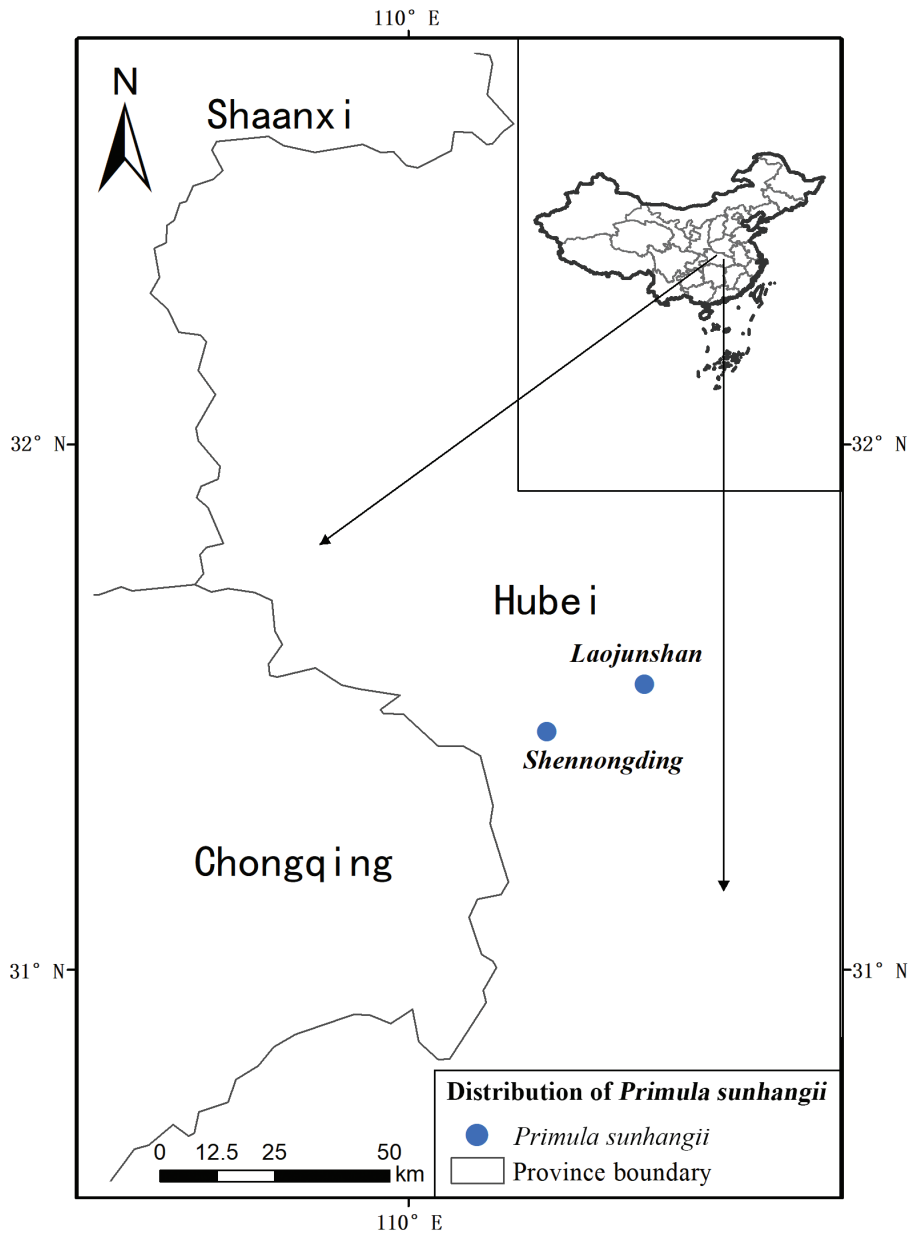


Figure 3. Distribution of *Prunus sunhangii* T. Deng, D. G. Zhang & Jiao Sun.

Table I. Diagnostic morphological characters comparison between *Primula sunhangii* and *P. involucrata*.

Characteristics	<i>P. sunhangii</i>	<i>P. involucrata</i>
Base of leaves	truncate or acute	cuneate, rounded to slightly cordate
Sepal	glabrous	glandular ciliolate
Calyx shape	cylindrical	campanulate
Capsule shape	cylindrical	oblong
Ratio of scape to leaves	less than 3 times	3–5 times

Table 2. Primers used in PCR amplification and sequencing.

DNA fragments	Primer name	5'-3' Sequences
ITS	ITS1	GTCCACTGAACCTTATCATTTAG
	ITS4	TCCTCCGCTTATTGATATGC
rbcL	rbcLa_f	ATGTCACCACAAACAGAGACTAAAGC
	724R	TGCATGTACCTGCAGTAGC
matK	3F-KIM	CGTACAGTACTTTTGTGTTTACGAG
	XF	TAATTACGATCAATTCAATTC
trnH-psbA	trnH-05	CGCGCATGGTGGATTACAAATC
	psbA3	GTTATGCATGAACGTAATGCTC

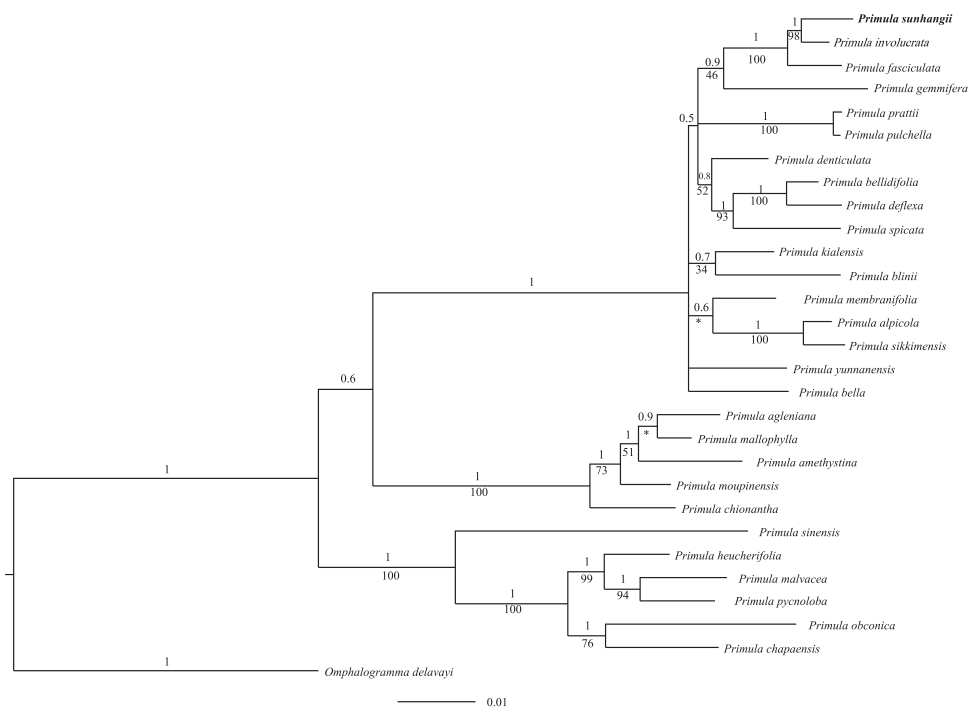


Figure 4. Bayesian tree of selected species in *Primula*, focused on Sect. *Aleuritia*. Based on a combined sequences from nuclear ITS and *rbcL*, *matK* of cpDNA genes. Numbers above the branches represent the Bayesian posterior probabilities, and below showing the maximum likelihood values. (* multifurcation in Maximum-likelihood tree).

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Supplementary material I

Taxa, specimen collection information, GenBank accession numbers of all studied samples

Author: Jiao Sun

Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.156.49137.suppl1>

***Geum sunhangii* (Rosaceae), a new species from Hubei Province, China**

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Abstract

Geum sunhangii – first discovered in Shennongjia National Nature Reserve, Hubei Province, China – is described as a new species of Rosaceae. Compared to all known Chinese *Geum* species, the new species differs by possessing jointed styles, imbricate petals and a reniform radical leaf terminal leaflet. Most significantly, the jointed style is curved at an obtuse or a right angle. In addition, the inclusion of this species within the genus *Geum* was supported by phylogenetic analysis using the sequence data of a nuclear ribosomal internal transcribed spacer (nrITS) and a chloroplast *trnL–trnF* intergenic spacer. The new species was found to be closely related to *G. rivale* and *G. aleppicum*.

Keywords

anatomical, morphology, phylogeny, taxonomy

* The authors contributed equally to this study.

Introduction

The genus *Geum* L. (1753: 500) (Rosaceae), also known as Avens, contains ca. 56 species distributed throughout the temperate, tropical and arctic regions of the world and is found mainly in the Northern Hemisphere (Gajewski 1959). The morphology of *Geum* is very complex. Most species are herbaceous perennials that form rosettes consisting of imparipinnate leaves and a thick caudex, but a few species are small shrubs. In addition, most species in this genus have fish-hook shaped fruits (Rafinesque 1833). Only three species *G. aleppicum* Jacq., *G. rivale* L. and *G. japonicum* var. *chinense* F.Bolle are present in China (Li et al. 2003). Bolle (1933) divided *Geum* into several genera, including *Novosieversia* F.Bolle and *Oncostylus* F.Bolle (Bolle, 1933), amongst others. However, based on cytological data, Gajewski (1957) concluded that *Geum* is a polyploid complex and did not support any previous circumscriptions of *Geum*; instead, Gajewski recognised most of the previously segregate genera as subgenera within the genus *Geum* (Gajewski 1957, 1968). Finally, his point of view was further supported by later morphological and molecular studies (Schulze-Menz 1964; Hutchinson 1967; Robertson 1974; Kalkman 1988; Smedmark and Eriksson 2002).

The Shennongjia National Nature Reserve is located in the northwest of China's Hubei Province. It is a world diversity hotspot defined by its unique geographical location and complex topography (Hu et al. 2004). The Nature Reserve is also characterised by its high species diversity (including many rare and endangered animals and plants) and relict plant species (Fan et al. 2017). Correspondingly, it has recently attracted considerable attention from many researchers (Ma 2016; Xie et al. 2017). Recently, several new angiosperm taxa have been discovered there, including *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun (Deng et al. 2013), *Mazus sunhangii* D.G. Zhang & T. Deng (Deng et al. 2016) and *Impatiens baokangensis* Q.L. Gan & X.W. Li (Gan and Li 2016).

During a biodiversity survey of the Shennongjia National Nature Reserve, we discovered an undescribed species belonging to the Rosaceae. This species was distinguished by the presence of a rosette of basal leaves, petals and jointed styles. Our morphological and molecular studies revealed that the newly-collected material belongs to an unknown *Geum* species which is described here.

Materials and methods

Plant materials. Specimens of the new species were collected from a site in Shennongjia National Nature Reserve, Hubei Province. Leaves of the new species were obtained for molecular studies. All herbaria used in this study were obtained from KUN (Herbarium, Kunming Institute of Botany, CAS).

Anatomical observation. Specimens of *G. sunhangii* were dissected and directly observed, before being placed under an anatomical lens for further observation under magnification. The cauline leaf, radical leaves, seeds, petal, style joint and stamen were inspected.

DNA sequencing and molecular analyses. Total DNA was extracted using a DP305 Plant Genomic DNA Kit (Tiangen, Beijing, China) and we selectively amplified the nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast *trnL-trnF* intergenic spacer (*trnL-trnF*) regions by Polymerase Chain Reaction (PCR). Successfully amplified DNA fragments were then sequenced commercially. Molecular analysis was performed using two outgroups (i.e. *Fallugia paradoxa* Endl. and *Sanguisorba officinalis* L.; Potter et al. 2007), one piece of material from the putative new species and fourteen samples with similar morphology. Partial sequences were obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank>); GenBank accession numbers for all species are listed in Table 2. Multiple sequence alignments were initially performed using CLUSTAL W ver. 1.4 (Thompson et al. 1994) and were manually adjusted using BioEdit ver. 7.0.5.3 (Hall 1999). We used SequenceMatrix ver. 1.7.8 to combine the sequences (Vaidya et al. 2011).

Phylogenetic trees were constructed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms. All characters were weighted equally, with gaps treated as missing data. MP analysis was conducted using PAUP ver. 4.0a (Swofford 2002). MP trees were obtained from a heuristic search of 1000 random addition replicates using tree bisection-reconnection (TBR). Finally, we obtained 1000 bootstrap values to evaluate the support for each branch.

ML analyses were conducted using the IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at/>) (Trifinopoulos et al. 2016). We performed 1000 replicates with the substitution model set to 'Auto'. Bayesian Inference was performed using Modeltest ver. 3.7 (Posada and Crandall 1998) to determine the best-fit models of nucleotide substitution. A comparison between the Akaike Information Criterion (AIC) values obtained with different tree models using the combined dataset revealed that TIMeF was the most appropriate (Posada 2004). However, the best-fit TIMeF model was substituted by the GTR + I model because TIMeF was not available for further analyses. Bayesian Inference was performed using MrBayes ver. 3.1.2 (Ronquist 2003). The programme was run for 10 million generations and sampling was performed every 1000 generations. Each tree used two independent Markov Chain Monte Carlo (MCMC) (Yang 1997) analyses with four chains. When the average standard deviation of split frequencies was less than 0.01, a consensus tree was calculated after discarding the first 25% of trees as burn-in. A Bayesian tree was constructed from the remaining trees with Posterior Probability (PP) values for each clade.

Results

Taxonomic treatment

***Geum sunhangii* D.G. Zhang, T. Deng, Z.Y. Lv & Z.M. Li, sp. nov.**

urn:lsid:ipni.org:names:77211169-1

Figures 1, 2

Type. CHINA. Hubei Province: Shennongjia National Nature Reserve, Nantianmen, alt. 2821 m, 11 July 2011, *zdg* 7313 (holotype: KUM!)

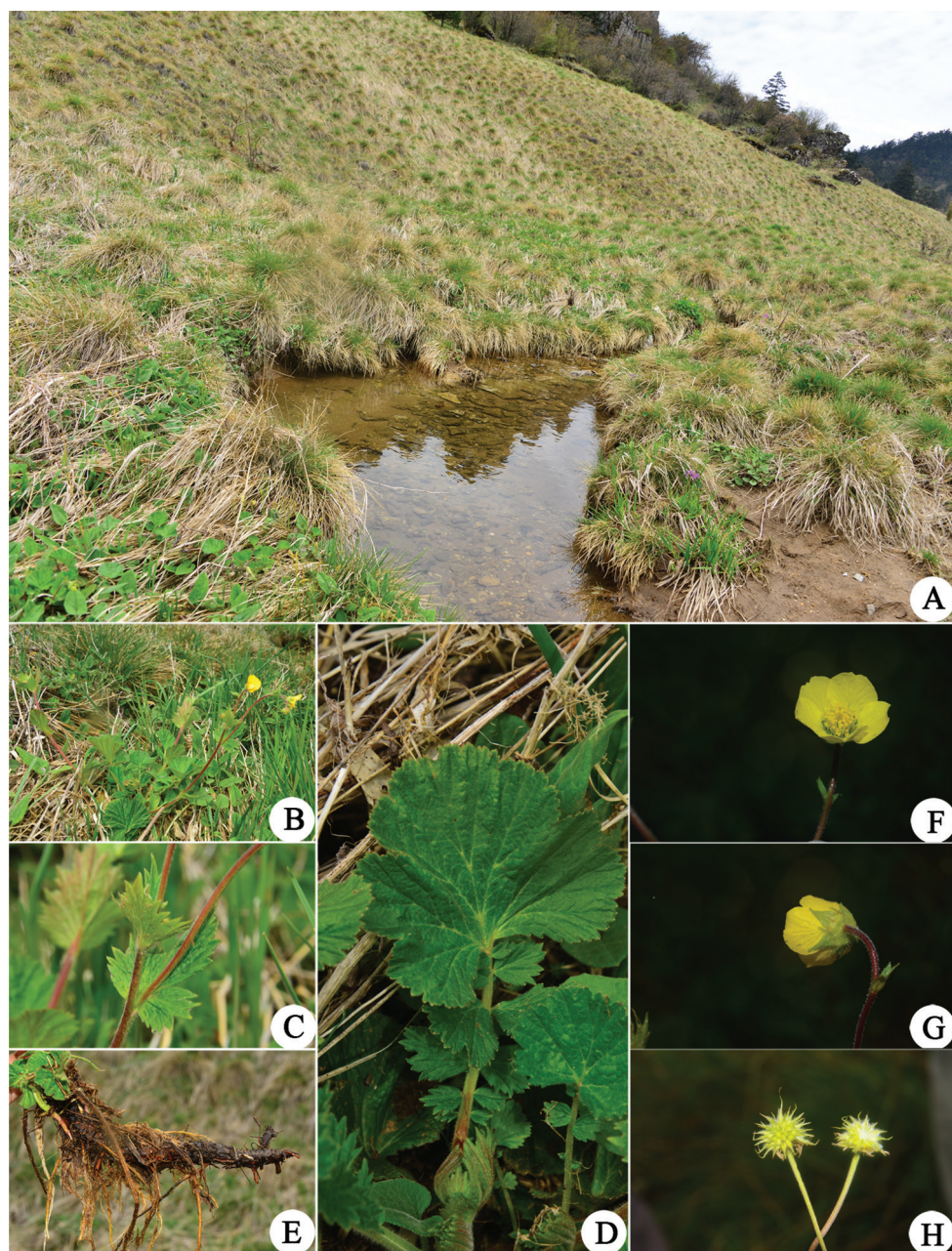


Figure 1. Images of *Geum sunhangii*. **A** Habitat **B** habit **C** cauline leaves **D** radical leaves **E** roots **F** corolla **G** sepals **H** infructescences.

Diagnosis. *Geum sunhangii* and *G. aleppicum* are most similar in morphology. However, *G. sunhangii* differs significantly from *G. aleppicum* by the entire or 3-lobed cauline leaf (vs. blade pinnate, sometimes repeatedly lobed), reniform radical leaf ter-

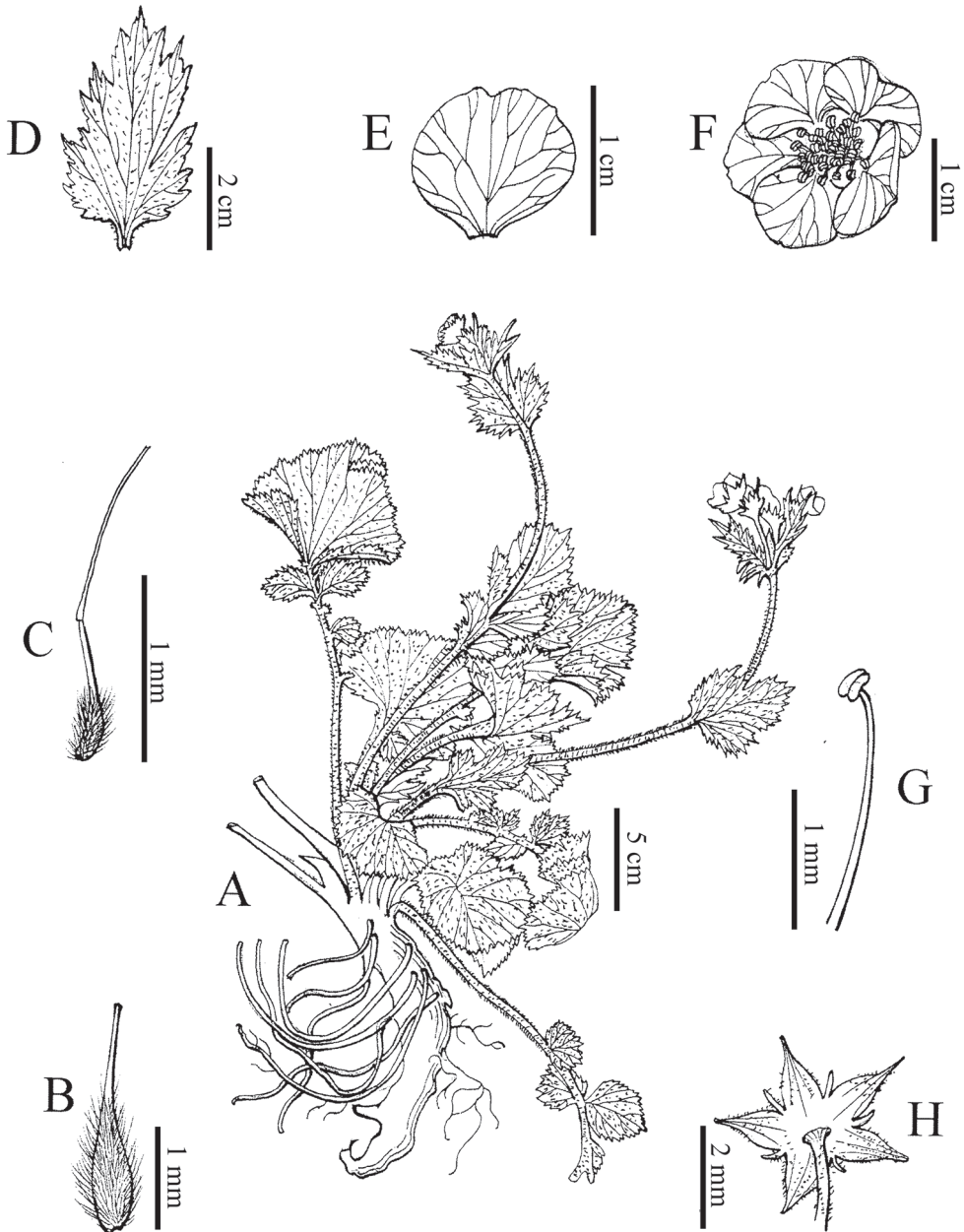


Figure 2. Holotype of *Geum sunhangii*. **A** Plant **B** achene **C** gynoecium **D** cauline leaf **E** petal **F** florets **G** filaments and anthers **H** calyx. Drawn by Ling Wang.

minial leaflet (vs. rhombic-ovate or compressed orbicular), nodding flowers (vs. erect), imbricate petals (vs. induplicate), curved at the obtuse or right angle at joint (vs. twisted), joint at ca. $2/3$ of style apex (vs. ca. $1/4$ way from apex). Detailed comparison information is in Table 1.

Table 1. Diagnostic morphological characters of *Geum sunhangii* compared to its three congeneric Chinese species.

	<i>G. sunhangii</i>	<i>G. aleppicum</i>	<i>G. japonicum</i> <i>var. chinense</i>	<i>G. rivale</i>
Cauline leaf	entire or 3-lobed	blade pinnate, sometimes repeatedly lobed	entire or 3-lobed	3-lobed or 3-parted
Shape of radical leaf terminal leaflet	reniform	rhombic-ovate or compressed orbicular	ovate or broadly obovate	rhombic-ovate
Arrangement of petals	imbricate	induplicate	induplicate	—
Petal shape	suborbicular	suborbicular	suborbicular	Semi-orbicular
Petal colour	yellow	yellow	yellow	yellow, purple-brown striate
Sepal colour	green	green	green	purplish
Sepal growth mode	spreading	spreading	spreading	erect
Flower	nodding	erect	erect	nodding
Style joint	obtuse or right angle curved	twisted	twisted	—
Style joint location	ca. 2/3 way from apex	ca. 1/4 way from apex	ca. 1/4 way from apex	—

Table 2. GenBank accession numbers for all species.

Species	Region	Accession	Region	Accession
<i>Geum sunhangii</i>	ITS	MT622526	<i>trnL-trnF</i>	MT614591
<i>Geum aleppicum</i>	ITS	KX645654	<i>trnL-trnF</i>	—
<i>Geum rivale</i>	ITS	AJ302352	<i>trnL-trnF</i>	AJ297338
<i>Geum andicola</i>	ITS	AJ302346	<i>trnL-trnF</i>	AJ297332
<i>Taihangia rupestris</i>	ITS	AJ302361	<i>trnL-trnF</i>	AJ297347
<i>Waldsteinia geoides</i>	ITS	AJ302362	<i>trnL-trnF</i>	AJ297348
<i>Coluria geoides</i>	ITS	AJ302343	<i>trnL-trnF</i>	AJ297329
<i>Geum bulgaricum</i>	ITS	AJ302347	<i>trnL-trnF</i>	AJ297333
<i>Geum calthifolium</i>	ITS	MG235321	<i>trnL-trnF</i>	AB219633
<i>Geum heterocarpum</i>	ITS	AJ302349	<i>trnL-trnF</i>	AJ297335
<i>Geum vernum</i>	ITS	AJ302355	<i>trnL-trnF</i>	AJ297341
<i>Geum reptans</i>	ITS	AJ302351	<i>trnL-trnF</i>	AJ297337
<i>Geum montanum</i>	ITS	AJ302350	<i>trnL-trnF</i>	AJ297336
<i>Fallugia paradoxa</i>	ITS	U90805	<i>trnL-trnF</i>	AJ297331
<i>Sanguisorba officinalis</i>	ITS	KR052188	<i>trnL-trnF</i>	AJ416465

Description. Roots fascicled, taproot terete, ca. 0.7 cm in diameter. Stems erect, 20–60 cm tall, pilose. Radical leaves lyrate-pinnate, 10–25 cm, with 2–4 pairs of leaflets, strigose; leaflets unequal, terminal leaflet largest, reniform, lobed, 2.5–6 × 3–10 cm, base cordate or truncate, margin irregularly coarsely serrate, apex rounded; cauline leaf ovate, herbaceous, 2–2.5 × 2.5–3.5 cm, leaf-like, leaf simple, entire or 3-lobed, margin irregularly coarsely serrate; veins bulge at leaf abaxially. Inflorescence terminal, corymb, bisexual, usually nodding, laxly 2–5 flowered. Flower actinomorphic, ca. 2.3 cm in diameter, pedicel densely pubescent and pilose; sepals triangular-ovate, green, apex acuminate; epicalyx elliptic or lanceolate, minute, ca. 1/3 as long as sepals, apex acuminate. Petals 5, yellow, imbricate, suborbicular, longer than sepals. Stamens numerous, yellow, ca. 0.22 cm; filament linear; anther yellow, ellipsoid. Style

terminal, ca. 0.24 cm in length, curved at an obtuse or right angle at joint, joint at ca. 2/3 of style apex, style glabrous and apex separating from joint at fruit maturity. Infructescence ovoid or ellipsoid, ca. 80 seeds; fruiting receptacle hirtellous; achenes hirtellous, hair ca. 0.3 mm, erect (Figs 1, 2).

Phenology. *G. sunhangii* flowers in May and sets fruit in August.

Distribution and habitat. *Geum sunhangii* is currently found in Nantianmen, Shennongjia National Nature Reserve, Hubei Province (Fig. 6). It grows on hillside meadows near water.

Conservation status. Based on the results of field investigations, *Geum sunhangii* was only discovered in Nantianmen, Shennongjia National Nature Reserve, where there is almost no human pressure on the distribution location of this species. About 60,000 individuals were present and the extent of occurrence is ca. 30,000 m². It is possible that additional populations may be discovered during future excursions. We initially define the category of *G. sunhangii* as Near Threatened (NT) by the Guidelines for Using the IUCN Red List Categories and Criteria (IUCN 2017).

Vernacular name. 神农花, shén nóng huā in Chinese Pinyin.

Etymology. The new species is named after the famous Chinese botanist Hang Sun, who made significant contributions to plant taxonomy, floral identification and biogeography in China.

Phylogenetic analyses

We conducted a phylogenetic analysis of 15 species, based on nrITS and *trnL-trnF* sequence data. The aligned combined data matrix included 1649 characters and 397 variables. The three topologies inferred by the Bayesian Inference analyses, MP analyses and ML analyses were similar. The Bayesian tree, including MP bootstrap (BP), ML bootstrap (LP) and PP values, is presented in Figure 5. Bayesian analysis of the combined dataset showed that samples were divided into three clades. Clade III included most species of the genus *Geum*, while *Coluria geoides* (Pall.) Ledeb., *Waldsteinia geoides* Willd. and *Taihangia rupestris* T.T. Yu & C.L. Li were grouped in Clade II. Clade I was a sister clade to Clades II and III and consisted of only one species, *Geum andicola* Reiche. Finally, *G. sunhangii* was nested into a monophyletic group (PP = 1, LP = 100%, BP = 100%; Fig. 5) with *G. rivale*, *G. aleppicum* and *G. montanum* Gouan ex Steud. The new species was clustered with *G. rivale*, but with a weak support (PP = 0.82, LP = 73%, MP = 67%; Fig. 5).

Discussion

Geum aleppicum and *G. japonicum* var. *chinense* are similar in morphology; they both have similar yellow petals and green sepals, but these species differ in receptacle hair (Fig. 4Aa, Ba; Table 1). In contrast, *G. rivale* is easily recognisable due to its purplish sepals and purple-brown striped petals (Table 1). The nodding flower of the new spe-

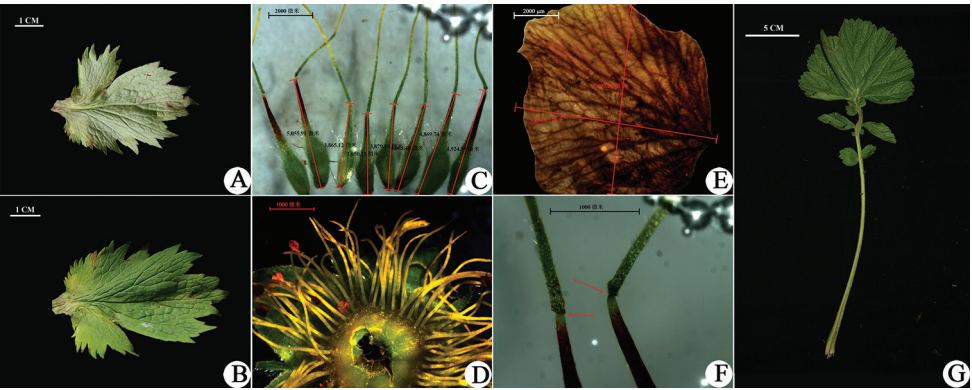


Figure 3. Anatomical characters of *Geum sunhangii*. **A, B** Cauline leaf **C** seeds **D** stamens **E** petal **F** style joints **G** radical leaf.

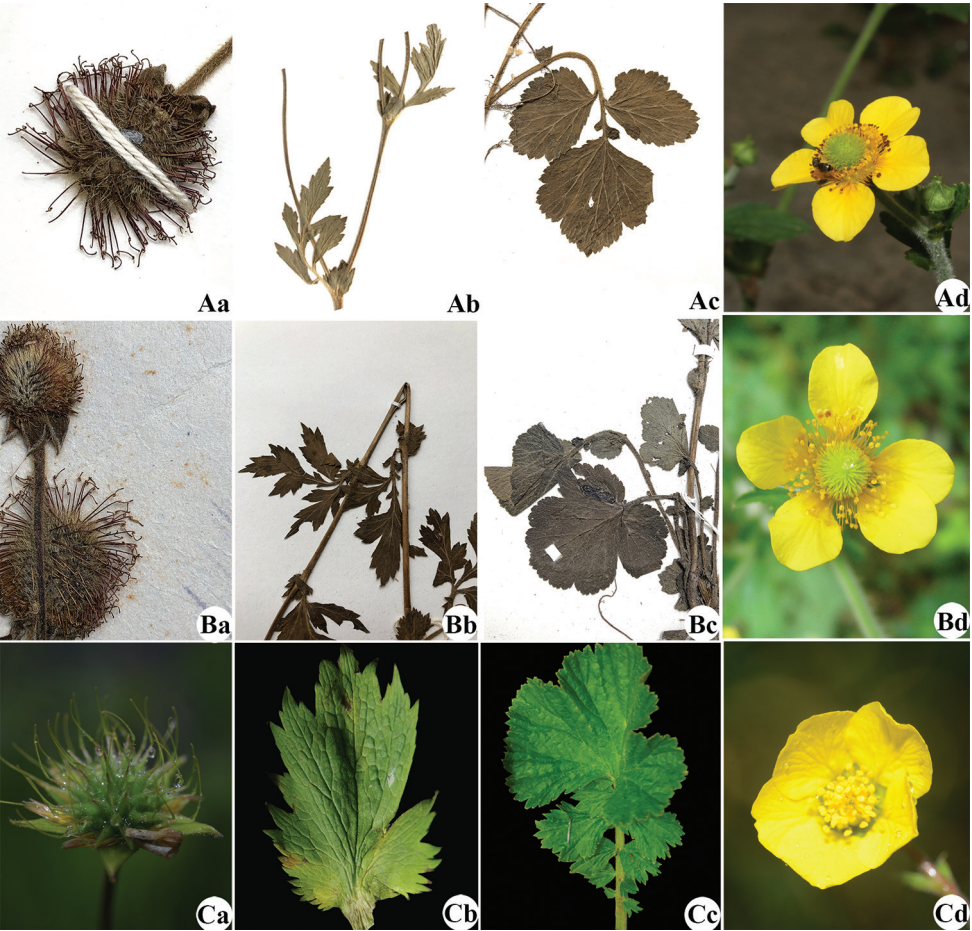


Figure 4. Morphological comparison of Chinese *Geum* species. **A** *G. aleppicum* **B** *G. japonicum* var. *chinense* **C** *G. sunhangii* (infructescences (a), cauline leaves (b) radical leaves (c) and flowers (d)).

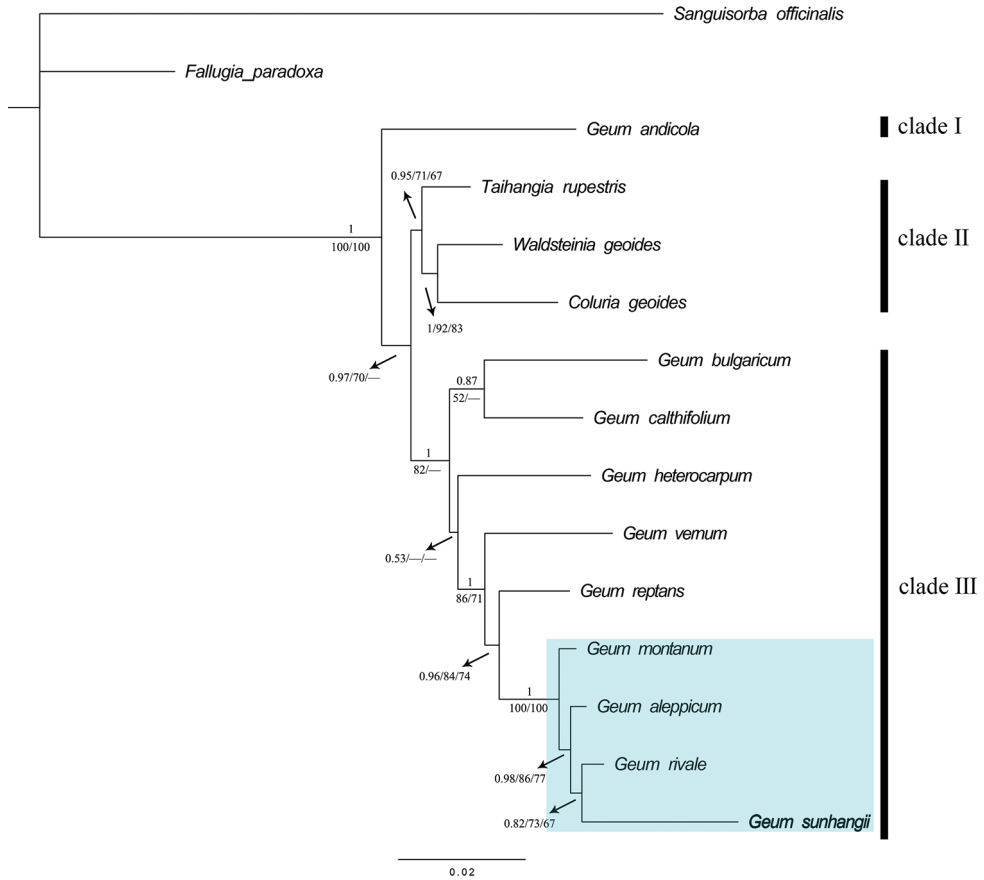


Figure 5. Bayesian consensus tree of the combined ITS and *trnL-trnF* sequence dataset. Numbers above branches indicate Bayesian posterior probability [PP], numbers below branches represent maximum likelihood bootstrap [LP] and maximum parsimony bootstrap [BP] values. The dash (–) indicate BP and LP < 50%. The new species is shown in bold. The monophyletic group with high support is framed by the blue rectangle.

cies is similar to that of *G. rivale*. Therefore, the combination of green sepals, nodding flower, yellow and imbricate petals is distinctly different from those found in the other three species present in China. In addition, the radical leaf and style joint of the new species are distinct in this genus. The terminal largest leaflet of the radical leaf is reniform (Fig. 3G) and different from the other species, which possess rhombic-ovate or compressed orbicular leaflets (Table 1). Curved styles are also important and recognisable features of the new species. Obtuse or right-angle curves of the style joint can be used to distinguish between the new species and its close relatives (Figs 3F, 4a). All distinguishing features are shown in Fig. 4 and Table 1.

We obtained samples of fifteen species for phylogenetic analysis. The topologies of the Bayesian, ML and MP trees were identical and were consistent with previous studies (Smedmark and Eriksson 2002). According to our results, *Geum* is probably a poly-

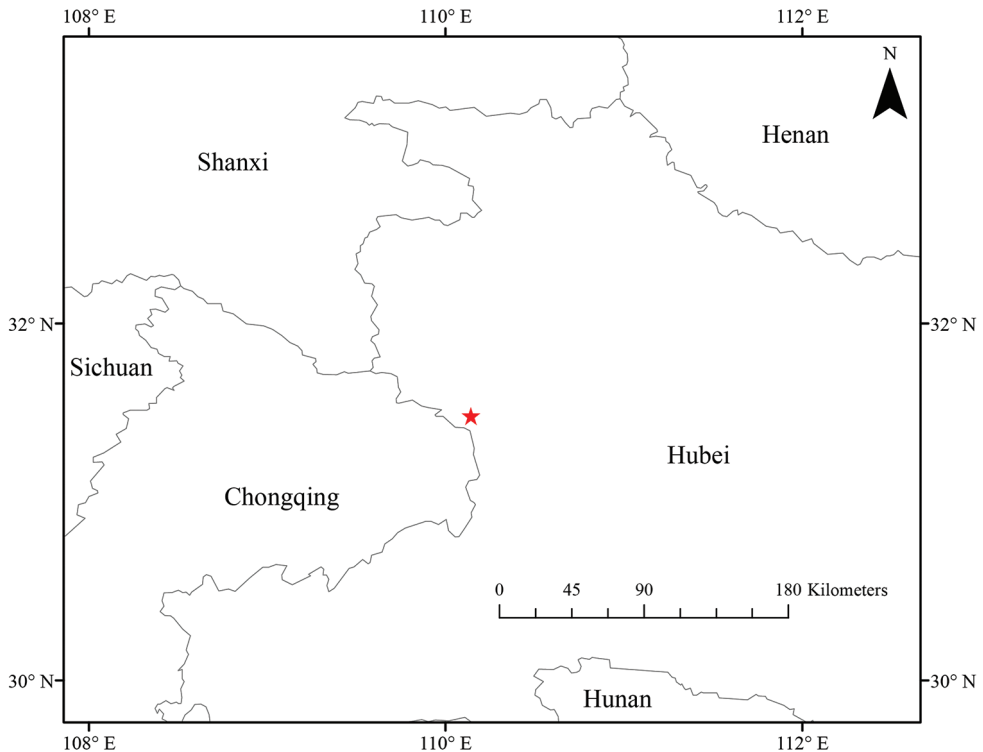


Figure 6. Distribution of *Geum sunhangii* in Hubei Province, China. Star indicates the type locality of *G. sunhangii*.

phyletic group and the boundary between *Geum* and species in Clade II is not clear (Fig. 5). At the same time, species in the genera *Taihangia* T.T. Yu & C.L. Li, *Coluria* R.Br. and *Waldsteinia* Willd. are likely congeneric to the *Geum* species. Therefore, further studies of the taxonomic and phylogenic relationships of *Geum*, *Taihangia*, *Coluria* and *Waldsteinia* species are needed. At present, the new species was confirmed as a member of *Geum*, since *G. sunhangii* was nested within a group of *Geum* species (PP = 1, LP = 100%, MP = 100%; Fig. 5) that form a monophyletic group (Fig. 5). In addition, the new species can be easily identified by its morphological features.

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Discovery of the first succulent bamboo (Poaceae, Bambusoideae) in a new genus from Laos' karst areas, with a unique adaptation to seasonal drought

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Abstract

Lush jungle flagship species, woody bamboos (Poaceae–Bambusoideae) are famed for their synchronous flowering as well as the extensive “bamboo forests” some species can form in tropical or temperate environments. In portions of their natural distribution, Bambusoideae members developed various adaptations to seasonality in environmental parameters, such as frost or seasonal drought. A new taxon, *Laobambos calcareus*, described here, is extremely novel in showing the first documented case of succulence in bamboos, with its ability to seasonally vary the volume of its stem depending on the quantity of water stored. Anatomical studies presented in this paper document this specificity at the cellular level. Though no flowers or fruits are known yet, unique morphological characteristics along with an investigation of its phylogenetic affinities using molecular data show that this new taxon should belong to a new genus herein described.

Keywords

Bambusinae, desiccation tolerance, genetic resources, xerophyte

Introduction

Bamboos (Poaceae – Bambusoideae) presently natively occur in every part of the world except Europe and Antarctica (Vorontsova et al. 2016). They form a very large culturally, economically and ecologically important group of organisms by sustaining entire endangered ecosystems, or as a source of food or materials for humans. Bamboos are famed cultural elements, used for virtually every part of the daily life in Asia, from food, cooking utensils, garden ornaments to house building material (Wong 2004). Bamboos are immediately recognizable by their typical habit while showing countless variations on the same model, from species with large woody culms, to small shrubby understory woody or herbaceous plants. The diversification of the group led to unique morphologies and adaptations, such as fire-resistance, as found in the South American genera *Actinocladum* McClure ex Soderst. (Soderstrom 1981), or *Glaziophyton* Franch. Bamboos even comprise truly climbing plants such as the Asian genus *Dinochloa* Buse, or the Malagasy *Sokinochloa* (Dransfield 2016). Complete seasonal deciduousness has been described in some instances, such as in Madagascar bamboos, occurring in tropical xeric conditions (Camus 1925) or correlated to cold tolerance in the temperate *Arundinaria appalachiana* Triplett, Weakley & L.G. Clark (Triplett et al. 2006). Some Mesioamerican *Otatea* taxa occur in extremely dry areas with annual precipitations as low as 350 mm (Ruiz-Sanchez 2015). However, “succulence”, i.e. the capacity to store water in a specific organ whose volume varies, has never before been documented for a bamboo.

The new taxon described herein, presenting a combination of both complete seasonal deciduousness and stem succulence, was collected in a karstic massif in Khammouane province in central Laos, during a Radeau des Cimes (“Canopy Raft”) expedition (Opération Canopée 2015). The climate is tropical, receiving more than 2000 mm of precipitation per annum, and characterized by a strong seasonality with a marked dry season from October to April/May (with virtually no rainfall during 4 months from November to February) and a very wet season from June to September with a monthly average of 400 mm of precipitation, and 25–30 rainy days monthly (World Weather Online 2020).

This plant was first spotted by the Canopy raft scouting expedition, during the dry season in January while the plants were leafless and deflated due to local extreme xeric conditions. In dormant state, the “deflated” aspect of the culms observed by the scouting expedition could easily be mistaken by a non-specialist as belonging to the orchid genus *Dendrobium* Sw., emphasizing the very peculiar wrinkled aspect of its culms and main branches when dormant. When subsequently visiting the same locality during the beginning of the rainy season, while the plants were in full leaf and the culms “inflated”, its “bambooness” was obvious in exhibiting typical architectural traits (Fig. 1). However, no fertile plants were located and only sterile specimens have been collected and documented. Several botanists later visited the area at various seasons to complement observations of seasonally expressed traits, but failed to find a fertile specimen. The authors also failed to locate any other specimens matching our new taxon in large international collections representative of the area visited.



Figure 1. *Laobambos calcareus*. **A** Adult plant in habitat at the type locality during rainy season **B** detail of foliage leaves **C** detail of the waxy marks on fresh inflated culms, revealing the ridges present on the dried-state culm during the dormant period **D** detail of a node and a branch complement **E** view of base of the clump showing the greenish inflated fresh live culms, along with ridged dehydrated brown dead culms. (Photo credit Thomas Haevermans 05/2012).

While bamboo species descriptions based on sterile material only are generally avoided, there do, however, exist precedents in the literature such as *Oldeania ibityensis* (A.Camus) D.Z.Li, Y.X.Zhang & Haev. (Zhang et al. 2017), originally described as *Arundinaria ibityensis* A.Camus, based merely on sterile syntypes from Madagascar (Camus 1960), but supported by recent molecular evidence. *Aulonemia nitida* Judz. described from sterile material, from the Pakaraima mountains in Guyana, South America, is another example of description based on non-fertile material (Judziewicz 2005).

The present paper is thus an exception in describing a new bamboo species, in a new genus, based solely on macro-morphological and anatomical studies of sterile material (herbarium specimens and field observations) and DNA sequence comparison.

Methods

Specimens were collected in Laos according to national and international regulations, and dried on-site with hot air; silica gel preserved leaf fragments were prepared during collection for subsequent DNA sequencing for use in comparative studies. Original specimens were deposited in Laotian national facilities for permanent storage, and duplicates were distributed to collaborating institutes for further reference and dissemination of the results. Drawings illustrating the description were prepared from the specimens.

Anatomical preparations of the leaves and the culms were performed from dry herbarium specimens. Freehand sections of subsequently rehydrated culms were done (without chemical treatments) to understand the distribution of the water storage elements in the plant. Additionally, leaf and culm sections from dried herbarium samples were remounted with heated 3% aqueous KOH, fixed with FAA 70%, sectioned with a Ranvier microtome, stained with Astra blue and Safranin red, and finally mounted in glycerinated water, to better describe the anatomical structure.

Supplementary video file. Rehydration of a dead dried stem section of *Laobambos calcareus* with distilled water. The stem section was 1.5–2.0 mm thick. Scale marks represent 1 mm. The real-time video lasts for 1:13min. The video is available from Figshare <https://doi.org/10.6084/m9.figshare.11919003> (video credit Dulce Mantuano).

Results

Figure 2A, B show that the leaf blades are not succulent and are essentially not distinct from typical bamboos leaves. The transverse section of a mature leaf (Fig. 2A) shows an adaxial epidermis presenting bulliform cells, chlorenchyma with two abaxial layers with arm cells, fusoid cells in the middle, one adaxial layer of arm cells, and an abaxial epidermis with papillae and trichomes. The stem shrinks in diameter during the dry season, forming grooves in the outer surface, as can be also be seen on herbarium specimens. The absolute water content of the mature culm was 1.63 g.g⁻¹. The rhizome (extremely reduced in proportion to the plant) does not show the typical succulence and shrinkage wrinkles of the culm when dry (Fig. 3G, H).

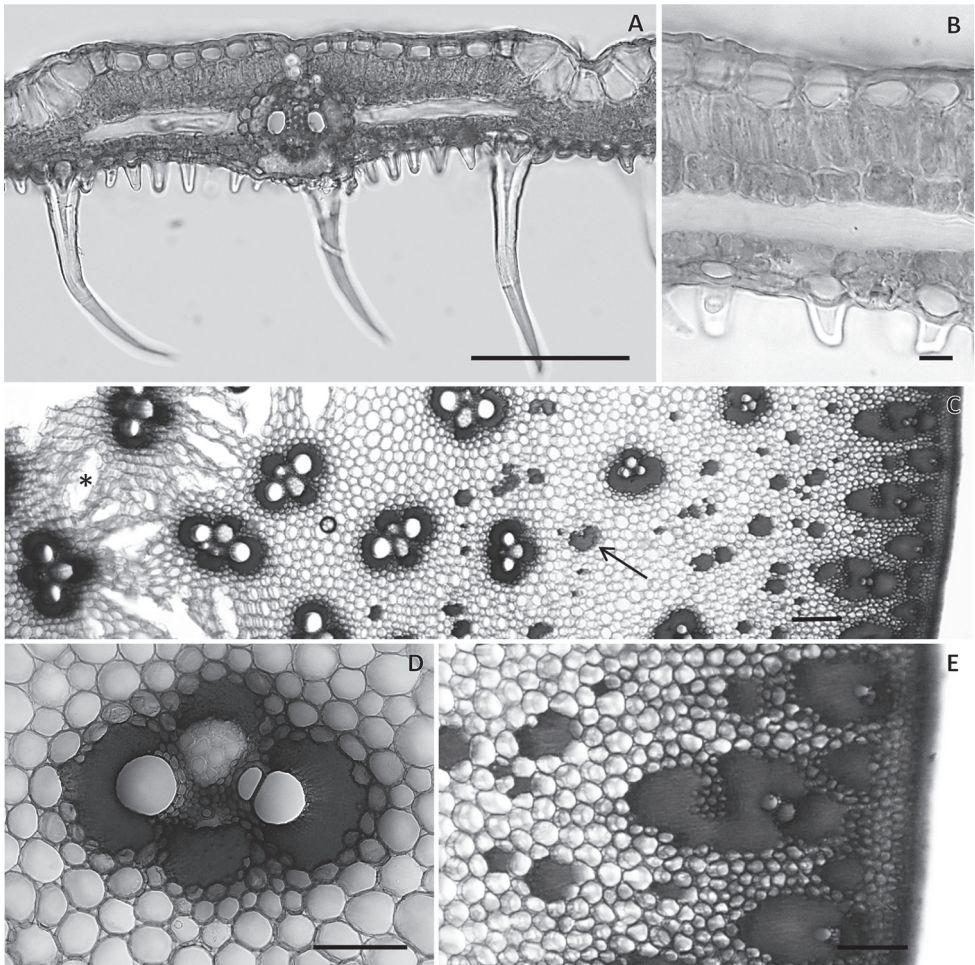


Figure 2. Transverse sections of leaf and culm of *Laobambos calcareus*. **A** Transverse section of a mature foliage leaf **B** mesophyll showing chlorenchyma with arm cells, fusoid cells and abaxial epidermis with papillae and trichomes **C** culm transverse section showing a medullary region (left side) with typical vascular bundles and cavities (asterisk) and a cortical region (right side) with isolated fiber bundles (arrow) **D** central vascular bundle in detail **E** cortical region in detail. Scale bars: 100 μm (**A**, **D**, **E**); 10 μm (**B**); 200 μm (**C**). (Photo credit Dulce Mantuano).

The culm and branches are solid throughout, presenting numerous vascular bundles and isolated non-vascular fiber bundles embedded in the parenchymatous ground tissue. Cavities can be found in the pith region (Fig. 2C). Subepidermal parenchyma is present. Vascular bundles did not present a transition between central and peripheral morphologies. Vascular bundles in the central region presented four discontinuous sclerenchymatic fiber sheaths associated with two metaxylem vessels, phloem, and intercellular space (Fig. 2D). Vascular bundles in the peripheral region are radially oriented, protoxylem and metaxylem are surrounded by sclerenchyma, and phloem can be absent (Fig. 2E).



Figure 3. *Laobambos calcareus*. **A** Plant in habitat, growing in karst crevices typically along with *Euphorbia antiquorum* L. and *Dracaena cochinchinensis* (Lour.) S.C.Chen **B** leaf complement **C** plant in dormant state, rhizome leaves removed to show the structure **D** rhizome with year+1 shoot cross-section, and young shoots **E** petiole insertion with inner and outer ligules (view from below) **F** culm sheath, ventral view. Scale bars: 1 m (**A**); 2 cm (**B**); 5 cm (**C**, **D**); 2 mm (**E**); 1 cm (**F**). (Illustration credit Agathe Haevermans).

Discussion

This unique bamboo, to be named *Laobambos calcareus*, possesses solid succulent culms bearing single-branched branch-complements, culm leaves that are persistent, coriaceous throughout, and devoid of a blade, developed auricles, or oral setae. Its unequal culm-nodes pattern and branching architecture (Figs 4; 1D) is very unusual for a Paleotropical bamboo, and though unrelated to, reminds of some Neotropical Arthrostylidiinae possessing a first elongated internode followed by reduced ones (*Arthrostylidium schomburgkii* Munro or *Glaziophyton*) as documented in Tyrrell et al. (2012). The genus *Myriocladus* Swallen, also in this Neotropical Bambuseae group, expresses a pattern of internodes heterogeneity similar in some ways to *Laobambos*, but the two differ otherwise in terms of their respective architectures (Afonso et al. 2019).

The succulence of the culm, i.e. its capacity to store water by modifying its diameter, evident from macromorphological observation, is further informed by an anatomical cross-section suggesting the capacity for the bamboo to store water in zones distributed throughout the solid culm. Measured succulence (absolute water content) for mature culms is higher than previously reported for young, one year-old bamboo shoots; which usually varies between 1.2–1.3 g.g⁻¹ (Rao 1985). Succulence of the culm is mainly due to the water-holding capacity of parenchyma cells, as no specific water-storage cells were identified. Surprisingly, a rapid rehydration could be observed even when the tissue is dead. A video in real time is provided as supplementary online material to show the speed at which a culm cross-section from a herbarium specimen can take water in. The ability to rapidly absorb water is probably linked to an adaptation to water pulses in the karstic rock habitat, desiccation tolerance (DT) being adaptively optimal for plants growing on substrates impenetrable to roots or experiencing seasonally dry conditions (Proctor and Tuba 2002). Variation in moisture content of different parts of culms were found to be clearly associated with the proportion of parenchyma cells in the tissue system (Liese and Grover 1961). Our anatomical findings corroborate this observation, although the culm structure of *Laobambos calcareus* does not show, as is typical of bamboos, a dense vascular bundle transition layer (Grosser and Liese 1971), but rather sparsely distributed vascular tissues with a high proportion of parenchyma, mechanically supported by isolated non-vascular fibers. Fibers non-related to the vascular bundles in the culms seem to be a particular feature.

Molecular analyses (Zhou et al. 2017) demonstrate that *Laobambos* belongs to core Bambusinae, the largest paleotropical woody bamboo subtribe, and is the closest relative to two other (facultatively) karst dwelling taxa; *Laobambos* being the sister group (Fig. 5) of the genera *Temochloa* S.Dransf. + *Neomicrocalamus* Keng f. (represented by *N. prainii* (Gamble) Keng f. in the study), with which it does not share any notable morphological characteristics (*Neomicrocalamus* and *Temochloa* are scrambling bamboos, with hollow to solid culms, culm-leaves apices papery and thin, multiple-branched branch complements with a main dominant branch and sub-equal internodes). *Laobambos calcareus* bears some overall resemblance in habit with another Asian karst dweller, *Bonia saxatilis* var. *solida* (C.D.Chu & C.S.Chao) D.Z.Li, to

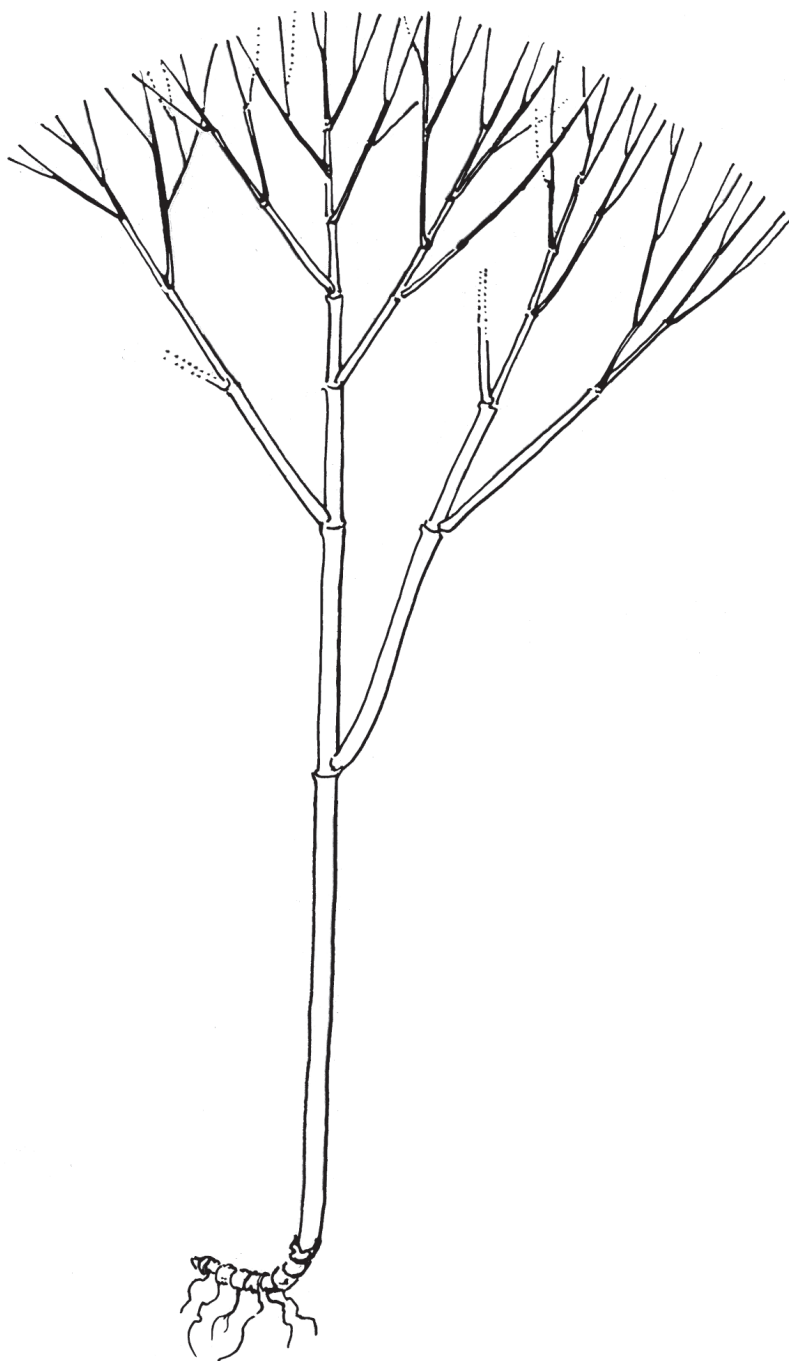


Figure 4. Scheme detailing *Laobambos calcareus* culm architecture illustrating the much elongated first internode, alternate branching with branch diameter matching the diameter of adjacent culm segment, and length of branches not exceeding the total length of the culm. (Illustration credit Agathe Haevermans).

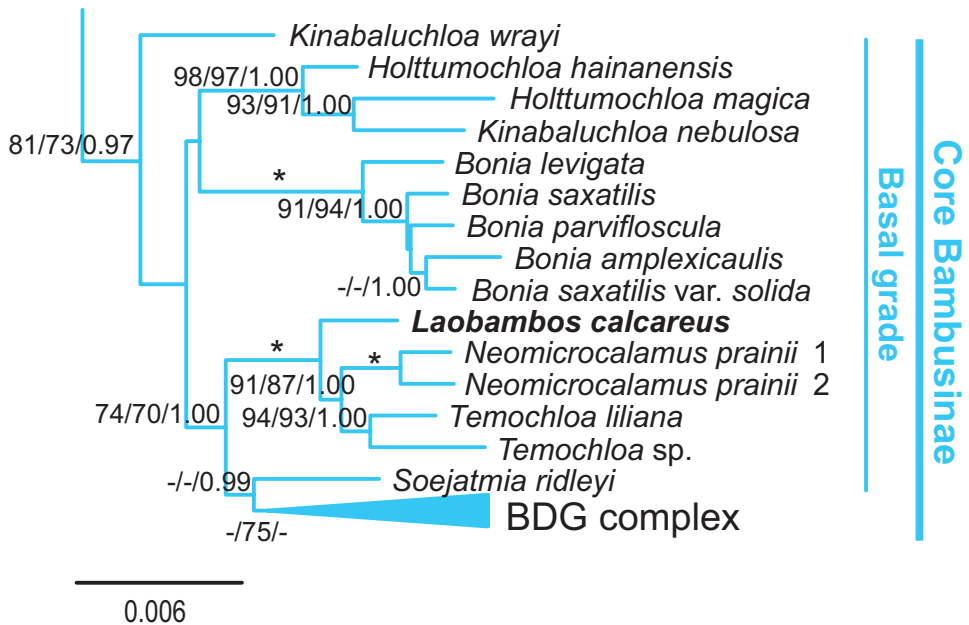


Figure 5. Phylogenetic placement of *Laobambos calcareus*, adapted from Zhou et al. 2017 (the original “Genus indet” has been replaced here by the newly described name).

which it is not immediately related (Fig. 5), while also belonging to core Bambusinae (Zhang et al. 2016; Zhou et al. 2017). *Bonia saxatilis* var. *solida*, while single-branched, differs significantly from *Laobambos* by culm leaf characters such as the persistence of the culm sheath and the presence of a developed blade, auricles and oral setae.

For woody bamboos, flowering cycles can vary greatly from a couple of years to several decades (up to unknown, in several instances), thus offering no guarantee that this taxon may ever be found flowering in the researchers’ lifetime. The species is undoubtedly new, as a thorough search in the literature of the Indo-Burma biodiversity hotspot area does not mention a bamboo possessing this combination of characters, especially reviewing the literature concerning taxa growing in similar karstic habitats in neighboring countries. The architecture presented by this bamboo is also extremely peculiar (see Fig. 4), and even if the inflorescences, flowers and caryopsis are unknown, we can confidently describe this taxon as new, as no other extant bamboo possesses this combination of characters.

Its generic affinities were clarified when this taxon was placed within a molecular phylogeny (the accession referred to as “Genus indet” in Zhou et al. (2017: fig. 1); the accessions we sequenced resolved as the sister group to two other karst-dwelling taxa: *Neomicrocalamus prainii* (Gamble) Keng f. and *Temochloa liliana* S.Dransf. (Dransfield 2000), with which it shares no morphological characteristics (Fig. 5).

Its morphology is so distinct from other known bamboos that, after consulting several Asian bamboo systematics authorities during the latest symposium on bamboo

systematics and ecology (held during the XIXth International Botanical Congress in Shenzhen, RPC, July 2017), they agreed that in such cases there is no reason to further delay its description, due to its singular morphology, even if no fertile material is known.

Taxonomic treatment

***Laobambos* Haev., Lamxay & D.Z.Li, gen. nov.**

urn:lsid:ipni.org:names:77211170-1

Type species. *Laobambos calcareus* Haev., Lamxay & D.Z.Li.

***Laobambos calcareus* Haev., Lamxay & D.Z.Li, sp. nov.**

urn:lsid:ipni.org:names:77211171-1

Type. LAOS • sterile; Khammouane province, Phou Hin Boun, Ban Natan (ບ້ານນາຕັນ) area, in bare-rock cracks on slopes of karstic area dolines close to the Canopy Raft camp; 17°59.01'N, 104°48.01'E; elev. 265 m; May 2012; *T. Haevermans, V. Lamxay, P. Blanc & F. Hallé, TH852* (holotype HNL!; isotypes: P! (mounted on 4 sheets), K!, KUN!); .

Diagnosis. Similar to *Bonia saxatilis* var. *solida* in habit but differs in its succulent culms, persistent culm-sheaths, the unequal structure of its internode pattern, and the absence of culm leaf blade, auricles, or oral setae.

The generic name indicates that the new taxon is restricted to Laos, and the specific epithet emphasizes that the species is restricted to bare karstic crevices.

Shrubby, clumping (non-running) bamboo exclusively growing in karstic rock crevices. Clumps 2–3 m high, 2–3 m in diameter, often with more than 50 culms simultaneously alive per mature clump, with several persistent withered dry old culms; rhizomes pachymorph, short-necked, non-succulent; culms straight, each internode slightly wavy, 6–10 mm in diameter when full of water, cross-section rounded (4–6 mm in diameter when dormant and dry, cross-section star-shaped), solid, and storing water within its mass, deep-green with longitudinal lines of whitish bloom corresponding to the wrinkles (tips of the cross-section star-shape arms) when the bamboo culm shrinks in the dry season; when dried with hot-air for preserving the sample as a herbarium specimen, a thin black wax layer flakes off the culm and greases the paper used for drying; culm leaf, 57 × 12 mm, acute, apical part slightly pubescent but devoid of auricles, cilia or a blade, persisting on culms; no transition between the culm leaf (devoid of blade) and foliage leaf (with inner and outer ligule, pulvinus and fully developed blade). Plant architecture: each culm with a very long first internode, followed by successively shorter and shorter ones (typically 60 cm/40 cm/25 cm/etc., cf. Fig. 4); branch complement always consisting of a single intravaginal branch (Fig. 1D); alternate branching pattern with each branch being roughly as long as the sum of

the remaining segments of the main culm, and with the same diameter as the adjacent internode just after the branching (cf. Fig. 4); internode length variation likely dependent on the availability of water during elongation; leaf-complement consisting of around 6–7 non-succulent foliage leaves (Fig. 1B); foliage leaves consistently deciduous during the resting dry season, blades 12–16 mm wide, (50–)80–130(–140) mm long, pseudo-petiole 2–3 mm long with a slightly swollen pulvinus at the base, inner and outer ligules bearing cilia 1 mm long, shoulders asymmetrical, auricles absent. Inflorescences, flowers and caryopsis presently unknown.

Other specimens examined. LAOS • sterile; Khammouane province, Haute vallée de la Hin Boun; 16 January 2012; *F. Hallé et al.* 4966 (HNL!, P!) • sterile; Khammouane province, Pan a'm, the hill behind Ban Natan, Konglor Cave, 08 April 2013, *T. Zhang et al.* 13CS6294 (KUN!).

Conclusion

DNA sequences comparison informs us that this unique karst-restricted desiccation tolerant bamboo taxon is related to *Neomicrocalamus* and *Temochloa* (Fig. 5). However, no morphological characters unite it to these taxa and thus provides no justification for describing it as a species in one of these two sister genera. We decided to accommodate this species in a new genus to take into account its unique morphology and DNA-based phylogenetic relationships. Further work is needed as no other specimens (fertile or not) have been found in local and international herbaria such as the Paris herbarium (P), which holds a very large representative collection of bamboo specimens from this area. While its formal conservation status cannot be assessed for now due to data deficiency (it is thus tentatively rated Data Deficient), this taxon appears rare and quite restricted in range and habitat as only one population has been located by us or collaborators and thus likely belongs to one of the IUCN threat categories. Extensive fieldwork is required to try to locate other populations of this taxon as well as to collect flowering material if ever possible. Anatomical and developmental studies based on live plants are also necessary to document and understand the water storage cycle in this taxon and determine its abilities and limits in terms of desiccation tolerance. Being a wild relative of economically important tropical bamboos, further research into the adaptations of *Laobambos* toward desiccation tolerance may bring further breeding possibilities and genetic resources for commercial bamboo growers in seasonally dry areas hit by climate change.

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Cimes expedition organizers according to national and international regulations. Subsequent additional fieldwork funding was provided by the Muséum national d'histoire naturelle PPF grants “Biodiversité actuelle et fossile”, and the Germplasm Bank of Wild Species, Kunming Institute of Botany, Kunming, China. Molecular experiments were supported by a grant of the National Natural Science Foundation of China (no. 31670396). Working visits to China were jointly funded by the French Embassy in Beijing and the Chinese Ministry of Science and Technology (MOST) (TH was the recipient of grants from scientific cooperation programs Xu Guangqi (徐光启) 2012, and “Jeunes Talents France-Chine” 2017) to visit Kunming Institute of Botany and herbarium KUN, as well as South China Botanical Garden Herbarium (IBSC) in Guangzhou for this research.

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Sonerila cardamomensis (Melastomataceae), a new species from Cambodia

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Abstract

Sonerila cardamomensis, a new species of family Melastomataceae from the Central Cardamom Protected Area in Koh Kong province of southwestern Cambodia, is described and illustrated. The species is similar to *S. violifolia* Hook.f. ex Triana, but is readily distinguished by its cordate leaf base, lack of setae at the nodes, longer pedicels, smaller petals, smaller hypanthiums and smaller capsules.

Keywords

Cambodia, Central Cardamom Protected Area, new species, *Sonerila*

Introduction

Melastomataceae Juss. consists of approximately 166 genera and 4,200–4,500 species which are distributed in both the New World (about 2,950 species) and the Old World (1,275–1,550 species) (Renner 1993, Michelangeli et al. 2013). In Cambodia, Melastomataceae are currently represented by 34 species and 6 varieties for a total of 40 taxa in 9 genera (Cho et al. 2015, Tagane et al. 2015, Cho et al. 2016a, Cho et al. 2016b). The most species-rich genera in Cambodia are *Memecylon* L. (18 species), *Osbeckia* L. (7 species), and *Melastoma* L. (5 species).

In Indochina, there are around 22 species of *Sonerila* and the present count includes 9 species from Vietnam, 13 species from Thailand and 4 from Laos (Guillaumin 1913, Ho 1999, Renner et al. 2001, Chen and Renner 2007, Newman et al. 2007). In Cambodia, at the beginning of the 20th century, only one species was described, namely *Sonerila quadrangularis* (synonym of *S. maculata* Roxb.). Three species are reported in the present account, namely *S. bokorensis* S.H. Cho & Y.D. Kim, *S. maculata* Roxb., and *S. plagiocardia* Diels (Cho et al. 2016a).

During the recent floristic survey, one species of *Sonerila* was collected at Thmor Bang District of the Central Cardamom Protected Area in Koh Kong province of southwestern Cambodia that does not appear to be similar to previously described species (Fig. 1, 2). It is the most similar to *Sonerila violifolia* Hook.f. ex Triana, but detailed examination of the morphology revealed that it differs from *S. violifolia*. Therefore, it is described here as a new species.

Taxonomy

Sonerila cardamomensis S.H.Cho, sp. nov.

urn:lsid:ipni.org:names:77211172-1

Figures 1, 2

Type. CAMBODIA. Koh Kong Province, Central Cardamom Protected Area, sandstone rocky area in evergreen forest, 11°42'08.0"N, 103°26'22.5"E, a.s.l. 427 m, 9 August 2018, with flowers, Cho S.H., Shin J.S., Song B.K., Chhang Phourin CB-4467 (holotype K^B!, isotypes K^B!, K^I!, P^I!).

Diagnosis. *Sonerila cardamomensis* is most similar to *S. violifolia* Hook.f. ex Triana, which is distributed in Myanmar and Thailand but is readily distinguished from the latter by the cordate leaf base, lack of setae at the nodes, longer pedicels, smaller petals, smaller hypanthia and smaller capsules (Table 1).

Description. Perennial, herbs, terrestrial, 4–7 cm high, 1 (or rarely 2) shoot from rhizome. Rhizome short, bulbous, 0.4–1.0 cm in diam. Stems 4-sided, tinged purplish, the internodes almost absent. Leaves simple, membranous, opposite, clustered at base, those of a pair isomorphic; petioles 0.4–2.5 cm, glandular trichome; leaf blade ovate, 1.0–4.0 × 0.9–2.7 cm, apex acute, rarely obtuse, base cordate, upper surface covered with glandular trichome, lower surface slightly covered with glandular trichome, lateral primary vein 2–3 pairs, departing at the base, margin serrate. Inflorescences pedunculate, 1–5[7] scorpioid cymes with 5 to 11 flowers; peduncle [1.5]3.0–5.0 cm (up to 8.0 cm long when fruiting), angular with glandular trichome. Pedicel ca. 4.0 mm long (up to 5.0 mm long when fruiting), with glandular trichomes. Flowers bisexual, 3-merous. Hypanthium campanulate, 3-angled, 6-ribbed, 3.5 × 1.3–1.5 mm, with glandular trichomes. Calyx lobes broadly triangular 0.7 × 1.0 mm, apex acute. Petals 3, thin, 5.5–6.0 × 3.5–4.0 mm, ovate-obovate, apex acute to acuminate, pink to purplish pink, abaxially glandular trichome on midveins, adaxially glabrous. Stamens 3, isomorphic; filaments distinct, 3.7–4.0

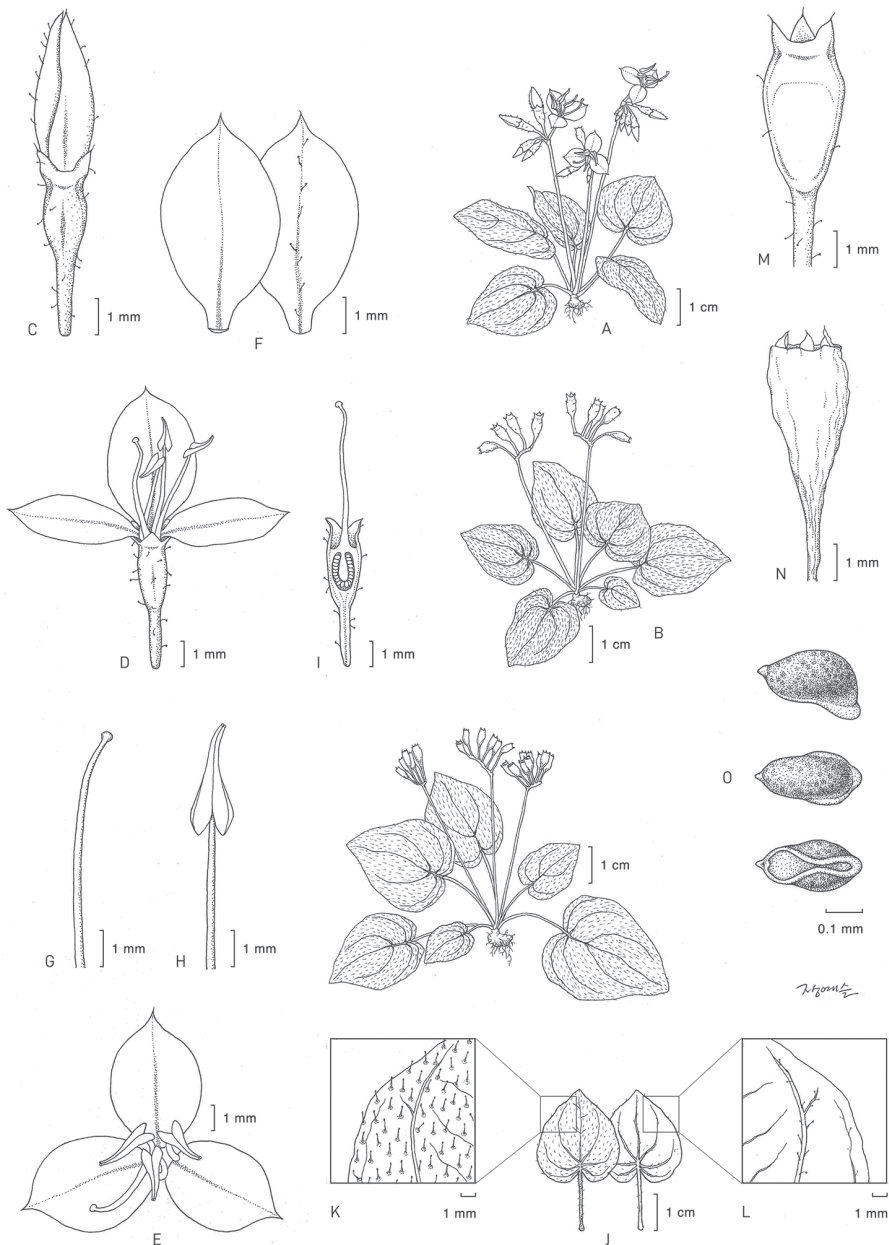


Figure 1. *Sonerila cardamomensis* **A** Flowering individual **B** fruiting individual **C–D** developing flower **E** mature flower **F** petals (right: abaxial, left: adaxial) **G** style and Stigma **H** filament and Anther **I** gynoecium **J** leaf **K** upper surface of leaf **L** lower surface of leaf **M** immature capsule **N** mature capsule **O** seeds: Cho et al. CB-4467. Illustration by Ye-Seul Jang.

mm, glabrous, pinkish; anthers ca. 4.5 mm, lanceolate, deeply cordate to sagittate at the base, apex acute, yellow, dehiscence poricidal. Ovary inferior, urceolate, apex with a membranous 3-lobed crown, ovules numerous, placentation axillary; style

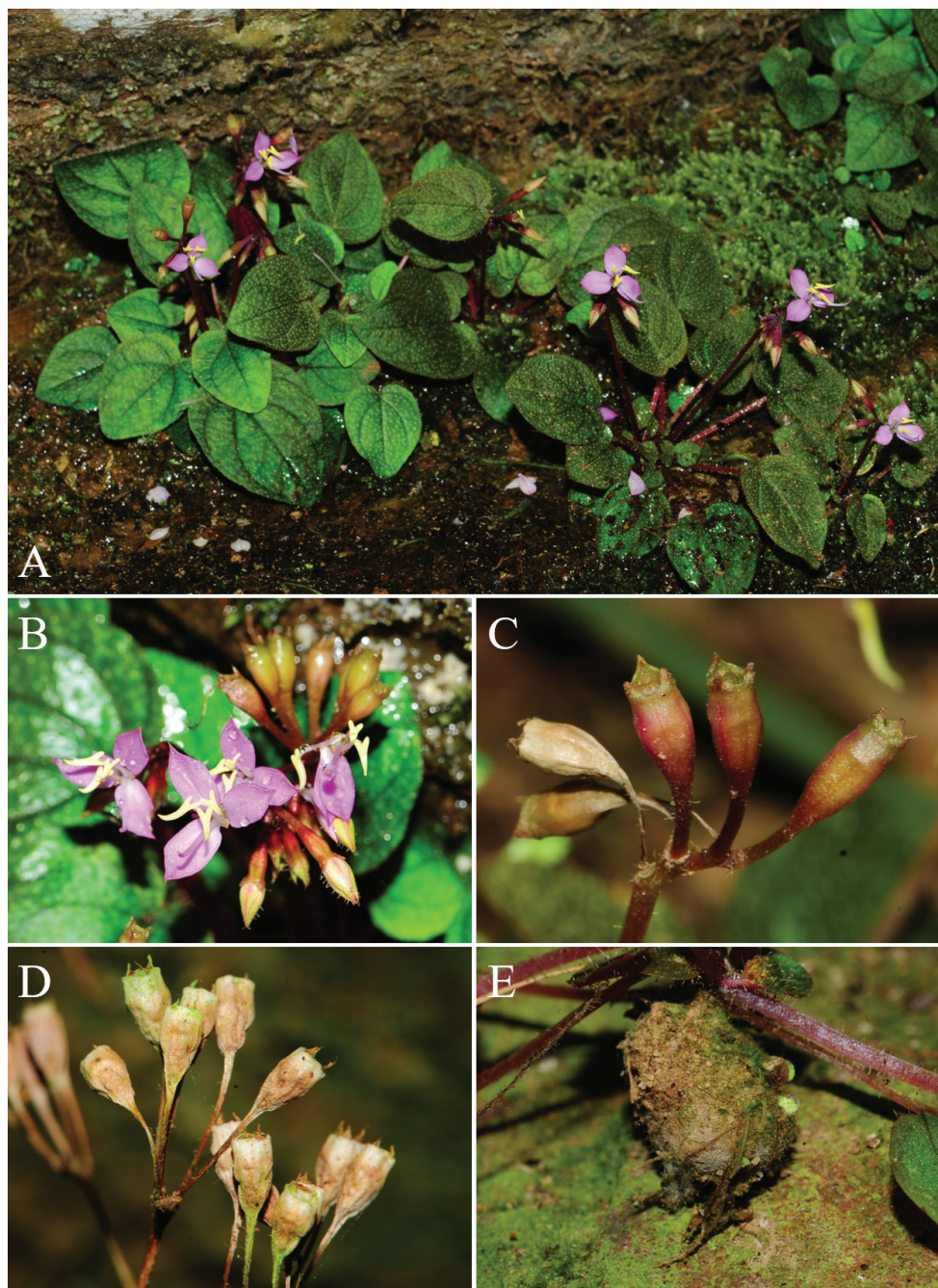


Figure 2. A–E *Sonerila cardamomensis* **A** habit **B** flowers **C** immature capsules **D** mature capsules **E** short bulbous rhizome: Photos by Seong-Hyun Cho.

Table 1. Comparison of key features of *Sonerila cardamomensis* and *S. violifolia*.

Taxonomic traits	<i>Sonerila cardamomensis</i>	<i>S. violifolia</i>
Stem setae at the nodes	Absent	reddish brown, 4.0–6.0 mm long
Leaf upper surface base	glandular trichomes cordate	spars minute bristly hairs obtuse or cuneate
Pedicel	ca.4.0 mm long	2.0–3.0 mm long
Hypanthium	ca. 3.5 mm long	5.0–6.0 mm long
Petal	5.5–6.0 mm long	8.0–10.0 mm long
Capsule	3.5–4.0 mm long	6.0–7.0 mm long

filiform, 6.6–7.3 mm, glabrous, pink; stigma apiculate. Fruit a capsule, campanulate, 3.5–4.0 × 2.0–2.2 mm, 6 longitudinal ribs, glabrous. Seeds cuneate, numerous, ca. 0.3 mm, light brown.

Specimen examined. CAMBODIA. 9 August 2018, with flowers, Cho et al. CB-4469, 4471, 4473 (KB!), Won et al. 16499, 16506(DGU!); 15 January 2019, with fruits, Cho et al. CB-4614, 4615 (KB!)

Phenology. Fls July to August; Frts August to November.

Distribution and habitat. *Sonerila cardamomensis* grows on sandstone rocky area in evergreen forest from 420 to 600 m.a.s.l. Endemic to southwestern Cambodia, *S. cardamomensis* is at present known only in the Central Cardamom Protected Area in Koh Kong province.

Conservation status. *Sonerila cardamomensis* was collected in the Central Cardamom Protected Area of Koh Kong province in southwestern Cambodia. Until now, two big populations are known, each composed of more than ca. 1,000 individuals, and was discovered in the protected area. Therefore, it is preliminarily classified as data deficient (DD) according to the IUCN Red List criteria.

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