

The systematic position of puzzling Sino-Himalayan *Lophocolea sikkimensis* (Lophocoleaceae, Marchantiophyta) is identified

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Abstract

Lophocolea sikkimensis, a little-known Sino-Himalayan species, was collected in North Vietnam and its taxonomic position was identified by molecular genetic techniques. The species is characterized by generally narrowly pointed leaves, which are not seen in other representatives of Lophocoleaceae. We found that it belongs to the recently described genus *Cryptolophocolea*, although it is clearly morphologically dissimilar to other members of the genus. We propose a corresponding nomenclature combination: *Cryptolophocolea sikkimensis* comb. nov. This species is the only one in its genus with a predominantly Sino-Himalayan distribution; the vast majority of congeners are distributed in the Southern Hemisphere (mostly in Australasia). Reports of this species in Vietnam further confirm the close phylogeographic relationships of the flora of northern Indochina with those of the Sino-Himalayas and suggest that this species is found in other parts of the Hoang Lien Range and the southern Hengduan Range.

Keywords

Lophocolea, Lophocoleaceae, molecular phylogenetic, Sino-Himalaya, taxonomy, Vietnam

Introduction

Lophocolea sikkimensis (Steph.) Herzog & Grolle is a poorly known Lophocoleaceae species but is so different from other known members of the family that it appears to belong to a different genus. Kitagawa (1974) discussed similar considerations based on the first impressions of this taxon (l.c.: 32). The specific features (mostly not unique to *Lophocolea* s.l.) include ovate leaves with acute to obtuse apices, almost rounded underleaves distinctly connate to leaves on both sides, and biseriate antheridium stalk. Kitagawa (1974) described the history of the taxonomic interpretation of the species in detail, eventually concluding that the placement of the species in *Lophocolea* was appropriate. This point of view was adopted by Söderström et al. (2016). After we collected this species in Vietnam (Bakalin et al. 2018), we were impressed by its unusual appearance and decided to review the taxonomic position of this species, including molecular genetic methods that were, of course, unavailable to N. Kitagawa fifty years ago. This attempt seemed particularly appropriate in light of recent perturbations of Lophocoleaceae systematic over the last 10–15 years, which are clearly described by Söderström et al. (2013: 36): “Lophocoleaceae is a family with a turbulent history and many taxa have been moved back and forth among genera”. Thus, the goal of this work was to determine the taxonomic position of the species known as *Lophocolea sikkimensis*.

Historical background

Initially, *Lophocolea sikkimensis* was placed in the genus *Herpocladium* as *H. sikkimense* (Stephani 1922); this genus contained heterogeneous elements (at least 4 genera) and is synonymous with *Herbertus* by its type species. Since the species was “hidden” within a contradictory treated genus, the same taxon was independently described 17 years later as *Lophocolea trollii* by Herzog (1939). This name was synonymized with *Herpocladium sikkimense* until the species was transferred to *Lophocolea* 20 years later (Herzog and Grolle 1958).

After reviewing the characteristics previously mentioned (plant color, large entire underleaves, dorsally secund leaves, biseriate antheridium stalk, etc.), Kitagawa (1974) concluded that some were not unique to that genus (as it was treated in that time) or were subject to variability within the species. After this comprehensive work, no additional special taxonomic investigations of this species were conducted. However, the generic position of this species was changed due to the broad genus concept of the Lophocoleaceae family (or that of the broadly defined Geocalycaceae family). Engel and Schuster (1985) proposed a broad concept: the *Chiloscyphus*-*Lophocolea* complex that fused both genera under an older name (*Chiloscyphus*), and therefore renamed the species in question as *Chiloscyphus sikkimensis* (Steph.) J.J. Engel & R.M. Schust. This concept was received somewhat critically and was only limitedly accepted.

In the mid-2000s, important molecular-genetic comparisons were carried out on Lophocoleaceae, providing a new perspective on the old problem. Research by Hentschel

et al. (2006a, 2006b, 2007) made it clear that Engel and Schuster (1985) were correct: it is impossible to distinguish only two genera in the *Chiloscyphus-Lophocolea* complex because the second genus then becomes clearly polyphyletic. The solution may be to either unite both genera (as done by Engel and Schuster 1985) or to divide *Lophocolea* into several genera. Hentschel et al. pursued the latter method. The most important entity segregated based on the molecular-genetic approach was *Cryptolophocolea*, which was described in 2013 (Söderström et al. 2013). This genus is characterized by a number of features, some of which (bifid, opposite to subopposite leaves, 2–4(–6)-lobed underleaves) are not observed in *Lophocolea sikkimensis*; other features of this genus, such as the biseriate antheridial stalk, large underleaves connate with leaves at both sides, indicate a possible relationship between it and *Lophocolea sikkimensis*.

Methods

Taxon sampling

We analyzed two specimens of *Lophocolea sikkimensis* in our molecular phylogenetic study using nucleotide sequence data from ribosomal operon of nuclear DNA (ITS1–2) and *trnL-F* of chloroplast DNA. In addition to Lophocoleaceae, the analysis included sequence data from genetically related Jungermanniales families (Cooper et al. 2011; Patzak et al. 2016). The outgroups in the ITS1-2-based tree were Herbertaceae species (*Herbertus dicranus* (Gottsche, Lindenb. & Nees) Trevis., *Triandrophyllum subtrifidum* (Hook.f. & Taylor) Fulford & Hatcher), and Lepicoleaceae species (*Lepicolea attenuata* (Mitt.) Steph., *Lepicolea scolopendra* (Hook.) Dumort. ex Trevis., *Lepicolea ochroleuca* (Spreng.) Spruce) for the *trnL-F* tree that correspond to the topologies in Patzak et al. (2016). All sequences except those newly obtained were downloaded from GenBank.

There were too few *trnG*-intron sequences from Lophocoleaceae in GenBank to construct a reliable phylogenetic tree for this marker that establishes the position of *Lophocolea sikkimensis*.

Therefore, new *trnG*-intron sequences were obtained for this taxon but not analyzed properly.

Specimen voucher details, as well as newly identified and previously identified sequences, are listed in Table 1.

DNA isolation, amplification, and sequencing

DNA was extracted from dried liverwort tissues using the NucleoSpin Plant II Kit (Macherey-Nagel, Germany). Amplification of ITS1–2, *trnL-F*, and the *trnG*-intron was performed using an Encyclo Plus PCR kit (Evrogen, Moscow, Russia) with the primers listed in Table 2.

The polymerase chain reaction was performed in a total volume of 20 µl, including 1 µl of template DNA, 0.4 µl of Encyclo polymerase, 5 µl of Encyclo buffer, 0.4 µl of

Table 1. The list of voucher details and GenBank accession numbers for the specimens used in phylogenetic reconstructions in the present paper (* *trnG*-intron GenBank accession number: OK562104; ** *trnG*-intron GenBank accession number: OK562105). Newly obtained sequences are in bold.

Initial species name	Accepted name	Label	GenBank accession number	
			ITS1–2	<i>trnL</i> -F
<i>Bragginsella anomala</i> R.M. Schust.	<i>Bragginsella anomala</i> R.M. Schust.	New Zealand, M. von Konrat & J.J. Engel, L1129 (F)	–	KJ802081
<i>Chiloscyphus austrigenus</i> (Hook. f. & Taylor) J.J. Engel & R.M. Schust.	<i>Pachyglossa austrigena</i> (Hook. f. & Taylor) L. Söderstr.	Chile, Hyvönen et al. 5793 (JE)	AM282805	–
<i>Chiloscyphus ciliolatus</i> (Nees) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea ciliolata</i> (Nees) L. Söderstr., Crand.-Stotl., Stotler & Váňa	Indonesia, Gradstein, 10327 (GOET)	AM491286	–
<i>Chiloscyphus connatus</i> (Sw.) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea connata</i> (Sw.) L. Söderstr. & Váňa	Costa Rica, Gradstein, 9404 (GOET)	AM282806	–
<i>Chiloscyphus costatus</i> (Nees) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea costata</i> (Nees) L. Söderstr.	Malaysia, Schäfer-Verwimp & Verwimp, 18724/A (JE)	AM282807	–
<i>Chiloscyphus cucullistipulus</i> (Steph.) Hässel	<i>Clasmatocolea cucullistipula</i> (Steph.) Grolle	Chile, Drehwald, 970184 (GOET)	AM491287	–
<i>Chiloscyphus cuspidatus</i> (Nees) J.J. Engel & R.M. Schust.	<i>Lophocolea cuspidata</i> (Nees) Limpr.	Germany, Hentschel, Bryo 01411 (JE)	AM491604	–
<i>Chiloscyphus fragmentissimus</i> (R.M. Schust.) J.J. Engel & R.M. Schust.	<i>Lophocolea fragmentissima</i> R.M. Schust.	Venezuela, Frahm, 97/5/N (GOET)	AM282809	–
<i>Chiloscyphus fragrans</i> (Moris & De Not.) J.J. Engel & R.M. Schust.	<i>Lophocolea fragrans</i> (Moris & De Not.) Gottsche, Lindenb. & Nees	Azores, Schwab, 113 (JE)	AM282810	–
<i>Chiloscyphus fulvellus</i> (Hooker f. & Taylor) Nees	<i>Clasmatocolea fulvella</i> (Hook. f. & Taylor) Grolle	Chile, Hyvönen, 5313 (GOET)	AM491288	–
<i>Chiloscyphus gayanus</i> (Mont.) Gottsche & al.	<i>Clasmatocolea gayana</i> (Mont.) Grolle	Chile, Holz & Franzaring, CH 00-151a (GOET)	AM491289	–
<i>Chiloscyphus gottscheoides</i> (Besch. & C. Massal.) J.J. Engel & R.M. Schust.	<i>Pachyglossa gottscheoides</i> (Besch. & C. Massal.) L. Söderstr.	Chile, Drehwald & Mues, 3239 (GOET)	AM282811	–
<i>Chiloscyphus guadalupensis</i> (Steph.) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea guadalupensis</i> (Steph.) L. Söderstr. & Váňa	Costa Rica, Gradstein & Mues, 9630 (GOET)	AM282813	–
<i>Chiloscyphus helmsianus</i> (Steph.) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea helmsiana</i> (Steph.) L. Söderstr.	New Zealand, Engel & von Konrat, 28439	–	FJ173297
<i>Chiloscyphus humilis</i> (Hook. f. & Taylor) Hässel	<i>Clasmatocolea humilis</i> (Hook. f. & Taylor) Grolle	Chile, Holz & Franzaring, CH 00-44B (GOET)	AM491290	–
<i>Chiloscyphus itoanus</i> (Inoue) J.J. Engel & R.M. Schust.	<i>Lophocolea itoana</i> Inoue	China, 1999 Piippo, 60709	–	AY149868
<i>Chiloscyphus japonicus</i> (Steph.) J.J. Engel & R.M. Schust.	<i>Cryptolophocolea compacta</i> (Mitt.) L. Söderstr.	China, 1998 Koponen et al. 50238	–	AY149869
<i>Chiloscyphus lentus</i> (Hook. f. & Taylor) J.J. Engel & R.M. Schust.	<i>Lophocolea lenta</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	New Zealand, Engel & von Konrat, 24002	–	FJ173298
<i>Chiloscyphus leucophyllus</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	<i>Cryptolophocolea leucophylla</i> (Hook. f. & Taylor) L. Söderstr.	New Zealand, Engel & von Konrat, 24319	–	FJ173299

Initial species name	Accepted name	Label	GenBank accession number	
			ITS1–2	trnL-F
<i>Chiloscyphus liebmannianus</i> (Gottsche) J.J.Engel & R.M.Schust.	<i>Lophocolea liebmanniana</i> Gottsche	Mexico, Burghardt Bryo, 01655 (GOET)	AM282816	–
<i>Chiloscyphus martianus</i> (Nees) J.J.Engel & R.M.Schust.	<i>Cryptolophocolea martiana</i> (Nees) L. Söderstr., Crand.-Stotl. & Stotler	Ecuador, Gradstein, 10119 (GOET)	AM282817	–
<i>Chiloscyphus massalongoanus</i> Steph.	<i>Cryptolophocolea massalongoana</i> (Schiffn.) L. Söderstr.	Indonesia, Schaefer-Verwimp & Verwimp, S-V 25279	AM491292	–
<i>Chiloscyphus minor</i> (Nees) J.J.Engel & R.M.Schust.	<i>Lophocolea minor</i> Nees	Germany, Hentschel Bryo, 01006 (JE)	AM282818	–
<i>Chiloscyphus minor</i> (Nees) J.J.Engel & R.M.Schust.	<i>Lophocolea minor</i> Nees	China, Hunan Province, Zhangjiajie, 1999 Rao, 58428	–	AY149864
<i>Chiloscyphus muricatus</i> (Lehm.) J.J.Engel & R.M.Schust.	<i>Lophocolea muricata</i> (Lehm.) Nees	Australia, Streimann, 51629 (JE)	AM282819	–
<i>Chiloscyphus novae-zeelandiae</i> (Lehm. & Lindenb.) J.J.Engel & R.M.Schust.	<i>Lophocolea novae-zeelandiae</i> (Lehm. & Lindenb.) Nees	Australia, Eggers, AUS 3/81 (JE)	AM282820	–
<i>Chiloscyphus novae-zeelandiae</i> var. <i>grandistipulus</i> (Schiffn.) J.J.Engel	<i>Lophocolea novae-zeelandiae</i> var. <i>grandistipula</i> (Schiffn.) Vána	New Zealand, Engel & von Konrat, 24120	–	FJ173300
<i>Chiloscyphus obvolutus</i> (Hook. f. & Taylor) Hässel	<i>Clasmatocolea obvoluta</i> (Hook. f. & Taylor) Grolle	Chile, Hyvoenen, 2827 (GOET)	AM491293	–
<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	Germany, Thuringia, Hentschel Bryo, 01418 (JE)	AM282821	–
<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	Bulgaria, Hentschel Bryo, 0772 (JE)	AM282825	–
<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	<i>Chiloscyphus pallescens</i> (Hoffm.) Dumort.	Poland, 1993 A. Stenel (W-4)	–	AY149871
<i>Chiloscyphus perissodontus</i> (Spruce) J.J.Engel & R.M.Schust.	<i>Cryptolophocolea perissodonta</i> (Spruce) L. Söderstr.	Guyana, Gradstein, 4890 (GOET)	AM282826	–
<i>Chiloscyphus perissodontus</i> (Spruce) J.J.Engel & R.M.Schust.	<i>Cryptolophocolea perissodonta</i> (Spruce) L. Söderstr.	Guyana, Gradstein, 5042 (GOET)	AM282827	–
<i>Chiloscyphus platensis</i> J.J.Engel & R.M. Schust.	<i>Lophocolea platensis</i> C. Massal.	Bolivia, Churchill et. al., 20950 (JE)	AM491295	–
<i>Chiloscyphus platensis</i> J.J.Engel & R.M. Schust.	<i>Lophocolea platensis</i> C. Massal.	Bolivia, Churchill et. al., 22090 (GOET)	AM491294	–
<i>Chiloscyphus polyanthos</i> (L.) Corda	<i>Chiloscyphus polyanthos</i> (L.) Corda	Slovakia, Hentschel Bryo, 0318 (JE)	AM282831	–
<i>Chiloscyphus polyanthos</i> (L.) Corda	<i>Chiloscyphus polyanthos</i> (L.) Corda	Finland, 2000 He-Nygren & Piippo, 1469	–	AY149873
<i>Chiloscyphus polychaetus</i> (Spruce) J.J. Engel & R.M. Schust.	<i>Heteroscyphus polychaetus</i> (Spruce) Hentschel & Heinrichs	Ecuador, Gradstein & Mandl, 10139 (GOET)	AM491296	–
<i>Chiloscyphus profundus</i> (Nees) J.J.Engel & R.M.Schust.	<i>Lophocolea profunda</i> Nees	Germany, Hentschel Bryo, 01414 (JE)	AM282832	–
<i>Chiloscyphus profundus</i> (Nees) J.J.Engel & R.M.Schust.	<i>Lophocolea profunda</i> Nees	Finland, 2000 & Piippo, 1470	–	AY149874

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			ITS1–2	trnL-F
<i>Chiloscyphus randii</i> (S.W.Arnell) J.J.Engel & R.M.Schust.	<i>Lophocolea randii</i> S.W. Arnell	Prince Edward Isles, Gremmen, 98-63 (JE)	AM282833	–
<i>Chiloscyphus sabuletorum</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	<i>Lophocolea sabuletorum</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	Argentina, Hyvönen, 3233 (JE)	AM282834	–
<i>Chiloscyphus sabuletorum</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	<i>Lophocolea sabuletorum</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	Chile, Busch et al. Bryo, 01396 (JE)	AM282835	–
<i>Chiloscyphus semiteres</i> (Lehm.) Lehm. & Lindenb.	<i>Lophocolea semiteres</i> (Lehm.) Mitt.	Australia, Streimann, 58464 (GOET)	AM282836	–
<i>Chiloscyphus semiteres</i> (Lehm.) Lehm. & Lindenb.	<i>Lophocolea semiteres</i> (Lehm.) Mitt.	The Netherlands, Stieperaere, 8611 (JE)	AM282837	–
<i>Chiloscyphus semiteres</i> (Lehm.) Lehm. & Lindenb.	<i>Lophocolea semiteres</i> (Lehm.) Mitt.	New Zealand, Engel & von Konrat, 27982	–	FJ173301
<i>Chiloscyphus spinifer</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	<i>Cryptolophocolea spinifera</i> (Hook. f. & Taylor) L. Söderstr.	New Zealand, Schäfer-Verwimp & Verwimp, 13808 (JE)	AM282838	–
<i>Chiloscyphus spinifer</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	<i>Cryptolophocolea spinifera</i> (Hook. f. & Taylor) L. Söderstr.	New Zealand, Engel & von Konrat, 28452	–	FJ173302
<i>Chiloscyphus trachypopus</i> (Hook. f. & Taylor) Hässel	<i>Clasmatocolea trachypopa</i> (Hook. f. & Taylor) Grolle	Chile, Hyvoenen, 5933 (GOET)	AM491298	–
<i>Chiloscyphus vermicularis</i> (Lehm.) Hässel	<i>Clasmatocolea vermicularis</i> (Lehm.) Grolle	Ecuador, Sauer & Gradstein, MS-E065 (GOET)	AM491299	–
<i>Clasmatocolea ctenophylla</i> (Schiffn.) Grolle	<i>Clasmatocolea ctenophylla</i> (Schiffn.) Grolle	Chile, Engel, 25779	–	FJ173304
<i>Clasmatocolea humilis</i> (Hook.f. & Taylor) Grolle	<i>Clasmatocolea humilis</i> (Hook.f. & Taylor) Grolle	Chile, Engel, 25274	–	FJ173305
<i>Clasmatocolea obvoluta</i> (Hook.f. & Taylor) Grolle	<i>Clasmatocolea obvoluta</i> (Hook.f. & Taylor) Grolle	Chile, Engel, 25696	–	FJ173306
<i>Cyanolophocolea echinella</i> (Lindenb. & Gottsche) R.M. Schust.	<i>Heteroscyphus echinellus</i> (Lindenb. & Gottsche) J.J. Engel & X.L. He	New Zealand, Lewington, 1140 (H)	–	FJ919297
<i>Cyanolophocolea echinella</i> (Lindenb. & Gottsche) R.M. Schust.	<i>Heteroscyphus echinellus</i> (Lindenb. & Gottsche) J.J. Engel & X.L. He	New Zealand, Engel, 27818 (F)	–	FJ919304
<i>Herbertus dicranus</i> (Taylor ex Gottsche, Lindenb. & Nees) Trevis.	<i>Herbertus dicranus</i> (Taylor ex Gottsche, Lindenb. & Nees) Trevis.	H3230549 (H)	KU523784	KU523718
<i>Heteroscyphus argutus</i> (Reinw., Blume & Nees) Schiffn.	<i>Heteroscyphus argutus</i> (Reinw., Blume & Nees) Schiffn.	Nepal, D.G. Long, 30333 (JE)	–	AY149861
<i>Heteroscyphus aselliformis</i> (Reinw., Blume & Nees) Schiffn.	<i>Heteroscyphus aselliformis</i> (Reinw., Blume & Nees) Schiffn.	Indonesia, Gradstein, 10240 (GOET)	AM180588	–
<i>Heteroscyphus biciliatus</i> (Hook. f. & Taylor) J.J. Engel	<i>Heteroscyphus biciliatus</i> (Hook. f. & Taylor) J.J. Engel	New Zealand, Frahm, 20-6 (GOET)	AM491300	–
<i>Heteroscyphus coalitus</i> J.J. Engel	<i>Heteroscyphus coalitus</i> J.J. Engel	Nepal, D.G. Long, 17402 (JE)	AM282839	–
<i>Heteroscyphus coalitus</i> J.J. Engel	<i>Heteroscyphus coalitus</i> J.J. Engel	Nepal, D.G. Long, 30316 (JE)	–	AY149865
<i>Heteroscyphus cuneistipulus</i> (Steph.) Schiffn.	<i>Heteroscyphus cuneistipulus</i> (Steph.) Schiffn.	New Zealand, Frahm, 9-15 (GOET)	AM282840	–
<i>Heteroscyphus fissistipus</i> (Hook.f. & Taylor) Schiffn.	<i>Heteroscyphus fissistipus</i> (Hook.f. & Taylor) Schiffn.	Ireland, D.G. Long, H4064 (JE)	AM282841	–

Initial species name	Accepted name	Label	GenBank accession number	
			ITS1–2	trnL-F
<i>Heteroscyphus inflatus</i> (Steph.) S.C. Srivast. & A. Srivast.	<i>Heteroscyphus inflatus</i> (Steph.) S.C. Srivast. & A. Srivast.	Nepal, D.G. Long, 30457 (JE)	–	AY149875
<i>Heteroscyphus planus</i> (Mitt.) Schiffn.	<i>Heteroscyphus planus</i> (Mitt.) Schiffn.	Japan, 1992 Mizutani, 15828	–	AY149872
<i>Heteroscyphus splendens</i> (Lehm. & Lindenb.) Grolle	<i>Heteroscyphus splendens</i> (Lehm. & Lindenb.) Grolle	Papua New Guinea, 1989 Hoffmann, 89-749	–	AY149876
<i>Heteroscyphus zollingeri</i> (Gottsche) Schiffn.	<i>Heteroscyphus zollingeri</i> (Gottsche) Schiffn.	China, 1998 Koponen et al. 57927	–	AY149879
<i>Hygrolembidium acrocladum</i> (Berggr.) R.M. Schust.	<i>Hygrolembidium acrocladum</i> (Berggr.) R.M. Schust.	Australia, Curnow, 5587	–	AY463560
<i>Leiomitra lanata</i> (Hook.) R.M. Schust.	<i>Leiomitra lanata</i> (Hook.) R.M. Schust.	New Zealand, Glenney s.n., 2001	–	AY463565
<i>Lepicolea attenuata</i> (Mitt.) Steph.	<i>Lepicolea attenuata</i> (Mitt.) Steph.	New Zealand, South Island, Stotler & Crandall-Stotler, 4586 (ABSH)	–	JF316578, AY507540
<i>Lepicolea ochroleuca</i> (Spreng.) Spruce	<i>Lepicolea ochroleuca</i> (Spreng.) Spruce	Chile, Hyvonen, 2938	–	AY463566
<i>Lepicolea scolopendra</i> (Hook.) Dumort. ex Trevis.	<i>Lepicolea scolopendra</i> (Hook.) Dumort. ex Trevis.	Australia, Streimann, 55445	–	AY463568
<i>Leptophyllopsis laxa</i> (Mitt.) Hamlin	<i>Leptophyllopsis laxa</i> (Mitt.) Hamlin	Australia, Streimann, 43810 (JE)	AM491301	–
<i>Leptoscyphus amphibolius</i> (Nees) Grolle	<i>Leptoscyphus amphibolius</i> (Nees) Grolle	Brazil, Schafer-Verwimp, Schafer 14748	–	EU350474
<i>Leptoscyphus gibbosus</i> (Taylor) Mitt.	<i>Leptoscyphus gibbosus</i> (Taylor) Mitt.	Dominican Republic, Schafer-Verwimp (herb. Schafer), 17647	–	DQ176702
<i>Leptoscyphus gibbosus</i> (Taylor) Mitt.	<i>Leptoscyphus gibbosus</i> (Taylor) Mitt.	Costa Rica, Herbarium Schafer-Verwimp, SV/H-0364	–	EU350480
<i>Leptoscyphus porphyrius</i> (Nees) Grolle	<i>Leptoscyphus porphyrius</i> (Nees) Grolle	Ecuador, Schafer-Verwimp (herb. Schafer), 23229/a	–	DQ176707
<i>Leptoscyphus porphyrius</i> (Nees) Grolle	<i>Leptoscyphus porphyrius</i> (Nees) Grolle	Ecuador, Herbarium Schafer-Verwimp, Schafer 24214/a	–	EU350481
<i>Lophocolea bidentata</i> (L.) Dumort.	<i>Lophocolea bidentata</i> (L.) Dumort.	Poland: Silesian upland, K. Jedrzejko & A. Stenel (W-58)	–	AY149862
<i>Lophocolea cuspidata</i> (Nees) Limpr.	<i>Lophocolea bidentata</i> (L.) Dumort.	China, Hunan Province, Sang-Zhi Co., Koponen et al. 48430	–	AY149866
<i>Lophocolea heterophylla</i> (Schr.) Dumort.	<i>Lophocolea heterophylla</i> (Schr.) Dumort.	USA, Indiana, ML Sargent's culture collection, #481	–	AF231899
<i>Lophocolea martiana</i> Nees	<i>Cryptolophocolea martiana</i> (Nees) L. Söderstr., Crand.-Stodl. & Stotler	French Guiana, Kourou, Gradstein, 6265	–	AY149870
* <i>Lophocolea sikkimensis</i> (Steph.) Herzog & Grolle	* <i>Cryptolophocolea sikkimensis</i> (Steph.) Bakalin et Maltseva	Vietnam, Lao Cai Province, V.A. Bakalin & K.G. Klimova, V-12-17-17 (VBGI)	OK523503	OK562106
** <i>Lophocolea sikkimensis</i> (Steph.) Herzog & Grolle	** <i>Cryptolophocolea sikkimensis</i> (Steph.) Bakalin et Maltseva	Vietnam, Lao Cai Province, V.A. Bakalin, V-3-86-16 (VBGI)	OK523504	–
<i>Mastigophora woodsii</i> (Hook.) Nees	<i>Mastigophora woodsii</i> (Hook.) Nees	China, D.Long, 33696 (E)	–	JF316581
<i>Mastigophora woodsii</i> (Hook.) Nees	<i>Mastigophora woodsii</i> (Hook.) Nees	Australia, Frahm, CANB639918, 2000	–	AY463574

Initial species name	Accepted name	Label	GenBank accession number	
			ITS1–2	<i>trnL</i> -F
<i>Pachyglossa tenacifolia</i> (Hook. f. & Taylor) Herzog & Grolle	<i>Pachyglossa tenacifolia</i> (Hook. f. & Taylor) Herzog & Grolle	New Zealand, Bartlett 196 (JE)	AM491297	–
<i>Pedinophyllum interruptum</i> (Nees) Kaal.	<i>Pedinophyllum interruptum</i> (Nees) Kaal.	Germany, Schaefer-Verwimp, 35485 (M)	KT992498	–
<i>Pedinophyllum interruptum</i> (Nees) Kaal.	<i>Pedinophyllum interruptum</i> (Nees) Kaal.	Russia, N.A. Konstantinova & A.N. Savchenko, k508/7-07 (F)	–	KJ802073
<i>Plagiochila alternans</i> Lindenb. & Gottsche	<i>Plagiochila alternans</i> Lindenb. & Gottsche	Bolivia, Heinrichs et al. GP 16 (GOET)	AY550130	–
<i>Plagiochila asplenoides</i> (L.) Dumort.	<i>Plagiochila asplenoides</i> (L.) Dumort.	Finland, Nuuksio National Park, He-Nygren and Piippo 1467	–	AY149858
<i>Plagiochila fruticella</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	<i>Plagiochila fruticella</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	New Zealand, Engel & von Konrat, 23943 (GOET)	AM180613	–
<i>Plagiochila pleurata</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	<i>Plagiochila pleurata</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	New Zealand, Schaefer-Verwimp & Verwimp, 13777 (GOET)	AM180615	–
<i>Plagiochila porelloides</i> (Torr. ex Nees) Lindenb.	<i>Plagiochila porelloides</i> (Torr. ex Nees) Lindenb.	Germany, Schaefer-Verwimp, 31077 (M)	KX896587	–
<i>Plagiochila porelloides</i> (Torr. ex Nees) Lindenb.	<i>Plagiochila porelloides</i> (Torr. ex Nees) Lindenb.	USA, Alaska, B. Shaw, F955/1 (DUKE)	–	KF943056
<i>Plagiochila sichotensis</i> Bakalin & Vilnet	<i>Plagiochila sichotensis</i> Bakalin & Vilnet	Russia, Russian Far East, Primorsky Territory, V.A. Bakalin & G.A. Arutinov, Arutinov 1-25-13 (VBGI)	MF947695	MF947697
<i>Plagiochila xerophila</i> Bakalin & Vilnet	<i>Plagiochila xerophila</i> Bakalin & Vilnet	China, Sichuan Province, V.A. Bakalin & K.G. Klimova, China-46-2-17 (VBGI)	–	MK123266
<i>Tetracymbaliella cymbalifera</i> (Hook. f. & Taylor) Grolle	<i>Tetracymbaliella cymbalifera</i> (Hook. f. & Taylor) Grolle	New Zealand, M.A.M. Renner, 6139 (NSW)	KT992470	–
<i>Tetracymbaliella cymbalifera</i> (Hook. f. & Taylor) Grolle	<i>Tetracymbaliella cymbalifera</i> (Hook. f. & Taylor) Grolle	New Zealand, Frahm 1-23 (MO-5131915)	–	DQ026625
<i>Triandrophyllum subtrifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	<i>Triandrophyllum subtrifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	Bolivia, Churchill et al. 22800	AJ972455	–
<i>Triandrophyllum subtrifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	<i>Triandrophyllum subtrifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	Venezuela, Ricardi, 9730/T	–	JF316580
<i>Trichocolea tomentella</i> (Ehrh.) Dumort.	<i>Trichocolea tomentella</i> (Ehrh.) Dumort.	China, He-Nygren, 1137	–	AY463590
<i>Trichotemnoma corrugatum</i> (Steph.) R.M. Schust.	<i>Trichotemnoma corrugatum</i> (Steph.) R.M. Schust.	New Zealand, Glennly 8426	–	AY463591
<i>Zoopsis argentea</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	<i>Zoopsis argentea</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	Australia, Streimann, 51704	–	AY463595
<i>Zoopsis argentea</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	<i>Zoopsis argentea</i> (Hook. f. & Taylor) Gottsche, Lindenb. & Nees	New Zealand, J.J.Engel, 23962	–	JF316577

DNA isolation, amplification, and sequencing. DNA was extracted from dried liverwort tissues using the NucleoSpin Plant II Kit (Macherey-Nagel, Germany). Amplification of ITS1–2, *trnL*-F, and the *trnG*-intron was performed using an Encyclo Plus PCR kit (Evrogen, Moscow, Russia) with the primers listed in Table 2.

Table 2. Primers used in polymerase chain reaction (PCR) and cycle sequencing.

Locus	Sequence (5'-3')	Direction	Annealing temperature (°C)	Reference
ITS 1–2 nrDNA	CGTTGTGAGAAGTTCATTAAACC	forward	64	Feldberg et al. 2016
ITS 1–2 nrDNA	GATATGCTTAAACTCAGCGG	reverse	58	Milyutina et al. 2010
<i>trnL</i> -F cpDNA	CGAAATTGGTAGACGCTGCCG	forward	62	Bakalin et al. 2021
<i>trnL</i> -F cpDNA	ATTTGAACTGGTGACACGAG	reverse	58	Taberlet et al. 1991
<i>trnG</i> -intron cpDNA	ACCCGCATCGTTAGCTTG	forward	56	Pacak and Szweykowska-Kulinska 2000
<i>trnG</i> -intron cpDNA	GCGGGTATAGTTTAGTGG	reverse	54	Pacak and Szweykowska-Kulinska 2000

dNTP-mixture (included in Encyclo Plus PCR Kit), 13.4 µl (for *trnL*-F and the *trnG*-intron)/12.4 µl (for ITS1–2) of double-distilled water (Evrogen, Moscow, Russia), 1 µl of dimethylsulfoxide/DMSO (for ITS1–2) and 0.4 µl of each primer (forward and reverse, at a concentration of 5 pmol/µl). Polymerase chain reactions were carried out using the following program: 180 s initial denaturation at 95 °C, followed by 30–40 cycles of 30 s denaturation at 94 °C, 20 (for *trnL*-F) – 30 s (for ITS1–2, *trnG*-intron) annealing at 56 °C (*trnG*-intron) or 58 °C (*trnL*-F and ITS1–2), and 30 s elongation at 72 °C. Final elongation was carried out in one 5-min step at 72 °C. Amplified fragments were visualized on 1% agarose TAE gels by EthBr staining and purified using the Cleanup Mini Kit (Evrogen, Moscow, Russia). The DNA was sequenced using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with further analysis of the reaction products following the standard protocol on an ABI Prism 3100-Avant Genetic Analyser (Applied Biosystems, USA) in the Genome Center (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow).

Phylogenetic analyses

The datasets were produced for the ITS1–2 and *trnL*-F loci. Both datasets were aligned using MAFFT (Katoh and Standley 2013) with standard settings and then edited manually in BioEdit ver. 7.2.5 (Hall 1999). All positions of the final alignments were included in the phylogenetic analyses.

Phylogenies were reconstructed under three criteria: maximum parsimony (MP) with Mega X (Kumar et al. 2018), maximum likelihood (ML) with IQ-tree ver. 1.6.12 (Nguyen et al. 2015) and Bayesian inference (BA) with MrBayes ver. 3.2.7 (Ronquist et al. 2012).

MP analysis for both datasets included 1,000 bootstrap replicates, default settings for all other parameters, and treated gaps as partial deletions with a site coverage cut-off of 95%.

For the ML analysis, the best fitting evolutionary model of nucleotide substitutions according to the BIC value was TIM3+F+I+G4 for the ITS dataset and TVM+F+I+G4

for the *trnL*-F dataset as determined by IQ-tree. Consensus trees were constructed with 1000 bootstrap replicates.

Indels for both datasets were coded with FastGap ver. 1.2 (Borchsenius 2009) and then added to the nucleotide matrices in the Bayesian analyses. Bayesian analyses were performed by running two parallel analyses using the GTR+I+G model. For both datasets, the analysis consisted of four Markov chains. Chains were run for five million generations, and trees were sampled every 500th generation. The first 2,500 trees in each run were discarded as burn-in; thereafter, 15,000 trees were sampled from both runs. Bayesian posterior probabilities were calculated from the trees sampled after burn-in. The average standard deviation of split frequencies between two runs was 0.0017 for ITS1–2 and 0.0068 for *trnL*-F.

The infrageneric and infraspecific variability of ITS1–2 and *trnL*-F were quantified as the average pairwise *p*-distances calculated in Mega X (Kumar et al. 2018) using the pairwise deletion option for counting gaps.

Results

Five new sequences from *Lophocolea sikkimensis* specimens were deposited in GenBank: two for ITS1–2, one for *trnL*-F and two for *trnG*-intron cpDNA. ITS1–2 alignment of the 55 specimens consisted of 955 character sites, and the *trnL*-F alignment of 53 specimens consisted of 612 character sites. The parameters of the tested alignments are shown in Table 3.

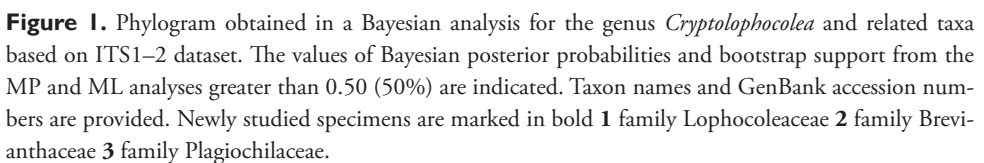
Table 3. The characteristics of ITS1–2, and *trnL*-F nucleotide sequence alignments.

Locus	Total sites	Conservative sites		Variable sites		Parsimony-informative sites	
		base pairs	%	base pairs	%	base pairs	%
ITS1–2	955	376	39.37	376	39.37	431	45.13
<i>trnL</i> -F	612	325	53.11	325	53.11	208	33.99

The MP analysis for ITS1–2 yielded a single parsimonious tree with CI = 0.364388 and RI = 0.619946. The ML criterion recovered a bootstrap consensus tree with a log-likelihood = -10978.46. The arithmetic means of the log likelihoods in Bayesian analysis for each sampling run were -11026.6 and -11028.06.

The MP analysis for *trnL*-F yielded five equally parsimonious trees with CI = 0.480315 and RI = 0.697248. The ML criterion recovered a bootstrap consensus tree with a log-likelihood = -4951.09. The arithmetic means of log likelihoods in the Bayesian analysis for each sampling run were -4993.95 and -4989.08.

The trees constructed for each dataset by the three different methods appeared highly congruent. Fig. 1 shows the phylogenetic tree based on the ITS1–2 dataset retained under Bayesian analysis, along with bootstrap support (BS) values from the



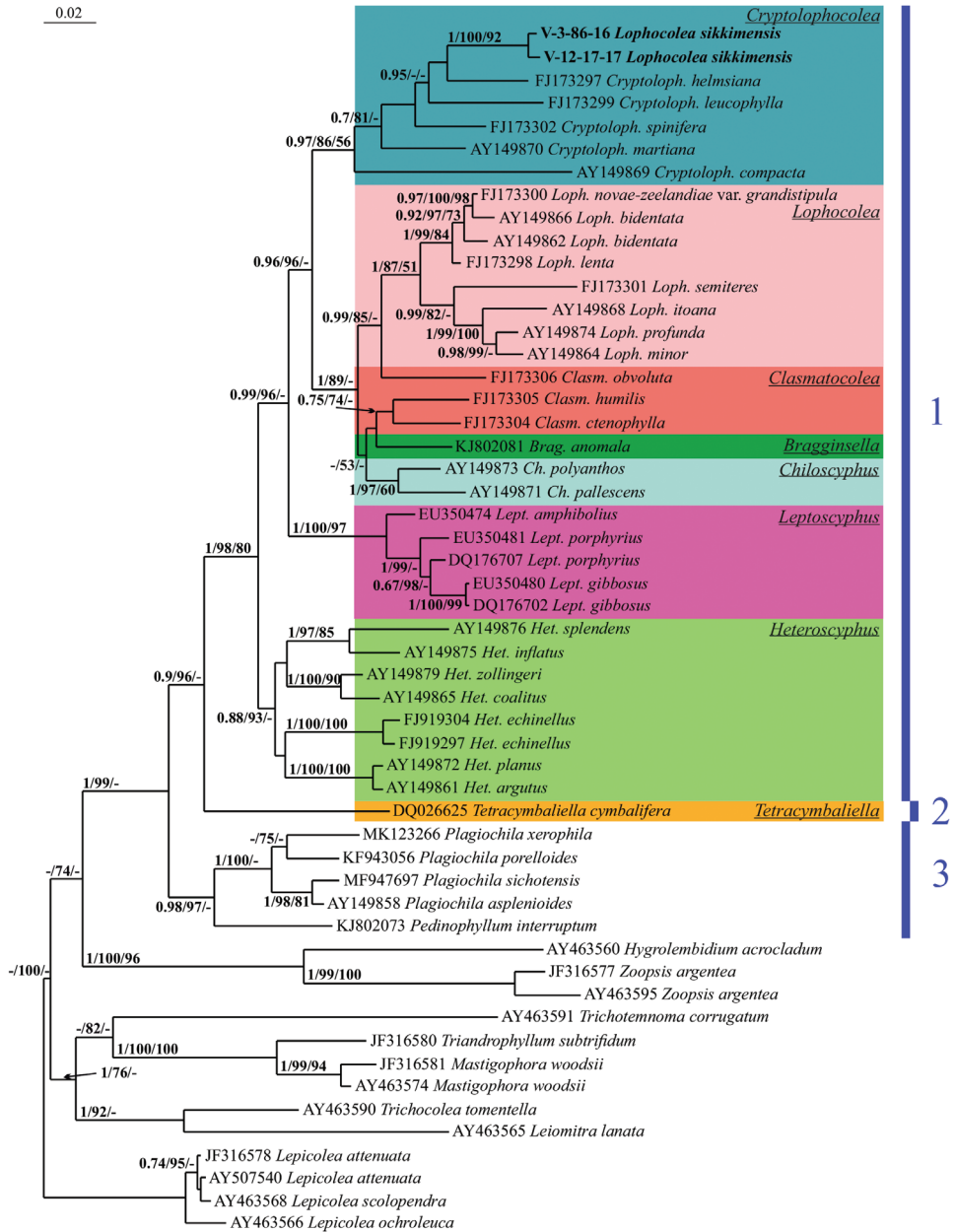


Figure 2. Phylogram obtained in a Bayesian analysis for the genus *Cryptolophocolea* and related taxa based on *trnL*-F dataset. The values of Bayesian posterior probabilities and bootstrap support from the MP and ML analyses greater than 0.50 (50%) are indicated. Specimen names and GenBank accession numbers are provided. Newly studied specimens are marked in bold **1** family Lophocoleaceae **2** family Brevianthaceae **3** family Plagiochilaceae.

Table 4. Inter- and Infrageneric *p*-distances, ITS1–2 and *trnL*-F. The number of base differences per site from averaging over all sequence pairs within and between each group are shown. The upper triangle – data for ITS 1–2, the lower triangle – data for *trnL*-F; — – data are absent.

<i>trnL</i> -F, %	Taxon	<i>Cryptolophocolea</i> <i>sikkimensis</i> +	<i>Lophocolea</i> <i>sikkimensis</i>	<i>Lophocolea</i> <i>Crypto-</i> <i>lophocolea</i>	<i>Lophocolea</i>	<i>Clasmatocolea</i>	<i>Chiloscyphus</i>	<i>Leptoscyphus</i>	<i>Hetero-scyphus</i>	<i>Pachyglossa</i>	ITS 1–2, %
0.061	<i>Lophocolea</i> <i>sikkimensis</i> + <i>Crypto-lophocolea</i>		–	–	0.164	0.150	0.153	–	0.167	0.156	–
0	<i>Lophocolea sikkimensis</i>	–		0.128	0.142	0.129	0.130	–	0.154	0.138	0.011
0.070	<i>Cryptolophocolea</i>	–	0.058		0.168	0.155	0.158	–	0.170	0.161	0.135
0.040	<i>Lophocolea</i>	0.092	0.085	0.094		0.088	0.096	–	0.163	0.100	0.074
0.058	<i>Clasmatocolea</i>	0.082	0.069	0.088	0.068		0.074	–	0.146	0.075	0.063
0.037	<i>Chiloscyphus</i>	0.081	0.069	0.087	0.062	0.056		–	0.147	0.079	0.013
0.021	<i>Leptoscyphus</i>	0.086	0.081	0.087	0.094	0.086	0.090		–	–	–
0.055	<i>Heteroscyphus</i>	0.088	0.080	0.091	0.091	0.085	0.083	0.074		0.151	0.135
–	<i>Pachyglossa</i>	–	–	–	–	–	–	–	–		0.044

MP and ML analyses and the Bayesian posterior probabilities (PP) for each node. Fig. 2 shows the BA tree based on the *trnL*-F dataset as well as the BS from the MP and ML calculations and the PP for each node.

The topologies obtained here are quite similar to previously published phylogenies in the reinstatement of Lophocoleaceae (Hentschel et al. 2006a), the identification of the systematic position of *Pachyglossa* and *Clasmatocolea* by Hentschel et al. (2007), and the reconstruction of the phylogeny of Lophocoleaceae-Plagiochilaceae-Brevianthaceae by Patzak et al. (2016). The two studied specimens of *Lophocolea sikkimensis* formed a strongly supported subclade in both calculations (100/100/1.00, 1/100/92 Figs 1, 2), which was placed within robustly supported clade of the genus *Cryptolophocolea* (1/88/73 in ITS1–2, 0.97/86/56 in *trnL*-F). The position of *Clasmatocolea obvoluta* was unstable: based on the *trnL*-F reconstruction, it belongs in *Lophocolea*, but based on the ITS1–2 sequences, it should remain in *Clasmatocolea*, possibly, it could be associated with sequence origin from different specimens and their identification.

The intergroup average distance between *Lophocolea sikkimensis* and *Cryptolophocolea* (Table 4) is lower than most of its distances to other genera of Lophocoleaceae (although the average infrageneric distance in *Cryptolophocolea* is maximum compared to other Lophocoleaceae genera).

Therefore, according to the estimated phylogenetic relationships and level of genetic differences, *Lophocolea sikkimensis* should be transferred to the genus *Cryptolophocolea*.

Due to the obvious position of the studied specimens in the *Cryptolophocolea* clade, we provide the corresponding new combination for *Lophocolea sikkimensis*:

***Cryptolophocolea sikkimensis* (Steph.) Bakalin & Maltseva, comb. nov.**

Basionym. *Herpocladium sikkimense* Steph., Sp. Hepat. (Stephani) 6: 349, 1922 (= *Lophocolea sikkimensis* (Steph.) Herzog & Grolle, Rev. Bryol. Lichénol. 27 (3/4): 164, 1958 [1959])

Discussion**Morphology**

Söderström et al. (2013) list 20 genera in Lophocoleaceae, excluding *Conoscyphus*, which was later transferred to Acrobolbaceae by Dimon et al. (2018). The genera in this large and morphologically variable family have several common features (with several exceptions), including generally obliquely to very obliquely inserted leaves, rhizoids mostly from the small-celled area near the underleaf bases (thus the stem is free of rhizoids), trigonous perianth (exceptions are common) and generally bilobed leaves (the entire leaves are sparsely distributed across the family). *Cryptolophocolea sikkimensis* is distinguished by generally entire underleaves that are widely connate on both sides with the leaves. These leaves (along with entire leaves), generally occur in *Heteroscyphus*, the basal clade of *Lophocoleaceae* (Figs 1, 2). However, the entire leaves of *Cryptolophocolea sikkimensis* are quite different from the entire leaves in *Heteroscyphus*. In *Heteroscyphus*, *Chiloscyphus*, *Cryptolophocolea* and *Lophocolea*, which generally have entire leaves, the leaves are lingulate but not ovate, with an apiculate to obtuse apex. The leaf shape of *Cryptolophocolea sikkimensis* is therefore similar to the leaf shape of *Cuspidatula contracta* (Reinw., Blume & Nees) Steph. (Adelanthaceae) and is unlikely to occur in Lophocoleaceae.

However, the morphology of *Cryptolophocolea sikkimensis* is highly variable, and along with well-developed plants with ovate leaves and entire underleaves, modifications with shortly bilobed to bidentate leaves and underleaves may be observed. Indeed, Kitagawa (1974: 36) wrote, “Plants of Taiwan (Fig. 3) are so markedly different from the typical ones that I felt some hesitation in regarding them as conspecific with the Himalayan plants. Most leaves are distinctly bilobed, and underleaves are often emarginate”. These features may have an atavistic nature.

Notably, the Vietnamese populations, located between the extreme flanks of the species range, sometimes exhibit an intermediate morphology: plants with bilobed leaves and emarginate underleaves are often found. However, these plants are usually smaller than the well-developed individuals and characterized by distanced leaves and underleaves; they generally provide an impression of weakly developed or “suppressed” shoots. This intermediate morphology corresponds to the observations by Kitagawa (1974) that plants from Thailand are characterized by a smaller size. Our well-developed plants are distinctly larger, and the mats from drier habitats contain smaller plants. Additionally, the rounded apex of the leaf is frequently found in Himalayan plants. In contrast, Indochinese plants (both from Thailand and Vietnam) as

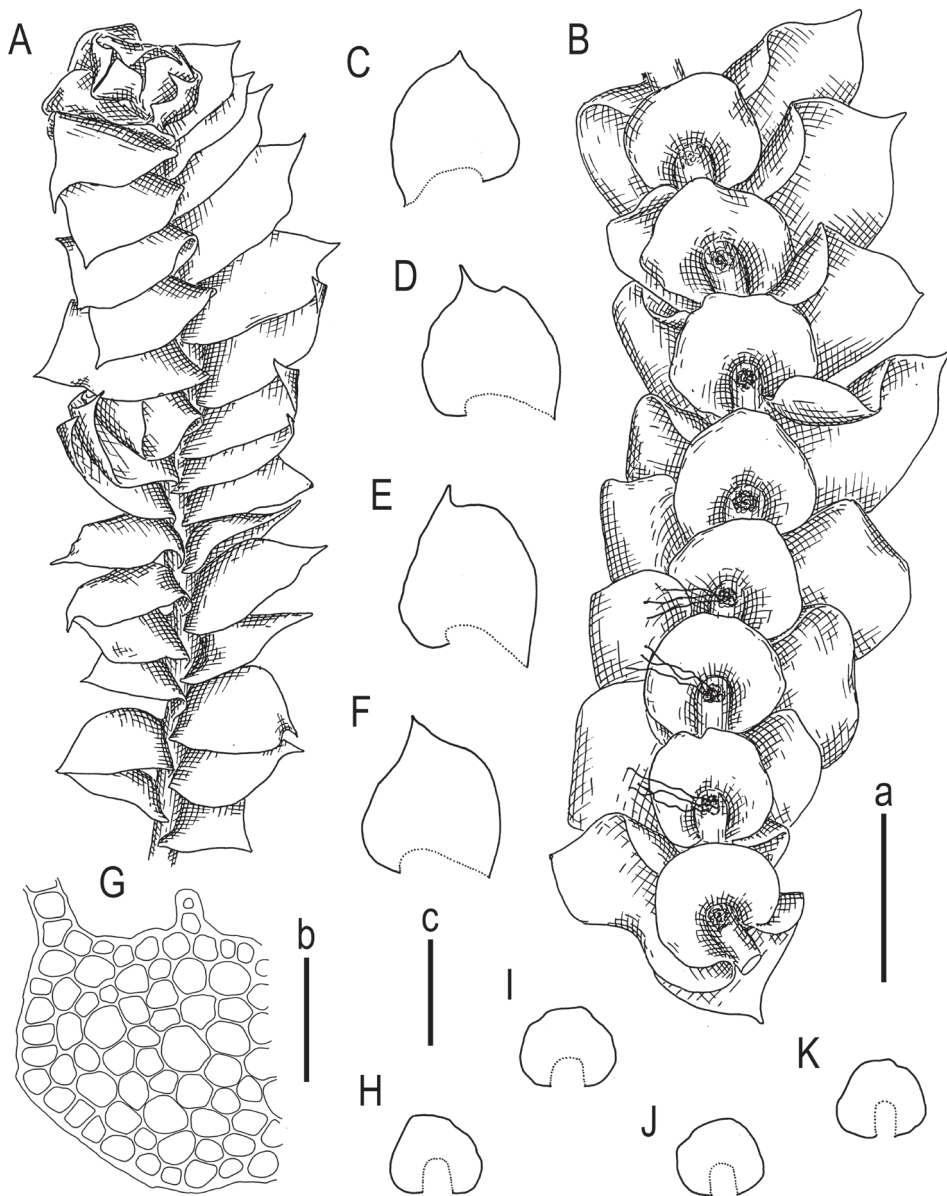


Figure 3. *Cryptolophocolea sikkimensis* (Steph.) Bakalin & Maltseva, comb. nov. **A** plant habit, fragment, dorsal view **B** plant habit, fragment, ventral view **C–F** leaves **H–K** underleaves **G** stem cross-section, fragment. Scale bars: 1 mm **a** (**A**, **B**); 100 μ m **b** (**G**); 1 mm **c** (**C–F**, **H–K**). All from V-8-54-17 (VBGI).

well as Bornean plants (according to Kitagawa (1974) and our own observations) never exhibit a rounded apex; instead, the leaf apex is generally acute (rarely bicuspidate in smaller plants). Thus, the observed morphological variability, clearly correlating with geographic longitude, is likely associated with genetic infraspecific variability. However, we can neither prove nor disprove this assumption.

Ecology

Kitagawa (1974: 35) noted that “Plants occur on various substrata (rocks, rotten logs, humus) but usually do not grow directly on such substrata but creeping larger bryophytes”. In general, the same can be said about the ecological preferences of Vietnamese plants. Meanwhile, it should be noted that in Thailand (in the apical part of Mt. Doi Inthanon in Chiangmai Province, where the species is observed), known specimens of the species (only two, both cited by Kitagawa 1974) are restricted to tree branches and trunks (Kitagawa 1974). Moreover, the species is confined to stony substrates in its only locality in Yunnan, adjacent to Indochina from the north, where it was found in the vicinity of Lijiang (Piippo et al. 1998). Thus, the known variation in habitat in Vietnam exceeds that known in China’s Yunnan Province and Thailand and corresponds to the general variation across species range (detailed information is included in the specimens examined section). The species associates in Vietnam include a lot of liverwort taxa, such as *Mnioloma fuscum* (Lehm.) R.M. Schust., *Scapania ciliatospinosa* Horik., *Lepidozia* cf. *subtransversa* Steph., *Herbertus dicranus* (Gottsche, Lindenb. & Nees) Trevis. and *Riccardia* sp.

Description (based on plants from Vietnam)

Plants yellowish green, greenish and whitish yellowish to yellowish brownish, sometimes grading to grayish brownish in the herbarium, gentle, very fragile and glistening when dry, forming loose pure patches over other bryophytes or rarely intermixed with *Riccardia* sp., *Herbertus dicranus*, *Plicanthus*, *Scapania ciliatospinosa*, *Mnioloma fuscum*; creeping to loosely ascending (very rarely suberect in dense patches); normally developed shoots 1.1–2.0 mm wide (narrower, depauperate plants are commonly occurring) and 8–20(–30) μm long. Rhizoids regular, in erect or upraise spreading fascicles, originating from a small-celled initial zone of the underleaf adjacent to the stem in the axial part of the underleaf, fascicles 0.1–0.5 mm long. Stem rarely intercalary (lateral, from the middle part of the sinus) branched; cross section slightly transversely ellipsoidal, ca. 170–200 $\times$ 220–250 μm , external wall distinctly thickened, cell in 1(–2) marginal rows thick-walled, with large (sometimes loosely confluent) concave trigones, 17–27 μm in diameter, inner cells thin-walled, trigones moderate to large, concave, 23–27 μm in diameter. Leaves contiguous to distant in depauperate shoots, obliquely spreading, very obliquely to obliquely inserted (insertion line 20–45° with stem axis), barely decurrent dorsally, very ventral end of the insertion line subtransverse, dorsally leaves alternate to subopposite with a somewhat adjacent one to another dorsal bases, ventrally widely connate with underleaves; in general outline slightly convex to concave (never flat), with leaf apex commonly turned to the apical part of the shoot, when flattened in the slide widely ovate to obliquely ovate and widely ovate-triangular, widest very near to the base, apex acute to apiculate, rarely shortly bilobed with unequal to subequal lobes (bilobed apex mostly present in small shoots), normally developed leaves 900–1200 $\times$ 950–1100 μm . Underleaves loosely canaliculate, if looking from

the ventral side, widely connate with leaves in both sides, transversely ellipsoidal, with apex entire, rarely emarginate to shortly bilobed (with sinus semicrescentic), insertion line arcuate (sinuate), $400\text{--}600 \times 700\text{--}850 \mu\text{m}$. Midleaf cells subisodiametric, $22\text{--}33 \mu\text{m}$ in diameter or shortly oblong, to $38 \mu\text{m}$ long, thin-walled, trigones large, mostly triangle to slightly concave or slightly convex, cuticle virtually smooth; cells along leaf margin subisodiametric (subquadrate), $21\text{--}25 \mu\text{m}$ in diameter to slightly elongate along the margin, to $25\text{--}27 \mu\text{m}$ long; oil bodies in the midleaf cells 2–5 per cell, finely granulate, irregularly oblong, ellipsoidal to shortly fusiform, $8\text{--}17 \times 5\text{--}7(-8) \mu\text{m}$, grayish (Figs 3–5).

Specimens examined (North Vietnam). VIETNAM • Lao Cai Province, Sa Pa District, San Sa Ho Commune, Hoang Lien Range, Hoang Lien National Park, one of the ways to the Phan Xi Pan Peak; $22^{\circ}18.8'N$, $103^{\circ}45.933'E$; 2727 m a.s.l.; 3 Apr. 2018; V.A. Bakalin & K.G. Klimova leg.; thickets of *Sinobambusa* with many rocky outcrops

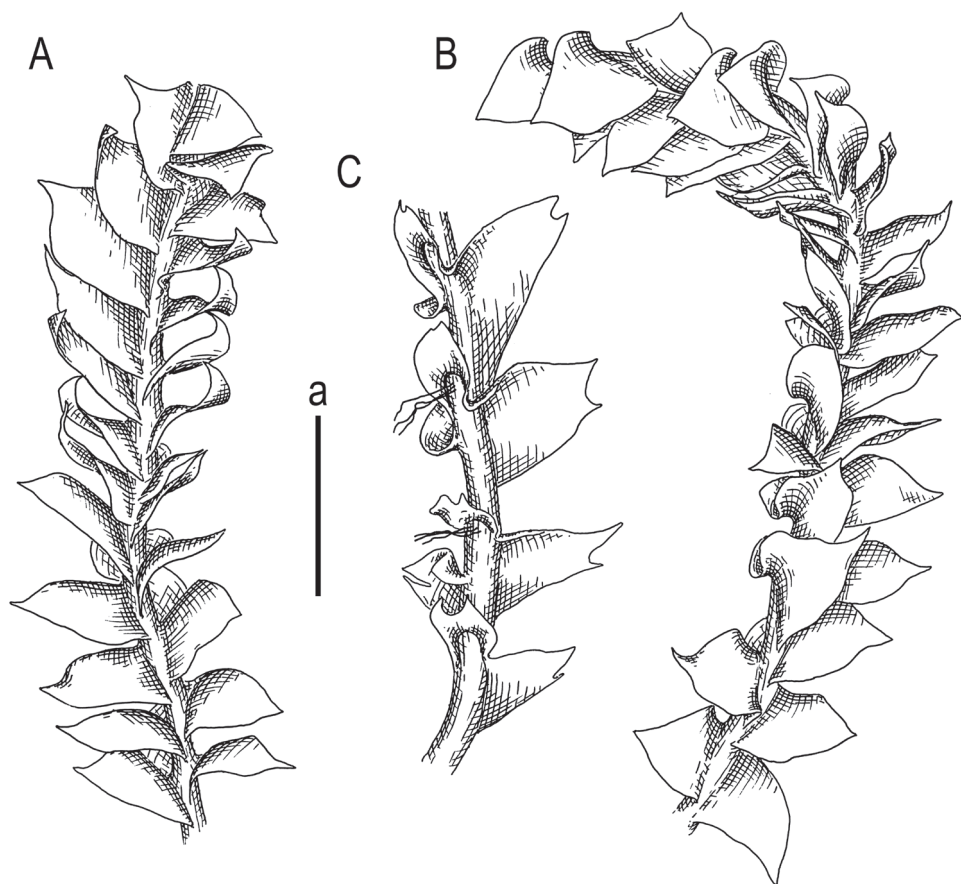


Figure 4. *Cryptolophocolea sikkimensis* (Steph.) Bakalin & Maltseva, comb. nov. **A, B** plant habit, fragment, dorsal view **C** plant habit, fragment, ventral view. Scale bars: 1 mm **a** (**A–C**). All from V-8-54-17 (VBGI).

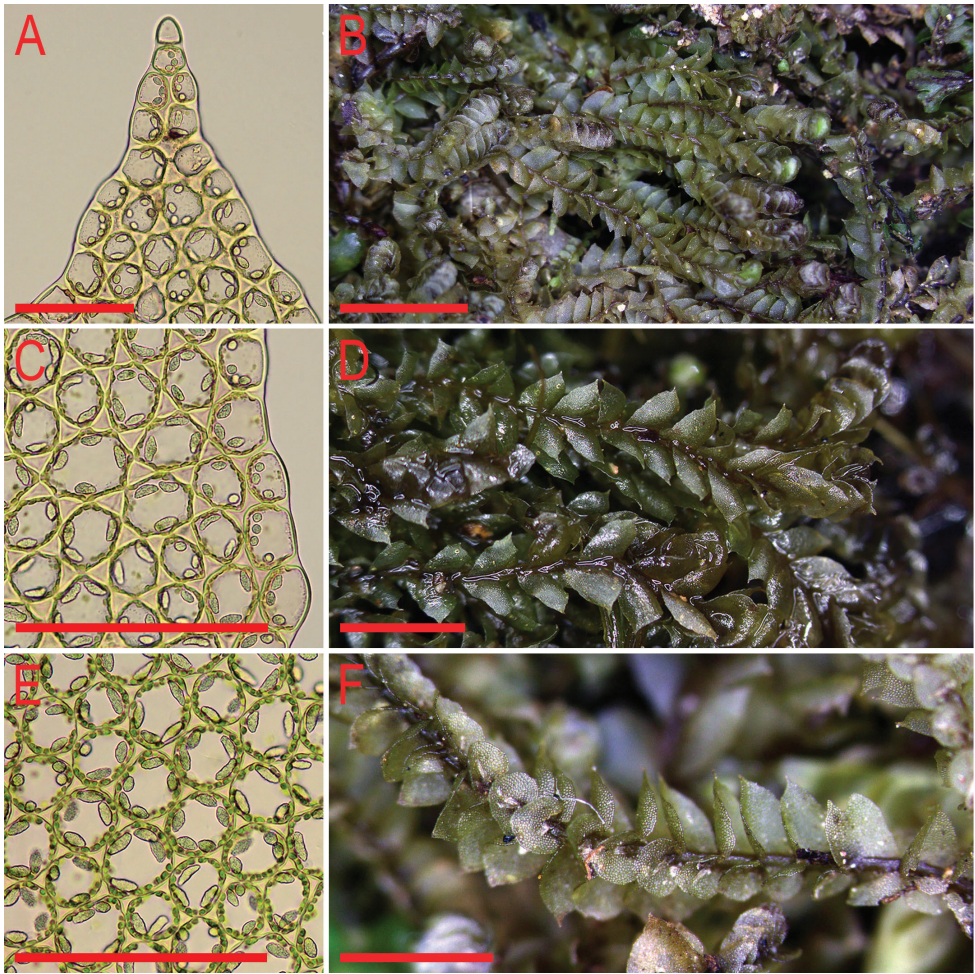


Figure 5. *Cryptolophocolea sikkimensis* (Steph.) Bakalin & Maltseva, comb. nov. **A** oil bodies in apical part of the leaf **B** mat **C** oil bodies in leaf margin cells **D** shoots, fragment, dorsal view **E** oil bodies in midleaf cells **F** shoot, fragment, ventral view. Scale bars: 100 μ m (**A**, **C**, **E**); 5 mm (**B**); 2 mm (**D**, **F**). All from V-8-54-17 (VBGI).

and *Rhododendron* trees, partly shaded moist cliff, over *Sphagnum* mat; VBGI V-16-6-18 • same collection data as for preceding; 22°19.2'N, 103°46.183'E; 2610 m a.s.l.; 22 Apr. 2017; V.A. Bakalin & K.G. Klimova leg.; evergreen south subtropical mountain forest with bamboo thickets and many rocky outcrops, open moist cliff; VBGI V-12-17-17 • same collection data as for preceding; 22°18.45'N, 103°46.567'E; 2900 m a.s.l.; 20 Apr. 2017; V.A. Bakalin & K.G. Klimova leg.; *Rhododendron* dominated forest with bamboo thickets and many rocky outcrops, moist cliff in part shade; VBGI V-9-22-17; • same collection data as for preceding; 22°18.25'N, 103°46.5'E; 3050 m a.s.l.; 20 Apr. 2017; V.A. Bakalin & K.G. Klimova leg.; *Rhododendron* dominated forest with bamboo thickets and many rocky outcrops, moist open cliffs; VBGI V-8-14-17, V-8-29-

17, V-8-32-17, V-8-52-17, V-8-53-17, V-8-54-17, V-8-62-17, V-8-73-17, V-8-13-17 • same collection data as for preceding; 22°18.183'N, 103°46.517'E; 3100 m a.s.l.; 17 Mar. 2016; V.A. Bakalin leg.; evergreen south subtropical mountain forest over the peak, partly shaded moist cliffs; VBGI V-3-86-16, V-3-91-16, V-3-61-16, V-3-81-16 • same collection data as for preceding; Lai Châu Province, Ta Leng Commune, Pu Ta Leng Mt. summit; 22°25.367'N, 103°36.233'E; 3050 m a.s.l.; 30 Mar. 2018; V.A. Bakalin & K.G. Klimova leg.; rhododendron trees with a dense bamboo understory, partly shaded moist decaying decorticated fallen tree trunk; VBGI V-11-45-18, V-11-16-18 • same collection data as for preceding; partly shaded mesic trunk of a living tree; VBGI V-11-36-18.

Distribution

Cryptolophocolea sikkimensis has a pronounced Sino-Himalayan distribution. Its range stretches from Nepal to Taiwan and Borneo. Specifically, the species is found in China (Yunnan and Taiwan Provinces), North Borneo, Bhutan, Nepal, India (Sikkim, Darjeeling), North Thailand, and Vietnam (Kitagawa 1974; Long and Grolle 1990; Piippo 1990; Piippo et al. 1998; Bakalin et al. 2018; the present paper). Thus, the species ranges from the “*Rhododendron* flora” and “*Metasequoia* flora” (Wu and Wu 1996; Chen et al. 2018) in East Asia, to the Indochinese floristic region in the Palaeotropics (although in the upper mountain belts in areas with widely distributed Sino-Himalayan taxa) and to the Malesian floristic region. The reports from the Malesian region (both specimens cited by Kitagawa are from Kinabalu Mt.) are restricted to the upper belts (2900–3000 m a.s.l.), but distant from the area with widely distributed Sino-Himalayan taxa. The distribution of *Cryptolophocolea sikkimensis* in Taiwan is far less surprising. This island has, in a floristic sense, very close relationships with the Sino-Himalayan flora, and is the eastern outpost for some exclusively (or at least predominantly) Himalayan species: *Acrobolbus ciliatus*, *Anastrepta orcadensis*, *Bazzania imbricata*, *Bazzania sikkimensis*, *Frullania gaudichaudii*, *Gymnomitrium rubidum*, *Odontoschisma grosseverrucosum*, and many others (Wang et al. 2011).

As described in the following section, the distribution of *Cryptolophocolea sikkimensis* is quite unusual within the genus. The vast majority of taxa principally exhibit a different distribution pattern. The phylogenetic tree shows that *Cryptolophocolea sikkimensis* forms a sister branch to all other taxa widespread in Southeast Asia (widely irrigated to Melanesia) and one pantropical species (*Cryptolophocolea connata*). This somewhat correlates with the distinctly different distribution and unique morphology of *C. sikkimensis*. The available data are insufficient for determining the morphological evolution pathways and distribution history within the genus. However, *C. sikkimensis* is assumed to belong to an isolated and morphologically specialized branch. The taxon probably had a wide range in the past that is now disjunctively distributed; in fact, the species is ‘locked’ in the mountainous regions from the Sino-Himalaya to Borneo, considering its ecological preferences.

Within Vietnam, the distribution of the species is limited to the peak surroundings of Phan Xi Pan Mt., a refugium containing a number of Sino-Himalayan species

(Bakalin et al. 2018). Although we have visited several highest points in North Vietnam over the last five years, we have not found this taxon, despite the ease of recognition of this species in the field and its large size. On the one hand, this indicates the rarity of the taxon in Vietnam; on the other hand, it confirms the disjunctive relict range of the species.

Geographical patterns in the genus

Cryptolophocolea includes 32 species (including the newly transferred *C. sikkimensis*), of which the species status is questionable for eight (one star in the World Liverwort Checklist, Söderström et al. 2016). The highest taxonomic diversity is found in Australasia and South America. Africa, the tropical zone of Asia, and other regions contain fewer species. In general, based on the distribution data available at <https://www.catalogueoflife.org/> (last accessed 12/15/2021), the distribution is as follows:

Australasia and New Zealand contain 10 species, including four restricted to New Zealand and adjacent islands (*Cryptolophocolea aculeata* (Mitt.) L. Söderstr., *C. helmsiana* (Steph.) L. Söderstr., *C. spinifera* (Hook.f. & Taylor) L. Söderstr., *C. tuberculata* (J.J. Engel) L. Söderstr.), one taxon restricted to Tasmania (*C. connatifolia* (J.J. Engel) L. Söderstr.), three restricted to Southeast Australia and New Zealand (*C. trialata* (Gottsche) L. Söderstr., *C. subopposita* (J.J. Engel) L. Söderstr., *C. pallida* (Mitt.) L. Söderstr.), and two restricted to Tasmania, New Zealand, Antipodean Islands and some other small adjacent islands (*C. leucophylla* (Hook.f. & Taylor) L. Söderstr., *C. mitteniana* (Colenso) L. Söderstr.).

Cryptolophocolea chiloscypioidea (Lindenb.) L. Söderstr. & Crand.-Stotl. is broadly distributed in Australasia, South America, and the subantarctic islands (and also recorded in India, but that record may be doubted for phytogeographic reasons). South America contains four taxa (in addition to the one mentioned above): *C. fleischeri* (Steph.) L. Söderstr. (also in Mexico), *C. proteus* (Herzog) L. Söderstr., *C. pycnophylla* (Spruce) L. Söderstr., *C. tricolorata* (Hässel) Crand.-Stotl. & Stotler.

Cryptolophocolea connata (Sw.) L. Söderstr. & Vána is broadly distributed from Africa to Malesia, Australasia, the Neotropics and Polynesia (Hawaii). Africa and South America contain two species that extend beyond this large region: *C. martiana* (Nees) L. Söderstr. (also in the southern part of the U.S.A.) and *C. pallidovirens* (Hook.f. & Taylor) L. Söderstr. (also circumsubantarctic by subantarctic island). Africa has a restricted distribution of *C. lilliana* (Steph.) L. Söderstr. (Kenya only) and *C. regularis* (Steph.) L. Söderstr. (Madagascar, Réunion, and Mauritius). South Asia contains *C. fleischeri* (Steph.) L. Söderstr. (Sri Lanka only). *C. compacta* (Mitt.) L. Söderstr. is strictly found in temperate East Asia (East China, Korea, Japan, also a questionable record from Thailand).

The large region stretching from Southeast Asia (Indochina) to Melanesia contains eight species, with three species distributed across this large area: *C. ciliolata* (Nees) L. Söderstr., Crand.-Stotl., Stotler & Vána (also in southeast China (Hainan, Taiwan), Sri Lanka in south Asia and Hawaii in Polynesia), *C. costata* (Nees) L. Söderstr. (also in

Taiwan) and *C. edentata* (J.J. Engel) L. Söderstr. (also in Taiwan). Melanesia has a restricted distribution of *C. explanata* (Mitt.) Váňa & Crand.-Stotl. (New Caledonia and Samoa). Malesia and Melanesia have a restricted distribution of *C. levieri* (Schiffn.) L. Söderstr. Malesia additionally contains three species: *C. massalongoana* (Schiffn.) L. Söderstr., *C. stephanii* (Schiffn.) L. Söderstr. (Java only), *C. thermarum* (Schiffn.) L. Söderstr. (Java only). Finally, Polynesia contains *C. whittieriana* (Inoue & H.A.Mill.) L. Söderstr. (Caroline Island only).

Therefore, the highest taxonomic diversity is found in New Zealand and adjacent islands (and, to some extent, Tasmania); a less prominent taxonomic ‘peak’ can be found in the southern part of South America, and the third-most taxonomically diverse area is Malesia to Melanesia. The Indochina Peninsula (north Thailand only) contains four species. Northwards of Indochina, the distinctly East Asian *Cryptolophocolea compacta* and predominantly Paleotropical *C. ciliolata* (reaching Yunnan Province in China) are found. None of the species listed are referred to as Sino-Himalayan floral elements. Therefore, *C. sikkimensis* is the first known species whose area core is distinctly Sino-Himalayan.

Conclusions

Cryptolophocolea sikkimensis possesses generally narrowly pointed ovate leaves that are unique in the genus. Its phylogenetic affinity could not be clearly identified without molecular genetic investigations. In the present study, such bright and easily noted leaf features were the only possible variants of morphological pathways that occurred in the genus, whereas the underleaves (widely connate with the leaves), and biseriate antheridium stalk show much stronger taxonomic value. The species’ atavistic traits are generally typically evidenced by depauperate plants with bidentate leaves and underleaves. The unique morphology of *C. sikkimensis* is associated with its unique distribution – the species has the only predominantly Sino-Himalayan distribution in the genus.

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New *Piper* species from the eastern slopes of the Andes in northern South America

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Abstract

We describe four new species of *Piper* from the Amazonian slopes of the northern Andes. *Piper hoyoscardozi* is distinguished from similar climbing species, *P. dryadum* and *P. flagelliscuspe*, by its longer peduncles. The Amazonian species *Piper indiwasii* is distinguished from *P. scutilimbum* from Panama and northern Colombia by the narrowly spatulate leaf base extension. *Piper nokaidoyitau* is characterised by the presence of larger leaves and longer spikes than similar species, *P. anonifolium* and *P. hostmannianum*. Finally, *P. velae* is characterised by cordulate leaf bases in all nodes, petioles 0.8–1.5 cm long and pubescent fruits, which easily distinguish it from the related species, *P. holdridgeanum*.

Keywords

Colombia, Ecuador, north-western Amazon, Peru, *Piper hoyoscardozi*, *Piper indiwasii*, *Piper nokaidoyitau*, *Piper velae*, Piperaceae

Introduction

Piper L. is the most diverse and representative genus in Piperaceae, encompassing ca. 2600 species (Callejas-Posada 2020). *Piper* represents an extraordinary diversification amongst early diverging angiosperms. It is a Pantropical group (Jaramillo and Manos 2001) and its highest diversity lies in the Neotropics (ca. 1800 species; *vide* Ulloa-Ulloa et al. 2018 onwards). The growth forms of the genus are very variable and are most commonly perennial shrubs and suffrutices, growing mainly in the understorey of rainforests. Additionally, other *Piper* species are lianas, rarely caulescent herbs, hemi-epiphytes or trees not taller than 15 m (Callejas-Posada 2020). *Piper* is characterised by simple, alternate leaves and jointed stems with enlarged nodes; many species produce pearl bodies on the leaves or stems (Tepe et al. 2004), but the most distinctive morphological feature of the genus is the production of solitary spikes, upright or pendant, which contain dozens to thousands of apetalous flowers. Many *Piper* species are rich in essential oils, which can be found in many tissues and organs, including the fruits, seeds, leaves, branches, roots and stems (Salehi et al. 2019). Although very few species have significant global economic value, many are locally important for their use by native populations for medicinal and culinary purposes (Colvard et al. 2006; Cáceres and Kato 2014).

Piper has an extensive history of taxonomic and nomenclatural instability. The leading causes for that uncertainty are the challenges of interpreting morphological characteristics and the limited geopolitical circumscription of taxonomists' work (Ramírez-Amezcuca 2016; Callejas-Posada 2020). *Piper* flowers are small and morphologically homogeneous and the number of stamens and their position, characters of diagnostic importance, are challenging to see or interpret. Not all stamens develop at anthesis (Tucker 1982a, b) and the anthers, articulated at the filaments, fall off quickly after anthesis (Valentin-Silva et al. 2018). Many *Piper* species present foliar dimorphism and many specimens do not include leaves of both monopodial and sympodial axes. Description of new species, based on incomplete specimens, has led to the publication of superfluous names that later need to be submerged in synonymy (Ramírez-Amezcuca 2016). Furthermore, because floristic treatments are often limited to specific countries, many species are given several names across borders (Ramírez-Amezcuca 2016). Extensive fieldwork and comparisons with collections (including digitised collections) are essential to improve the taxonomy of this species-rich genera.

Observing species in the field is imperative to ensure detailed descriptions and complete specimens that support an excellent *Piper* taxonomy. Numerous essential characters cannot be included or are lost in dry specimens. Fortunately, in the last decade, W. Trujillo has collected and studied more than one thousand (1000) specimens from the Amazonian slopes of the Colombian Andes. Some of them have become type specimens of recently-described taxa (Trujillo and Callejas 2015; Trujillo and Jaramillo 2019, 2021). Continued work by the authors will bring to light more diversity in the future.

The present contribution describes four new *Piper* species from the Amazonian slopes of the Andes in Colombia, Ecuador and Peru. We use the nuclear ribosomal

internal transcribed spacer (ITS) to determine their phylogenetic relationships within *Piper*. We increase to 419 the number of *Piper* species known from Colombia (Bernal et al. 2015).

Materials and methods

Fieldwork was conducted along the Amazonian slopes of the Andes in southern Colombia, in the Department of Caquetá, during 2010–2020. We collected silica gel-dried leaf tissue samples for DNA extractions. Detailed observations in the field and examination of available herbarium collections were used to describe growth habits and phenological stages accurately. We deposited botanical specimens in COAH, COL, HUAZ and HUA (acronyms according to Thiers 2019). Detailed comparisons with morphologically similar species allowed us to recognise the four new species. Besides reviewing the literature (Trelease 1936; Trelease and Yuncker 1950; Brako and Zarucchi 1993; Jørgensen and León-Yáñez 1999; Jørgensen et al. 2014; Jardim Botânico do Rio de Janeiro 2020), we examined specimens through visits to Herbaria COAH, COL, HUA, HUAZ, GH, NY, PMA, RSA and US and digitised plant specimens available on the web (e.g. JSTOR Global Plants, <https://plants.jstor.org/>). Measurements included here were taken from specimens collected in Colombia. To describe leaf architecture, we used terminology proposed in the Manual of Leaf Architecture (Ellis et al. 2009). To assess conservation status, we calculated area of occupancy (AOO) and extent of occurrence (EOO) using R and the package ConR (Dauby et al. 2017).

We extracted DNA from all the new species (Table 1) using a CTAB method (Doyle and Doyle 1987). We amplified the nuclear ribosomal internal transcribed spacer (ITS) according to Jaramillo and Manos (2001) and aligned sequences using previous alignments as a guide (Jaramillo et al. 2008). First, we included the new sequences in our 900+ *Piper* ITS alignment, to determine the relationships of the new *Piper* species. This preliminary analysis (not shown) served to select representatives of each major clade of *Piper* (Jaramillo et al. 2008) for the analysis presented here. Forty-two species of *Piper*, 35 from the Neotropics and seven from Asia were selected to provide comparisons with the new taxa. Maximum Likelihood (ML) phylogenetic and bootstrap (100 replicates) analyses were conducted using RAXML (Stamatakis 2014) using species from the Asian tropics to root the phylogeny.

Results

Phylogenetic analyses identified the relationships of the new species described here (GenBank accession numbers are provided in Table 1). The ML tree placed *Piper hoyoscardozii* and *Piper nokaidoyitau* within the *Radula* clade, *Piper indiwassii* species within *Oxodium* (= *Schilleria*, Callejas-Posada 2020) and *Piper velae* forms a clade with *Piper holdridgeanum* W. C. Burger (1971: 144–145), sister to the *Macrostachys* clade (Fig. 1).

Table 1. GenBank accessions for new species and reference taxa. Other GenBank accessions are available in original manuscripts (Jaramillo et al. 2008). New species in bold.

Taxon	Voucher	GenBank Accession ITS	Collection *	Publication
<i>Piper abalienatum</i> Trel.	MAJ 552	EU581075	DUKE	Jaramillo et al. (2008)
<i>Piper abbreviatum</i> Opiz	MAJ 203	EU581076	DUKE	Jaramillo et al. (2008)
<i>Piper aduncum</i> L.	MAJ 76	AF275157	DUKE	Jaramillo & Manos (2001)
<i>Piper alatabaccum</i> Trel. & Yunck.	AL 1177	KJ930372	INPA	Molina-Henao et al. (2016)
<i>Piper albispicum</i> C. DC.	MAJ 388	AY572317	DUKE	Jaramillo & Callejas (2004)
<i>Piper albozonatum</i> C. DC.	MAJ 697	AY326195	DUKE	Jaramillo & Callejas (2004)
<i>Piper amalago</i> L.	MAJ 561	AF215186	DUKE	Jaramillo & Manos (2001)
<i>Piper amplum</i> Kunth	MAJ 804	EU581096	RB	Jaramillo et al. (2008)
<i>Piper anonifolium</i> Kunth	AL 1242	EU581084	MG	Jaramillo et al. (2008)
	EJT 527	EU581101	MU	Jaramillo et al. (2008)
<i>Piper arboretum</i> Aubl.	MN 1565	EU581106	RB	Jaramillo et al. (2008)
<i>Piper biolleyi</i> C DC.	CD 10896	EU581128	SRP	Jaramillo et al. (2008)
<i>Piper brachypodon</i> (Benth.) C. DC.	MAJ 757	AY326198	DUKE	Jaramillo & Callejas (2004)
<i>Piper breviamentum</i> C.DC.	MAJ 221	EU581134	DUKE	Jaramillo et al. (2008)
<i>Piper callosum</i> Ruiz & Pav.	MJK 161	EU581142	SPF	Jaramillo et al. (2008)
<i>Piper candollei</i> Sodirol	EJT 1449	EF056237	MU	Jaramillo et al. (2008)
<i>Piper capense</i> L.	CD 11004	EU581143	SRP	Jaramillo et al. (2008)
<i>Piper cavendishoides</i> Trel. & Yunck.	MAJ 70	AF275153	DUKE	Jaramillo & Manos (2001)
<i>Piper cinereum</i> C. DC.	MAJ 66	AF275190	DUKE	Jaramillo & Manos (2001)
<i>Piper cocornarum</i> Trel. & Yunck.	RC 12493	AY326203	HUA	Jaramillo & Callejas (2004)
<i>Piper cordulatum</i> C. DC.	BCI-EL	EU581167		Jaramillo et al. (2008)
<i>Piper darienense</i> C. DC.	MAJ 103	AF275181	DUKE	Jaramillo & Manos (2001)
<i>Piper dilatatum</i> Rich.	MAJ 858	EU581179	RB	Jaramillo et al. (2008)
<i>Piper dryadum</i> C. DC.	EJT 1047	EU581186	MU	Jaramillo et al. (2008)
<i>Piper excelsum</i> G. Forst.	N. V.	EF635476		Jaramillo et al. (2008)
<i>Piper filistilum</i> C. DC.	MAJ 157	AF275155	DUKE	Jaramillo & Manos (2001)
<i>Piper flagellicuspe</i> Trel. & Yunck.	MAJ 65	AF275154	DUKE	Jaramillo & Manos (2001)
<i>Piper hartwegianum</i> (Benth.) C. DC.	MAJ 781	AY326207	DUKE	Jaramillo & Callejas (2004)
<i>Piper hispidum</i> Sw.	MCS 304	EU581241	RB	Jaramillo et al. (2008)
<i>Piper holdridgeanum</i> W. C. Burger	CD 10865	EU581247	SRP	Jaramillo et al. (2008)
	JF 9128	EU581248	MO	Jaramillo et al. (2008)
<i>Piper hostmanianum</i> C. DC.	SM 25228	EU581251	NY	Jaramillo et al. (2008)
	EJT 573	EU581249	MU	Jaramillo et al. (2008)
<i>Piper boyoscardozi</i>	WT 4127	OK235346	UMNG	This study
	WT 4004	OK235347	UMNG	
	WT 4099	OK235348	UMNG	
<i>Piper imberbe</i> Trel. & Yunck.	AB 983	EU581255	SEMO	Jaramillo et al. (2008)
<i>Piper indiwasi</i>	ET 7254	OK235342	JBB	This study
	EJT 1438	EU581390	MU	Jaramillo et al. (2008)
<i>Piper maxonii</i> C. DC.	EJT 370	EF056270	MU	Jaramillo et al. (2008)
<i>Piper multiplinervium</i> C. DC.	MAJ 139	AF275168	DUKE	Jaramillo & Manos (2001)
<i>Piper nokaidoyitau</i>	WT 4171	OK235339	COAH	This study
	WT 4258	OK235340	COAH	
	ET 7249	OK235341	COAH	
<i>Piper novogranatense</i> C. DC.	MAJ 71	EU581317	DUKE	Jaramillo et al. (2008)
<i>Piper peltatum</i> L.	MAJ 564	EU581335	DUKE	Jaramillo et al. (2008)
<i>Piper retrofractum</i> Vahl.	MAJ 395	AF275196	DUKE	Jaramillo & Manos (2001)
<i>Piper sanctum</i> (Miq.) Schtdl. ex C. DC.	AB 744	EU581382	SEMO	Jaramillo et al. (2008)
<i>Piper scutilimbium</i> C. DC.	WT 4151	OK235343	UMNG	This study
<i>Piper scutifolium</i> Yunck.	MAJK 281	EU581389	SPF	Jaramillo et al. (2008)
<i>Piper sorgosonum</i> C. DC.	MAJ185	AY572320	DUKE	Jaramillo & Manos (2001)
<i>Piper spoliatum</i> Trel. & Yunck.	MAJ 60	AF275179	DUKE	Jaramillo & Manos (2001)

Taxon	Voucher	GenBank Accession ITS	Collection *	Publication
<i>Piper subscutatum</i> C. DC.	EJT 1604	EU581406	MU	Jaramillo et al. (2008)
<i>Piper trianae</i> C. DC.	MAJ 662	EU581413	DUKE	Jaramillo et al. (2008)
<i>Piper truncatum</i> Vell.	MAJ 937	EF056291	RB	Jaramillo et al. (2008)
<i>Piper tuberculatum</i> Jacq.	MAJ 710	AY326225	DUKE	Jaramillo & Callejas (2004)
<i>Piper umbellatum</i> L.	MAJ 35	EU581433	DUKE	Jaramillo et al. (2008)
<i>Piper umbricola</i> C. DC.	EJT 1014	EU581435	MU	Jaramillo et al. (2008)
<i>Piper velae</i>	WT 4058	OK235344	UMNG	This study
	WT 3995	OK235345	UMNG	
<i>Piper viçosanum</i> Yunck.	MAJ 809	EU581440	RB	Jaramillo et al. (2008)
<i>Piper yanaconasense</i> Trel. & Yunck.	MAJ 774	AY326229	DUKE	Jaramillo & Callejas (2004)

*Collections: COAHH, Herbario Amazónico Colombiano; DUKE, Duke University Herbarium, UMNG: Herbario Universidad Militar Nueva Granada; HUA, Herbario de la Unversidad de Antioquia; INPA, Instituto Nacional de Pesquisas da Amazônia; MG, Museu Paraense Emílio Goeldi; MU, Miami University Herbarium; NY, New York Botanical Garden Herbarium; RB, Herbario Jardim Botânico do Rio de Janeiro; SEMO, Southeast Missouri State University Herbarium; SPF, Herbario da Universidade de São Paulo; SRP, Snake River Plains Herbarium (University of Idaho).

Taxonomic treatment

Piper hoyoscardozii W. Trujillo-C & M. A. Jaram., sp. nov.

urn:lsid:ipni.org:names:77303983-1

Figs 1, 2, 3 and 4

Type. COLOMBIA. Caquetá: Florencia, vereda Sucre, 1076 m elev., 1°47'50"N, 75°38'50"W, 18 Oct 2020 [fr], *F. Hoyos 049* (Holotype COL, Isotype COAH, HUA, UMNG)

Diagnosis. *Piper hoyoscardozii* W. Trujillo & M. A. Jaram. is similar to *P. dryadum* C. DC. (1891:221) and *P. flagellicuspe* Trel. & Yunck. (1950:59) from which it is easily distinguished by peduncles 2–3 cm long, spikes long-apiculate and fruit with stigmas sessile vs. peduncle 0.5–1 cm long, spikes not-apiculate and fruit with stigmas on a short style in *P. flagellicuspe* and *P. dryadum*.

Description. *Shrub* with sarmentose branches. Internodes (1)3–7 cm long, smooth, green, pubescent, trichomes pluricellular, uniseriate, 1–2.3 mm long, idioblasts not visible. *Prophylls* caducous, 1.2–1.5 cm long, green-whitish, pubescent, trichomes pluricellular, uniseriate, 0.2–1.0 mm long, dispersed on the abaxial surface, idioblasts not visible. *Petioles* uniform in size along all axes, 0.5–0.8 cm long, vaginate on the basal half, smooth and pubescent. *Leaf-blades* membranaceous, drying black, uniform in shape and size on all nodes, (5)6–7 × (13)15–17 cm, elliptic, symmetric, base cordate to rounded, apex acuminate; leaf blade smooth, pubescent on both surfaces, trichomes pluricellular, uniseriate, 0.5–2.3 mm long, dispersed on the adaxial surface, along first and second order nerves and dispersed on the areolas and third order nerves of the abaxial surface, eciliate; pinnately nerved from the lower 1/3, 2–3 nerves on each side, with spacing uniform or decreasing and angle increasing gradually towards the base, eucamptodromous, tertiary veins percurrent. *Inflorescence* and *infructescence* a simple spike, erect; peduncle 2–3 cm long, pubescent, green; rachis in flower 4–7 cm long, rachis in fruit 7–9 cm long, rachis with a 10–15 mm long, sterile green apical extension, fruits densely grouped along the rachis. *Floral bracts* cucullate, reddish in flower, triangular from above, 0.4–0.6 × 0.7–0.8 mm, glabrous on the adaxial surface, margin fimbriate, bracts

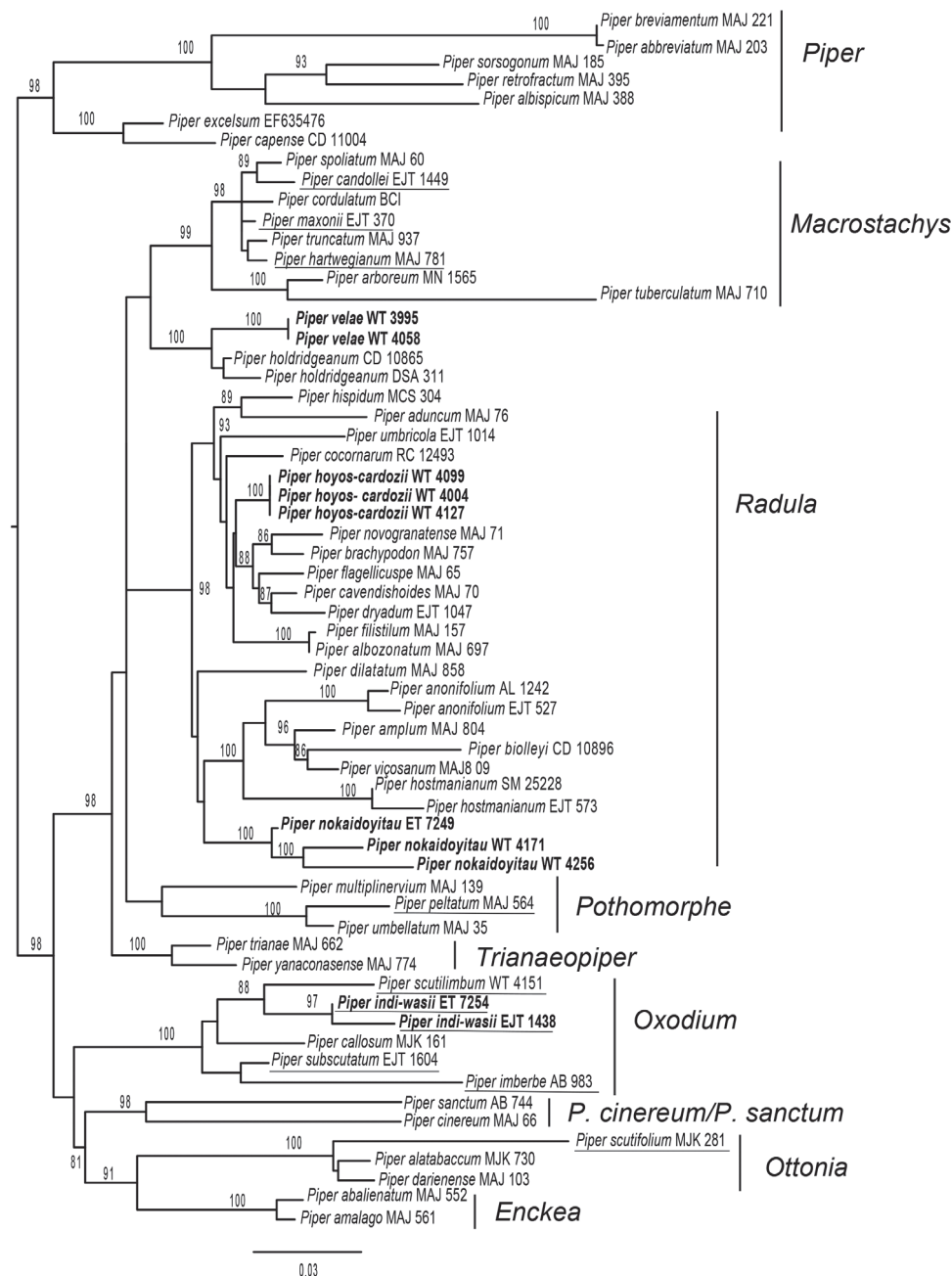


Figure 1. Phylogenetic relationships of species described in this manuscript, based on Maximum Likelihood analysis of nrITS sequences. New species are shown in bold. Species with peltate leaves are underlined. Numbers above branches are ML bootstrap support values (> 80).

forming bands around the spike. **Flowers** with four stamens, filaments 0.6–0.8 mm long, anthers $0.4\text{--}0.6 \times 0.5\text{--}0.7$ mm, longitudinally dehiscent, dithecal, with connective not protruding, glabrate, idioblasts not evident, black when dried. Sessile stigmas. **Fruits** rectangular, laterally compressed, green when alive and black when dry, $0.9\text{--}1.2 \times 1.5\text{--}1.9$ mm, pubescent, partially immersed in the rachis, with persistent sessile stigmas, 0.05–0.1 mm. Seeds oblong, laterally compressed, brown, smooth, $0.8\text{--}1 \times 0.8\text{--}1.1$ mm.

Distribution and habitat. *Piper hoyoscardozii* is a shade-loving sarmentose shrub that grows on trees and rocks. It is known from the Amazonian slope of the Andes in southern Colombia and Ecuador, between 1000–1500 m in elevation (Fig. 2).

Phenology. Flowering specimens were collected in July. Fruiting specimens were collected in August.

Etymology. This species name is dedicated to Fernando Hoyos Cardozo, a great companion during our floristic explorations of the Amazonian foothills and who collected the type specimen of this species in Caquetá.

Conservation status. This species is known from six specimen collections representing two subpopulations. The locations where it occurs are threatened by deforestation and expansion of the agriculture frontier, especially extensive cattle ranching. The extent of occurrence (EOO) of 876 km² and area of occupancy (AOO) of 16 km² are small, which, together with the continuing decline in quality of habitat, suggests it is Endangered [EN B1a+B2a].

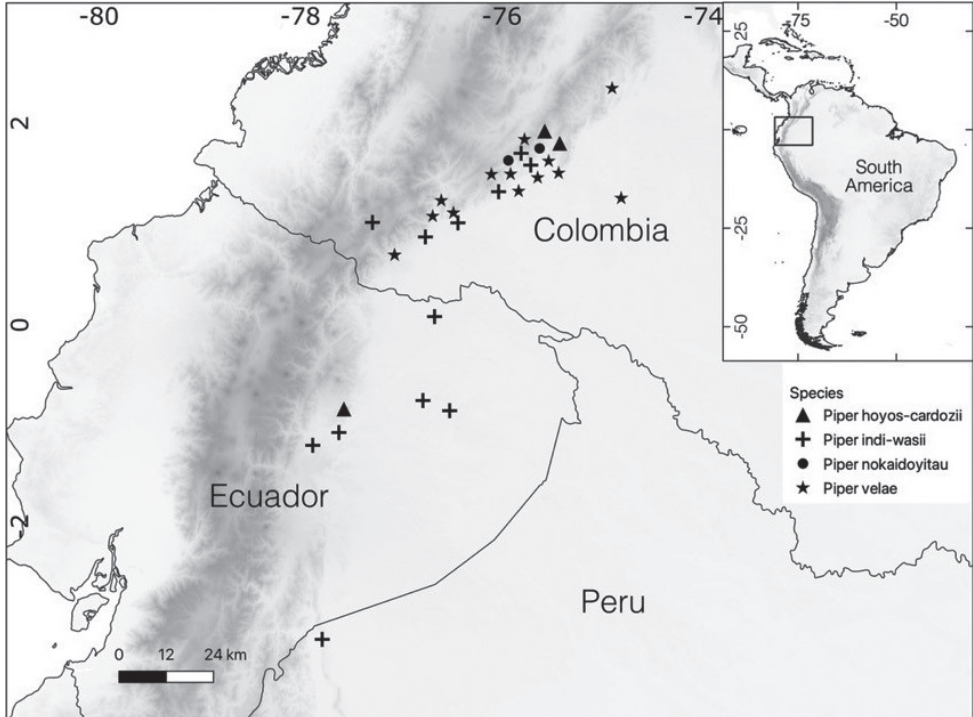


Figure 2. The locality of four new *Piper* species from the Andes eastern slopes of Colombia, Ecuador and Peru.

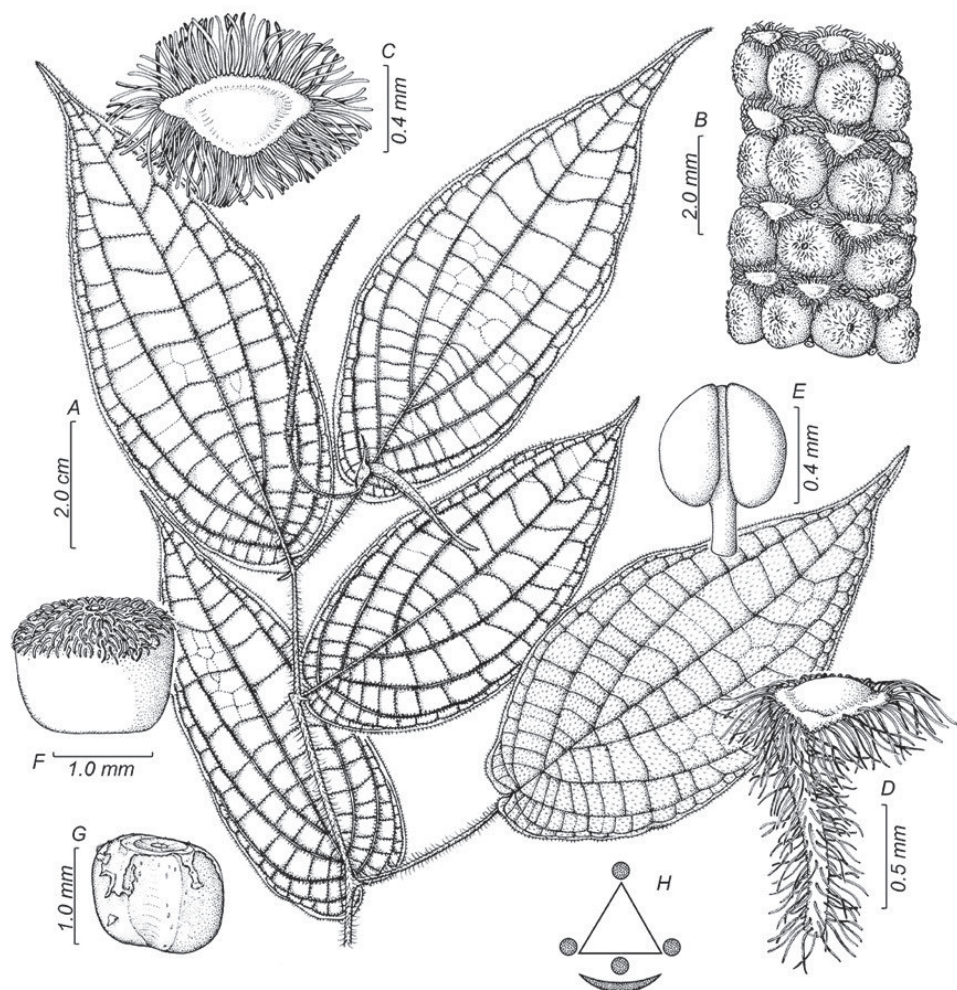


Figure 3. *Piper hoyoscardozii* **A** sympodial branch **B** magnified view of the infructescence **C** floral bract view from above **D** floral bract side view **E** anthers and distal portion of the filament **F** fruit side view **G** seed **H** floral diagram. Illustration by Marcela Morales based on *F. Hoyos 049* COAH.

Phylogenetic relationships. *Piper hoyoscardozii* belongs to the large clade *Radula*. A group of medium-size shrubs, mostly self-supporting, but some species are herbs or lianescent shrubs; leaves are plinnerved or pinnately nerved. Flowers are densely arranged in spikes forming banding patterns and inflorescences can be erect or distally curved. Furthermore, this species is closely related to the clade of sarmentose shrubs occurring in wet tropical forests in Central America and the western slopes of the west Cordillera in Colombia (the latter corresponds largely to the Chocó Region) that includes *P. brachypodon* C. DC. (1869:327), *P. cavendishoides* Trel. & Yunck. (Trelease and Yuncker 1950: 85), *P. dryadum*, *P. flagelliscuspe*, *P. ottoniifolium* C. DC. (1866:213), *P. oxystachyum* C. DC. (1898:255) and *P. novogranatense* C. DC. (1869:313) (Jaramillo et al. 2008).

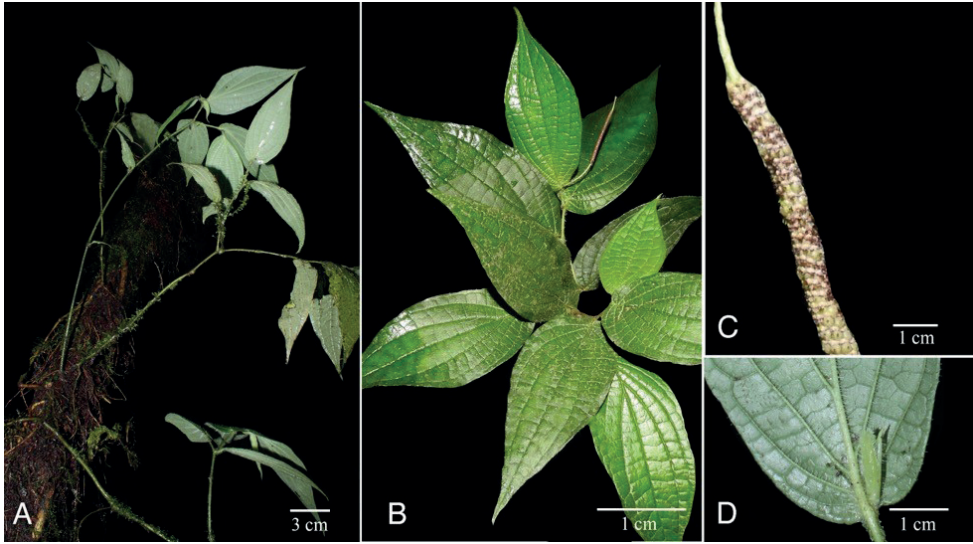


Figure 4. *Piper hoyoscardozii* **A** growth form, scandent shrub **B** upper leaf surfaces and spike **C** infructescence **D** leaf base and prophyll. Photos from *F. Hoyos 049* by F. Hoyos

Discussion. *Piper hoyoscardozii* is a sarmentose shrub, a habit not commonly observed amongst *Piper* species in the study region (eastern slope of the Andes). The phylogeny (Fig. 1) places *P. hoyoscardozii* sister to other climbing *Piper* species occurring on the western slope of the Andes and wetter parts of Mesoamerica. Here we provide a comparative table for the climbing *Piper* species included in the phylogeny (Table 2). *P. hoyoscardozii* is easily differentiated because its spikes (in flower and fruit) have a long peduncle and a long apiculate apex.

Specimens examined. **COLOMBIA:** Caquetá, Florencia, vereda Tarqui, monumento Divino Niño, 1570 m elev., 1°50'0.3"N, 75°39'52.8"W, 30 Aug 2020 [fl], *W. Trujillo* & *F. Hoyos 4120* (COAH, UMNG).; vereda Tarqui, quebrada Tarqui, 1530 m elev., 1°50'28"N, 75°39'42"W, 20 Aug 2020 [42], *W. Trujillo 4099* (COAH); corregimien-

Table 2. Comparison of *Piper hoyoscardozii* with related species of scandent habit.

Species	Internodes indument	Secondary nerves branch	Peduncle length	Rachis length	Spike a pex	Distribution
<i>P. brachypodon</i> (Benth.) C. DC.	glabrous	from the lower half	1 cm	5–7 cm	obtuse	Chocó Region
<i>P. cavendishoides</i> Trel. & Yunck.	tomentulose	from the lower one-fourth or one-third	1 cm	7–8 cm	obtuse	Chocó Region
<i>P. dryadum</i> C. DC.	pubescent	from the lower third	0.5–1 cm	5–6 cm	obtuse	Mesoamerica and Chocó Region
<i>P. flagelliscuspe</i> Trel. & Yunck.	velvety	from the lower half	0.5 cm	4 cm	obtuse	Chocó Region
<i>P. novogranatense</i> C. DC.	glabrous	from the lower third	0.5 cm	4 cm	obtuse	Chocó Region
<i>P. ottoniaefolium</i> C. DC.	glabrous	near the base	0.5–1 cm	6–8 cm	obtuse	Chocó Region
<i>P. xanthostachyum</i> C. DC.	glabrous	from the lower third	0.5–1 cm	5–6.5	obtuse	Mesoamerica and western slope of the Andes
<i>P. hoyoscardozii</i> W. Trujillo & M. A. Jaram.	pubescent	from the lower third	2–3 cm	7–9 cm	long apiculate	Eastern slope of the northern Andes

to El Caraño, vereda Sucre. 1076 m elev., 1°47'50.8"N, 75°38'50.5"W, 8 Jul 2014 [fl], *W. Trujillo 3130* (COAH); vereda Sucre, Finca campamento Sucre. 1°46'52"N, 75°39'5.1"W. 1050 m elev., 5 Jul 2012 [fl]. *W. Trujillo & C. Malambo 2400*; vereda Sucre, vía antigua Florencia-Huila, 1°47'50.8"N, 75°38'50.3"W, 1000 m elev., 24 Sep 2020 [fl], *F. Hoyos 042* (COAH). **ECUADOR.** Napo, Parque Nacional Sumaco-Galeras, 0°50'S, 77°34'W, 1090 m elev., 27 Oct 2005 [fr], *J. Homeier & M.A. Chinchero 2000* (MO).

***Piper indiwassii* W. Trujillo-C & M. A. Jaram., sp. nov.**

urn:lsid:ipni.org:names:77303984-1

Figs 1, 2, 5 and 6

Type. COLOMBIA. Caquetá, municipio de San José del Fragua, ronda de bosque cerca al balneario Villa Collazos sobre el río Fragua, 1°20'04"N, 75°59'28"W, 395 m elev., 14 May 2020, *M. Angulo 1550* (Holotype COL, Isootype COAH, HUA, HUAZ, UMNG).

Diagnosis. *Piper indiwassii* W. Trujillo & M. A. Jaram. can be distinguished from *P. scutilimum* C. DC. (1920a:242) by many attributes. *Piper indiwassii* has 1–1.7 cm long petioles, 7–8 pairs of secondary veins and a narrowly spatulate leaf base extension, 0.4–0.9(1.5) cm wide, vs. *P. scutilimum*, which has a 4–6 cm long petioles, 10–12 secondary veins and an obtuse and rounded leaf-base extension, 2.5–4 cm wide. *Piper indiwassii* occurs in the Amazon watershed, on the eastern foothills of the Andes, while *P. scutilimum* occurs west of the Andes in Panama and extends to Sierra Nevada de Santa Marta in northern Colombia.

Description. *Shrub*, up to 2 m tall. Internodes 2–4(5) cm, canaliculate, green, glabrous. *Prophylls* not seen. *Petioles* are uniform in size along all axes, 1–1.7 cm long, vaginate along their entire length, canaliculate, glabrous. *Leaf-blades* coriaceous, drying grey to brown, uniform in size along all axes, (4.5)6–8.5 × (11)14–18 cm, elliptic, symmetric, leaf-base peltate, symmetric, with a narrowly spatulate extension, 0.4–0.9 × (0.5)1–2.3(2.5) cm, the leaf-base extension covering the petiole on sympodial nodes and orientated towards the axis on monopodial nodes, apex acuminate; leaf-blade glabrous on both surfaces, eciliate; pinnately nerved throughout, the nerves 7–8 on each side, brochidodromous, with spacing decreasing towards the base and angle uniform throughout, tertiary veins random reticulate. *Inflorescences and infructescences* a solitary spike, erect; peduncle 0.9–1.5 cm long, glabrous, green; rachis length in fruit (5)6–20 cm, fruits loosely grouped along the rachis. *Floral bracts* cucullate, triangular from above, 0.3–0.5 × 0.5–0.8 mm, glabrous on the adaxial surface, margin eciliate, not forming bands around the spike. *Flowers* with three stamens, filaments 0.5–0.8 mm long, anthers 0.3–0.5 × 0.2–0.3 mm long, longitudinally dehiscent, ditheous, with connective not protruding, glabrate, idioblasts not evident; stigmas 3, 0.05–1.5 mm long, sessile. *Fruits* obpyriform, green when alive and brown when dry, 0.6–0.8 × 0.9–1.2 mm, glabrous, partially immersed in the rachis, with stigmas persistent, 0.05–1.5 mm long, sessile. *Seeds* obpyriform, black.

Distribution and habitat. *Piper indiwassii* is known from the Amazonian slopes of the Andes in Colombia (Departments of Putumayo, Caquetá and

Guaviare), Ecuador (Provinces of Guayas, Napo, Orellana, Pastaza and Sucumbíos) and Peru (Provinces of Amazonas), from 200 to 1,608 m elevation (Fig. 2). It occurs in lowland (sometimes along riverbanks) and lower montane forests. It is a shade-loving species that grows in the understorey and the edges of trails of preserved forests.

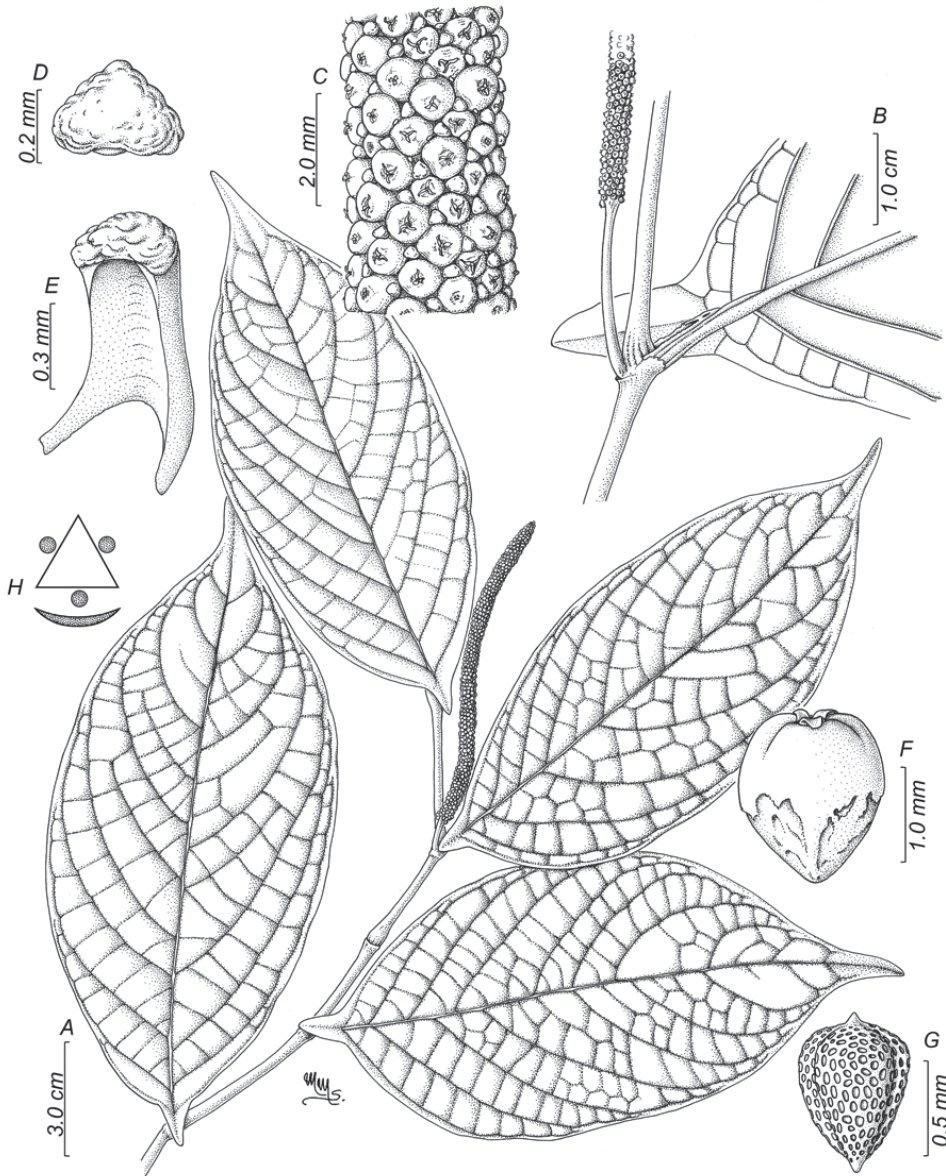


Figure 5. *Piper indiwassii* **A** sympodial branch **B** lower leaf surfaces and details of the petiole **C** magnified view of the infructescence **D** floral bract view from above **E** floral bract side view **F** fruit side view **G** seed side view. Illustration by Marcela Morales based on *Angulo 1550*, COAH.

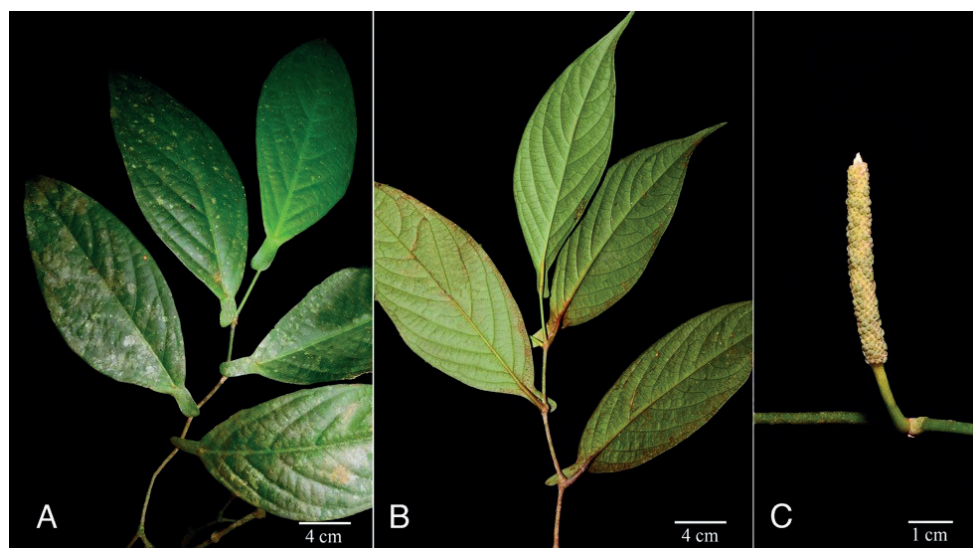


Figure 6. *Piper indiwassii* **A** sympodial branch upper leaf surfaces **B** sympodial branch lower leaf surfaces **C** erect spikes. Photos from *E. Trujillo* 7249 by G. Delgado.

Phenology. Fruiting specimens were collected from December to June and August to October. Flowering samples were collected in March, April, May and December.

Etymology. *Piper indiwassii* is named after the Inga word meaning “House of the Sun”. Ingas or Inganos are an indigenous group belonging to the Quechua linguistic family. The Ingas of the Amazon foothills are made up of migratory groups from the Peruvian and Ecuadorian Amazon, the Mocoas and some survivors of the Andaquíes. The clans are united by their location, cosmovision and the culture of “yajé” (*Banisteriopsis caapi*). Furthermore, the type specimen of this species was collected in the Alto Fragua Indi-Wasi National Park, located in San José del Fragua, Caquetá-Colombia.

Conservation status: *Piper indiwassii* is not endangered. It is known from 11 sub-populations and 12 localities; it has an EOO of 1,478,359 km² and an AOO of 52 km². According to IUCN guidelines, it is of Least Concern (LC) as the region where it occurs is threatened by deforestation and its conservation status should be monitored.

Phylogenetic relationships. *Piper indiwassii* belongs to the Neotropical clade *Oxodium*. Species in this group are shrubs, sometimes sarmentose. They have plinerved or pinnately nerved leaves, leaf bases are often acute or cordate and they have lax inflorescences with loosely arranged flowers (Jaramillo et al. 2008). *Piper indiwassii* is closely related to *P. scutilimbum*. Nucleotide difference between the two *Piper indiwassii* ITS accession is 5 bp out of 641 (0.8%), while these sequences have a 27 bp (4%) dissimilarity with *P. scutilimbum* (collected in the type locality). Sequence data and leaf material were available for five species of Neotropical *Piper* with peltate leaves. *P. subscutatum* C. DC. (1869:321) and *P. imberbe* Trel. & Standl. (Standley and Steyermark 1952:303–304) are in the *Oxodium* clade as *P. indiwassii* and *P. scutilimbum*;

P. scutifolium Yunck. (1966:123–124) is part of the *Ottonia* clade; and *P. maxonii* C. DC. (1920b:16), *P. hartwegianum* (Benth.) C. DC. (1869: 369) and *P. candollei* Sodiro (1905:202) are members of the *Macrostachys* clade. Neotropical *Piper* species with peltate leaves do not form a monophyletic group and are part of at least four clades: *Oxodium*, *Pothomorphe*, *Macrostachys* and *Ottonia* (Fig. 1). Further studies are needed to shed light on the convergence of this trait.

Uses by communities. Various common names are used for *P. indiwassii* amongst indigenous communities in Ecuador: a) “*náhuí tapa panga*” (closed eye leaf) (D. Irvine & L. Cejua 1125, F, QCA); when a patient is sick and the eyes are closed even when awake, leaves are wrapped around tobacco and the smoke blown over the eyes of the patient; b) “*uchi-ampar*” a Shuar name, plant used against parasites, leaves and roots are used (Guerrero 171; Herrera 288, MO, QCNE); c) “*palu sera aula*” or the grandmother of *palu sera*, which is used for relieving toothache (D. Irvine 959, F, QCA).

Discussion. After reviewing the specimens identified as *P. scutilimum* from Panama and northern Colombia vs. specimens from the eastern (Amazonian) Andes slope in Colombia, Ecuador and Perú (Fig. 2), we found a consistent difference in the leaf base shape between collections from both regions. The obtuse and rounded leaf base extension of the type specimen from the Sierra Nevada de Santa Marta coincides with that of specimens collected in Panama in contrast to the narrowly spatulate base extension seen in *P. indi-wasi*. The leaf base shape, combined with other morphological characters and geographical distribution, clearly allows *Piper indiwassii* to be proposed as a new species. We provide a comparative table of morphological characters for species of *Piper* with peltate leaves that belong to the *Oxodium* clade (see Table 3).

Specimens examined. COLOMBIA. Caquetá, San José del Fragua, ronda de bosque cerca al balneario Villa Collazos sobre el río Fragua, 1°20'04"N, 75°59'28"W, 400 m elev., 29 Jun 2011 [fr], W. Trujillo et al. 1999 (COAH); Belén de los Andaquies, vereda las verdes, cerro Monserrate, entrada por dos quebradas, 1°36'38"N, 75°53'23"W, 700 m elev., 24 Jun 2011 [fr], W. Trujillo et al. 1990 (COAH); Belén de los Andaquies, Parque Natural Municipal Andaqui, cabeceras del río pescado, 1°41'52"N, 75°54'15"W, 1608 m elev., 25 Jan 2017 [fr], N. Castaño et al. 8734 (COAH, HUA); Belén de los Andaquies, Parque Natural Municipal Andaqui, sector

Table 3. Comparison of *Piper indiwassii* with species of *Piper* with peltate leaves belonging to the *Oxodium* clade.

Species	Leaf dimorphism*	Leaf base extension width	Leaf base extension shape	Leaf width	Geographic distribution
<i>P. imberbe</i> Trel. & Standley	all leaves peltate	1.2–1.5 cm	rounded	4.5–8 cm	Mesoamerica
<i>P. scutilimum</i> C.DC.	all leaves peltate	(2)2.5–4 cm	obtuse and rounded	8–14 cm	Mesoamerica and northern Andes
<i>P. subscutatum</i> (Miq.) C. DC	present	2–4 cm	obtuse and rounded	25–28 cm	Eastern slope of the northern Andes and Amazonia
<i>P. indiwassii</i> W. Trujillo & M. A. Jaram.	all leaves peltate	0.4–0.9 cm	narrowly spatulate	4–18 cm	Eastern slope of the northern Andes

* Leaves can be peltate or not.

entre filo seco y la bocana de la quebrada las verdes, 1°37'13"N, 75°53'46"W, 600–800 m elev., 7 Feb 2017 [fr], *N. Castaño et al.* 9659 (COAH, HUA); Florencia, vereda Damas Arriba, finca el Mirador, 1°37'56"N, 75°41'49"W, 750 m elev., 14 Feb 2002 [fr], *M. Correa et al.* 2853 (COAH, UDBC); Guaviare, El Retorno, cerca del Retorno, granja de la Corporación Araracuara, zona ligeramente disectada, bosque intervenido, 1 Mar 1994 [fl], *P. Stevenson* 1168 (COAH); Putumayo, Villagarzón, corregimiento la Castellana, vereda la Pradera, finca el Cairo, bosque intervenido a borde de quebrada, 0°52'23"N, 76°45'27"W, 600 m elev., 10 Dec 1999 [fr], *C. Marín & D. Cárdenas* 1997 (COAH); Villagarzón, vereda la Kofaina, 1°01'00"N, 77°17'00"W, 550–700 m elev., 2 Sep 1993 [fr], *A. Cogollo et al.* 6830 (COAH, JAUM, MO); **ECUADOR**. Napo, Estación Biológica Jatun Sacha, 1°04'00"S, 77°37'00"W, 450 m elev., 10 Oct 2007 [fr], *J. Homeier et al.* 2834 (MO, QCA, QCNE, GOET); Estación Biológica Jatun Sacha, río Napo, 8 km debajo de Misahualli, 1°04'00"S, 77°36'00"W, 450 m elev., 17 Jan – 6 Feb 1987 [fr], *C. Cerón* 638 (MO, HUA); Estación Biológica Jatun Sacha, río Napo, 8 km debajo de Misahualli, 1°04'00"S, 77°36'00"W, 450 m elev., 19–28 Mar 1987 [fr], *C. Cerón* 1063 (HUA, MO); 9 km río debajo de puerto Misahualli y 2 km al sur de la cuenca del río Chinguipino, 1°05'00"S, 77°36'00"W, 430 m elev., 10 Mar 1985 [fr], *D. Neill et al.* 6054 (ECUAMZ, HUA, MO, NY, QCNE, US); Tena, Estación Biológica Jatun Sacha, río Napo, 8 km E of puerto Misahualli, 1°04'00"S, 77°36'00"W, 400 m elev., 18 May 1985 [fr], *W. Palacios* 421 (AAU, HUA, MO, NY, QCNE, US); Tena, Estación Biológica Jatun Sacha, along S bank of río Napo, 8 km E of puerto Misahualli, 1°04'00"S, 77°36'00"W, 450 m elev., 1 Apr 1992, *T. Croat* 73366 (HUA, MO) [fr]; along road between Tena and Puyo, 61.5 km N of Puyo, 1°11'36"S, 77°52'34"W, 500 m elev., 22 Dec 1979 [fl], *T. Croat* 49657 (MO); Archidona, Parque Nacional Galeras a 1.5 km de la comunidad Santa Rosa de Arapino, 00°51'00"S, 77°31'00"W, 1230 m elev., 3 Apr 1996 [fr], *H. Vargas & P. Grefa* 951 (HUA, MO, QCNE); San José de Payamino 40 km W of Coca, 00°30'S, 77°20'W, 26 Apr 1984 [fl], *D. Irvine & H. Jipa* 959 (F, QCA); 1 May 1984, *D. Irvine & L. Cejua* 1125 (F, MO, QCA); Sucumbíos, Dureno, comunidad Cofan al sur del río Aguarico, 20 km al este de Lago Agrio, 00°05'00"N, 76°40'00"W, 350 m elev., 27 Dec 1988 [fr], *C. Cerón et al.* 5824 (MO, HUA); Dureno, comunidad Cofan al sur del río Aguarico, 20 km al este de Lago Agrio, 00°05'00"N, 76°40'00"W, 350 m elev., 27 Dec 1988 [fr], *C. Cerón et al.* 5827 (MO, HUA); Francisco de Orellana, Orellana, comunidad Shuar Tiguanó al sur del Coca por la vía al Pindo, 00°44'58"S, 76°46'55"W, 300 m elev., 6–12 May 2004 [fl], *W. Guerrero & A. Herrera* 171 (MO, QCNE); Orellana, comunidad Shuar Tiguanó al sur del Coca por la vía al Pindo, 00°44'58"S, 76°46'55"W, 300 m elev., 11 May 2004 [fr], *A. Herrera & W. Guerrero* 288 (MO, QCNE); Parque Nacional Yasuni, Río Tiputini al noroeste de la confluencia con el Río Tivacuno, este de la carretera Repsol-YPF, km 32 hacia NPF, Sendero Botánico Guiyero, 00°38'S, 76°30'W, 200–300 m elev., 26 Feb 2002 [fr], *G. Villa* 1350 (F, QCA, US); **PERU**. Amazonas, Condorcanqui, Santiago, Cerros Kampankis, Serranía entre los Ríos Santiago y Morona, desde Río Marañón hasta frontera con Ecuador, Campamento 1: Pongo Shenin, 03°07'01.52"S, 77°46'55.14"W, 520 m elev., 3 Aug 2011 [fr], *I. Huamantupa* 15217 (USM, F).

***Piper nokaidoyitau* W. Trujillo-C & M. A. Jaram., sp. nov.**

urn:lsid:ipni.org:names:77303985-1

Figs 1, 2, 7 and 8

Type. COLOMBIA, Caqueta; Florencia, corregimiento el Caraño, vereda Sucre, 01°47'50.8"N, 75°38'50.5"W, 1020 m elev., 25 Oct 2020 [fr], *F. Hoyos & W. Trujillo 046* (Holotype COL, Isotype COAH, UMNG, HUAZ, HUA)

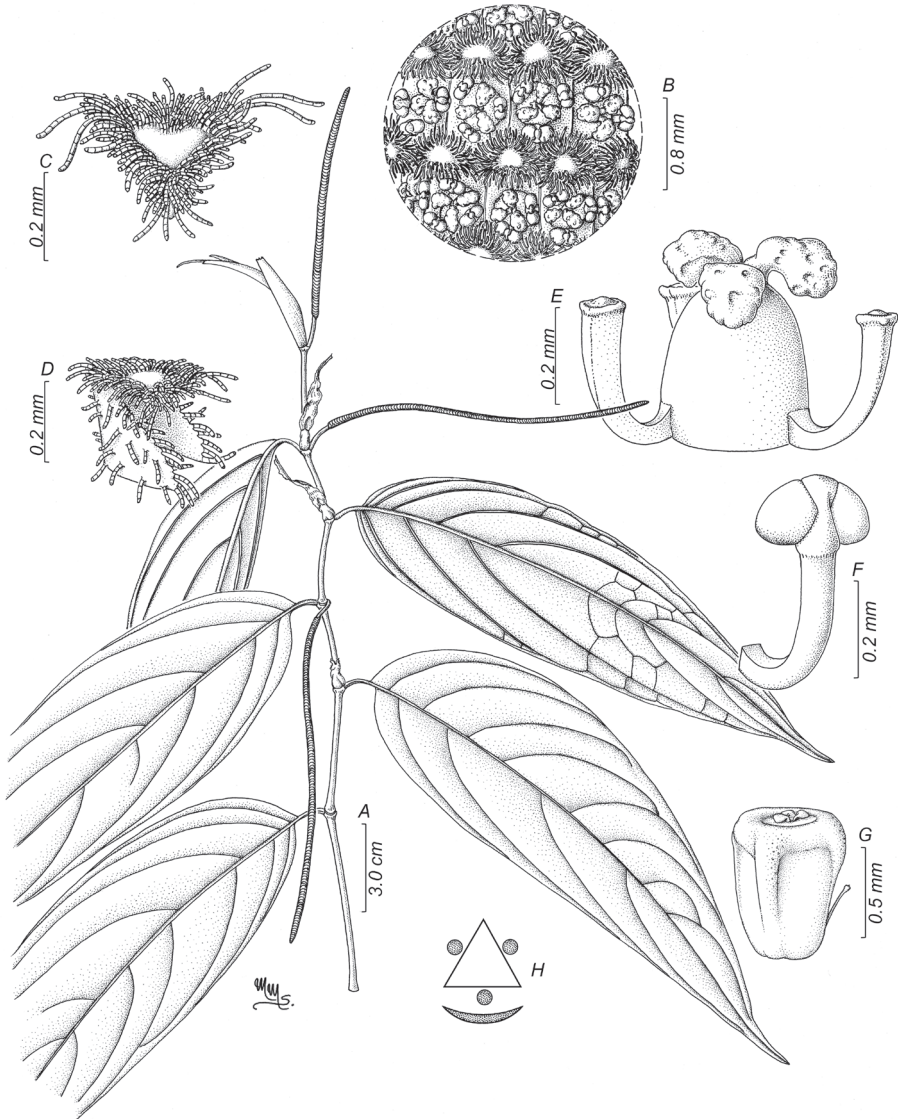


Figure 7. *Piper nokaidoyitau* **A** sympodial branch **B** details of the inflorescence **C** floral bract viewed from above **D** floral bract side view **E** flower side view **F** stamen with glabrous connective **G** fruit side view **H** floral diagram. Illustration by Marcela Morales based on *F. Hoyos 046* (COL).

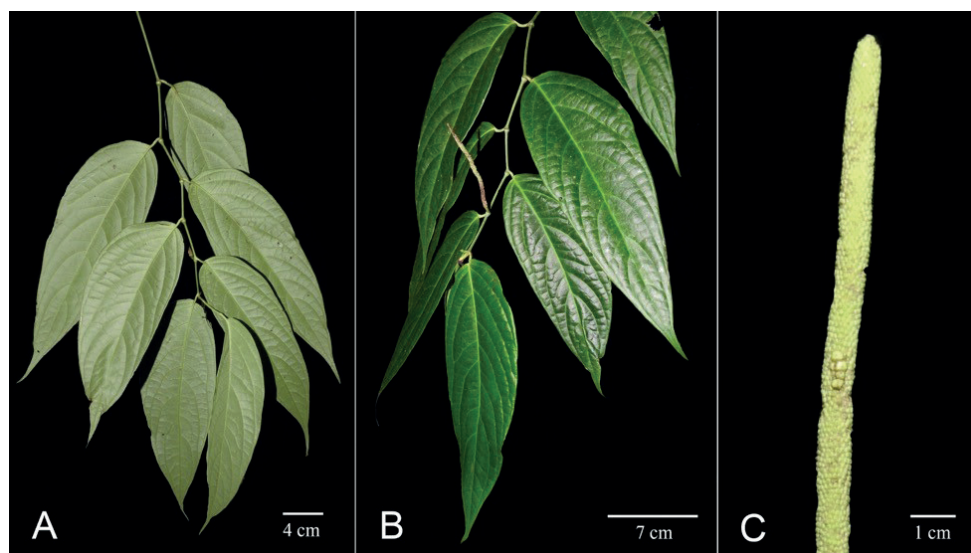


Figure 8. *Piper nokaidoyitau* **A** sympodial branch, lower leaf surfaces **B** sympodial branch, upper leaf surfaces and spikes **C** single infructescence. Photos from *F Hoyos & W Trujillo 046* by F Hoyos

Diagnosis. *Piper nokaidoyitau* W. Trujillo & M. A. Jaram., can be separated from the similar species *P. hostmannianum* (Miq.) C. DC. (1869:287), by its prophylls up to 2.4 cm long, leaves 12–20 cm long vs. prophylls 2.8–3.5 cm long, leaves 21–26 cm long in *P. hostmannianum*.

Description. *Shrub* up to 3 m tall; internodes (2.5–)3–4.5 cm long, canaliculate superficially, green, glabrous. **Prophylls** 2.8–3.2 cm long, green, glabrous, caducous, swollen in the basal portion (observable in live plants). **Petioles** uniform in size along all nodes, (0.7–)1–1.2(–1.5) cm long, sheathing at the base, smooth, glabrous. **Leaf-blades** coriaceous, drying black, uniform in shape and size along all axes, (4.5–)5–8 × (18) 21–26 cm, ovate, asymmetric, base rounded, glabrous on both surfaces, eciliate; pinnately nerved throughout, 4–5 ascending nerves on each side, eucampitodromous, with spacing decreasing and angle increasing towards the base, tertiary veins random, reticulate; apex acuminate. **Inflorescences** simple spikes, erect; peduncle 1–1.5 cm long, glabrous, green; rachis (7.5)10–12 × 0.3 cm in flower, 11–13 × 0.4–0.5 cm in fruit, flowers densely grouped along the rachis, forming bands around the spike. Floral bracts cucullate, heart-shaped from above, 0.2–0.35 × 0.4–0.7 mm, glabrous centrally on the abaxial surface, margin densely fimbriate. **Flowers** with three stamens, filaments 0.3–0.5 mm long, anthers 0.1–0.25 × 0.2–0.3 mm, transversally dehiscent, ditheous, with connective not protruding, glabrate, idioblasts not evident, colour black when dried; stigmas 3, 0.1–0.25 mm long, sessile. **Fruits** obpyriform in side view and triangular from above, green when alive, black when dry, 0.5–0.7 × 0.8–0.9 mm, glabrous, partially immersed in the rachis, with stigmas sessile and persistent.

Distribution and habitat. *Piper nokaidoyitau* is known from the lower montane forests in the eastern slopes of the Andes in Colombia, ca. 1,100 m elevation (Fig. 2),

the Department of Caquetá. It is a shade-loving species that grows in the understorey of preserved forest.

Phenology. Flowering specimens were collected in September and October. Fruiting specimens in October.

Etymology. *Piper nokaidoyitau* is named after the Huitoto name for *Piper* plants, “Nokaido yitau”. It means “the powers of the toucan” because these are sacred and medicinal plants used against fever, body and headaches and as anti-inflammatories.

Phylogenetic relationships. *Piper nokaidoyitau* belongs to the *Radula* clade of Neotropical *Piper* (Jaramillo et al. 2008). Specifically, *P. nokaidoyitau* is sister to the *Isophyllon* subclade, within *Radula*. *Isophyllon* species are mostly self-standing shrubs with coriaceous leaves, pinnately nerved, acute or obtuse bases and flowers densely arranged in erect inflorescences. *Isophyllon* species occur in the Atlantic Forest, Central America and the Amazon Region.

Conservation status. This species is known from four specimen collections representing two subpopulations. The locations where it occurs are threatened by deforestation and expansion of the agriculture frontier. The area of occupancy (AOO) of 8 km² is small, which, together with the continuing decline in habitat quality, suggests it is Endangered [EN B1a+B2a].

Comments. Leaves of *Piper nokaidoyitau* are pinnately nerved throughout with 4–5 ascending secondary veins on each side. This characteristic is shared by other species belonging to the *Radula* clade, specifically subclade *Isophyllon* (Jaramillo et al. 2008). We compared *P. nokaidoyitau* with similar species that occur on the eastern slope of the northern Andes (Table 4).

Table 4. Comparison of *Piper nokaidoyitau* with related species that have leaves pinnately nerved throughout and occur in the eastern slope of the northern Andes.

Species	Prophylls length	Leaf shape	Leaf base shape	Leaf length	Infructescence length	Geographic distribution
<i>P. anonifolium</i> Kunth	0.5–0.8 cm	elliptic	cuneate	(10)14–17 cm	3–3.5 cm	Amazonia
<i>P. hostmannianum</i> C. DC.	1–2 cm	elliptic	rounded	12–20 cm	10–11 cm	Amazonia
<i>P. nokaidoyitau</i> W. Trujillo & M. A. Jaram.	3–3.5 cm	ovate	rounded	21–26 cm	11–13 cm	Eastern slope of the northern Andes
<i>P. mastersianum</i> C. DC.	0.4–0.8 cm	ovate	rounded	9–12 cm	4–9 cm	Amazonia

Specimens examined. COLOMBIA. Caquetá, municipio de Florencia: corregimiento El Caraño, vereda El Caraño, finca Las Brisas, 01°44'14.7"N, 75°40'35.3"W, 1116 m elev., 18 Oct 2013 [fl], W. Trujillo et al. 3005 (COL); Corregimiento El Caraño, finca Las Brisas, 01°44'14.7"N, 75°40'35.3"W, 1116 m elev., 18 Oct 2013 [fl], W. Trujillo et al. 3022 (COL); Municipio de Belén de los Andaquíes: río Pescado, Parque Natural Andaquí, sector sur, 01°36'31"N, 75°55'16"W, 950 m elev., 25 Jun 2013, W. Trujillo et al. 2791 (HUAZ). Cauca. Municipio de Piamonte, corregimiento de Miraflores, vereda La Florida, camino a la reserva La Cristalina, 01°04'59.6"N, 76°28'08.2"W, 1146 m elev., 06 Jan 2021 [fr], E. Trujillo et al. 7249 (CUVC, JBB).

***Piper velae* W. Trujillo-C & M. A. Jaram., sp. nov.**

urn:lsid:ipni.org:names:77303989-1

Figs 1, 2, 9 and 10

Type. COLOMBIA, Caquetá, Belén de los Andaquies, corredor resguardo La Cerinda, PNN Alto Fragua Indiguazi, etnia Embera Katio, 1°36'08.6"N, 75°51'49.1"W, 470 m elev., 03 Oct 2007, *W. Trujillo et al.* 905 (Holotype COAH, Isotype HUAZ).

Diagnosis. *Piper velae* W. Trujillo & M. A. Jaram. can be distinguished from the related species *P. holdridgeanum* W.C. Burger by its elliptic leaves with cordulate leaf bases at all nodes, petioles 0.8–1.5 cm long, fruits cylindrical and pubescent vs. leaves cordate to elliptical with leaf bases that are rounded at fertile nodes and cordate at sterile nodes, petioles that are variable in size from 1–5 cm long, fruits rounded and glabrous in *P. holdridgeanum*. It can be separated from similar species *P. cornifolium* Kunth (1815 [1816]:52) because it has leaves pinnately nerved in the lower half of the blade and pubescent fruits vs. leaves pinnately nerved in the lower third of the blade and glabrous fruits in *P. cornifolium*.

Description. *Shrub* up to 1.5 m tall. Internodes 2–8.5 cm long, smooth, green, tomentulose, idioblasts not evident. **Prophylls** 1.2–2 cm long whitish, tomentulose, caducous. **Petioles** variable along all axes; on monopodial axes 1–1.5 cm long, vaginate to 3/4 of the length, smooth, tomentulose; on sympodial axes 0.8–1.2 cm long, vaginate at the base, smooth, tomentulose. **Leaf-blades** coriaceous, drying black, uniform in shape and size along all axes, 6–7(11) × 12–15(19) cm, elliptic, symmetric, base cordulate, basal extension asymmetrical; leaf blade smooth, tomentulose on the abaxial surface and glabrous adaxially, eciliate; pinnately nerved from the lower half, 4–5 ascending nerves on each side, festooned brochidodromous, with spacing decreasing and angle increasing towards the base, tertiary veins percurrent; apex acuminate. **Inflorescences** and infructescence a solitary spike, terminal, erect; peduncle 0.8–1.5 cm long, tomentulose, green; rachis in flower not seen, rachis in fruit 6–8.5 cm long, fruits densely grouped along the rachis. **Floral bracts** cucullate, triangular from above, 0.15–0.25 × 0.3–0.4 mm, glabrous on the adaxial surface, margin fimbriate, not forming bands around the spike. **Flowers** with four stamens, filaments 0.2–0.4 mm long, anthers 0.2–0.3 × 0.15–0.25 mm, longitudinally dehiscent, dithecal, shorter than filament, with connective not protruding, glabrate, idioblasts not evident, colour not seen. Stigmas 3, on a short style. **Fruits** cylindrical, laterally compressed, green when alive and black to brown when dry, 0.8–1.2 × 1–1.3 mm, pubescent on the tip, partially immersed in the rachis, with stigmas persistent, 0.07–0.12 mm long, on a short style, 0.1–0.3 mm long. **Seeds** 0.4–0.6 × 0.9–1.1 mm, rectangular, laterally compressed, obtuse, black.

Distribution and habitat. *Piper velae* occurs in the eastern slopes of the Andes, from 250–1,500 m in elevation, spreading from wet lowland to wet premontane forests. It occurs in the Colombian Departments of Caquetá, Meta, Cauca and Putumayo. In lowland forests, it occurs in dense *terra firme* forests. In premontane forests, it grows mostly on moderate slopes, sometimes occurring on steep slopes and rocky substrates. It is a shade-loving species, growing in the understorey and it is also found in forest gaps.

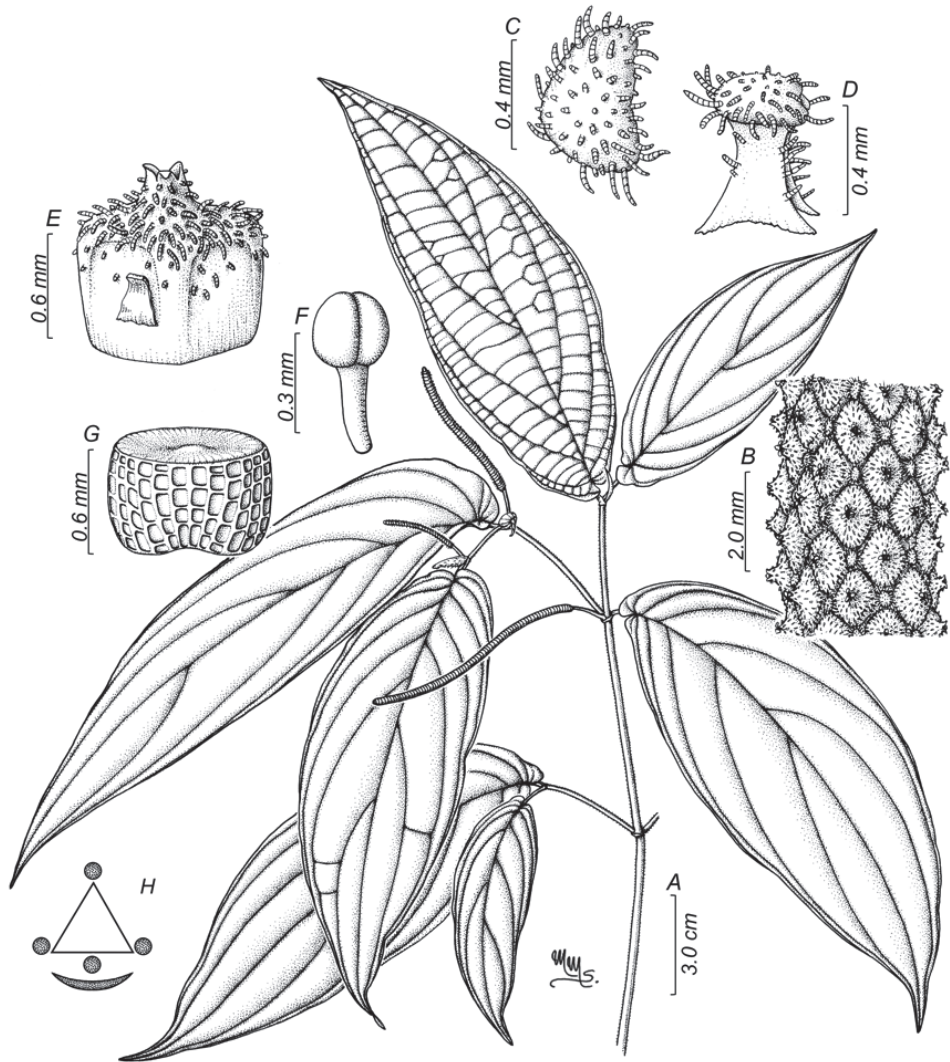


Figure 9 *Piper velae* **A** sympodial branch **B** magnified view of the infructescence **C** floral bract view from above **D** floral bract side view **E** fruit side view **F** stamen side view **G** Seed side view **H** floral diagram. Illustration by Marcela Morales based on *W Trujillo 905*

Phenology. Flowering specimens were collected in February, April, May, June, July and October. Fruiting specimens in January, April, June, July, September, October and December.

Etymology. *Piper velae* is named in honour of Huber Fernando Vela, M.D., a social and environmental leader of Caquetá who was murdered in 2021. Dr Vela and sponsored *Piper* collections by WT during 2020. Huber Fernando was the leader of the Nature Reserve Romi Kumu, where 30 ha of forest were restored in 2020. The type specimen of *P. velae* occurs in the region that Dr Vela loved and helped conserve and restore.

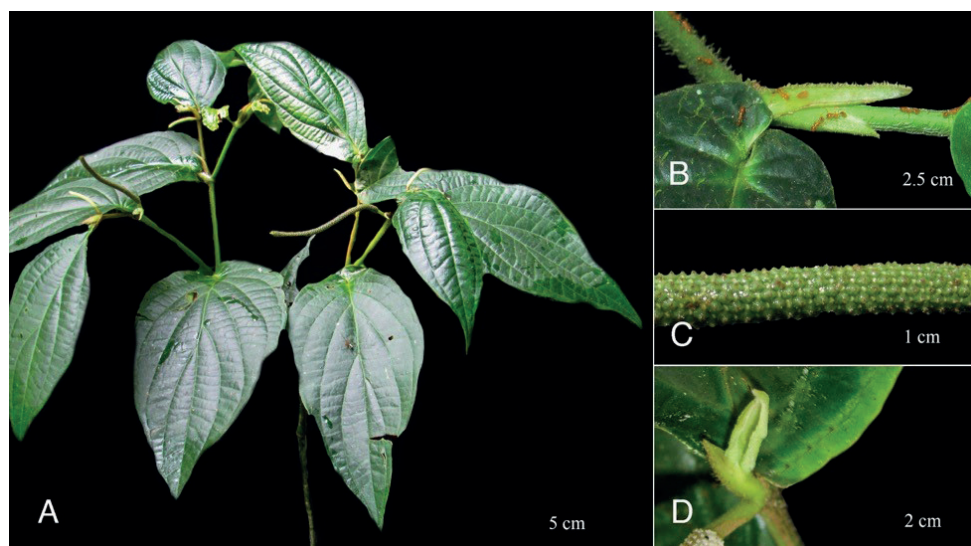


Figure 10 *Piper velae* **A** sympodial branch upper leaf surfaces and spike **B** magnified view of the leaf base and prophyll **C** magnified view of the spike **D** magnified view of the prophyll. Photos from W. Trujillo 2039 by William Trujillo.

Conservation status. This species is known from 41 specimen collections representing 12 subpopulations. It occurs in 18 locations threatened by deforestation. The extent of occurrence (EOO = 40,810 km², below the EOO to be considered Vulnerable, VU) and area of occupancy (AOO = 96 km²), suggest it is of Near Threatened [NT B1a+B2a].

Phylogenetic relationships. *P. velae* is sister to *P. holdridgeanum* and these form a clade sister to *Macrostachys* (Jaramillo et al. 2008). *P. velae* and *P. holdridgeanum* have sheathing petioles to $\frac{3}{4}$ of their length and tightly-arranged flowers. The marked foliar dimorphism between leaves on sterile (monopodial) and fertile (sympodial) nodes distinctive of *P. holdridgeanum* have obscured its relationships (Callejas-Posada 2020). Here, we present phylogenetic evidence for its placement sister to *Macrostachys*. Both species, *P. velae* and *P. holdridgeanum* require further study to understand their morphological affinities.

Discussion. *Piper velae* can be confused with *P. cornifolium*, because of its cordulate leaf base; however, these taxa are distinguished, based on the leaf venation pattern and fruit pubescence (see Table 5). We also compare *P. velae* to closely-related *P. holdridgeanum*; further studies will help us corroborate this relationship and find morphological similarities.

Specimen examined. COLOMBIA: **Caquetá:** Belén de los Andaquíes: Parque Bosque Microcuenca La Resaca, sendero Alto Sarabando, 800 m elev., 1°27'29"N, 75°53'1.8"W, 26 Oct 2010, D. Cárdenas et al. 40791 (COAH); vereda Las Verdes, río Pescado, margen izq. Parque Natural Municipal Andakí, 700 m elev., 1°36'7.2"N, 75°54'10"W, 28 Oct 2010, D. Cárdenas et al. 40896 (COAH); sector Paramillo, camino entre Acevedo - Belén de Andaquíes, 1400 m elev., 1°40'58"N, 75°54'21"W, 23 Jul 2011, D. Cárdenas et al. 41848 (COAH); cerca del río Pescado, vereda Los Angeles, 1225 m elev., 1°34'49.7"N, 75°54'17"W, 9 Jul 2011, L. Martínez 24 (COAH); río Pescado, Parque Natural Andakí, sector Sur, 950 m elev., 1°36'31"N, 75°55'16"W, 25 Jun 2013, W. Trujillo et al. 2792

(COAH). Cartagena del Chairá: vereda Laguna del Chairá, 240 m elev., 1°15'23.9"N, 74°48'49.8"W, 28 Sep 2007, *W. Trujillo et al.* 853 (COAH). Florencia: corregimiento El Caraño, vereda Alto Brasil, camino hacia la quebrada Yumal, 321 m elev., 1°39.543'N, 75°36.128'W, 22 Oct. 2012, *A. Jimenez et al.* 4 (HUAZ); río Hacha, vereda El Caraño, finca Marsella, 500 m elev., 1°41'47"N, 75°37'16"W, 26 Jun 2010, *D. Cárdenas et al.* 24884 (COAH, HUAZ); vereda El Paraíso, antigua vía Florencia-Neiva, zona de cordillera baja, 756 m elev., 1°45'7.4"N, 75°39'56.9"W, 20 Oct 2010, *D. Cárdenas et al.* 40568 (COAH); vereda El Paraíso Bajo, vegetación de cordillera baja, 800 m elev., 1°45'0.2"N, 75°37'8.2"W, 22 Oct 2010, *D. Cárdenas et al.* 40595 (COAH); Centro de investigaciones Macagual, 258 m elev., 1°29'59.8"N, 75°39'22.6"W, 13 Dec 2008, *W. Trujillo et al.* 1214 (COAH); vereda El Caraño, 1116 m elev., 1°44'14.7"N, 75°40'35.3"W, 18 Oct 2013, *W. Trujillo et al.* 3002 (COAH). La Montañita: vereda Los Morros, reserva Las Dalias, 300 m elev., 1°29'21.5"N, 75°24'17.6"W, *A. Meneses* 15 (HUAZ); vereda Itarca, Reserva Natural Itarca, 340 m elev., 1°32'53"N, 75°28'20"W, 30 Oct 2010, *D. Cárdenas et al.* 40956 (COAH); vereda Itarca, Reserva Natural Itarca, 330 m elev., 1°32'34.5"N, 75°28'19"W, 26 Apr 2011, *N. Castaño et al.* 3142 (COAH); Reserva Las Dalias, 382 m elev., 1°29'21.6"N, 75°24'17.6"W, 41050, *W. Trujillo et al.* 2086 (COAH). San Vicente del Caguán: inspección Guacamaya, vereda La Música, margen izquierda del río Caguán, 600 m elev., 2°20'46"N, 74°54'12.4"W, 12 Jul 2015, *D. Cárdenas et al.* 44401 (COAH). **Cauca:** Piamonte: La Libertad, 700 m elev., 1°6'41.78"N, 76°28'46.5"W, 22 Feb 2010, *W. Trujillo et al.* 1271 (COAH). Santa Rosa: Inspección de Santa Marta, vereda Diamante Alto, 1150 m elev., 1°14'N, 76°36'W, 22 Jun 2002, *B. Ramírez* 16061 (COAH). **Meta:** San Juan de Arama: sector del chorro Santo Domingo, Parque Nacional Natural Sierra de La Macarena, 520 m elev., 3°15'23"N, 73°57'50"W, 27 Oct 2019, *D. Cárdenas et al.* 52002 (COAH); vereda Monserrate Bajo, finca El Paraíso, cerca al caño Las Ninfas, 590 m elev., 3°20'33.75"N, 73°57'3.06"W, 4 Apr 2004, *L. Carvajal et al.* 173 (COAH). **Putumayo:** Mocoa: vereda Medio Afán, camino Serranía El Churumbelo, sector Nororiental, 900 m elev., 1°10'39"N, 76°38'47"W, 4 Oct 2000, *D. Cárdenas et al.* 12221 (COAH); vereda San José del Pepino, 1°5'40.17"N, 76°37'49.05"W, 21 Jun 1997, *R. López et al.* 2514 (COAH); vereda San José del Pepino, 1°5'40.17"N, 76°37'49.05"W, 21 Jun 1997, *R. López et al.* 2610 (COAH). Orito: Santuario de Flora y Plantas Medicinales Ingui-Ande, vereda Líbano, sector de don Reinaldo, 900 m elev., 0°41.62'N, 77°3.789'W, 1 Oct 2015, *D. Cárdenas et al.* 45416 (COAH); Santuario de Flora y Plantas Medicinales Ingui-Ande, 1004 m elev., 0°41.627'N, 77°3.801'W, 27 Sep 2015, *D. Cárdenas et al.* 45454 (COAH); vereda El Líbano, 832 m elev., 0°38'15"N, 77°4'17.9"W, *D. Cárdenas et al.* 51291 (COAH).

Table 5. Comparison of *Piper velae* with closely-related species *P. holdridgeanum* and morphological similar species *P. cornifolium*.

Species	Petiole length	Leaf shape	Leaf base	Secondary nerves branched	Fruit shape	Fruit pubescence	Geographic distribution
<i>P. cornifolium</i> Kunth	0.8–1.2 cm	obovate-elliptic	cordulate	from the lower third	rounded	pubescent	Northern Andes
<i>P. holdridgeanum</i> W.C. Burger	1–5 cm	cordate to elliptic	rounded to cordate	from the lower half	rounded	glabrous	Mesoamerica
<i>P. velae</i> W. Trujillo & M. A. Jaram.	0.8–1.5 cm	elliptic	cordulate	from the lower half	cylindrical	pubescent	Eastern slope of the northern Andes

Acknowledgements

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Campanula luzhijiangensis (Campanulaceae), a new species from Yunnan, southwest China

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Abstract

Campanula luzhijiangensis (Campanulaceae: Campanuleae) is described and illustrated as a new species from Yunnan, southwest China. The new species is mainly characterized by its relatively gracile stems polyphyllous, small and oblanceolate leaves, and flowers and fruits with small size within Chinese *Campanula*. It is only known from a single locality in the valley of the Luzhijiang River, usually occurring in the rock crevices, xerophilous scrubs or grasslands. A table of morphological characters comparing the new species with its closest relatives is provided along with a key to the species of *Campanula* from Yunnan Province, as well as a preliminary conservation assessment of *C. luzhijiangensis* under the IUCN criteria.

Keywords

Campanula mekongensis, Campanuleae, endemism, Luzhijiang Valley, morphology, taxonomy

Introduction

The tribe Campanuleae, comprising more than 620 species, is the largest tribe in the Campanulaceae *s. str.* (excluding Cyphiaceae, Cyphocarpaceae, Lobeliaceae, and Nemacladaceae) (Lammers 2007a, b; Xu and Hong 2020). Within this tribe, the generic classification still remains contentious, especially in the delimitation of genus *Campanula s. l.*, which was found to be polyphyletic by the recent molecular phylogenetic analyses (Roquet et al. 2008; Lakušić et al. 2013; Crawl et al. 2016; Liveri et al. 2019; Xu and Hong 2020). *Campanula s. l.* consists of about 420–600 species

(Lammers 2007b), most of which are perennials with alternate cauline leaves, flowers with radial flora symmetry and composed of a calyx with five persistent sepals, campanulate, tubular-campanulate, or funnelform corolla with five lobes, filaments dilated, an inferior ovary, and the capsule dehiscent at side. Members of this genus are widely distributed in temperate regions of the Northern Hemisphere (Borsch et al. 2009), and are especially abundant in the Mediterranean region and the Middle East (Fedorov and Kovanda 1978; Contandriopoulos 1984). They inhabit a wide range of habitats, including meadows, woodland-edges, moorlands, and cliffs, as well as steppe and mountainous habitats (Fedorov 1957; Kovacic 2004).

There are 22 species of *Campanula* in China, of which 11 are endemic (Wang and Hong 2000; Hong et al. 2011). During recent field investigations in Yunnan province of southwestern China, we found an unknown species of *Campanula*. After detailed comparison with morphologically similar taxa and extensive analysis of the relevant literature, it became clear it represents an undescribed species.

Materials and methods

This study followed the normal practice of plant taxonomic survey and herbarium taxonomy. Morphological studies of the new species were based on observation of living plants and specimens from the Luzhijiang Valley in Yimen County, Yunnan Province, southwest China. Morphological features were studied under a stereomicroscope (Olympus SZX2, Tokyo, Japan), and measurements were made using a ruler or a micrometer. Digital images of type specimens of its congeners available at the JSTOR Global Plants (<http://plants.jstor.org/>) and the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>), as well as relative collections housed at CDBI, KUN, PE, PYU and YUKU (acronyms according to Thiers 2022), were examined and compared with the new species. Pertinent taxonomic literature (e.g. Hong 1983, 2015; Huang 1991; Hong et al. 2011) were extensively consulted.

Taxonomy

***Campanula luzhijiangensis* Huan C. Wang & T. T. Wang, sp. nov.**

urn:lsid:ipni.org:names:77303990-1

Figs 1–3

Type. CHINA. Yunnan Province: Yimen County, Luzhi Town, Luzhijiang Valley, Xiaoluzhi, 24°40'53"N, 101°58'19"E, elev. 1450 m, 25 September 2021, *H. C. Wang et al.* YM15319 (holotype YUKU!, isotypes YUKU!).

Diagnosis. *Campanula luzhijiangensis* is most similar to *C. mekongensis* Diels ex C. Y. Wu, but clearly distinguished from the latter by its stems with numerous leaf scars at base, leaves usually oblanceolate, relatively small, 0.3–2.0 cm long, 0.1–0.3 (– 0.5) cm

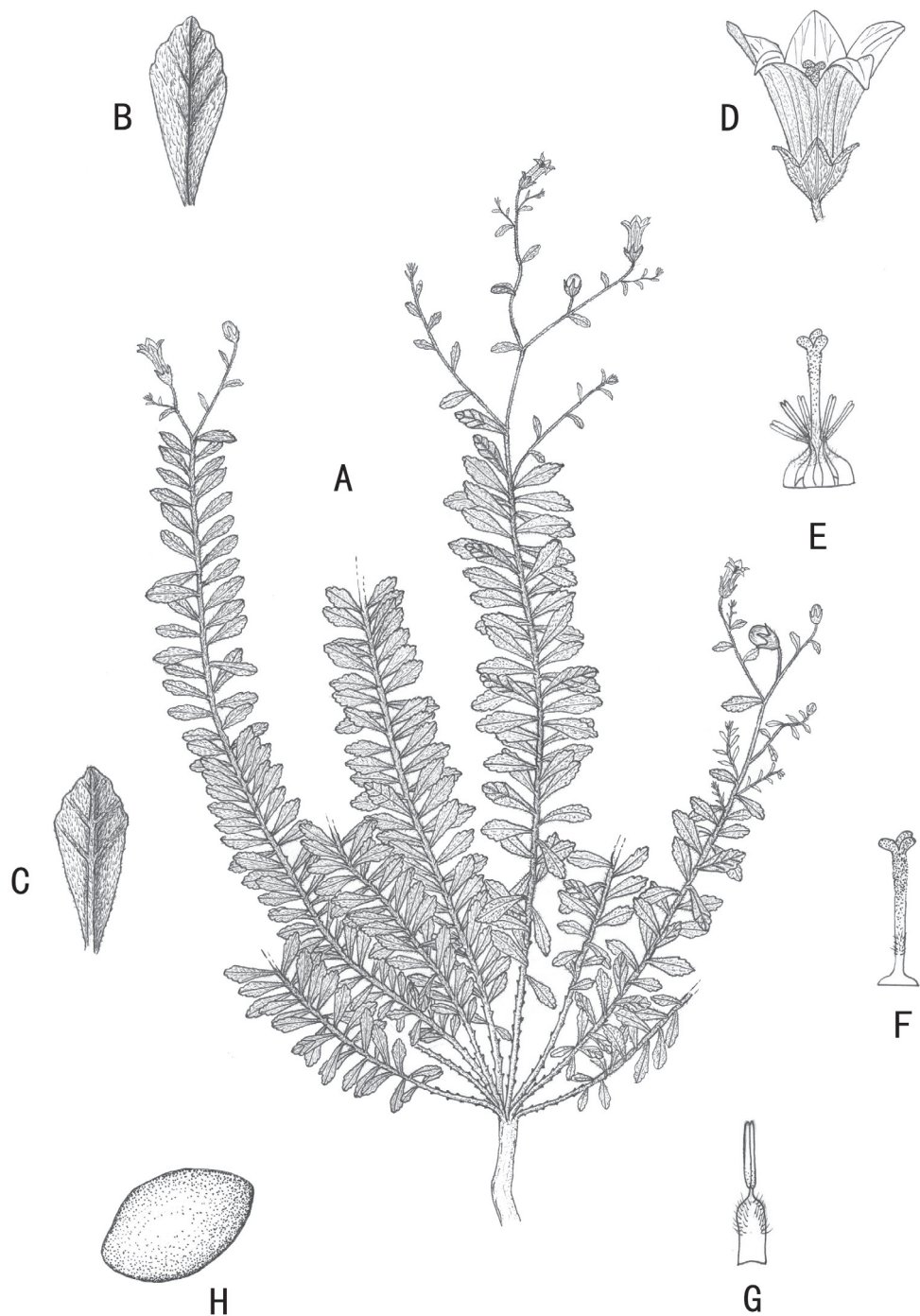


Figure 1. *Campanula luzhijiangensis* **A** habit **B** adaxial surface of leaf **C** abaxial surface of leaf **D** flower **E** style and stamens **F** style **G** stamen **H** seed.

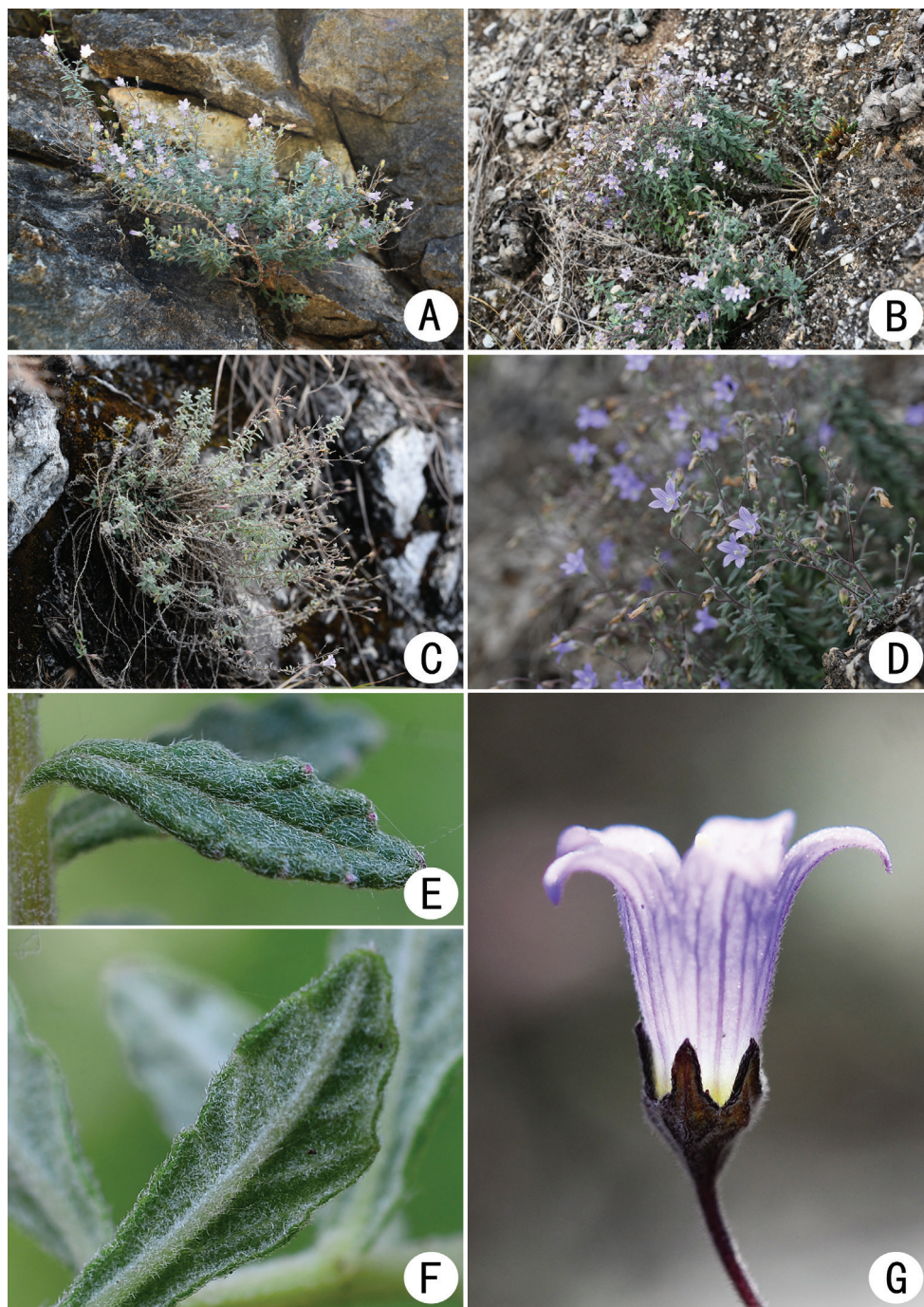


Figure 2. *Campanula luzhijiangensis* **A, B** habit **C** plants in fruiting stage **D** plants in flowering stage **E** adaxial surface of leaf **F** abaxial surface of leaf **G** flower (side view).

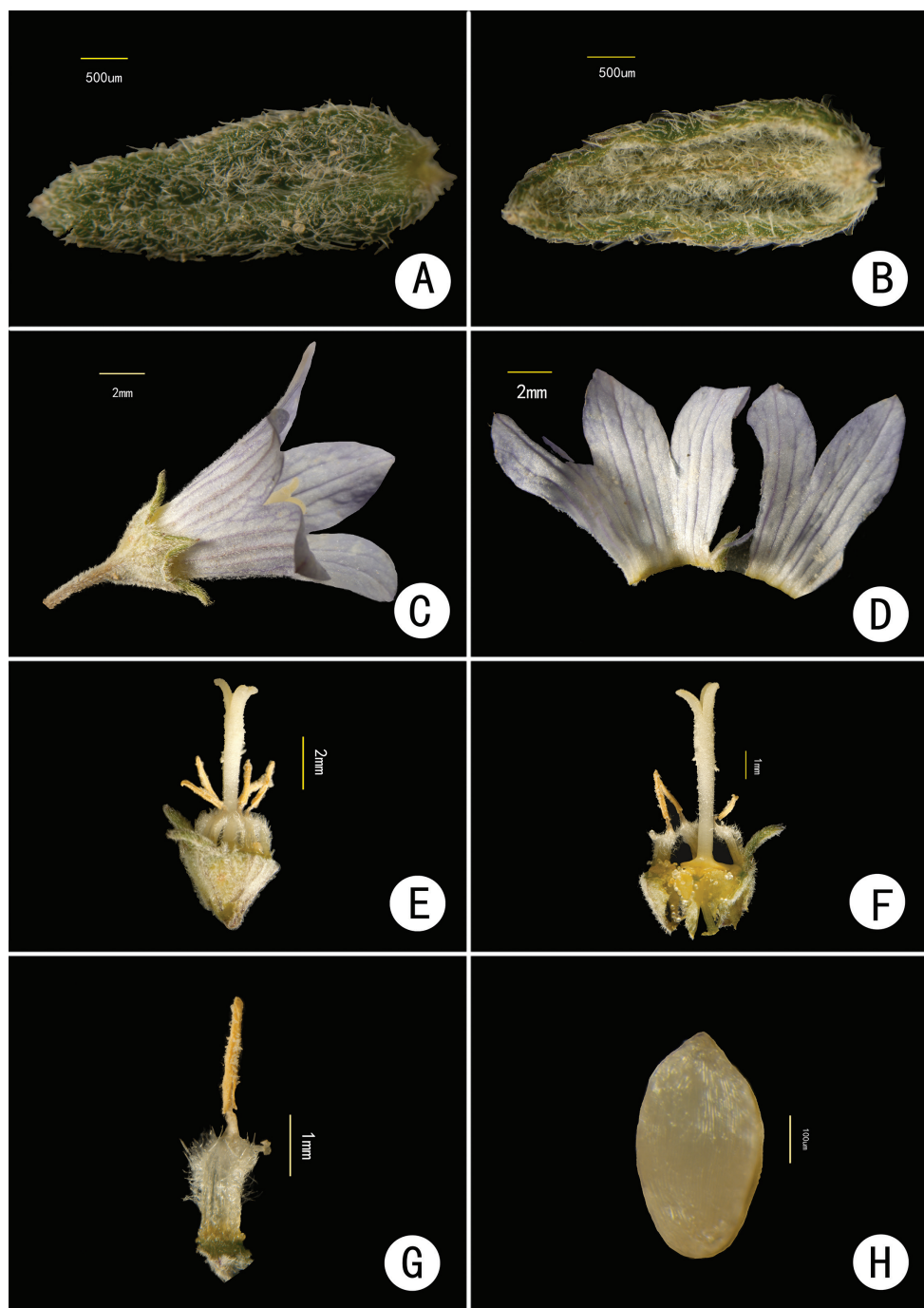


Figure 3. *Campanula luzhijiangensis* **A** adaxial surface of bract **B** abaxial surface of bract **C** flower (side view) **D** corolla dissected **E** stamens and pistil **F** style, stamens and dissected hypanthium **G** stamen **H** seed.

wide, margin subentire or sparsely crenate, slightly recurved, hypanthium densely villous throughout, calyx lobes usually ovate, 1–2 mm long, 1.0–1.5 mm wide, corolla tubular-campanulate, tube 2–4 mm in diameter. In contrast, *C. mekongensis* has stems without leaf scars at base, leaves oblong, narrowly obovate or oblanceolate, 0.5–3.0 cm long, 0.3–1.2 cm wide, margin not recurved, serrate, hypanthium only hispid along ribs, calyx lobes subulate, 2–4 mm long, less than 1 mm wide, corolla campanulate, tube 6–10 mm in diameter.

Description. Herbs perennial, caespitose. Rootstock woody, naked, with numerous ascending stems. Stems polyphyllous, usually simple at base, rarely branched, slightly lignified, purplish, densely white villous, 10–30 cm long. Leaves alternate, sessile or subsessile, basal leaves withering or caducous; blades usually oblanceolate, rarely elliptic, 0.3–2.0 cm long, 0.1–0.3 (–0.5) cm wide, abaxially densely villous, adaxially appressed pubescent, apex obtuse to acute, margin subentire or sparsely crenate, slightly recurved, base cuneate. Inflorescences terminal, thyriform; rachis and branches gracile, indumentum similar to that of the stems; bracts oblanceolate, lanceolate to linear, 0.1–0.2 cm long, 0.7–0.9 mm wide. Flowers erect or ascending, rarely reflexed; pedicels gracile, villous, 0.5–1.0 cm long, 0.2–0.3 mm in diameter; hypanthium obconic, longitudinally ribbed, densely spreading villous, base cuneate, calyx lobes ovate, 1–2 mm long, 1.0–1.5 mm wide at base, acute to acuminate at apex, margin slightly reflexed, serrulate. Corolla blue, blue-white or lilac, tubular-campanulate, 5–10 mm long, externally pubescent, internally glabrous; tube subconic, 4–8 mm long, 2–4 mm in diameter; corolla lobes ovate to ovate-lanceolate, or nearly oblong, 2.5–7.0 mm long, acute at apex. Stamens 5, included, shorter than style; filaments ca. 3 mm long, base dilated into flakes, dilated part nearly elliptic, densely villose, connivent around the style at the anthesis; anthers clavate, ca. 2 mm long, light yellow. Style slightly exerted, ca. 6 mm long, base glabrous, middle and lower part with hairs, upper part papillose; stigma 3-fid, 1.0–1.3 mm long. Capsule obconic, 3-poricidal toward base, apical calyx lobes persistent. Seed elliptic, shiny, 0.4–0.5 mm long, 0.2–0.3 mm wide.

Phenology. *Campanula luzhijiensis* has a relatively long flowering period; it usually flowers from August to January of the following year, and fruits from September to February.

Etymology. The specific epithet *luzhijiensis* is derived from the type locality of the new species, the Luzhijiang Valley, and the Latin suffix *ensis*, indicating the place of origin or growth.

Habitat and distribution. *Campanula luzhijiensis* appears to be a rare species endemic to Yunnan, southwest China. It is only known from the type locality in the valley of the Luzhijiang River, an upper tributary of the Hong (Red) River that flows from Yunnan in southwest China through northern Vietnam to the Gulf of Tonkin (Fig. 4). The climate in Luzhijiang Valley is semi-dry and hot, with an annual average temperature of 21.0 °C and a total annual precipitation of 822.8 mm. *Campanula luzhijiensis* usually occurs in the rock crevices, xerophilous scrubs or grasslands between 1250 and 1500 m elevation. Associated vegetation includes *Phyllanthus emblica* Linn. (Phyllanthaceae), *Paliurus orientalis* (Franch.) Hemsl. (Rhamnaceae), *Dalbergia yunnanensis* Franch. (Fabaceae), *Symphoricarpos sinensis* Rehd. (Caprifoliaceae),

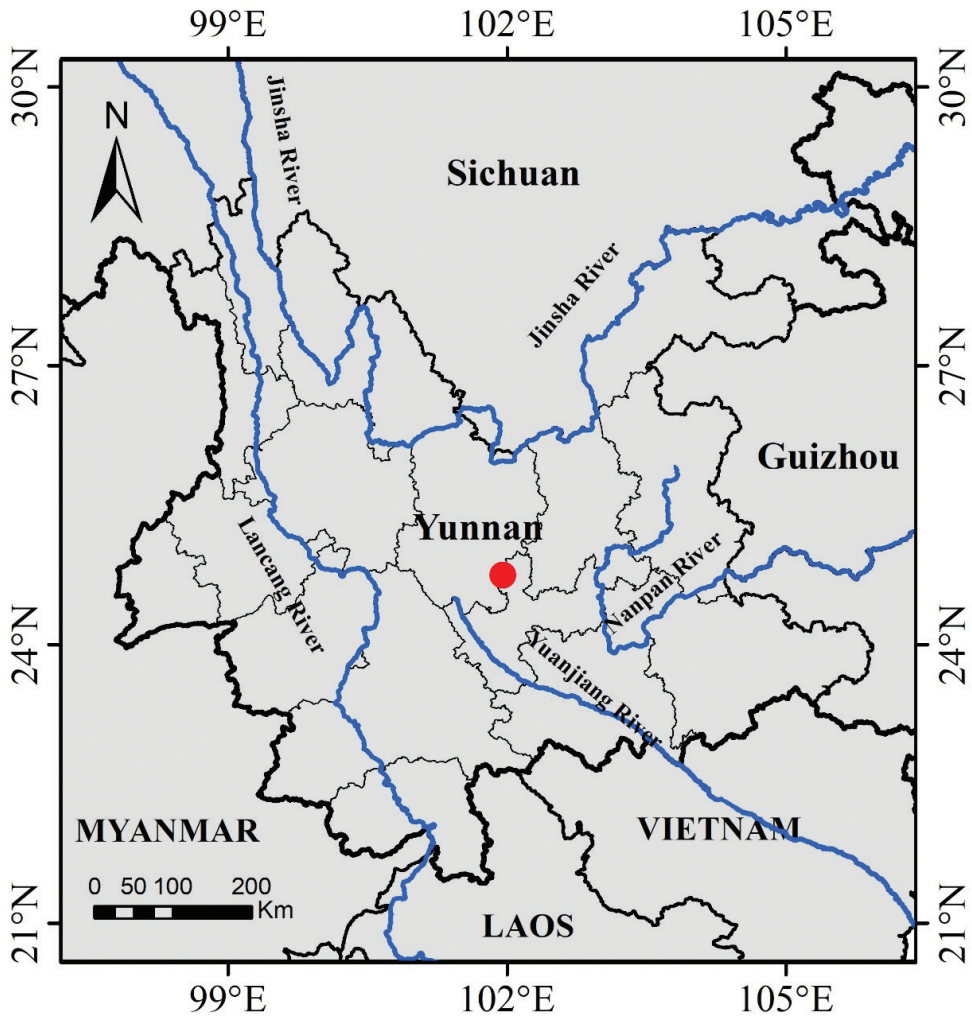


Figure 4. Geographical distribution of *Campanula luzhijiangensis* (red dot).

Duhaldea lachnocephala Huan C. Wang & Feng Yang (Asteraceae) (an endemic species described by Yang et al. (2022)), *Pterygiella luzhijiangensis* Huan C. Wang (Orobanchaceae), *Silene otodonta* Franch. (Caryophyllaceae), *Spodiopogon sagittifolius* Rendle (Poaceae), *Heteropogon contortus* (Linn.) Beauv. ex Roem. et Schult. (Poaceae) and *Themeda caudata* (Nees ex Hooker et Arnott) A. Camus (Poaceae).

Conservation status. *Campanula luzhijiangensis* is a rare species with a restricted distribution and small population size. Currently, it is only known from a single locality in the Luzhijiang River Valley in the Yimen County, southwest China, where the estimated area of occupancy (AOO) is less than 20 km². The total population size is estimated to be fewer than 500 mature individuals. Following the IUCN guidelines (IUCN 2012, 2022), this new species should be classified as Vulnerable [VU (D1, D2)].

Discussion

On the basis of living and herbarium materials, *Campanula luzhijiangensis* is well differentiated from the other species of the *Campanula* found in southwest China and the adjacent regions in several and significant features regarding the vegetative and reproductive structure. In particular, it is characterized by its relatively gracile stems polyphyllous, small and oblanceolate leaves, small flowers and fruits. Morphologically, *C. luzhijiangensis* is most similar to *C. mekongensis*, an endemic found in southwest (Xishuangbanna) and northwest (Nujiang Valley) Yunnan Province, southwest China (Hong 2015), and it is somewhat close to *C. cana* Wall. in sharing similar indumentum, and to *C. pallida* Wall., a variable and common species also found in southwest China, in inflorescence structure and shape of calyx lobe. Nevertheless, there are several morphological features distinguishing *Campanula luzhijiangensis* from the other three species (see Table 1). Species of *Campanula* found in Yunnan Province, southwest China, can be distinguished through the morphological characters presented in the following identification key modified from Hong (1983, 2015) and Hong et al. (2011).

Table 1. Morphological comparison of *Campanula luzhijiangensis*, *C. mekongensis*, *C. cana* and *C. pallida*.

Characters	<i>C. luzhijiangensis</i>	<i>C. mekongensis</i>	<i>C. cana</i>	<i>C. pallida</i>
Stem length (cm)	10–30	20–30	15–30	20–50 (–60)
Stem indumentum	densely white villous	pilose to sparsely villous	densely white villous, sometimes tomentose	hirsute to hispid
Leaf scars at lower part of stem	numerous	absent	absent or few	absent or few
Leaf shape	oblanceolate	oblong, narrowly obovate or oblanceolate	ovate, elliptic, oblanceolate or linear-lanceolate	elliptic, rhombic-elliptic or oblong
Leaf size (cm)	0.3–2.0 × 0.1–0.3 (–0.5)	0.5–3.0 × 0.3–1.2	0.4–2.5 × 0.3–1.0	1.0–4.0 × 0.3–1.5
Leaf apex	obtuse to acute	usually acute, rarely obtuse	obtuse to acute	acute to acuminate
Leaf margin	subentire or sparsely crenate, slightly recurved	serrate, not recurved	subentire, crenulate, or serrate, slightly recurved	denticulate or almost entire, not recurved
Flower	erect or ascending, rarely reflexed	ascending to nodding	erect or ascending, sometimes pendent	usually pendent
Length of flower pedicel (cm)	0.5–1.0	up to 2.0	0.5–3.0	0.4–2.0
Hypanthium	obconic, externally spreading villous	obconic or campanulate, only hispid along ribs	obdeltoid to broadly obconical, externally villous to tomentose	obconic or campanulate, externally hirsute to hispid
Shape of calyx lobes	ovate	subulate	subulate or narrowly triangular	deltoid, narrowly triangular, or subulate
Size of calyx lobes (mm)	1–2 × 1.0–1.5	2–4 mm long, less than 1 mm wide	3–5 × 2–3	2–8 × 2–5
Length of corolla (mm)	5–10	8	10–15	4–15
Diameter of corolla tube (mm)	2–4	6–10	2–7	5–10

Identification key to the species of *Campanula* found in Yunnan Province, China

- 1 Capsule poricidal toward base; stems with numerous flowers, solitary or in various types of inflorescences; leaves many and evenly distributed on stems; basal leaves usually wilted at anthesis **2**
- Capsule poricidal above middle; flowers solitary, terminal, or several terminal on main stems and branches; cauline leaves mostly toward base, upper cauline leaves sessile or nearly so, usually linear if present; basal leaves persistent at anthesis **8**
- 2 Annual herbs; rosulate basal leaves sometimes present at anthesis..... ***C. dimorphantha***
- Perennial herbs; basal leaves often absent at anthesis **3**
- 3 Stems with numerous leaf scars at base; flowers small, calyx lobes 1–2 mm long, corolla 5–10 mm long..... ***C. luzhijiangensis* sp. nov.**
- Stems with few or without leaf scars at base; flowers relatively large, calyx lobes 2–10 mm long, corollas more than 10 mm long **4**
- 4 Calyx tube hairy only along veins; lateral branches with several flowers; calyx lobes subulate, sinus between lobes truncate-obtuse..... ***C. mekongensis***
- Calyx tube densely hairy; lateral branches with a solitary flower or single simple inflorescence; calyx lobes subulate-triangular to deltoid, overlapping, or sinus acute **5**
- 5 Calyx lobes deltoid, with a pair of large teeth; stems long and prostrate; cauline leaves of lower half of stem wilted at anthesis, rest of leaves pannose abaxially, sessile, suborbicular..... ***C. yunnanensis***
- Calyx lobes subulate-triangular, rarely subdeltoid, with or without teeth; stems erect or diffuse; lower cauline leaves often present at anthesis; leaves sparsely hispid or densely pannose abaxially, elliptic, rhombic, or linear-elliptic **6**
- 6 Style strongly exserted; anthers completely or partially connivent ***C. chinensis***
- Style included; anthers completely free **7**
- 7 Calyx lobes narrowly triangular to subdeltoid, toothed or not; leaves often hispid, less frequently pannose abaxially; stems single or several from one caudex, erect or ascending..... ***C. pallida***
- Calyx lobes subulate-triangular to narrowly triangular, rarely toothed; leaves densely pannose abaxially; stems usually numerous from one caudex, often diffuse, less often ascending ***C. cana***
- 8 Plants with horizontal rhizomes; stems simple; stems and leaves glabrous ... **9**
- Plants without horizontal rhizomes; stems simple or branched; stems and leaves variously pubescent **10**
- 9 Hypanthium narrowly cylindrical; calyx lobes filiform, longer than corolla... ***C. aristata***
- Hypanthium obovoid or obconic; calyx lobes subulate or narrowly triangular, shorter than corolla..... ***C. modesta***

10	Flowers pendent.....	11
–	Flowers erect.....	12
11	Plants 20–50 cm tall; calyx lobes 1–5.5 mm; corolla lobes as long as tube	<i>C. delavayi</i>
–	Plants 6–33 cm tall; calyx lobes 3–12 mm; corolla lobes ca. 1/2 as long as tube	<i>C. crenulata</i>
12	Basal leaves cordate-reniform; stems slender but not filiform, hairy, lower half with cordate to ovate-lanceolate leaves; capsule 4–8 mm.....	<i>C. calcicola</i>
–	Basal leaves cordate; stems filiform, glabrous or subglabrous, with mostly linear leaves; capsule 9–19 mm	<i>C. chrysosplenifolia</i>

Additional specimens examined (paratypes): CHINA. Yunnan: Yimen County, Luzhi Town, Xiaoluzhi, 20 October 1965, *W. M. Zhu et al.* 1375 (YUKU); *ibid.*, 9 August 2016, *H. C. Wang et al.* YM1052 (YUKU); *ibid.*, 3 October 2016, *H. C. Wang et al.* YM1270 (YUKU); *ibid.*, 18 January 2018, *H. C. Wang et al.* YM8028 (YUKU); *ibid.*, 12 November 2019, *H. C. Wang et al.* YM8304, YM8327 (YUKU).

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A new species of *Quercus* genus (Fagaceae) from Son Tra Peninsula, Central Vietnam

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Abstract

A new species, *Quercus sontraensis* Ngoc, Binh & Son is described from Son Tra Nature Reserve, Son Tra Peninsula, Central Vietnam. We examined the morphology and constructed a highly resolved phylogeny of *Q. sontraensis* and its relatives (including *Q. langbianensis* and *Q. cambodiensis*) using Multiplex ISSR genotyping by sequencing (MIG-seq). The morphological analyses and molecular evidence support the distinction between the new species (*Q. sontraensis*) and its relatives.

Keywords

Da Nang City, flora, MIG-seq, phylogeny, taxonomy

Introduction

The genus *Quercus* contains more than 500 species worldwide, amongst which about 400 species from the Americas, Europe, North Africa and Macaronesia and about 125 species were reported from Asia (Govaerts and Frodin 1998; Borazan and Babaç 2003; Hubert et al. 2014), 50 of which are distributed in Vietnam (Ho 2003; Ban 2005; Li et al. 2016; Binh et al. 2018a, b, c; Binh et al. 2021). *Quercus* species are usually trees or sometimes shrubs that mostly occur in tropical montane forests in Southeast Asia and are often dominant in temperate deciduous forests in East Asia, Europe, North America, the Mediterranean

and in desert scrubs of North America (Nixon 1993; Huang et al. 1999; Phengklai 2008; Hubert et al. 2014; Valencia-A et al. 2016). The members of the genus are characterised by staminate inflorescences lax spikes or dichasia (“catkins”), carpellate flowers in stiff spikes (actually double spikes, but with reduced side flowers, leading to effective solitary flowers), subtended by an indehiscent cupule with cupule bracts being connate or forming concentric or spiral rings or free and imbricate cupule bracts; capitate or dilated stigma (Kaul 1985; Nixon 1993; Huang et al. 1999; Manos et al. 1999; Phengklai 2008).

Son Tra Nature Reserve is located on the Son Tra Peninsula of Da Nang City (Fig. 1) and was established in 1977 with a total area of 3.871 ha (Tran et al. 2019). The vegetation in the Son Tra Nature Reserve is characterised as a semi-evergreen seasonal forest (Wright 1996) with 1,032 species of vascular plants (483 genera from 145 families) recorded (Son et al. 2018).

During a field excursion to the Son Tra Nature Reserve carried out in 2016 and 2019, we collected specimens of *Quercus* from 340 m to 430 m altitude, which we were unable to allocate to a described species. Further studies, based on specimens in the herbaria such as DLU, FU, HN, P and VNM, the digitised specimen images on the website of JSTOR Global Plants and literature on related species (Camus 1936–1954; Huang et al. 1999; Ho 2003; Ban 2005; Phengklai 2008; Li et al. 2016; Binh et al. 2018a, b, c; Binh et al. 2021), showed that the samples were morphologically distinct from previously known taxa of the genus *Quercus*. In addition to using morphological evidence, the evidence from molecular analyses is helpful for delimiting species of the Fagaceae family. Specifically, Binh et al. (2018b), Ngoc et al. (2021) and Ngoc et al. (in press) successfully used molecular markers of both classic and next-generation sequencing methods to construct a highly resolved phylogenetic tree of the species of *Quercus* and *Lithocarpus*, as well as to describe new species from these genera. For the genus *Quercus*, recently, *Q. xuanlienensis* Binh, Ngoc & Bon (Binh et al. 2018a), *Q. trungkhanhensis* Binh & Ngoc (Binh et al. 2018c), *Q. baolamensis* Binh & Ngoc, *Q. bidoupensis* Binh & Ngoc and *Q. honbaensis* Binh, Tagane & Yahara (Binh et al. 2018b) have been described as new species, based on both morphological and molecular evidence.

By combining the molecular evidence and morphological observations, we describe and illustrate the unknown species from Son Tra as *Quercus sontraensis* Ngoc, Binh & Son. Additionally, this study used Multiplex ISSR genotyping by sequencing (MIG-seq; Suyama and Matsuki 2015) to determine the identities and phylogenetic relationship of new species from Son Tra and its close species from Vietnam and surrounding countries.

Materials and methods

Plant materials

A total of fifteen samples of *Quercus* from Vietnam and Cambodia including, *Q. sontraensis* and its related species, were collected for molecular analysis. Three samples of *L. dahuoaiensis*, *L. vuquangensis* and *L. vinhensis* were included as an outgroup in the

Table 1. Sample list for MIG-seq analysis in this study.

Country	Area	Voucher	Elevation	Species
Vietnam	Lam Dong	V9723	1930 m	<i>Quercus lanata</i>
	Lam Dong	V3172	890 m	<i>Q. setulosa</i>
	Cao Bang	V6066	767 m	<i>Q. trungkhanhensis</i>
	Da Nang	V3156	340 m	<i>Q. austrocochinchinensis</i>
	Da Nang	V3113	1310 m	<i>Q. poilanei</i>
	Lam Dong	V10132	1630 m	<i>Q. poilanei</i>
	Lam Dong	V9884	1637 m	<i>Q. braianensis</i>
	Lam Dong	V4445	1464 m	<i>Q. braianensis</i>
	Lam Dong	V10069	1867 m	<i>Q. bidouensis</i>
	Lam Dong	V10090	1884 m	<i>Q. bidouensis</i>
	Lam Dong	V5537	N/A	<i>Q. djiringensis</i>
	Lam Dong	V10061	1867 m	<i>Q. langbianensis</i>
	Lam Dong	V9972	1430 m	<i>Q. langbianensis</i>
	Da Nang	QC201	340 m	<i>Q. sontraensis</i>
	Ha Tinh	V5743	1518 m	<i>L. vuquangensis</i>
	Nghe An	V3787	1062 m	<i>L. vinhensis</i>
	Lam Dong	V3194	225 m	<i>L. dabuoaiensis</i>
Cambodia	Bokor	C4302	844 m	<i>Q. cambodiensis</i>

inference of the phylogenetic tree. The information on samples used for molecular analysis in this study is shown in Table 1.

In addition, the following specimen vouchers of *Q. cambodiensis*: *Poilane 215* (P [P00379257, P00379258] and NY [NY00253790]), *Poilane 270* (P [P06872434, P06872435]), *Tagane et al. C4302* (FU) and *Toyama et al. 1834* (FU), the following specimen vouchers of *Q. langbianensis*: *Chevalier 30029* (P [P00379254, P00379255, P00379256]), *Tagane et al. V4465, V4165, V4166, V3962* (DLU, FU) and the following specimen vouchers of *Q. sontraensis*: *Son et al. QC201* (DLU, VAFS: three duplicates), *Son H.T. QC202* (DLU, VAFS: two duplicates) and *Son H.T. QC203* (DLU, VAFS) were selected to measure the key morphological characters in the morphological analysis (a total 33 leaf of each species were measured).

Morphological analysis

To identify new samples that were collected from Son Tra Nature Reserve (Fig. 1), we referred to taxonomic literature of the genus *Quercus* in Vietnam and its surrounding countries (Camus 1936–1954; Soepadmo 1972; Huang et al. 1999; Ho 2003; Ban 2005; Phengklai 2008; Li et al. 2016; Binh et al. 2018a, b, c). Moreover, our specimens were also examined and compared with herbarium specimens at DLU, FU, HN, P and VNM, as well as images of type specimens on virtual herbaria websites (such as JSTOR Global Plants and the Chinese Virtual Herbarium).

Additionally, to provide strong evidence for the new species, we analysed and compared several key morphological characteristics including petiole length, leaf blade length and width and leaf blade aspect ratio, based on specimens of the new species

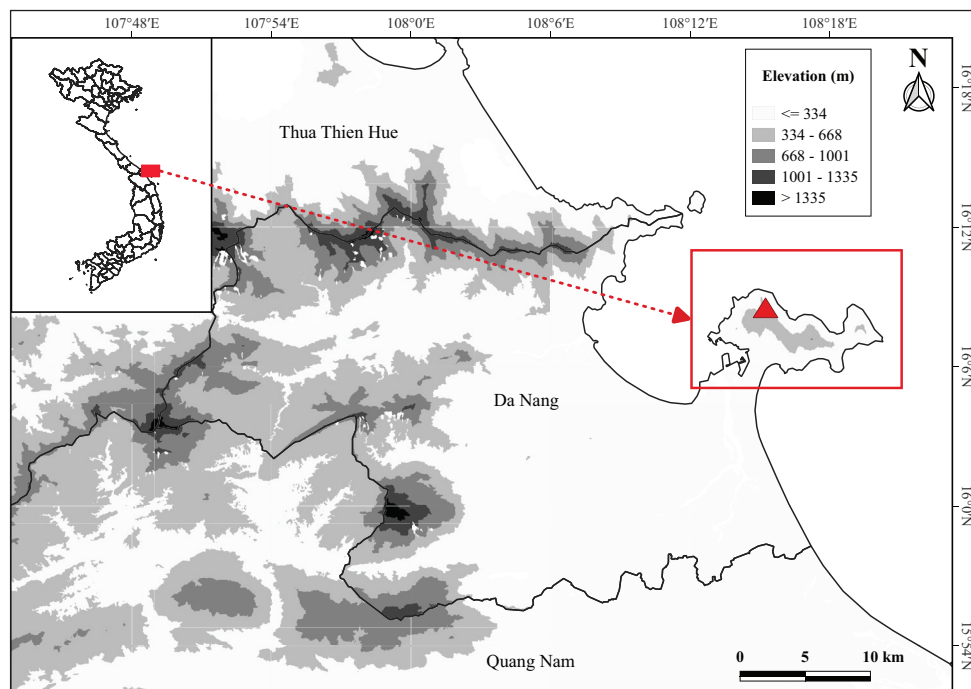


Figure 1. Study site: The area inside the red rectangle is Son Tra Peninsula; the red triangle is the type locality of *Quercus sontraensis* Ngoc, Binh & Son.

and its related species. We used ImageJ (Schneider et al. 2012) to measure the above-mentioned morphological characters.

To evaluate differences amongst species, analysis of variance (ANOVA) and Game-Howell post hoc test (Games and Howell 1976) were performed to confirm whether the differences were statistically significant (with the mean difference being significant when $p < 0.05$). The RStudio ver. 1.4.1106 (RStudio Team 2021) with ggstatsplot package (Patil 2021) and other built-in packages available in R were used to perform all statistical analyses in this study.

DNA extraction

For DNA extraction, we used the dried leaf material and milled them into fine powder with a QIAGEN TissueLyser. Then the powder was washed three times by 1 ml buffer solution (comprising 0.1 M HEPES, pH 8.0; 2% Mercaptoethanol; 1% PVP; 0.05% Ascorbic acid) (Toyama et al. 2015). Finally, DNA was isolated from the washed powder by using the CTAB method (Doyle and Doyle 1987) with a slight modification by Toyama et al. (2015).

Next-generation DNA sequencing

Fifteen DNA samples of eleven *Quercus* species were used to amplify thousands of short sequences by using the primers of “multiplexed ISSR genotyping by sequencing”

(MIG-seq: Suyama and Matsuki 2015). We performed two steps of PCR following the protocol of Suyama and Matsuki (2015) with minor modifications as in Binh et al. (2018b). PCR products of the 2nd PCR step were pooled as a single mixture library then we purified the mixture. Subsequently, we selected fragments of the size range 350–800 bp from the purified mixture by using a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Then approximately 10 pM of libraries were measured by quantitative PCR (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) and used for sequencing in an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit v.3 (150 cycles, Illumina) (Suyama and Matsuki 2015; Binh et al. 2018a, b).

Phylogenetic tree construction

The raw data of DNA sequence were pretreated following the published protocol (Suyama and Matsuki 2015; Binh et al. 2018b; Ngoc et al. 2021) by using the trimmomatic software version 0.40 (Bolger et al. 2014). The Stacks 2.41 pipeline (Catchen et al. 2013; Rochette et al. 2019) with the parameters set as described by Takata et al. (2019) and Ngoc et al. (2021) were used for de novo SNP discovery. The Maximum Likelihood tree was constructed by using the RAxML ver. 8.2 (Stamatakis 2014), based on the genome-wide SNPs dataset with the GTR+G model as selected by jMrModeltest 2.1.10 (Darriba et al. 2012) and examined its reliability by bootstrapping using 1000 replicates.

Results

Morphological comparison of the new species with its close species

Quercus sontraensis is morphologically most similar to *Q. cambodiensis* Hickel & A.Camus and *Q. langbianensis* Hickel & A.Camus. The comparison of key morphological characters amongst *Q. sontraensis*, *Q. cambodiensis* and *Q. langbianensis* are shown in Tables 2 and 3.

According to the original description of *Q. cambodiensis*, Hickel and Camus (1923) described this species, based on two collections by Eugène Poilane (*Poilane 215* and *Poilane 270*) from Cambodia. Of which, *Poilane 215* has three duplicates in P and NY Herbaria (P00379257, P00379258 and NY 00253790) and *Poilane 270* has two duplicates in P (P06872434 and P06872435). Amongst those specimens, only *Poilane 215* [P00379257] represents the diagnostic traits of nuts and cupules. The new species is most similar to *Q. cambodiensis* in lanceolate to elliptic leaves, densely golden hairy cupules with 7–8 rings and densely golden hairy nuts, but differs from *Q. cambodiensis* in having a distinctly serrate leaf margin in upper 1/3 (vs. leaf margin almost entire or with a few low teeth in upper 1/4) and cupules with a bract margin distinctly toothed in all rings, except two upper rings (vs. distinctly toothed only in two lower rings in *Q. cambodiensis*). *Quercus sontraensis* is also morphologically similar to *Q. langbianensis* Hickel & A.Camus (types: *Chevalier 30029*, P [P00379254, P00379255, P00379256]) in leaf

Table 2. Morphological comparison amongst *Quercus sontraensis* Binh, Ngoc & Son, sp. nov., *Quercus cambodiensis* Hickel & A.Camus and *Quercus langbianensis* Hickel & A.Camus.

Characters	<i>Q. sontraensis</i>	<i>Q. cambodiensis</i> ^(1,2,3)	<i>Q. langbianensis</i> ^(4,5,6)
Young shoot	Curly golden hairy	Golden tomentose	Golden tomentose
Leaf margin	Regularly and distinctly serrate on upper 1/3–1/4(–1/5)	Almost entire or with a few low teeth in upper 1/4	Distinctly serrate in upper 1/3
Length of petioles	(0.7–)1–1.5 cm	1–2.2 cm	1–2 cm
Number of secondary veins	(8–)11–14 pairs	7–11 pairs	10–12 pairs
Infructescence	Each infructescence with 1–3 acorns	Each infructescence with 4 acorns	Each infructescence with 2 acorns
Cupule shape	Bowl-shaped	Cup-shaped	Cup-shaped
Number of rings on cupule	7–8 rings	7–8 rings	6–9 rings
Margin of rings on cupule	Distinctly toothed in all rings, except two upper rings	Distinctly toothed in two lower rings	Distinctly toothed in all rings
Nut enclosure by cupule	Enclosing 1/3 of the nut	Enclosing < 1/2 of the nut	Enclosing 1/3 of the nut
Nut shape	Broadly ellipsoid	Obovoid to ellipsoid	Obovoid to ellipsoid
Base of the nut	Slightly convex	Slightly convex	Convex

⁽¹⁾ From the material *E. Poilane* 215 (P00379257) ⁽²⁾ From the original description in Hickel and Camus (1923)
⁽³⁾ From the material *Tagane et al. C4302* (FU) ⁽⁴⁾ From the original description in Ann. Sci. Nat., Bot. X, 3: 382 1921
⁽⁵⁾ From the material *Chevalier* 30029 (P) ⁽⁶⁾ From the material *Tagane et al. V4166* (DLU)

Table 3. The comparisons of mean (X) and standard deviation (SD) value of the leaf blade amongst *Quercus sontraensis*, *Q. cambodiensis* and *Q. langbianensis*. ¹Derived from type specimens, ²Derived from our collections, n = number of leaves measured in this study.

Parameters	<i>Q. sontraensis</i> ¹			<i>Q. langbianensis</i> ^{1,2}			<i>Q. cambodiensis</i> ^{1,2}		
	X	SD	n	X	SD	n	X	SD	n
Leaf blade length (cm)	9.27	1.93	33	10.54	2.02	33	7.76	2.25	33
Leaf blade width (cm)	2.89	0.59	33	3.77	0.80	33	2.57	0.66	33
Petiole length (cm)	1.0	0.24	33	1.62	0.43	33	1.61	0.51	33
Leaf blade aspect ratio	3.27	0.73	33	2.83	0.33	33	3.02	0.43	33

type specimens, ²Derived from our collections, n = number of leaves measured in this study.

shape, leaf margin distinctly serrate in upper 1/3 and cupules covering 1/3 of a nut. However, *Q. sontraensis* is distinct from *Q. langbianensis* in having bowl-shaped cupules (vs. cupules cup-shaped), cupule bract margin distinctly toothed in all rings, except two upper rings (vs. distinctly toothed in all rings), cupules bowl-shaped (vs. cup-shaped), nuts broadly ellipsoid (vs. obovoid to ellipsoid) and nut scar slightly convex (vs. more strongly convex). These general differences are shown in Table 2.

The morphological comparison of leaf traits amongst *Q. sontraensis* and *Q. cambodiensis* and *Q. langbianensis* (Table 3, Fig. 2) shows that the leaf blade length and width are significantly longer and relatively broader than those of *Q. cambodiensis* (9.27 ± 1.93 cm vs. 7.76 ± 2.25 cm and 2.89 ± 0.59 cm vs. 2.57 ± 0.66 cm, respectively), while significantly shorter and smaller leaf blade length and width compared to *Q. langbianensis*

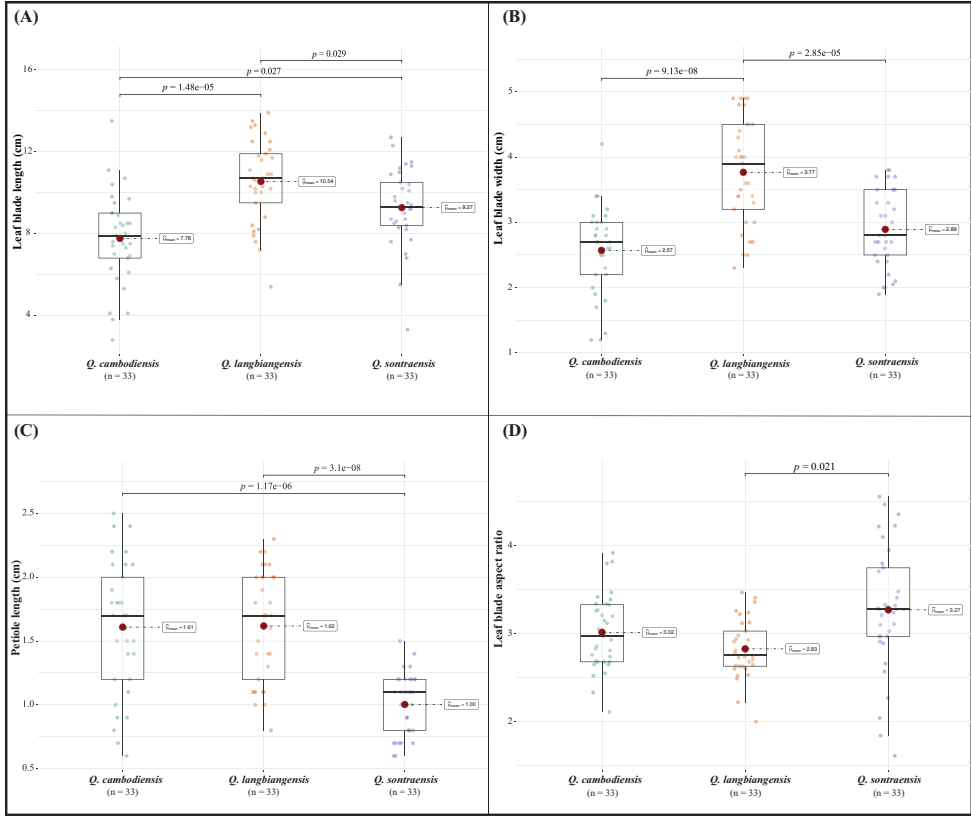


Figure 2. Morphological comparison of *Q. sontraensis* and its related species **A** box plot of leaf blade length **B** box plot of leaf blade width **C** box plot of petiole length **D** box plot of leaf blade aspect ratio. Red dots in the box plots indicate the mean value. Significant differences level (p) resulted from a Games-Howell post-hoc test (Comparison shown: only significant).

(9.27 ± 1.93 cm vs. 10.54 ± 2.02 cm and 2.89 ± 0.59 cm vs. 3.77 ± 0.80 cm, respectively). In addition, the new species has, on average, a significantly shorter petiole length than that of *Q. cambodiensis* and *Q. langbianensis* (1.0 ± 0.24 cm vs. 1.61 ± 0.51 cm and 1.62 ± 0.43 cm, respectively). Besides, the leaf blade aspect ratio of the new species is significantly greater than that of *Q. langbianensis* (3.27 ± 0.73 cm in *Q. sontraensis* vs. 2.83 ± 0.33 cm in *Q. langbianensis*), but there are no significant differences in the leaf blade aspect ratio between *Q. sontraensis* and *Q. cambodiensis* (Table 3, Fig. 2).

Phylogenetic tree using MIG-seq

The Maximum Likelihood tree, based on MIG-seq data for 15 samples of *Quercus*, strongly supports two major clades (except outgroup: *Lithocarpus vuquangensis*, *L. vinhensis* and *L. dahuoaiensis*) consisting of clade 1 and clade 2, with 100% bootstrap value (Fig. 3). Clade 1 consists of three species of section *Ilex* of subgenus *Cerris* with

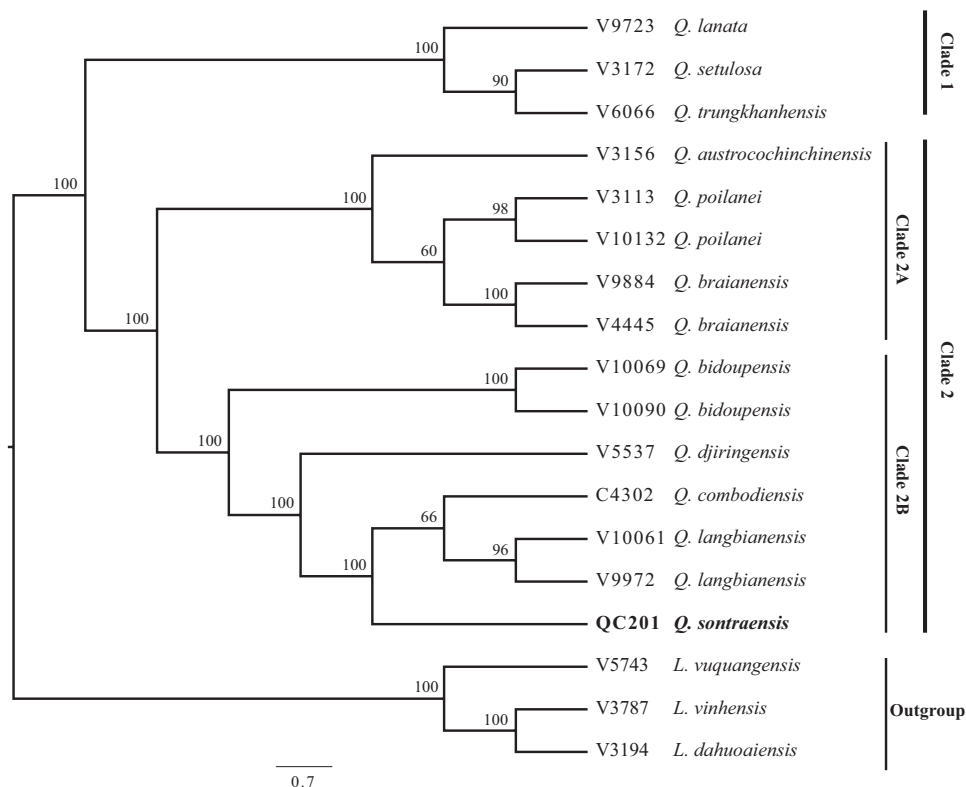


Figure 3. The phylogenetic relationship of the new species (Bold) with its related species, based on SNPs dataset from MIG-seq.

scale-cup oaks (Denk et al. 2017) (including *Q. lanata*, *Q. setulosa* and *Q. trungkhanhensis*) and clade 2 including 12 samples of eight species of section *Cyclobalanopsis* of subgenus *Cerris* with concentric lamellae-cup (Denk et al. 2017) (including *Q. austrocochinchinensis*, *Q. poilanei*, *Q. braianensis*, *Q. bidoupensis*, *Q. djiringensis*, *Q. cambodiensis*, *Q. langbianensis* and the new species as “*Q. sontraensis*”). The new species is placed in a highly supported monophyletic group (clade 2B with 100% bootstrap value) which included *Q. bidoupensis*, *Q. djiringensis*, *Q. cambodiensis* and *Q. langbianensis*. Amongst those five species of clade 2B, *Q. sontraensis* is placed together with *Q. cambodiensis* and *Q. langbianensis* with 100% bootstrap value. While the two accessions of *Q. langbianensis* are clustering together with 96% bootstrap support, a sister relationship of *Q. langbianensis* and *Q. cambodiensis* was only supported with 66%.

Discussion

The morphological examination and the MIG-seq tree support *Quercus sontraensis* as being closely related to *Q. langbianensis* and *Q. cambodiensis*. Morphological

differentiation of leaf characters is moderate and mainly shown through statistical measures (Fig. 2, Table 3), while simple comparisons might not be too helpful (Table 2). Cupule and nut characters seem to provide good qualitative characters to recognise *Q. sonraensis* (Table 2). In addition, *Q. sonraensis* was found at different elevations in Vietnam and Cambodia, respectively. While *Q. sonraensis* was collected at a lower elevation, around 340 m, the two other species were recorded at higher elevations, > 800 m. The combination of ecological differences and morphological and phylogenetic analysis provides good evidence for recognising the new species in this study.

Taxonomic treatments

Quercus sonraensis Ngoc, H.T.Binh & Son, sp. nov.

urn:lsid:ipni.org:names:77303991-1

Fig. 4

Diagnosis. *Quercus sonraensis* is morphologically similar to *Q. cambodiensis* and *Q. langbianensis* in leaf shape (lanceolate to elliptic), cupules with 6–9 rings and cupule enclosing less than $\frac{1}{2}$ of the nut. However, *Q. sonraensis* is distinguished from *Q. cambodiensis* by its leaf margin regularly and distinctly serrate on the upper $\frac{1}{3}$ – $\frac{1}{4}$ (– $\frac{1}{5}$) of the lamina (vs. almost entire or with a few low teeth in upper $\frac{1}{4}$), bowl-shaped cupule (vs. cup-shaped), cupule bract margin distinctly toothed in all rings, except two upper rings (vs. entire, except distinctly toothed in two lower rings) and differs from *Q. langbianensis* in having bowl-shaped cupule (vs. cup-shaped), cupule bract margin distinctly toothed in all rings, except two upper rings (vs. distinctly toothed in all rings), broadly ellipsoid nut (vs. obovoid to ellipsoid) and slightly convex nut scar (vs. convex).

Type. VIETNAM. Da Nang City, Son Tra Peninsula, Son Tra Nature Reserve, in evergreen forest, alt. 340 m, 16°08'22.90"N, 108°15'28.85"E, 9 October 2016, *Son H.T., Binh H.T., Ngoc N.V.* QC201 (holotype DLU!, isotypes HN!, VNM!, VAFS!).

Description. Tree, 12 m tall. Terminal and lateral buds ovoid, 3–5 mm long, 1.5–3 mm in diam., scales in 3–5 rows, imbricate, ovate-triangular, ca. 1.5×2 mm, apex obtuse, margin yellowish-brown ciliate, appressed hairy on both surfaces. Young twigs greyish-brown, 1.5–2 mm in diam., densely curly golden hairy, sometimes sulcate, old twigs greyish-brown, glabrous, lenticellate. Leaves alternate; blades elliptic to elliptic-lanceolate, 7 – 12.5×2.2 – 3.5 cm, acuminate or sometimes acute at apex, cuneate at base, margin regularly and distinctly serrate in the upper $\frac{1}{3}$ – $\frac{1}{4}$ (– $\frac{1}{5}$), densely golden hair on both surfaces when young, glabrescent, midrib sunken adaxially, prominent abaxially, lateral veins (8–)11–14 pairs, straight and running into the teeth of margin, slightly sunken adaxially, prominent abaxially, at an angle of 45–50 (–52) degrees from midrib, tertiary veins scalariform, faint on the upper surfaces and conspicuous on the lower surfaces; petioles (0.7–)1–1.5 cm long, densely curly golden hairy when young, soon glabrous. Male inflorescence 7–9 cm long. Female inflorescence 2.5–3.5 cm long, female flowers solitary. Infructescences axillary or terminal, erect, rachis 0.5–0.8 (–1.2) cm long, 3–4 (–6) mm in diam., densely golden hairy. Fruits 1–3, 3–3.3 cm long

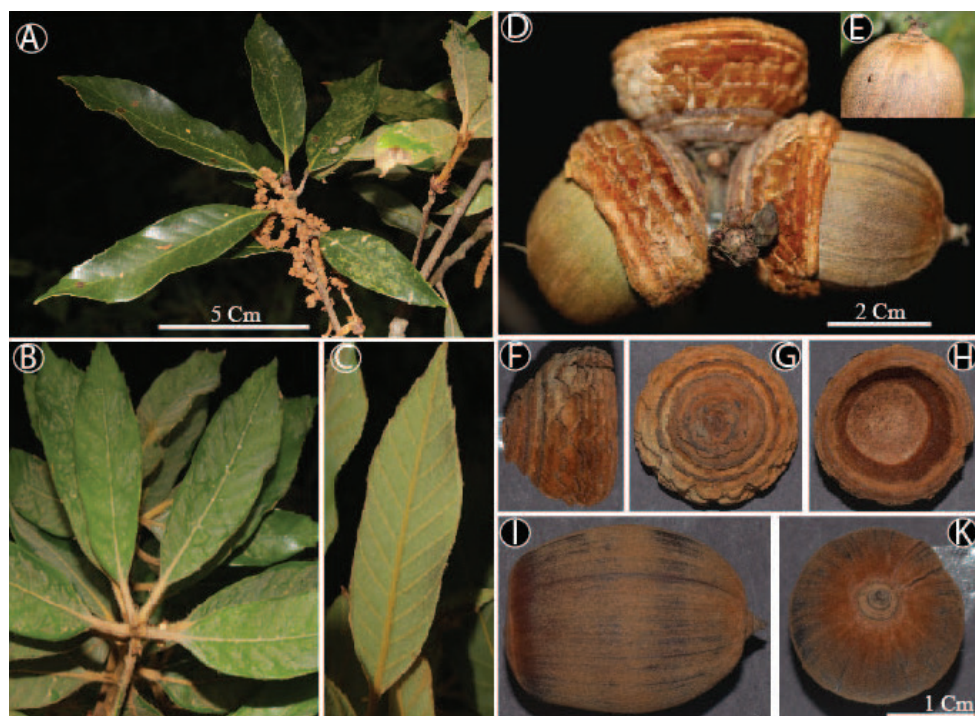


Figure 4. *Quercus sontraensis* Binh, Ngoc & Son **A** leafy twig with male inflorescences **B, C** young leaves covered with densely golden hairs **D** mature fruits **E** stylopodium **F, G** side and base of the cupule **H** inside of the cupule **I** nut (lateral view) **K** nut (top view). Materials from *Son et al. QC201*.

(including cupule), sessile; cupules bowl-shaped, 1.2–1.7 (–1.9) cm long, 1.3–2.2 cm in diam., enclosing 1/3 of the nut when mature, both outside and inside covered with densely appressed yellowish-brown hairs, wall ca. 1.5–3 mm thick, comprising scales, scales arranged in 7–8 rings, margin of the ring distinctly toothed in all rings, except two upper rings; nut broadly ellipsoid 2.3–2.6 cm long, 1.7–2.0 cm in diam., densely golden hairy, apex nearly flat, densely appressed curly golden hairs around stylopodia, stylopodia up to 1.5 mm long, basal scar 1.1–1.3 cm in diam., slightly convex, glabrous.

Distribution. Vietnam. Da Nang City, Son Tra Nature Reserve.

Etymology. The specific epithet is derived from the district name of the type locality, Son Tra Nature Reserve, Son Tra Peninsula, Da Nang City, Central Vietnam.

Vernacular name. Sồi Sơn Trà (suggested here).

Phenology. Flowering from January to March, fruiting specimens were collected from September to October.

Additional specimens examined. VIETNAM. Da Nang City, Son Tra Peninsula, Son Tra Nature Reserve, in evergreen forest, 385 m elev., 16°07'41.7"N, 108°15'55.7"E, 20 September 2019, *Son H.T. QC202* [fr.] (DLU!, VAFS!); *ibid.*, 428 m elev., 16°07'00.3"N, 108°17'40.5"E, 20 September 2019, *Son H.T. QC203* [fl.] (DLU!, VAFS!).

Preliminary conservation status. During our floristic survey inside the protected area of Son Tra Nature Reserve, less than 10 mature individuals of *Quercus sonraensis* were found in the evergreen forest, from 340 to 430 m altitude. According to the criteria established by the IUCN Red List (IUCN 2019), *Q. sonraensis* is qualified as Critically Endangered (CR), based on the extent of occurrence (EOO 1.47 km²) and area of occupancy (AOO 12.0 km²) [CR B1ab(i,ii,iii) 1 B2ab(i,ii,iii)].

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***Breynia hiemalis* (Phyllanthaceae, Phyllanthaeae), a new species from Yunnan, south-west China**

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Abstract

Breynia hiemalis Huan C. Wang & Feng Yang (Phyllanthaceae), of sect. *Cryptogynium* (Müll.Arg.) Welzen & Pruesapan in subg. *Breynia*, is described from Yunnan, south-west China. It is known from only a single locality in the valley of the Yuanjiang River, and usually occurs in the understory of the savanna vegetation. It is characterized by its broadly elliptic to orbicular leaf blades, shallowly plate-like calyces of the staminate flowers, ovaries with clearly erose rim and urceolate capsules. Morphological comparisons with similar species are also presented.

Keywords

Breynia sect. *Cryptogynium*, endemism, *Sauropus*, savanna, Yuanjiang River

Introduction

The family Phyllanthaceae, a segregate from Euphorbiaceae *sensu lato*, is a pantropical group of herbs, shrubs and treelets (Kathriarachchi et al. 2005; Hoffmann et al. 2006). It currently consists of about 2000 species, with more than 1200 placed in the largest tribe Phyllanthaeae (Govaerts et al. 2000; Hoffmann et al. 2006). Within the Phyllanthaeae, the generic classification is still contentious (Hoffmann et al. 2006;

Wagner and Lorence 2011; Pruesapan et al. 2012; Van Welzen et al. 2014; Jangid and Gupta 2016; Bouman et al. 2022). Recent molecular studies clearly demonstrated that *Phyllanthus* L. (in the traditional sense), the largest genus of Phyllanthaceae, with more than 800 species, is paraphyletic since *Glochidion* J. R. Forst. & G. Forst., *Breynia* J. R. Forst. & G. Forst. and *Synostemon* F. Muell. are nested within it (Bouman et al. 2021, 2022). Some authors suggested placing most species of the Phyllanthaceae in *Phyllanthus*. This would make *Phyllanthus* a large and morphologically heterogeneous group with more than 1200 species (e.g. Hoffmann et al. 2006; Wagner and Lorence 2011; Ralimanana et al. 2013; Kato and Kawakita 2017; Falcón-Hidalgo et al. 2020). Conversely, other authors (for example, Pruesapan et al. 2008; Pruesapan 2010; Van Welzen and Pruesapan 2010; Pruesapan et al. 2012; Bouman et al. 2018, 2021) prefer to divide *Phyllanthus* s. l. into several smaller, monophyletic genera that can be characterized morphologically. More recently, Bouman et al. (2022) split *Phyllanthus* s. l. into thirteen monophyletic and morphologically recognizable genera, where *Breynia* was kept as a separate genus.

Breynia and *Sauropus* Blume in the strict sense are closely related; both share bifid or emarginate styles, non-apiculate anthers, smooth seeds, and they generally possess sepal scales (Van Welzen et al. 2014). From the results of a well-sampled phylogenetic analysis, Van Welzen et al. (2014) expanded *Breynia* to include the south-east Asian species of *Sauropus* and reinstated the Australian genus *Synostemon*, previously relegated to a section of *Sauropus*, making each genus monophyletic and morphologically definable. The newly circumscribed *Breynia* is a moderately-sized genus of 85 to 90 species (Van Welzen et al. 2014; Bouman et al. 2022). It contains three infrageneric groups, including subg. *Breynia* and subg. *Sauropus* (Blume) Welzen & Pruesapan, with subg. *Breynia* subdivided into sect. *Breynia* and sect. *Cryptogynium* (Müll. Arg.) Welzen & Pruesapan. Members of *Breynia* are distributed mainly from Australia to tropical and subtropical Asia, with a center of diversity in south-east Asia (Van Welzen 2003; Bouman et al. 2022; POWO 2022). Twenty species of *Breynia* are found in China, including 15 species formerly placed in *Sauropus*, and most species occur in the southern and south-western regions (Li 1994; Li and Esser 2008; Li and Gilbert 2008). Recently, a new species of *Breynia* was described from Yunnan, south-west China by Yang et al. (2022). This brings the total number of Chinese species to 21, of which five species belong to sect. *Cryptogynium*, seven species belong to sect. *Breynia*, and nine species belong to subg. *Sauropus*.

During fieldwork in Yuanjiang National Natural Reserve, Yunnan Province (SW China), in December 2015 and January 2022, we came across a dwarf plant with discoid staminate flowers and 3 stigmas spreading horizontally from the top of obconical ovary, obviously belonging to *Breynia* according to the generic delimitation of Van Welzen et al. (2014) and Bouman et al. (2022). This could not be assigned to any previously known species from China (Li and Gilbert 2008), or from any of the adjacent south-east Asian countries, i.e. Vietnam, Laos, Thailand and Myanmar (Beille 1927; Pham 2003; Van Welzen 2003; Chakrabarty and Balakrishnan 2018; Van Welzen 2022; Van Welzen and Chayamarit 2022). Comparison with morphologically similar species supports recognizing this plant as an undescribed species.

Materials and methods

The new species was studied both in the field and in the herbarium. The collections of similar species housed at KUN, PE, XTBG, YUKU, and digital images available at the JSTOR Global Plants (<https://plants.jstor.org/>), the Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>), and the Global Biodiversity Information Facility (<https://www.gbif.org/>) were reviewed. Surveys of pertinent taxonomic literature (for example, Beille 1927; Li 1994; Pham 2003; Van Welzen 2003; Ma et al. 2006; Li and Gilbert 2008; Van Welzen et al. 2014; Chakrabarty and Balakrishnan 2018; Van Welzen 2022; Van Welzen and Chayamarit 2022) were carried out. Measurements were made under a stereomicroscope (Olympus SZX2, Tokyo, Japan) using a ruler and a metric vernier caliper.

Taxonomy

Breynia hiemalis Huan C. Wang & Feng Yang, sp. nov.

urn:lsid:ipni.org:names:77303992-1

Figs 1–3

Type. CHINA. Yunnan Province: Yuanjiang County, Pupiao, 600–700 m, 23°28'37"N, 102°10'37"E, in savanna on a mountain slope, 12 Jan. 2022, *H. C. Wang et al.* YJ16225 (holotype YUKU-02074690!; isotypes YUKU!, PE!, HITBC!).

Diagnosis. *Breynia hiemalis* can easily be distinguished from all morphologically similar species by plants glabrous throughout, by its broadly elliptic to orbicular and relatively small (4–21 × 4–17 mm) leaves, calyx of staminate flower shallowly plate-like, ovary rim conspicuously erose, and the urceolate capsule with a raised and lobed apical rim.

Description. Dwarf shrubs or subshrubs, 10–20 (–30) cm tall, monoecious, glabrous throughout, with phyllanthoid branching. Main stems more or less procumbent to ascending, brown, with 4 shallow ribs, sometimes rooting at the lower nodes; branches green, deciduous, ascending, 3–8 cm long. *Cataphylls* lanceolate, to 1 mm long, arranged spirally at the base of the plagiotropic branchlets. *Leaves* on ultimate branchlets distichous, simple; stipules triangular-lanceolate, usually auriculate basally, 1.5–2.0 mm long; *petiole* 1.2–1.6 × 0.3–0.5 mm; *blade* broadly elliptic to orbicular, rarely slightly ovate, papery, 4–21 × 4–17 mm, length/width ratio 1–1.5, base rounded to broadly cuneate, margin entire, flat, apex usually rounded, sometimes truncate, retuse, rarely mucronate, adaxially green, abaxially grey or slightly glaucous; venation pinnate, lateral veins 4 or 5 pairs, reticulate veins obscure. *Inflorescences* axillary, *peduncles* very short, ± 0.1 × 0.1 mm, with minute bracts, male or female flowers usually solitary, staminate flowers proximal, pistillate flowers usually distal. *Staminate flowers:* *pedicel* slender, ± 7 mm long; *calyx* shallowly plate-like, ± 4 mm in diam., red, 6-lobed; lobes biseriate, broadly obovate, slightly fleshy, 0.9–1.2 × 1.1–1.3 mm, apex obtuse or retuse, scales present; *stamens* 3, filaments connate, androphore ± 0.2 mm long, splitting horizontally, branches up to 0.5 mm long with anthers underneath, anthers ± 0.3 × 0.3 mm. *Pistillate flowers:* *pedicel* ± 3 mm

long, thickening upwards; calyx ± 6 mm in diam., greenish, whitish yellow, or pinkish, lobes biseriate, obovate, subcoriaceous, outer lobes $2.5\text{--}3.0 \times \pm 2.5$ mm, slightly longer and wider than the inner, inner lobes $\pm 2.5 \times 2.1\text{--}2.5$ mm, apex obtuse to truncate, shortly and abruptly acuminate; **ovary** obconical, 1.2–1.6 mm in diam., 3-locular, 2 ovules per locule, rim present at the apex, obviously erose; stigmas 3, spreading horizontally from top of ovary, apex split and recurved through $\pm 180^\circ$, sepals persistent and enlarged to $\pm 5 \times 4$ mm in fruit. **Capsules** urceolate, $\pm 4 \times 5\text{--}6$ mm, with a raised, lobed apical rim and persistent stigmas.

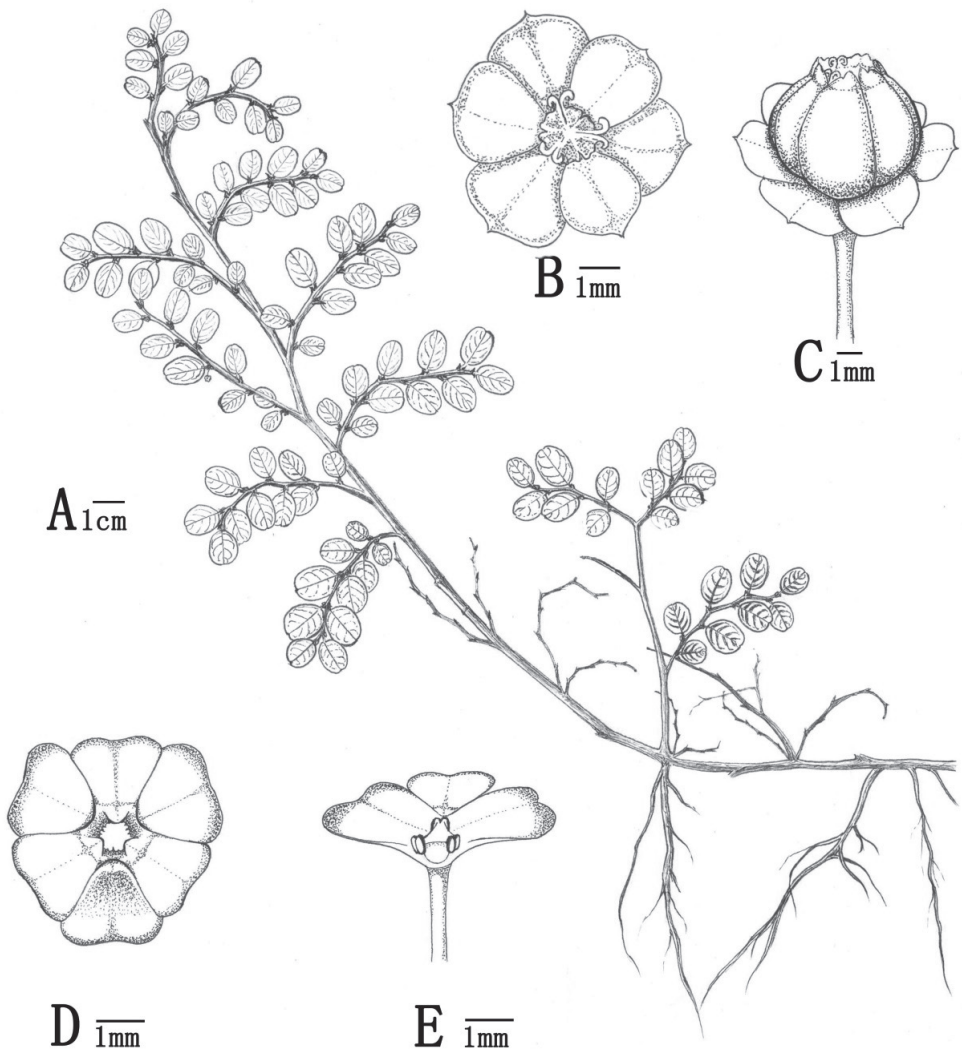


Figure 1. *Breynia hiemalis*. (Drawn by Jing-Yi Ye from type specimen *H. C. Wang et al. YJ16225*) **A** habit **B** pistillate flower (apical view) **C** fruit **D** staminate flower (apical view) **E** staminate flower (lateral view).

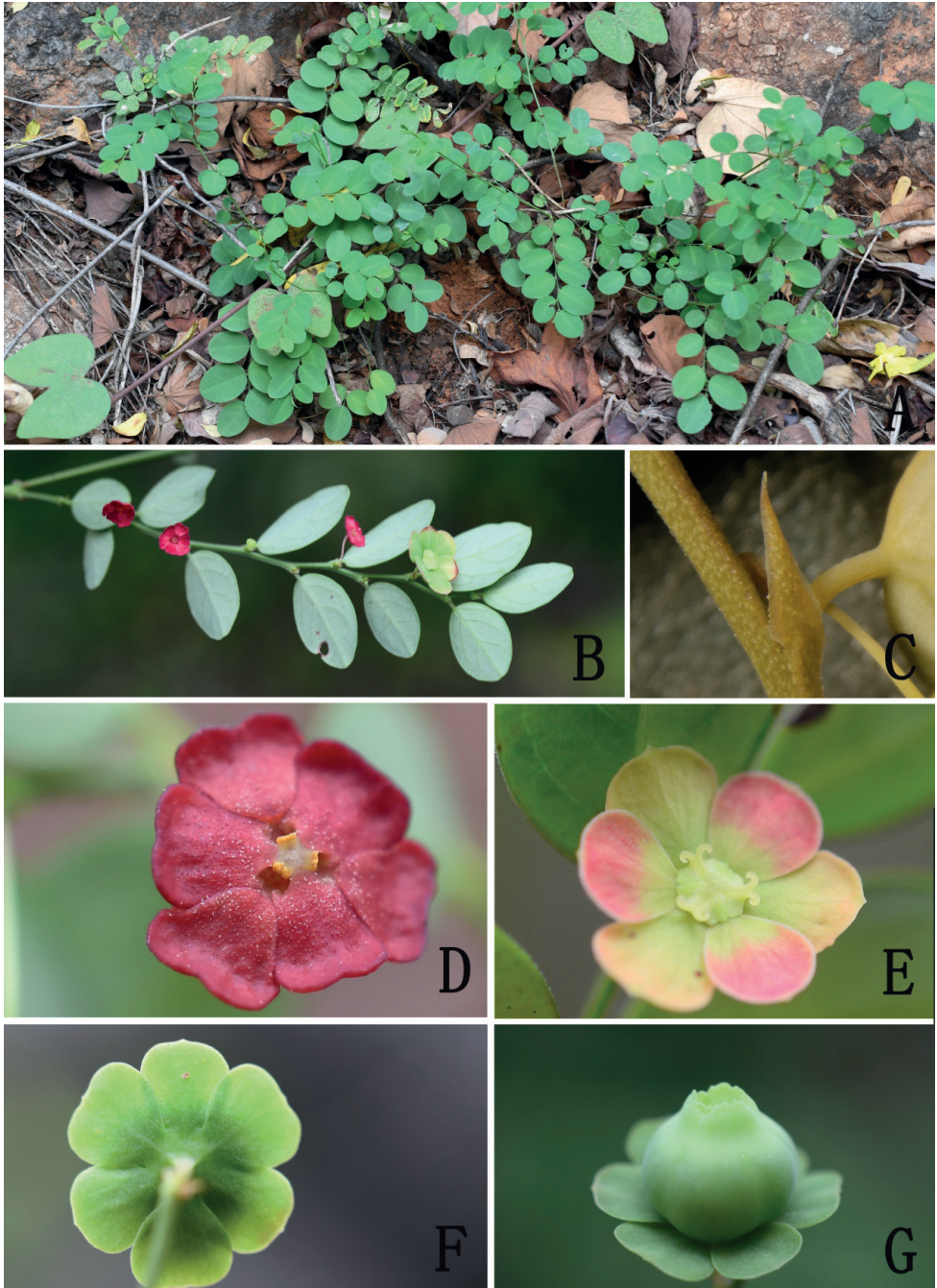


Figure 2. *Breynia hiemalis* **A** habit **B** phyllanthoid branch showing pistillate flower and staminate flowers **C** stipule **D** staminate flower (apical view) **E** pistillate flower (apical view) **F** calyx in fruit (dorsal view) **G** fruit. Photographed by H. C. Wang from type locality in January 2022.



Figure 3. Holotype of *Breyenia hiemalis* (YUKU-02074690).

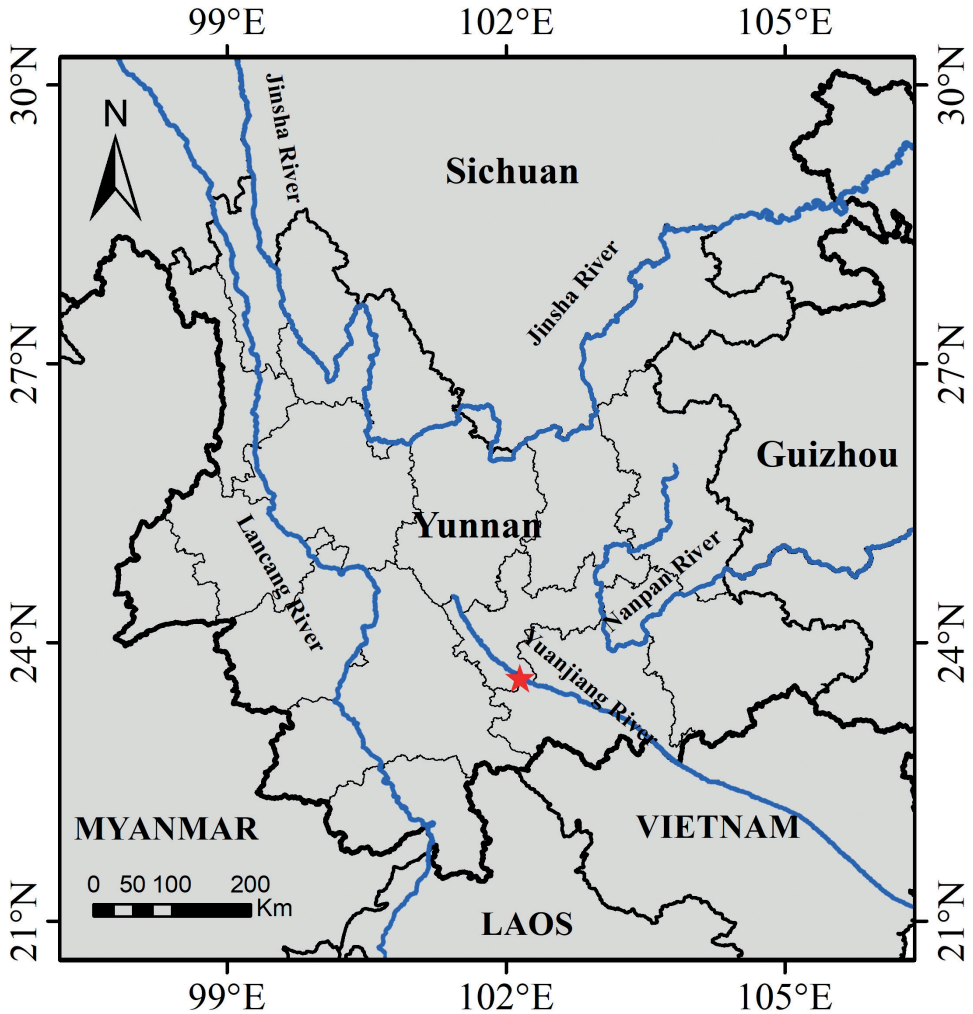


Figure 4. South-western China, showing the known distribution (red star) of *Breynia hiemalis*. The blue lines represent the rivers.

Phenology. Flowering from December to January, fruiting from January to February.

Etymology. The epithet “*hiemalis*” is Latin for “belonging to winter”, referring to the flowering period of this new species.

Distribution and habitat. *Breynia hiemalis* appears to be rare and is endemic to Yunnan, south-west China. It is known from only a single locality in the valley of the Yuanjiang River, which flows from Yunnan (south-west China) through northern Vietnam to the Gulf of Tonkin (Fig. 4). The climate in Yuanjiang valley is characterized by a long dry season (the dry season can be further divided into a cool dry season (November to February) and a hot dry season (March to April)), with an annual average

temperature of 24 °C and a mean annual evaporation capacity of 2700–3800 mm, that is three to six times higher than the mean annual precipitation (600–800 mm), and with 80–90% of the precipitation concentrated in the wet season (from May to October) (Jin 2002; Shen et al. 2010; Zhou et al. 2017). *Breynia hiemalis* grows in savanna on a mountain slope (Fig. 5) at elevations of 500–700 m, together with *Lannea coromandelica* (Houtt.) Merr. (Anacardiaceae), *Adina cordifolia* (Roxb.) Brandis (Rubiaceae), *Bauhinia brachycarpa* Wall. ex Benth. (Fabaceae), *Tephrosia purpurea* (L.) Pers. (Fabaceae), *Woodfordia fruticosa* (L.) Kurz (Lythraceae), *Waltheria indica* L. (Malvaceae), *Jasminum mesnyi* Hance (Oleaceae), *Searsia paniculata* (Wall. ex G. Don) Moffett (Anacardiaceae), *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult. (Poaceae), and others.

Additional specimens examined (Paratypes). **China. Yunnan:** Yuanjiang County, Pupiao, 17 Dec. 2015, *H. C. Wang et al.* YJ736 (YUKU).

Taxonomic notes. According to Van Welzen et al. (2014) and Bouman et al. (2022), *B. hiemalis* should be assigned to sect. *Cryptogynium* because of its horizontal anthers (the androphore splits apically into three horizontal arms with the anthers hanging underneath) and ovary with a rim. Prior to the present study, only five species of sect. *Cryptogynium* were recorded in China, namely *B. compressa* (Müll. Arg.) Chakrab. & N. P. Balakr. (a member of the *B. quadrangularis* (Willd.) Chakrab. & N. P. Balakr. complex which was recognized as a distinct species by Chakrabarty and Balakrishnan (2015)), *B. delavayi* (Croizat) Welzen et Priesapan, *B. pierrei* (Beille) Welzen et Priesapan, *B. similis* (Craib) Welzen et Priesapan and *B. tsiangii* (P. T. Li) Welzen et Priesapan (Li and Gilbert 2008; Van Welzen et al. 2014; Chakrabarty and Balakrishnan 2015). *Breynia hiemalis* shows some resemblance to *B. compressa* and *B. delavayi* in its dwarf habit and axillary inflorescences. However, *B. hiemalis* can be distinguished from *B. compressa* by several characters, namely stems more or less procumbent to ascending (vs. erect or arching in *B. compressa*), calyx of the staminate flower shallowly plate-like (vs. star-shaped), lobes broadly obovate (vs. suborbicular or squarish), apex obtuse or retuse (vs. emarginate-truncate to deeply bilobulate) (Fig. 6: D, E), ovary rim erose (vs. retuse) (Fig. 6: A, B), capsule urceolate (vs. ovoid), with raised and lobed apical rim (vs. with low and smooth apical rim). Additionally, *B. hiemalis* flowers in winter (from December to January), whereas *B. compressa* flowers from summer to autumn (from April to October). *Breynia hiemalis* differs strikingly from *B. delavayi* in its obscure reticulate veins (vs. reticulate veins elevated on both surfaces) and calyx of the staminate flower shallowly plate-like (vs. star-shaped) (Fig. 6: D, F).

Of the species of *Breynia* found in south-east Asia, *B. hiemalis* is also morphologically similar to *B. granulosa* (Airy Shaw) Welzen & Priesapan, from eastern Thailand. Nevertheless, *B. granulosa* differs from *B. hiemalis* in having obovate leaves (vs. broadly elliptic to orbicular, rarely slightly ovate in *B. hiemalis*), calyx of staminate flower campanulate (vs. shallowly plate-like), androphores ± 0.8 mm (vs. ± 0.2 mm) long, smaller pistillate flowers, usually 4–5 mm (vs. ± 6 mm) in diam., stigmas ascending (vs. horizontally spreading) (Fig. 6: A, C) and ovoid (vs. urceolate) capsules. *Breynia hiemalis* is also similar to *B. poilanei* (Beille) Welzen et Priesapan from Vietnam, but it clearly differs from the latter by its more or less procumbent to ascending stems (vs.

erect in *B. poilanei*), 0.1–0.2 (–0.3) m (vs. 1.5 m) tall, branches 3–8 cm (vs. 5–15 cm) long, stipules triangular-lanceolate (vs. triangular), 1.5–2.0 mm (vs. 0.5 mm) long, leaves broadly elliptic to orbicular, rarely slightly ovate (vs. ovate, rarely orbicular), capsules 4 mm (vs. up to 10 mm) wide. A key to distinguish the members of *Breynia* sect. *Cryptogynium* in China is given below.

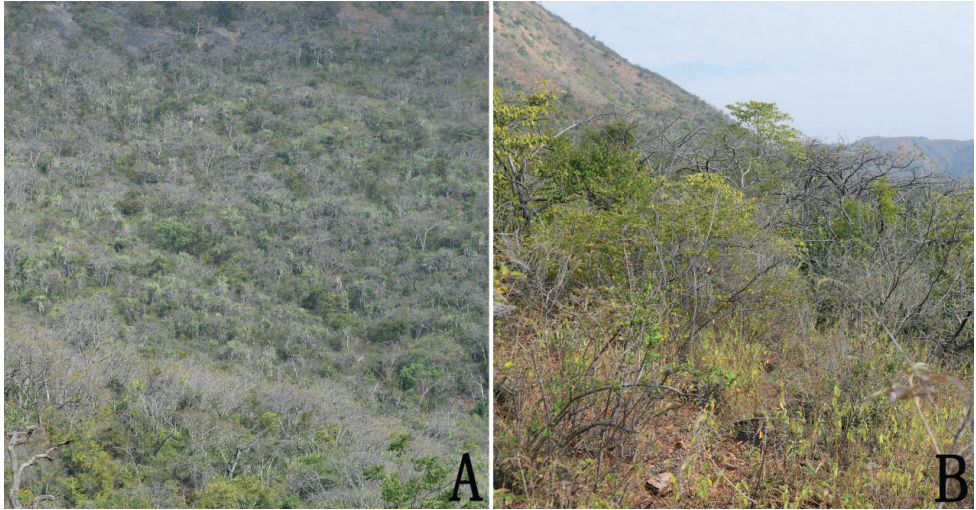


Figure 5. Habitat of *Breynia hiemalis* **A** distant view of the type locality **B** nearby view.

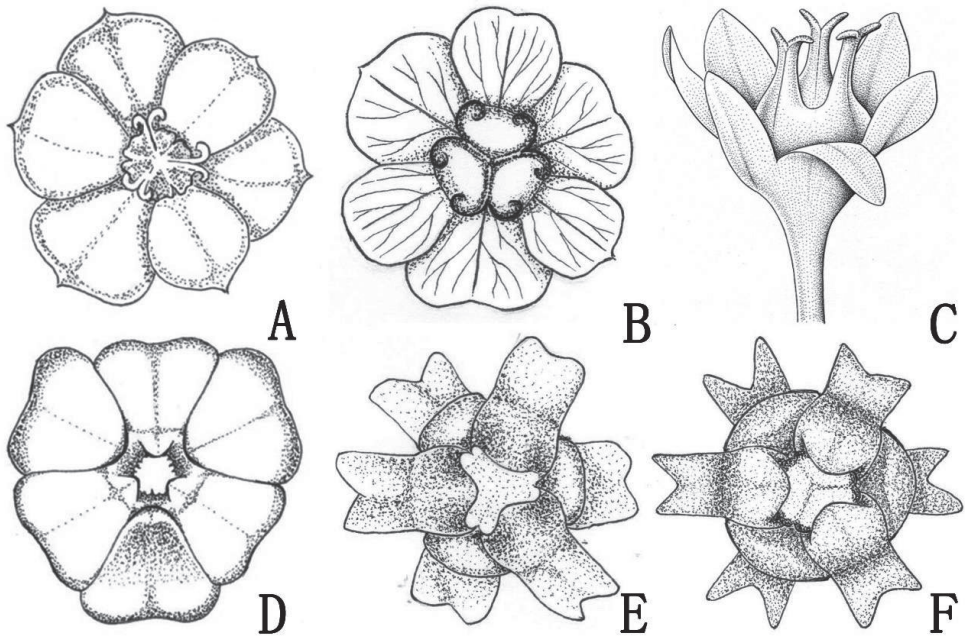


Figure 6. *Breynia hiemalis* (**A**, **D**), *B. compressa* (**B**, **E**), *B. granulosa* (**C**), *B. delavayi* (**F**). **A–C** pistillate flowers **D–F** staminate flowers.

Key to Chinese species of *Breynia* sect. *Cryptogynium*

- 1 Shrubs 1–3 m tall **2**
- Dwarf shrubs or subshrubs 0.1–1.0 m tall **4**
- 2 Leaves ovate, 0.7–3.7 × 0.5–1.9 cm *Breynia similis* **3**
- Leaves ovate to lanceolate, 6–10 × 3–4 cm **3**
- 3 Leaves usually ovate, rarely ovate-lanceolate, papery, base rounded, apex caudate-acuminate *B. tsiangii*
- Leaves ovate-lanceolate to lanceolate, coriaceous, base cuneate, apex acuminate *B. pierrei*
- 4 Calyx of staminate flower shallowly plate-like *B. hiemalis*
- Calyx of staminate flower star-shaped **5**
- 5 Branches pubescent, leaves orbicular, base shallowly cordate, truncate or rounded, lateral veins robust, reticulate nerves prominent *B. delavayi*
- Branches glabrous; leaves ovate, elliptic or nearly rounded, base rounded or broadly cuneate, lateral veins slender, reticulate nerves obscure ... *B. compressa*

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Seed variability of *Sisymbrium polymorphum* (Murray) Roth (Brassicaceae) across the Central Palaearctic

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Abstract

This study presents the results of investigation of the micromorphology and variability of *Sisymbrium polymorphum* seeds collected in 49 localities in the core range and isolated populations of Central Europe, Eastern Europe and Central Asia. In addition, we compared the ultrastructure of the seeds of *S. polymorphum* with that of the closely-related species *S. loeselii* and *S. linifolium*. The seeds were measured with a stereoscopic microscope and a biometric programme (ImageJ) and micromorphological studies were performed by scanning electron microscopy (SEM). The seed variability showed intraspecific stability of the ultrastructure and low variability of metric features within the studied range. Studied species showed differences in the ultrastructure, which will be valuable for diagnostic purposes. We present and describe, for the first time, the ultrastructure of *S. polymorphum* seeds.

Keywords

Brassicaceae, latitude variability, micromorphology, scanning electron microscopy (SEM), seed coat, taxonomy, 3D ultrastructure

Introduction

The differences in the structure of seeds allow for distinguishing individual units at different levels of hierarchy, making them useful in plant taxonomy and identification (Saadaoui et al. 2013; Rewicz et al. 2017, 2020; Martín-Gómez et al. 2019a, 2019b; Ullah et al. 2019). The properties of seeds, such as sculpture variability, are the subject of many scientific studies (Vaughan and Whitehouse 1971; Stace 1993; Gamarra et al. 2010; Kaya et al. 2011; Meyer et al. 2015; Rewicz et al. 2016). Seed shape is crucial in some taxonomic research and, next to sculpture, is one of the most constant characteristics of species (Gamarra et al. 2007; Hadidchi et al. 2020). Moreover, the biometric analysis of individuals from different localities covering a wide range provides objective data on a given taxon's morphological (as well as habitat and climatic) variability (Skrajna et al. 2012; Rewicz et al. 2017; Kostrakiewicz-Gierał et al. 2022). Such analyses help to determine the influence of various environmental factors on the phenotypic variability of plants and phylogenetic inference (Bacchetta et al. 2011; Rewicz et al. 2016). Modern imaging methods, for example, scanning electron microscopy (SEM), allow for a detailed analysis of seed ultrastructure, relevant in carpological research (Heywood 1969; Hadidchi et al. 2020). The development of new mathematical models and statistical methods also allow for a more precise metric analysis of the tested objects or a more accurate description of shapes (Saadaoui et al. 2013).

The Brassicaceae (Cruciferae) family, commonly known as the mustards, the crucifers or the cabbage family, is widespread worldwide, including 4050 species and 341 genera (Kiefer et al. 2014; Chen et al. 2019). These are primarily herbaceous, annual or perennial plants (Schmidt et al. 2001). Species within the family are centred in regions with a temperate climate; most representatives grow in the Northern Hemisphere (Warwick et al. 2002).

The endo- and exomorphological features of Brassicaceae seeds have been the subject of many taxonomic studies (Musil 1948; Murley 1951; Corner 1976; Koul et al. 2000; Pinar et al. 2007; Karaismailoğlu and Erol 2018; Karaismailoğlu 2019, 2022). Vaughan and Whitehouse (1971) studied the macro- and micromorphology of seeds within 90 genera, including about 200 species of Brassicaceae. Moreover, Kasem et al. (2011) and Antkowiak et al. (2018) paid particular attention to the relationship between the seed structure and the taxonomy of selected species from this family, for example, *Nocca* spp. and *Thlaspi* spp.

Sisymbrium polymorphum (Murray) Roth is a sub-Irano-Turanian species that occurs in eastern and south-eastern Europe and reaches as far as Mongolia, the vicinity of Irkutsk and Lake Baikal, where it has a diffused range (Kaźmierczakowa and Zarzycki 2001). The western border of its range in Central Europe has some isolated populations in the Pannonian Plateau and the Polish Uplands (Kaźmierczakowa et al. 2014). *Sisymbrium polymorphum* is currently one of the rarest plants in its westernmost range, probably along with those from the early Holocene migration of steppe plants from the Podolia refuge (Towpasz and Trzcińska-Tacik 1998; Bauer and Somlyay 2007). In Poland, locations are concentrated in the south-eastern part of the Nidziańska Basin in

the Małopolska Upland, where relic patches of steppe vegetation have survived (Towpasz and Trzcińska-Tacik 1998). Along with the core part of the range, species vary in abundance: common in the Ukrainian steppe and forest-steppe zones and rare in the Roztochchia-Opillia. It also has some populations in the Polissia and Transcarpathia, where the species can be considered as alien (Ilyinska et al. 2007).

The genus *Sisymbrium* L. (comprising about 50 species) typifies a lack of clear generic boundaries and unique synapomorphies (Al-Shehbaz 1988, 2012, 2015; Warwick et al. 2002; Warwick and Al-Shehbaz 2003; Rūrāne and Roze 2015). The first monograph (Fournier 1865) on the genus listed 166 species, while Schulz (1924), at the beginning of the 20th century, accepted 77 species within the genus and divided it into 14 sections. *Sisymbrium polymorphum*, together with *S. volgense* M. Bieb. ex E. Fourn., *S. irio* L., *S. austriacum* Jacq. and *S. loeselii* L., were included in The Old World section *Irio* (Schulz 1924, 1936). Molecular research by Warwick et al. (2002), based on the ITS nuclear region, showed that these species appear in five different terminal clades. The classification proposed by Schulz (1924, 1936) appears utterly incongruent with the above-mentioned molecular studies. *Sisymbrium polymorphum* appears to be the genetically closest related to *S. loeselii* L. and *S. linifolium* (Nutt.) (Warwick et al. 2002). Further recent studies by Žerdoner Čalasan et al. (2021) involving five nuclear encoded and three chloroplast encoded molecular loci confirm a close relationship with *S. linifolium* and *S. polymorphum*; however, they found *S. polymorphum* paraphyletic or they found some cryptic or pseudo-cryptic species identified as *S. polymorphum* used in their study. Chen et al. (2019) suggest that only molecular identification can help in distinguishing *S. polymorphum* from *S. linifolium*. Our literature review showed a scarcity of data regarding seed size of *S. polymorphum* and a complete lack of information describing the morphological variation of these seeds. Moreover, there is no available information on seed ultrastructure of these closely-related species. Thus, there is no information on whether the genetic closeness of these species is reflected by similarity of the ultrastructure of the seeds.

The presented study aims to: i) analyse differences in the ultrastructure of the seed surface and morphological features of *S. polymorphum* from the core range in Eastern Europe and Central Asia and from isolated localities in Central Europe; ii) indicate differences and similarities between the analysed localities; iii) analysing the variability of the examined morphological traits of seeds and iv) analysing differences in the ultrastructure of the seed surface between *S. polymorphum* and two most closely-related species, *S. loeselii* and *S. linifolium*.

Materials and methods

Biometric analyses of seeds

We analysed the seeds of *S. polymorphum* (Suppl. material 2: Appendix S1; Fig. 1) from 49 localities. Seeds were loaned out from the following Herbaria: **KRA** (Jagiellonian

University), **KW** (M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine), **KWHA** (M.M. Gryshko National Botanical Garden, National Academy of Sciences of Ukraine), **MHA** (Main Botanical Garden, Russian Academy of Sciences), **PKM** (Penza I.I. Sprygin Herbarium, Penza State University), **PAV** (Institute of the Ecology of the Volga River Basin, Russian Academy of Sciences), **YALT** (Nikitsky Botanical Garden, National Scientific Centre, National Academy of Agrarian Sciences of Ukraine).

For statistical analyses, 20–30 seeds from each locality were measured. We used a Nikon SMZ-800 DS-Fi optical stereomicroscope (Nikon Instruments, Europe B.V.), connected to a Coolview camera 2274 (Nikon) for observation and documentation. To explore the variation in micromorphology, the following features were quantified: seed length (SL), seed width (SW). The terminology follows Bojnanský and Fargašová (2007) and Barthlott et al. (1984).

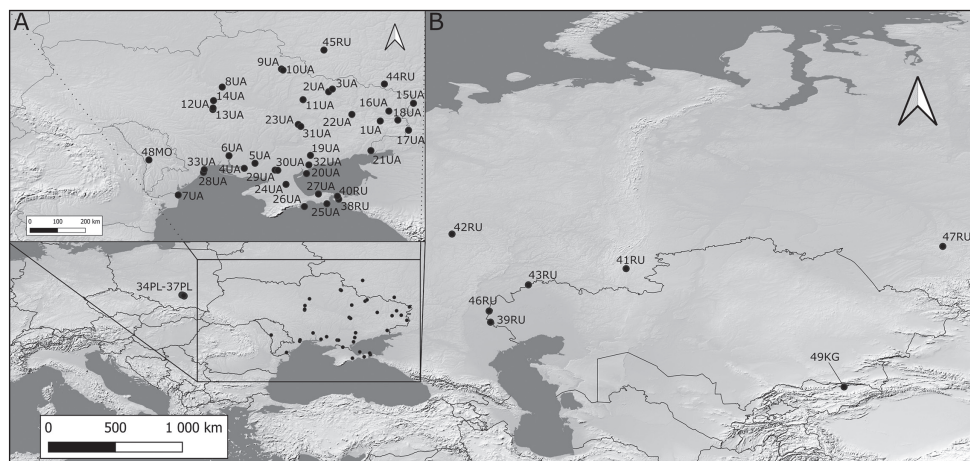


Figure 1. Localities of *Sisymbrium polymorphum* samples (and zoom window on Ukraine and parts of Russia (A) and the rest of the study area (B). Codes of localities according to Suppl. material 2: Appendix S1.

Scanning electron microscopy (SEM)

Micromorphological pictures by scanning electron microscopy were obtained in the facilities: Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Poland (Phenom Pro X) and at the Centre of Electron Microscopy of the M. G. Kholodny Institute of Botany, NAS of Ukraine (JSM-6060 LA). The seeds were fixed on brass tables and were sputter-coated with a 4 nm layer of gold. The SEM photos were taken for 30 tested locations (Suppl. material 2: Appendix S1).

To check if seed ultrastructure showed interspecific differences, we used *Sisymbrium loeselii* seeds from the Herbarium Universitatis Lodzensis (LOD): 085854, 097669, 107817, 153192 and *S. linifolium* from the M.S. Turchaninov memorial collections (KW). The length and width of 25 cells were measured for each species (with

the length of the seed). We used SEM pictures (from 18 randomly selected locations) with the same magnification and orientation of the seed.

Three-dimensional models of the ultrastructure of the surface of the seed of *S. polymorphum* were made using 3D Roughness Reconstruction software for the Phenom Electron Microscope.

Statistical analyses

The following features were calculated: arithmetic average ($\bar{x}$), maximum and minimum values (max and min) and coefficient of variation for traits of *S. polymorphum* (Suppl. material 3: Appendix S2). The ordination of localities according to seeds' biometric parameters were analysed using a PCA (Principal Components Analysis) scatter plot (van Emden 2019). The relationship between altitude and seed traits for every population was determined with Pearson's correlation coefficient (r): the following values according to Meissner (2010) were adopted: less than 0.20 = correlation very poor, 0.21–0.39 = weak correlation, 0.40–0.69 = moderate correlation, 0.70–0.89 = strong correlation and above 0.89 = correlation very strong. Cluster analyses showing the morphological similarity of *Sisymbrium polymorphum* populations according to seed traits were grouped using the UPGMA method.

The Shapiro-Wilk and Kolmogorov-Smirnov tests were conducted to check for a normal distribution of the data; both were not normal; therefore, the Kruskal-Wallis test (for $p \leq 0.05$) was used as a non-parametric alternative to ANOVA. The post-hoc test (Dunn's test) was used to show which populations differed significantly by the statistics. The software package STATISTICA PL. ver. 13.1 (Stat-Soft Inc. 2011) was used for all the numerical analyses (van Emden 2019).

Description of study species

Sisymbrium polymorphum (Murray) Roth (*Brassica polymorpha* Murray, *S. junceum* M. Bieb.) is a perennial herb. It grows in grasslands and steppes and disturbed biotopes on the slopes of ravines, gullies, river banks, roadsides, fields and railway embankments. This continental species occurs primarily on gypsum soils, but is currently also encountered on secondarily disturbed biotopes, outcrops or even moderately saline soils (Każmierczakowa and Zarzycki 2001; Atlas NBN 2022). The species occurs mainly in lowlands; however, some mountain locations have also been reported. The species' known altitude range is 300–1900 m a.s.l.

Individuals of *Sisymbrium polymorphum* grow from 20–80 cm and their leaves are medium in size; scattered-bristly on both sides of the petiole, glabrous above; lower – long-petiolate, broadly lanceolate-pinnately dissected or pinnately dissected, edge from toothed to acute triangular, apex pointed, wedge-shaped base; upper – short-petiolate, narrow, from lanceolate to linear, entire, occasionally unevenly toothed or at the base with two large acute triangular teeth, which turn into elongated lanceolate pointed segments, the base is convergent; succulent-scleromorphic. Stem erect, branched

(sometimes from the base), glabrous or below with long straight or twisted protruding hairs. The underground part is a caudex and has a rod-like root system. There are primrose flowers that whiten quickly after flowering. Inflorescence: compound raceme. Pollination: autogamy, entomogamy. Fruit: glabrous pod. Seed dispersal: anemochory and epizoochory. Reproduction can be vegetative (root sprouts) or generative and growth type is unitary and modular (Atlas NBN 2022; WFO 2022).

Results

Structure of seed surface

The analysis of *S. polymorphum* seeds showed no differences in their ultrastructure between the studied locations. The seeds were glossy and they were orange to brown. The shape of the seeds was observed to be polymorphous from ovate to angular with a subterminal (ST) hilum. The shape of the seeds was not constant within one locality. Pattern sculpture in all studied populations was reticulate and the cells were polygonal in shape (Table 1). We observed that the central part of the seed cells was most often rectangular, while the seeds had a polygonal shape on the sides.

Seed size

The analysis of average values of the studied biometric traits of *S. polymorphum* shows that the widest seeds characterise the most northern location in Russia (41, 42RU). The highest average seed width was observed in Asian and Eastern European localities in Russia (average 0.41 mm). The maximum and minimum width values analysis shows that the highest average width occurs in seeds growing in the Russian (42RU) population (average 0.68 mm) (Suppl. material 3: Appendix S2).

Comparatively high values of width are demonstrated in the Polish populations (average width 0.41 mm) and Kyrgyzstan's (49KG) population (average 0.41 mm). The longest seeds were measured in the Moldovian and Kyrgyzstan populations. The highest averages of length were observed in the following populations: Moldovian (48MO) (average 1.17 mm), as well as Kyrgyzstan's (49KG) (average 1.03 mm). The maximum values of length (1.43 mm) were recorded in Kyrgyzstan's (49KG) population (Suppl. material 3: Appendix S2). The minimum values of analysed traits were observed in the Ukrainian populations, with characteristics of the shortest and narrowest seeds in the analysis, with average width 0.36 mm and average length 0.84 mm. The Ukrainian (8UA) population has the smallest seeds both in terms of length (0.49 mm) and width (0.15 mm). The smallest seeds were recorded in the Ukrainian populations and the Russian population had the widest seeds. The features for Polish populations were between those of the other populations. The seeds of *S. polymorphum* displayed a range of variation between all populations.

The studied populations differ significantly in each of the analysed traits, i.e. seed length and width ($p < 0.5$, Kruskal-Wallis test, Fig. 2, Appendix S3 A, B). The *post-hoc* test showed that, in the case of seed length, the population that differs the most from

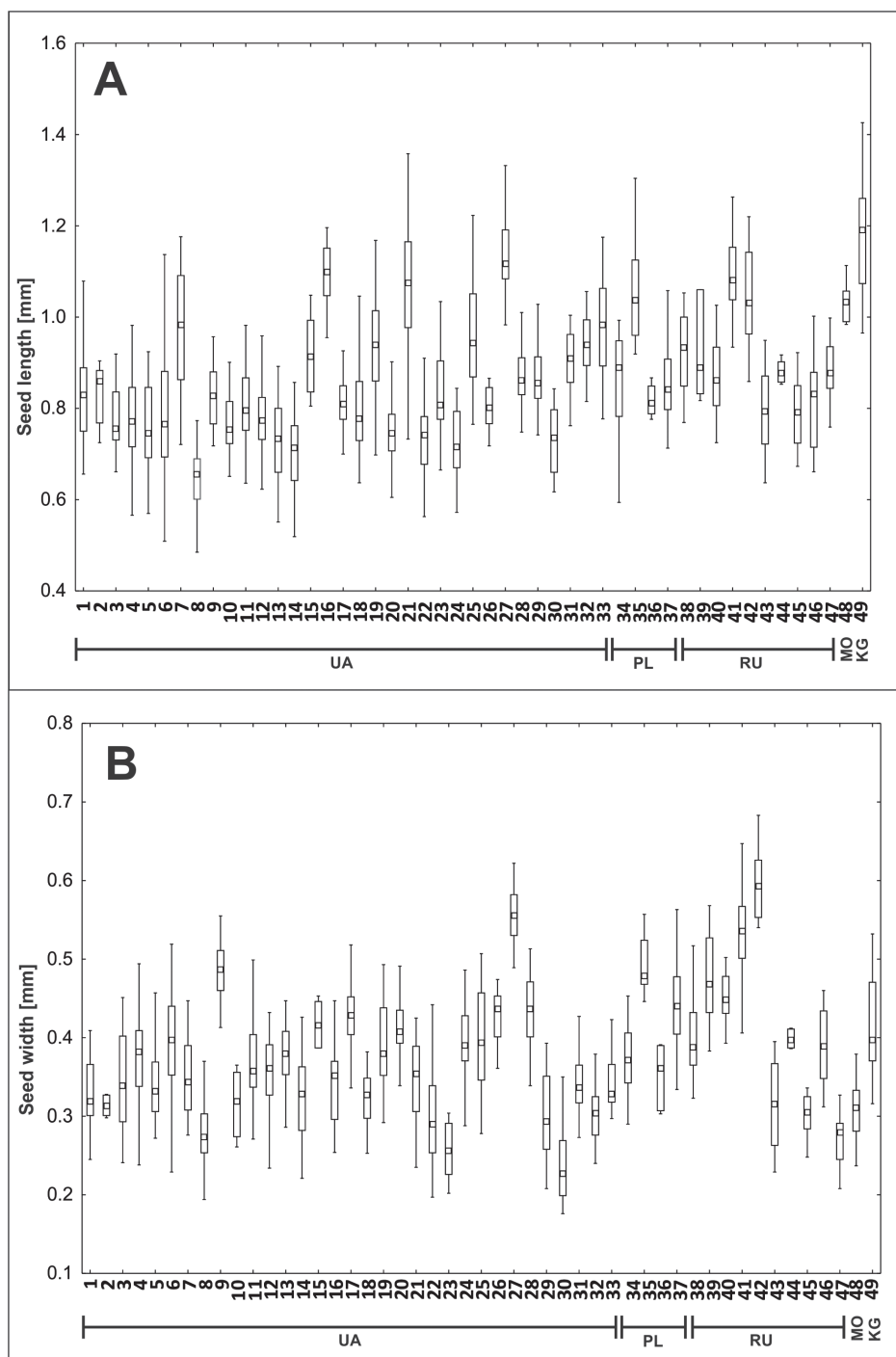


Figure 2. Ranges of variation of seed length **A** and seed width **B** of *Sisymbrium polymorphum*. The boxes represent the 25th–75th percentiles, the upper and lower whiskers extend the minimum and maximum data points and the square inside boxes indicate median. The order of localities is according to Suppl. material 2: Appendix S1.

the others is the population from Ukraine 8UA, which significantly differs statistically from the other 26 populations, mainly from Ukraine, but also from the three populations of Poland (34, 35, 37PL), seven from Russia (38–41, 44, 47RU), the one from Moldova (48MO) and the one from Kyrgyzstan (49KG); the 27UA population was significantly different with 19 populations and the 41RU population was different from 22 populations, mainly from Ukraine. On the other hand, the easternmost population of Kyrgyzstan differs from amongst 23 populations, mainly from Ukraine, but also from one population from Poland (37PL) and two from Russia (43, 45RU).

Principal Component Analysis (PCA) showed that the studied *S. polymorphum* populations from Russia (41RU, 42RU), Poland (35PL), Ukraine (16UA, 21UA, 27UA), Moldova (48MO) and Kyrgyzstan (49KG) are far from the centre of the PCA ordination space. Still, they cannot be said to form separate groups because the ranges of variability of the examined seed traits coincide (Fig. 3). The first component of PC1 explains 73.76% of the morphological variation, while the second component explains 26.24% of the variation. However, the biogeographical interpretation of both PCA axes is not clear due to the mixing of populations from different parts of the range in the diagram.

The similarity analysis performed by the cluster analysis showed that only a few Ukrainian populations (4, 5, 6, 8, 12, 13, 14, 20, 22, 24, 30UA) belong to the same cluster (Fig. 4). The analysis of the structure of the remaining clusters does not indicate a homogeneous separation of groups from individual countries or according to the

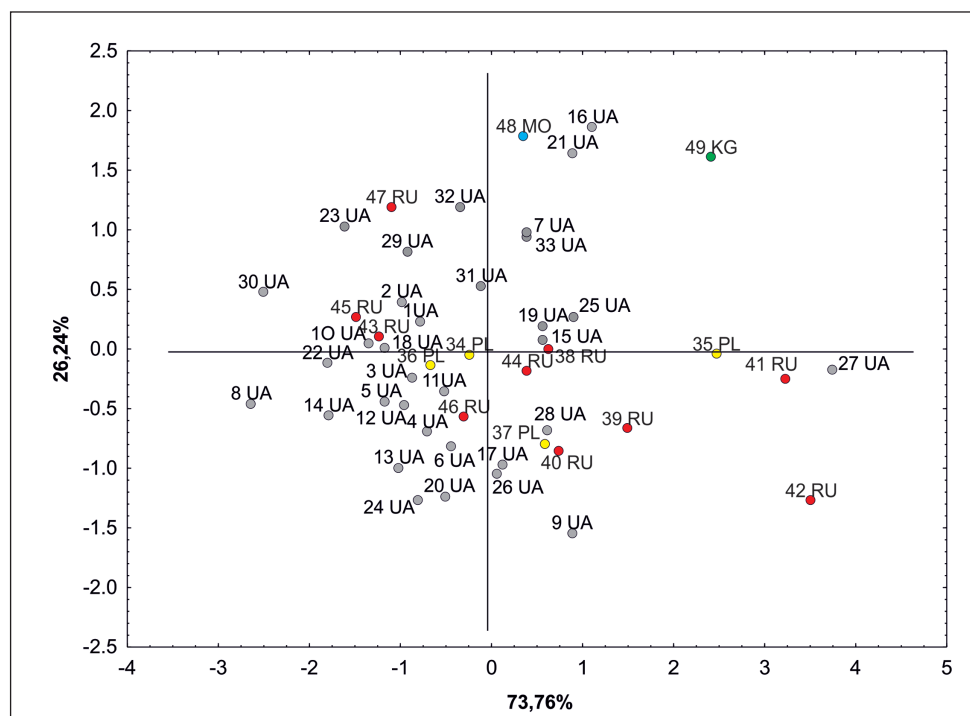


Figure 3. PCA ordination diagram of studied localities of *Sisymbrium polymorphum* according to the biometric traits of seeds. For codes of populations, see Suppl. material 2: Appendix S1.

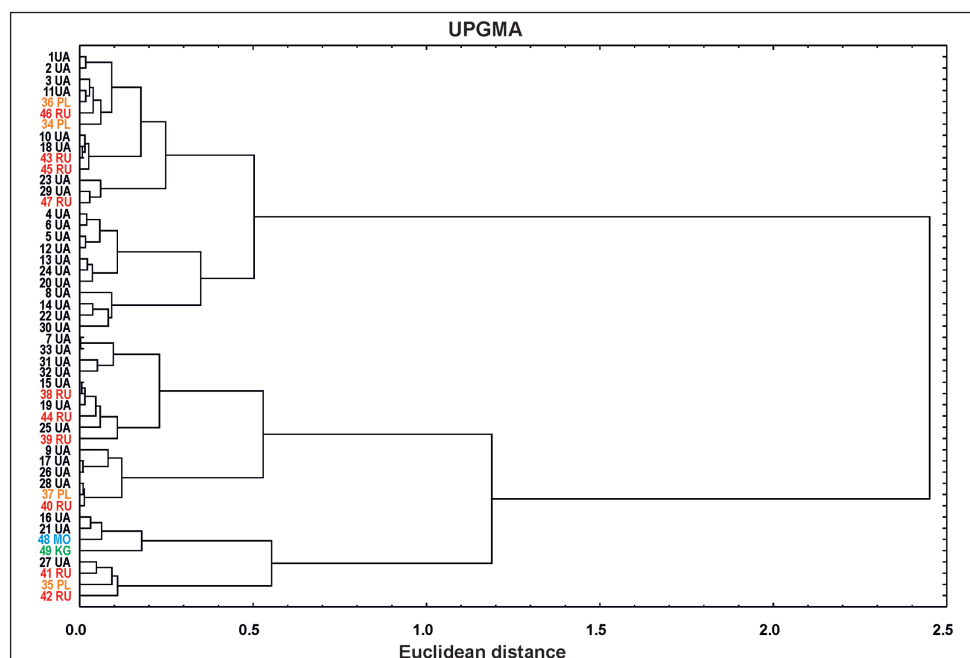


Figure 4. Dendrogram of similarities of *Sisymbrium polymorphum* populations according to seed traits. For codes of populations, see Suppl. material 2: Appendix S1.

geographical gradient. There is a grouping of populations within one cluster (which is confirmed by the PCA analysis) from Kyrgyzstan (49KG), Moldova (48MO) and particular ones from Russia (41, 42RU), Poland (35PL) and Ukraine (16, 21, 27UA).

The relationship between the biometric features of seeds and altitude showed a positive, very weak and weak correlation between the studied features. A weak correlation ($R = 0.39$) (Fig. 5) was demonstrated between seed length and altitude. The weak correlations obtained in our results may be due to the fact that the vast majority of the populations were located at lower altitudes.

The structure of seed surface amongst *Sisymbrium polymorphum*, *S. loeselii* and *S. linifolium*

The seed ultrastructure of the three analysed species showed interspecific differences in ornamentation (Figs 6–8). The species that differed most from the rest was *Sisymbrium linifolium*. Seeds of *S. linifolium* were characterised by acellate patterns, sculpture and circle shape of cells. In the case of *S. polymorphum* and *S. loeselii* seeds, the ultrastructure was quite similar. Both species had reticulate sculpture patterns and smooth anticlinal and periclinal walls. They differed in the shape of the cells, where it was polygonal in *S. loeselii* and from polygonal to rectangular in *S. polymorphum* (Table 1). The mean length and width of cells of the studied species were the highest in *S. linifolium* (41 μm length $\times$ 39 μm width) and *S. polymorphum* (43 μm $\times$ 30 μm) (Table 1).

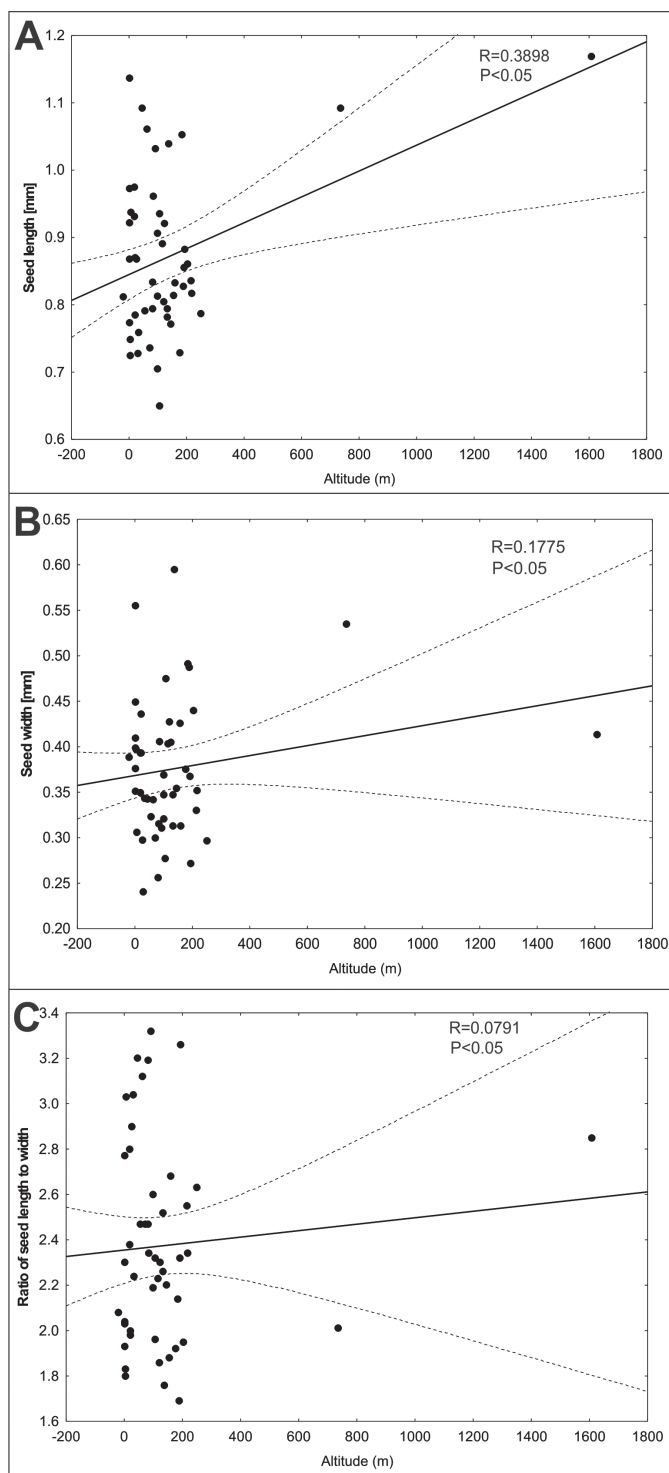


Figure 5. Correlation between **A** seed length **B** seed width **C** ratio of seed length to width of *Sisymbrium polymorphum* and altitude.

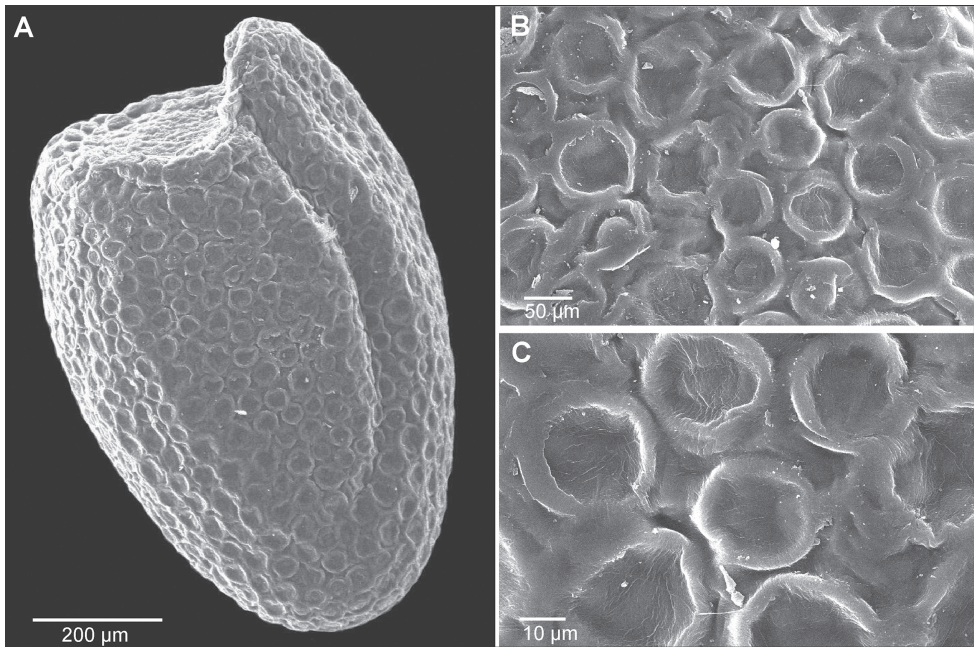


Figure 6. The seed surface of *Sisymbrium linifolium* **A** general view **B** cells **C** ornamentation (sculpture pattern).

Table 1. Seed traits of *S. polymorphum*, *S. loeselii* and *S. linifolium*.

Species	Colour	Shape of seeds	Sculptural pattern	Cell shape	Anticlinal wall	Periclinal wall	Length of cells (µm)	Width of cells (µm)
<i>S. polymorphum</i>	orange-brown, shiny	polymorphous from ovate to angular	reticulate	from polygonal to rectangular	smooth	smooth	43	30
<i>S. loeselii</i>	orange-brown,	oblong-ellipsoid	reticulate	polygonal	smooth	smooth	31	29
<i>S. linifolium</i>	light brown	oblong-ellipsoid	acellate	circular	smooth	smooth	41	39

Discussion

There is a scarcity of literature data on the seed micromorphology of *Sisymbrium polymorphum* (Săvulescu et al. 1955; Tutin et al. 1964; Bojňanský and Fargašová 2007; Karpova et al. 2010; Atlas NBN 2022; WFO 2022). Additionally, there seem to be no reports regarding the variability and ultrastructure of the seeds of these plants. Based on previous publications (e.g. Săvulescu et al. 1955; Tutin et al. 1964; Bojňanský and Fargašová 2007; Atlas NBN 2022; WFO 2022), the length of *S. polymorphum* seeds appears to be within the range 0.6–1.5 mm and the width between 0.3 and 1 mm. The seed dimensions (length, width) recorded in our study are similar to those reported in previous publications (Appendix S2). Our studies indicate lower values in terms of seed minimum

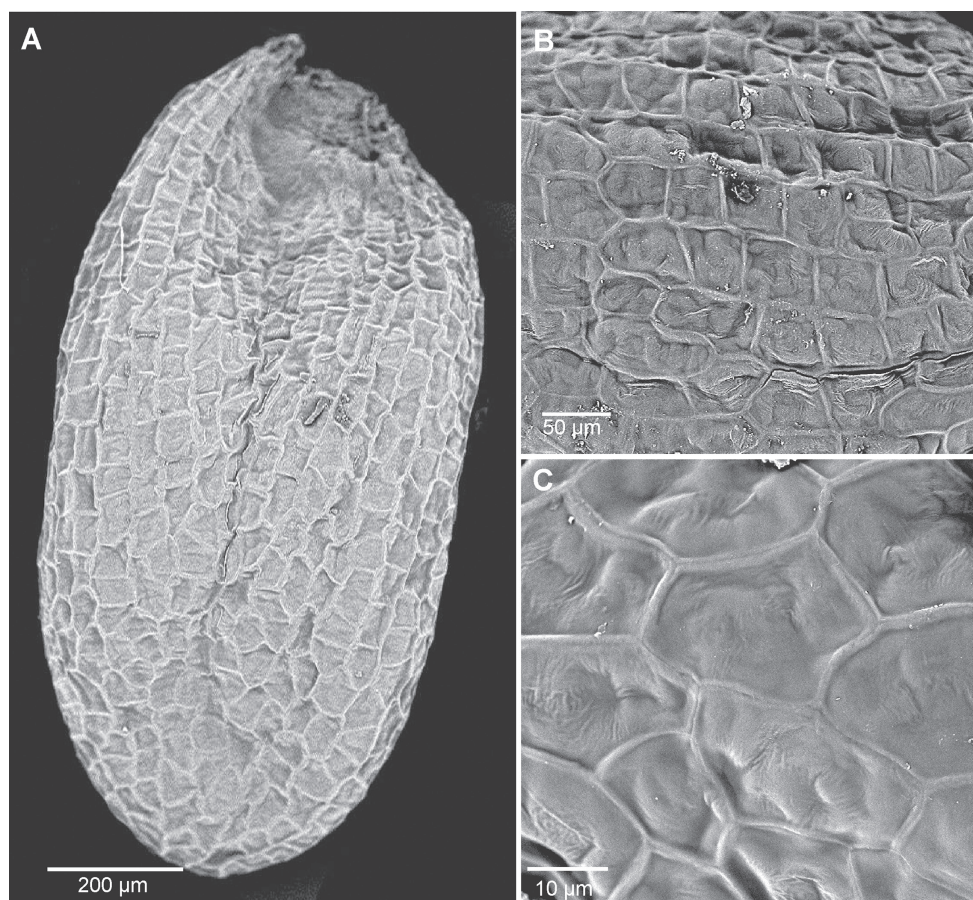


Figure 7. The seed surface of *Sisymbrium polymorphum* **A** general view **B** cells **C** ornamentation (sculpture pattern).

length and minimum width (0.49 mm, 0.18 mm, respectively). Moreover, the average seed width (0.24 mm) was lower than reported in previously-published data (0.3–1 mm) (Săvulescu et al. 1955; Tutin et al. 1964; Bojňanský and Fargašová 2007; www.efloras.org). Our results clearly show statistically significant differences between some populations regarding the analysed features. There were also differences in the coefficient of variation (length WZ 4.14–16.72%, width 4.06–23.93%) (Suppl. material 3: Appendix S2).

Our results did not show significant differences between the core range and isolated populations. However, the longest seeds belonged to the growing population in Kyrgyzstan (49KG) and the Polish populations (37PL), the most westward and second eastward localities. Thus, our research does not confirm that the seeds show variability with longitude change. The multivariate analysis of mean values of seed traits did not show any clear geographic pattern, which can be seen in the PCA analysis (Fig. 3). In our opinion, the size of seeds depends rather on the local habitat and climatic condi-

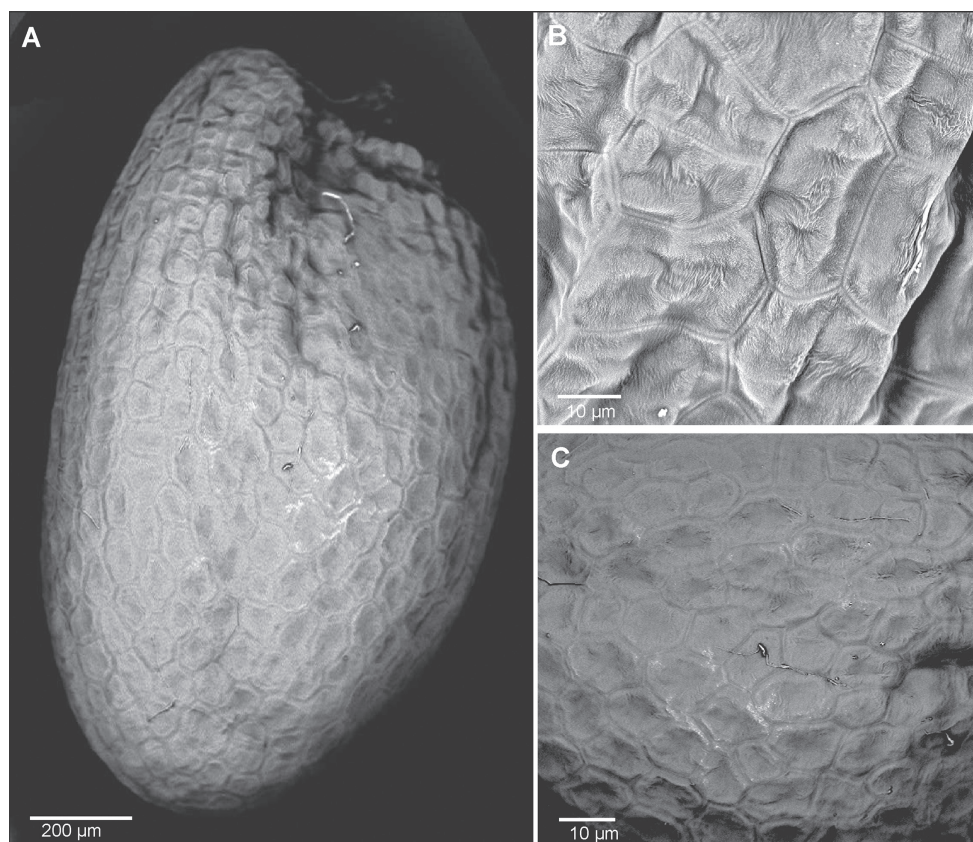


Figure 8. The seed surface of *Sisymbrium loeselii* **A** general view **B** cells **C** ornamentation (sculpture pattern).

tions in which a given population grows. Many previous studies (Sultan 2000, 2001; Fried et al. 2008; Skrajna et al. 2013; Szymura and Szymura 2013; Kostrakiewicz-Gierałt et al. 2022) confirm that the main factors affecting the growth and morphological variation of plants include: soil fertility, climate, topographical conditions or type of land use. *S. polymorphum* grows on various types of soil, i.e. rocky, gravel, sandy loams and, sometimes, on saline soils and plaster outcrops. It also occurs in very shallow initial rendzinas soil type made of gypsum in extremely xerothermic loose grasslands (Kaźmierczakowa and Zarzycki 2001). Depending on the region of occurrence, *S. polymorphum* may prefer other conditions. In Poland, localities are associated with gypsum outcrops (Kaźmierczakowa and Zarzycki 2001). There it occurs on steep slopes with inclinations ranging from 5 to 40 (and up to 70) degrees and prefers the top parts. Plants are also reported from gypsum ravines or on top of oblong gypsum hills. They can also appear in anthropogenic habitats: along railway tracks and embankments (Towpasz and Trzcińska-Tacik 1998). In Hungary, they occur mainly on rocky or sandy sites on river banks. However, throughout Eastern Siberia, they prefer sandy, gravel or rocky ground (Towpasz and Trzcińska-Tacik 1998).

The analysed species did not show a high plasticity in terms of shape and colour of the seeds, which is consistent with reports by other authors (Săvulescu et al. 1955; Gleason and Cronquist 1991; Bojňanský and Fargašová 2007; Cwener and Sudnik-Wojcikowska 2012; www.efloras.org). We observed seeds with polymorphous shapes – from ovate to angular and orange-brown colour. In addition, no variation was observed in the ultrastructure of seeds in the analysed populations. The seeds of the studied species have polygonal to rectangular cells (Suppl. material 1: Fig. S1). The tested seeds showed no variability in ornamentation, which confirms the high stability of this feature. According to Heywood (1974), each genotype has a specific range of plasticity for a given feature, so the analysed feature in some species may show a broader and narrower scale of variation. Knowledge about the plasticity of a given feature allows for the determination of its level and scope of variability and, thus, can indicate whether it might be helpful in the determination of a given species (Heywood 1974; Heywood and Dakshini 1971; Stace 1993). Our research confirms that seed ultrastructure of *S. polymorphum* is stable (has high “resistance” to environmental conditions) and has been shown to provide valuable characters for the delimitation of taxa. The ultrastructure of *S. polymorphum* seeds was here investigated by scanning electron microscopy for the first time.

Our research on the three closely-related *Sisymbrium* species revealed a heterogeneous seed surface structure between these species, the most distinct surface being that of *Sisymbrium linifolium*. The SEM data (shown in Figs 6–8) indicate that the cell-shaped seed surface (circle) can serve as a suitable diagnostic parameter at the species level within the taxa studied. In the case of the other two species (*S. polymorphum* and *S. loeselii*), the shape of the cells is quite similar (from polygonal to rectangular), which, in our opinion, does not allow for an unequivocal species definition. Chen et al. (2019) could not discriminate specimens of *S. linifolium* and *S. polymorphum* from China morphologically; however, molecular identification, based on ITS nuclear marker data, proved their delimitation as separate species in sister groups. Findings of Žerdoner Čalasan et al. (2021) showed that *S. linifolium* and *S. polymorphum* are phylogenetically closest and *S. loeselii* sister, but also a much wider group containing other species. However, our findings are not contradictory, as morphological features do not always have to follow phylogeny, based on molecular loci (Chaw et al. 1997; Donoghue and Doyle 2000). Our findings appear to support this relationship. There is a clear similarity of these three species, based on seed ultrastructural features. Žerdoner Čalasan et al. (2021) claim that *S. polymorphum* is a highly variable species, both morphologically and genetically. Two samples of *S. polymorphum* analysed in this work were assigned to two different subclades: the easternmost (21748), ca. 800 km SE from 47RU, and the third easternmost (25867), ca. 450 km east from 41RU compared to our data. It may be caused by the occurrence of cryptic (or pseudo-cryptic) species complex or paraphyly. We can also observe many incongruences between results from nuclear and chloroplast loci results. Moreover, Chen et al. (2019) could not discriminate morphologically between *S. linifolium* and *S. polymorphum* and only molecular data were able to prove that the Chinese specimens belonged to *S. linifolium*. Such studies once again demonstrate the importance of interdisciplinary integrative taxonomy. The reticulate type of seed coat

is common in other taxa of the Brassicaceae family (Koul et al. 2000; Moazzeni et al. 2007; Karaismailoğlu 2016). Other features of the seed coat surface, such as anticlinal and periclinal cell wall patterns, have been found to be helpful in the delimitation of taxa in Brassicaceae (Vaughan and Whitehouse 1971; Barthlott 1984; Moazzeni et al. 2007; Karaismailoğlu 2016). However, as shown in Figs 6–8, the here investigated species have similar outer periclinal and anticlinal walls, so we could not confirm that they can serve as adequate diagnostic parameters at the species level for the studied taxa. Previous studies have confirmed that seed colour can serve as a diagnostic feature at the generic and specific levels (Kasem et al. 2011; Kaya et al. 2011; Karaismailoğlu 2016, 2019). However, our research does not confirm these reports. Seed colour within the *S. polymorphum* population and between *S. polymorphum*, *S. loeselii* and *S. linifolium* varied from orange to brown to light brown. The addition of this set of micromorphological features to other morphological, biochemical and molecular characters should provide more robust information concerning the phylogenetic affinities between these taxa.

Conclusions

Our research proved that, despite the unclear taxonomic position of *S. polymorphum*, seed ultrastructure is a stable feature and may be useful in taxonomic studies. The study of the seed surface carvings of the other two species shows a difference in the shape and arrangement of cells, especially in the case of *Sisymbrium linifolium*, which confirms that, in the case of this type of carpological analysis, it can be used in diagnostics. Further integrative studies combining seed ultrastructure, other morphological features and molecular data, may finally solve many taxonomic question marks within the *Sisymbrium* genus.

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Supplementary material 1

Figure S1. General view of the seeds (SEM) of *Sisymbrium polymorphum* populations

Authors: Agnieszka Rewicz

Data type: morphological (.tiff file)

Explanation note: **Figure S1.** General view of the seeds (SEM) of *Sisymbrium polymorphum* populations: **A)** 35PL; **B)** 49KG; **C)** 41RU; **D)** 38RU; **E)** 5UA; cells seed 3D; **F)** 10UA; cells seed; **G)** 15UA; **H)** 21UA; **I)** 17UA; **J)** 34PL.

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Link: <https://doi.org/10.3897/phytokeys.206.85673.suppl1>

Supplementary material 2

Appendix S1. List of studied localities of *Sisymbrium polymorphum* included in this study

Authors: Agnieszka Rewicz

Data type: occurrences (.docx file)

Explanation note: List of studied localities of *Sisymbrium polymorphum* included in this study (* - seeds analyzed under SEM).

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Supplementary material 3

Appendix S2. Comparison of biometric traits of *Sisymbrium polymorphum* seeds in the analysed populations

Authors: Agnieszka Rewicz

Data type: morphological data (.docx file)

Explanation note: Comparison of biometric traits of *Sisymbrium polymorphum* seeds in the analysed populations: X arithmetic average; Min, Max maximum and minimum values; SD standard deviation; CV coefficient of variation; SL seed length; SW seed width.

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Supplementary material 4

Appendix S3. Result of the Test Kruskal-Wallis Test

Authors: Agnieszka Rewicz

Data type: morphological data (.docx file)

Explanation note: Result of the Kruskal-Wallis Test: A) seed length; B) seed width.

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A new propaguliferous species of *Pohlia* (Mielichhoferiaceae, Bryopsida) from Tibet, China

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Abstract

A new propaguliferous moss species, *Pohlia tibetana* X.R.Wang & X.M.Shao (Mielichhoferiaceae), from Tibet, southwest China, is described. The new species differs most saliently from other species of *Pohlia* by its combination of slender plants, loosely attached leaves and axillary solitary, and dark red and flower-like gemmae. In this paper, the line drawings, photographs, habit of the new species are provided and a morphological comparison of it with the similar species is made.

Keywords

Asexual reproduction, axillary gemma, Sygera Mountain

Introduction

Asexual reproduction is a remarkable feature and widespread in bryophytes (Frey and Kürschner 2011). Asexual propagules play important roles when sexual reproduction is not attainable (Imura 1994) and can be produced under more stressful conditions and germinate more rapidly in contrast to spores (Newton and Mishler 1994). Vegetative diaspores may come from caducous fragmentation of gametophytic parts (leaves,

leaf apices, shoots, branches and bulbils), specialized propagules (gemmae, protonemal brood cells and tubers) or clonal reproduction (Newton and Mishler 1994; Frey and Kürschner 2011).

A group of species in the genus *Poblia* Hedwig (Mielichhoferiaceae Schimp.) produce specialized asexual propagules and the characters of propagule were used to distinguish various species (Cao and Zhao 2009; Liu et al. 2018). The habitat and the gametophyte characters of these species are very similar. Shaw made a taxonomic revision of the propaguliferous species of *Poblia* in North America to better identify them (Shaw 1981a). In Czernyadjeva's (1999) study of propaguliferous *Poblia* founded in Russia and adjacent regions, the distribution with maps and habitat preferences of nine species was discussed. A taxonomic and descriptive study of seven propaguliferous species with axillary gemmae of *Poblia* in the Iberian Peninsula was made by Guerra (2007). In addition to providing the information on the habitat and distribution, Guerra also gave the photomicrographs of gemmae of each species. Suárez and Schiavone (2011a) meticulously compared the taxonomically important characters of American *Poblia* species in habits and morphology of stems, leaves, perichaetial leaves, setae, stomas, peristome and annulus. They revised the propaguliferous species of *Poblia* from Central and South America and presented the morphological illustrations and photomicrographs of six species with axillary gemmae and one with rhizoidal tubers (Suárez and Schiavone 2011a).

Liu et al. (2018) presented the taxonomic study of ten species of *Poblia* with axillary and rhizoidal propagules in China, including two new records: *P. andalusica* (Höhn.) Brotherus and *P. andrewsii* A.J. Shaw. with the photomicrographs of propagules and line drawings were provided. Wang et al. (2020) reported another newly recorded species with axillary gemmae to China from Tibet: *Poblia tundrae* A. J. Shaw.

Recently, the authors revised the genus *Poblia* in Tibet, China and found a collection different from any species previously known with axillary gemmae. It is characterized by the combination of slender plants, loosely attached leaves and solitary, dark red and flower-like gemmae, and it is here described as a new species.

Materials and methods

Microscopic examination was carried out using traditional methods. The collections of *Poblia* and relevant species in the herbarium of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Institute of Botany, the Chinese Academy of Sciences (PE), and China Agricultural University (BAU) were examined.

Authors observed the plants under the dissecting microscope and examined the leaves and gemmae under the compound light microscope. Light micrographs were photographed using a Motic BA210 digital microscope. All line drawings were made using the drawing tube attachments of these optical microscopes.

Taxonomic account

***Pohlia tibetana* X.R.Wang & X.M.Shao, sp. nov.**

Figs 1, 2

Type. CHINA. Tibet, Linzhi City, Sygera Mountain, Lulang Town, 29°49'0.96"N, 94°44'27.24"E, 3101 m a.s.l., 4 August 2017, *Wei Li & Li-wei Wang 20170804LL010* (**holotype**: BAU!).

Diagnosis. The new species differs most saliently from other species of *Pohlia* by the combination of slender plants (Figs 1A, 2A), loosely attached leaves (Figs 1A, 2A) and axillary solitary, dark red and flower-like gemmae (Figs 1A, F, 2A, F) (Table 1).

Description. Plants slender, light green, dull. Stems 0.5–1.2 cm. Leaves spreading, somewhat contorted when dry, lanceolate to ovate-lanceolate, 0.5–1.0 mm long, somewhat decurrent; margins serrulate to serrate in distal 1/2; costa ending 3–5 cells below leaf apices. Distal laminal cells rhomboidal, 35–70 µm long, 7–11 µm wide, walls thin; Median laminal cells linear-rhomboidal, 70–110 µm long, 5–11 µm wide, walls thin. Basal laminal cells rectangular, 23–65 µm long, 9–16 µm wide, walls thin. Axillary gemmae borne singly in upper leaf axils, 200–280 µm long, 140–230 µm wide, yellowish brown to deep cherry-red, opaque, rosebud shaped, with conspicuous, incurved, peglike to broadly triangular laminate leaf primordia scattered on the bulbiform body. Leaf primordia the same color as the body, arising as elongate, peglike outgrowths, but rapidly differentiating to form a laminate appearance. Sporophytes unknown in China populations.

Etymology. The specific epithet *tibetana* refers to the type locality in Tibet in southwestern China.

Distribution and habitat. Currently *Pohlia tibetana* is only known from the type locality. This species grows on loose soil of rocks in the forest of *Pinus armandii* Franch. It forms tufts mixed with *Pohlia flexuosa* Harvey, *Pohlia hisae* T.J.Kop. & J.X.Luo and *Calypogeia fissa* (L.) Raddi.

Chinese name. 西藏丝瓜藓 (xī zàng sī guā xiǎn)

Discussion

Gemmae, arising singly or clustered in the leaf axils, is very common in *Pohlia*. *P. inflexa* (Müll. Hal.) Wijk & Margad., *P. filum* (Schimp.) Mårtensson, *P. beringiense* A.J. Shaw, *P. rabunbaldensis* A.J. Shaw and *P. drummondii* (Müll. Hal.) A.L. Andrews are similar to the new species in the characteristic of having singly axillary gemmae (Shaw 1981a, 1982, 2006, 2015; Shevock and Shaw 2005; Guerra 2007; Uyar and Ören 2013; Wang et al. 2020). The detailed comparisons of plant and gemma morphological characters between them are shown in Table 1.

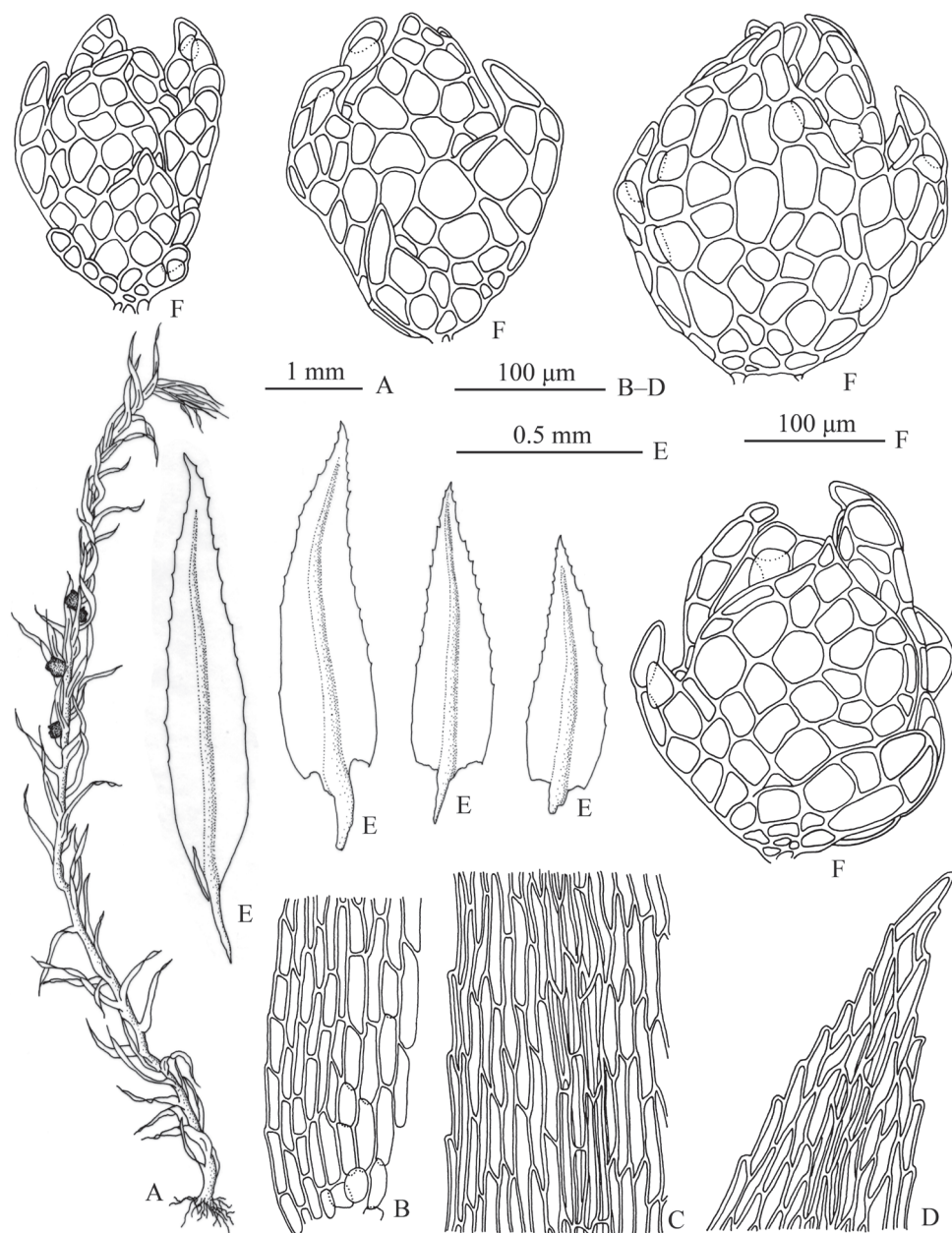


Figure 1. *Poblia tibetana*. **A** plant **B** proximal laminal cells **C** median laminal cells **D** apical laminal cells **E** leaves **F** gemmae. Drawn by Xiaorui Wang from the holotype (BAU!).

Among these species with singly axillary gemmae, *P. inflexa* and *P. filum* are most similar to *P. tibetana* in the features of plants (somewhat slender) and gemmae (subglobose). In *P. inflexa*, the gemmae are big (>500 µm long) and leaf primordia are conspicuous, pale to green, erect or somewhat incurved, while the gemmae are small (<300µm long) and leaf primordia are inconspicuous, the same color as the body and incurved in the new species.

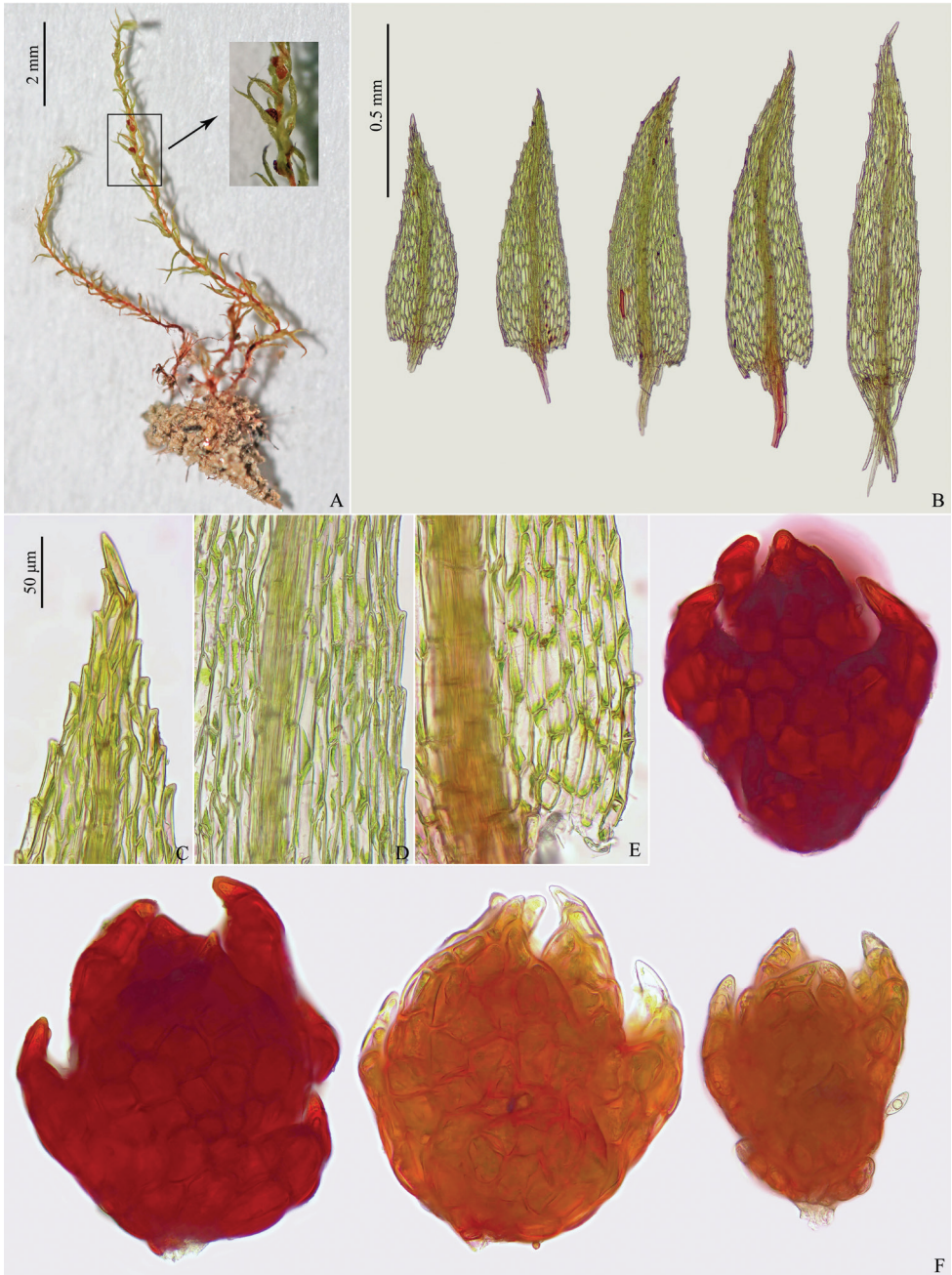


Figure 2. Light micrographs of *Pohlia tibetana*. **A** plants **B** leaves **C** apical laminal cells **D** median laminal cells **E** proximal laminal cells **F** gemmae. Photographed by Xiaorui Wang from the holotype (BAU!).

P. tibetana differs from *P. filum* by its yellowish brown or deep cherry-red, <300μm long (vs. orange or black and >300 μm long) gemmae and arising at apex as well as below, the same color as the body, incurved (vs. arising only in the apex, green to pale, erect) leaf primordia.

Table 1. Morphological comparison of characters distinguishing the similar species in *Pohlia* with single gemma per leaf axil.

Feature	<i>P. tibetana</i>	<i>P. inflexa</i>	<i>P. filum</i>	<i>P. beringiensis</i>	<i>P. rabunbaldensis</i>	<i>P. drummondii</i>
Plants	slender, light green, dull when dry	slender to medium-size,, whitish to yellow-green or green, ± glossy when dry	slender to medium-size, green to light green, slightly glossy when dry	slender, whitish green, glossy when dry	slender, green to light green, dull when dry	medium-size, dark-green, glossy when dry
shape of gemmae	Bulbiform	oblong-bulbiform	ovoid to elliptical or subglobose	bulbiform	narrowly bulbiform	oblong to cylindrical
color of gemmae	yellowish brown to deep cherry-red	deep cherry-red	orange to orange-brown, or black	red to black-red	orange or orange-red to reddish	dark red-brown
size of gemmae	200–280 µm long	>500 µm long	300–500(–550) µm long	500–650(–1000) µm long	400–750 mm long	350(500)–1000(1900) µm long
leaf primordia	inconspicuous, peglike to broadly somewhat triangular-laminate, at apex as well as below, the same color as the body, incurved	conspicuous, broadly lanceolate laminate, at apex and scattered lower on the body, pale to green, erect, somewhat incurved	inconspicuous, stiffly and triangular-lanceolate laminate, arising only in the apex and sometimes lower, green to pale, erect	conspicuous, stiffly and broadly laminate, at apex and more proximally, whitish green, erect	conspicuous, broadly laminate, at apex and lower, sometimes to base, green, flexuose	conspicuous, stiffly lanceolate laminate, scattered at the apex and below, green, erect

The gemmae of *P. tibetana* are rather like those of *P. andrewsii* from Arctic regions (Shaw 1981b, 2015; Liu et al. 2018). Nevertheless, *P. andrewsii* is distinguished from the new species by its glossy leaves and densely clustered gemmae.

The propaguliferous species of *Pohlia* occurring in Tibet are very alike in habit and generally grow together, forming dense or lax turfs on soil. *P. tibetana* grows on loose soil mixed with two species of *Pohlia* having clustered axillary gemmae: *P. flexuosa* and *P. hisae*. The gametophyte features of *P. tibetana*, such as slender plants and spreading leaves which are somewhat contorted when dry, are very similar to *P. flexuosa*. The two species are confused with each other in the absence of gemmae. However, *P. flexuosa* is distinguished from *P. tibetana* by its dimorphic gemmae in dense clusters (Shaw and Torne 2009; Liu et al. 2018).

Suárez and Schiavone have conducted systematic research on the genus *Pohlia* in Latin America and published a series of achievements (Suárez and Schiavone 2010, 2011a, 2011b). In the revision of the propaguliferous *Pohlia* species (Suárez and Schiavone 2011a), the morphological characters of *P. papillosa* (Müll. Hal. ex A. Jaeger) Broth., such as loosely arranged leaves on the sterile plants in watery habitats, oblong or obconical gemmae in leaf axils orange or reddish with leaf primordia and body of the same color, are consistent with those of *P. tibetana*. In the former species, the gemmae are numerous in each axil and variable from linear-vermicular to obconical, leaf primordia erect, whereas in *P. tibetana* the gemmae are singly in each axil and stable rosebud shaped, and leaf primordia are inconspicuous with incurved apices.

The biodiversity of bryophytes in Tibet, China is very abundant. Eighteen species of *Pohlia* distributed in Tibet were recorded in Flora Bryophytorum Sinicorum (Li 2006). Liu et al. reported that *P. drummondii* was also distributed in Tibet in their study of propaguliferous in China (Liu et al. 2018). Wang et al. (2020) reported a new

recorded species of this genus with axillary gemmae: *P. tundra* in Tibet. To date there are 20 species of *Pohlia* distributed in Tibet including 8 species with axillary gemmae.

Key to the *Pohlia* species with axillary gemmae in Tibet, China

- 1 Gemmae 1 per leaf axils **2**
- Gemmae numerous per leaf axils..... **3**
- 2 Plants medium-size, dark green, gemmae oblong to cylindrical, dark red-brown, 350–1000 µm long, leaf primordia conspicuous, stiffly lanceolate laminate, scattered at the apex and below, green, erect ***P. drummondii***
- Plants slender, light green, gemmae spherical, yellowish brown to deep cherry-red, 200–280 µm long, leaf primordia inconspicuous, broadly somewhat triangular-laminate, at apex as well as below, the same color as the body, incurved..... ***P. tibetana***
- 3 Plants with two different types gemmae: ellipsoidal and thread-like... ***P. flexuosa***
- Plants with only one type gemmae..... **4**
- 4 Gemmae spheroidal, leaf primordia inconspicuous ***P. camptotrachela***
- Gemmae obconic to filiform or cylindrical, leaf primordia conspicuous..... **5**
- 5 Leaf primordia laminate, clustered at apes and also scattered along the gemma body ***P. tundrae***
- Leaf primordia peg-like or rarely laminate, restricted to apex **6**
- 6 Gemmae obconic, leaf primordia approximately one to two times as long as the length of the gemma body ***P. hisae***
- Gemmae oblong to filiform, leaf primordia shorter than the length of the gemma body..... **7**
- 7 Plants dull when dry, gemmae shape is variable on a single plant, oblong or obconic, clavate to vermicular..... ***P. annotina***
- Plants glossy when dry, gemmae shape is uniform on a single plant, long filiform ***P. leucostoma***

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Ficus motuoensis (Moraceae), a new species from southwest China

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Abstract

A new climbing species, *Ficus motuoensis* Zhen Zhang & Hong Qing Li in Moraceae from southwest China has been described and illustrated in this paper. The new species resembles *F. disticha*, *F. diversiformis* and *F. hederacea*, but differs from these in the medium-sized acrophylls, shorter peduncle, as well as larger and spotted syconium. According to the morphological traits and phylogenetic placement, the new species belongs to *Ficus* subg. *Synoecea* sect. *Apiosycea*. Besides, the new species deviates from the common distribution pattern compared to the other members of sect. *Apiosycea*, indicating that it could be very useful for exploring the biogeography of sect. *Apiosycea*.

Keywords

climbing figs, fig tree, new taxon, Rosales, Sino-Himalaya

Introduction

Ficus L. is an extremely species-rich woody genus in the family Moraceae, mainly distributed in tropical and subtropical regions (Berg and Corner 2005; Pederneiras et al. 2018; Zhang et al. 2020). As the largest genus in Moraceae, *Ficus* features the syconium and mutualism relationship to fig wasps (Janzen 1979; Wiebes 1979; Bronstein and McKey 1989). To date, the number of species in *Ficus* has grown to almost 800 after recent frequent descriptions from South America, Southeast Asia and

so on (Chantarasuwan and Thong-Aree 2006; Berg and Homeier 2010; Whitfeld and Weiblen 2010; Berg 2012; Medina 2014; Machado and de Queiroz 2017; Pederneiras et al. 2017; Ezedin and Weiblen 2019; Rivera et al. 2020). Southwest China, especially the Himalaya-Hengduan Mountains, is one of twenty-five biodiversity hotspots worldwide and possesses abundant endemic species (Myers et al. 2000). Meantime, many gynodioecious fig trees are endemically distributed in the Himalaya-Hengduan Mountains or the Sino-Himalaya Region in *Ficus* subg. *Ficus* and subg. *Synoecia* (Miq.) Miq. However, relatively few species in *Ficus* have been described from these regions in two decades (Chen et al. 2020), indicating a possible underestimate of biodiversity.

Through two field investigations with an interval of seven years in Motuo County, Tibet, in China, we found an unrecorded climbing fig tree. The climbing species is rather distinct in the aspects of small leaves and spotted syconia compared to the other Chinese climbing figs. Based on specimen examination and phylogenetic analyses, we confirmed that it is a new species in subg. *Synoecia* and provided its taxonomical description and illustration.

Materials and methods

Morphological observations

The novel species was surveyed in Motuo County, Tibet, in China. The morphological characteristics were measured and then photographed by digital camera (Canon, D80) or stereomicroscope (SMZ25, Nikon). The type specimens have been stored in the Herbarium of the East China Normal University (HSNU). The morphological comparison between the new species and its congeners has also been examined.

Phylogenetic inference

Three samples represented the new species and three nuclear loci, internal transcribed spacer (ITS), external transcribed spacer (ETS) and glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) were used to verify its phylogenetic placement in *Ficus*. Simultaneously, two samples of its morphologically related species *F. hederacea* Roxb. were supplemented. The other taxa and sequences in subg. *Synoecia* were selected according to the work of Zhang et al. (2020) and their GenBank accession numbers can be found in Suppl. material 1: Table S1. In total, twenty-eight samples were involved in phylogenetic analyses together with two extra samples of *F. laevis* Blume as the outgroups.

Bayesian Inference (BI) and Maximum Likelihood (ML) analysis were implemented to reconstruct the phylogenetic trees. Bayesian Inference was carried out by MrBayes 3.2.6 (Ronquist et al. 2012) with 5,000,000 generations, sampling every 1,000 generations to ensure the convergence (average deviation of split frequencies less than 0.01 and the effective sample sizes over 200). The first 25% of sampling trees were treated as burn-in and the remainder were used to prepare the consensus tree and posterior probabilities. The IQ-TREE 2.1.3 (Nguyen et al. 2015) was used to reconstruct the Maximum Likelihood

tree with 10,000 ultrafast bootstrap to assess the confidence of the nodes. The nucleotide substitution models for both BI and ML were selected by ModelFinder (Kalyaanamoorthy et al. 2017) with the respective commands. The ML tree was chosen to show the topology after visualisation by Figtree 1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

Morphological observations

The novel species is a gynodioecious root-climbing taxon with obvious dimorphic leaves, thus it should belong to subg. *Synoecia*. Some key traits, including leaf dimorphy, rather small leaves, spotted syconia and sessile flowers, make the new species fairly distinct in subg. *Synoecia*. Four climbing fig trees, including *F. laevis*, *F. pubigera* var. *pubigera*, *F. pubigera* var. *maliformis* and *F. sarmentosa* Buch.-Ham. ex Sm., were sympatric with the new species in Motuo County, based on our field investigation. However, none of them resembles the new species. The bathyphylls of the new species are similar to those of *F. disticha* Blume (subg. *Synoecia*), except for the symmetric lamina and its round apex. Besides, the acrophylls (4.5–6.5 cm in length) and syconia (8–10 mm in diameter) of the new species are obviously larger than those of *F. disticha*. The new species also resembles *F. hederacea* and *F. diversiformis* in the aspect of acrophylls, whereas its syconia are different from these. The syconia of *F. hederacea* is globose with a 10–12 mm peduncle in length and those of *F. diversiformis* is basal constricted with a 3–12 mm peduncle. Both of them are clearly longer than the new species (1–2 mm). From a geographical point of view, the new species is also allopatric to *F. disticha*, *F. hederacea* and *F. diversiformis*. A comparison between the new species and its morphological allies are shown in Table 1.

Phylogenetic Inference

The phylogenetic tree indicates that three samples of the new species comprised a well-supported monophyletic group (posterior probability = 1 and ultrafast bootstrap value = 100, Fig. 1). The new species is phylogenetically sister to another widely distributed

Table 1. Morphological comparison amongst the new species and its three allies.

	<i>F. motuoensis</i>	<i>F. disticha</i>	<i>F. hederacea</i>	<i>F. diversiformis</i>
Bathyphylls size (cm)	1.5–2.5 × 0.8–1.2	1–2.5 × 0.8–1.5	4–8 × 2–3.5	1–2 × 0.5–1.5
Acrophylls size (cm)	4.5–6.5 × 2.5–3.5	2.5–5 × 2–5	6–11 × 3.5–5	1.5–5.5 × 1–3
Acrophylls shape	elliptical	variable, somewhat obovate	elliptical	obovate
Veins (pairs)	5–6	3–7	3–5	2–3
Peduncle length (mm)	1–2	0–4	10–12	3–12
Syconia size (mm)	8–10	3–6	7–14	10–13
Syconia shape	globose	globose to pyriform	globose	globose to pyriform
Syconia colour	green to red with variegation	green to red-brown to purplish	green to orange	unknown

species *F. hederacea* and both of them form a sister relationship to the Sri Lankan endemic species *F. diversiformis*. The closely-related genetic relationship amongst these three species is also supported by their morphological similarity. Another morphologically related species *F. disticha* is far from the new species in the phylogenetic tree.

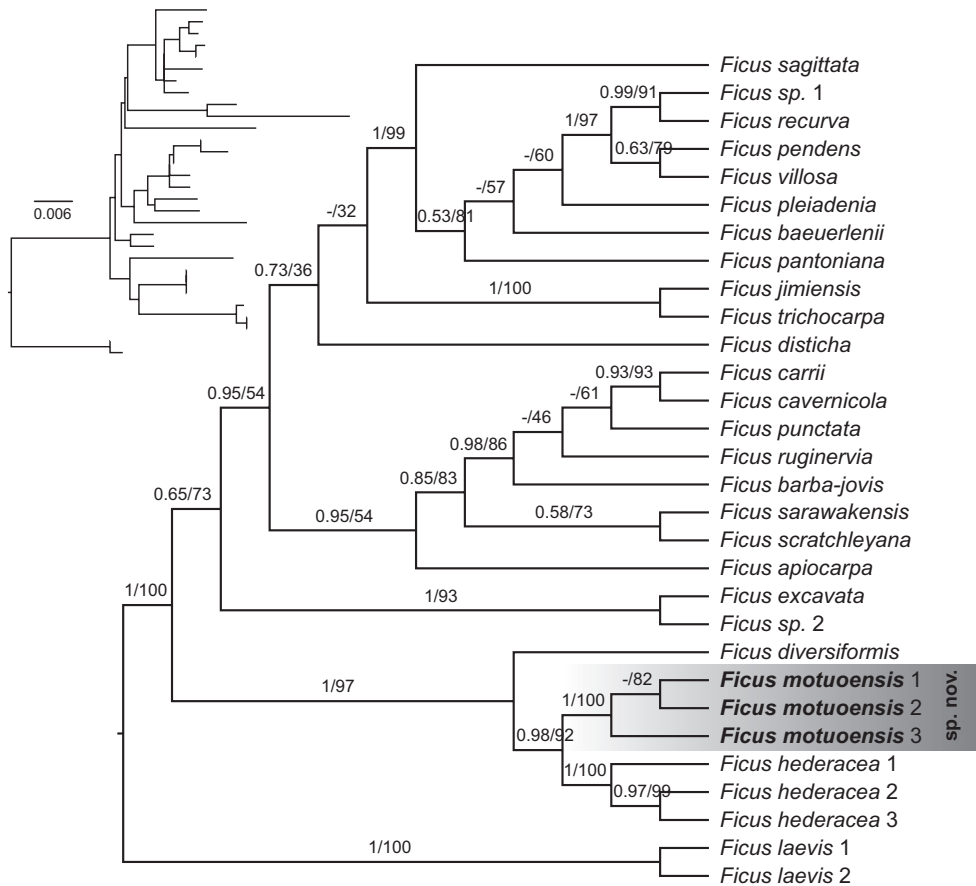


Figure 1. The Maximum Likelihood cladogram, based on three nuclear loci (ITS + ETS + *G3pdh*) with posterior probabilities and ultrafast bootstrap values shown on the branches. The phylogram of the tree shown in the upper left.

Taxonomic treatment

Ficus motuoensis Zhen Zhang & Hong Qing Li, sp. nov.

urn:lsid:ipni.org:names:77304424-1

Figs 2, 3

Type. CHINA. Tibet (Xizang): Linzhi, Motuo, Deergong. 25 Jun 2021, *Zhen Zhang & Jian-Hang Zhang* ZZ966 (holotype HSNU00079864!, isotype HSNU00079862!);

paratype Zhen Zhang & Jian-Hang Zhang ZZ905 (HSNU00079863!), Zhen Zhang & Jian-Hang Zhang ZZ955 (HSNU00079861!), Zhen Zhang & Jian-Hang Zhang ZZ962 (HSNU00079865!, HSNU00079866!, HSNU00079867!).

Diagnosis. *Ficus motuoensis* is similar to *F. disticha* in the shape and texture of the bathyphylls, but differs from the latter by its larger acrophylls (4.5–6.5 cm in *F. motuoensis* versus 2.5–5 cm in *F. disticha*) and larger syconia (8–10 mm in *F. motuoensis* versus 3–6 mm in *F. disticha*). The new species also resembles *F. hederacea* and *F. diversiformis* in the aspect of the acrophylls, but can be distinguished from these by its globose and spotted syconia (versus without spots in *F. hederacea* and *F. diversiformis*) with a shorter peduncle (1–2 mm in *F. motuoensis* versus 10–12 mm in *F. hederacea* and 3–12 mm in *F. diversiformis*).

Description. Gynodioecious root-climber. Branchlets densely pale pubescent, glabrous in biennial branches, with some lenticels in biennial branches. Stipules 2, 2–3 mm in length, long triangular-lanceolate, glabrous, reddish-brown, caducous; bathyphylls distichous, petiole 2.5–4 mm, greenish to light brown, adaxially furrowed, densely white pubescent at the both sides of furrow, lamina elliptical, 1.5–2.5 × 0.8–1.2 cm, symmetric, thinly chartaceous, base rounded, apex acute, margin entire, veins 4–5 pairs, abaxially slightly raised, basal vein up to 1/3 the length of the lamina, both surfaces glabrous, the abaxially surface tessellate; acrophylls distichous, petiole 5–10 mm, brown, subglabrous, adaxially furrowed, lamina elliptical, 4.5–6.5 × 2.5–3.5(–4) cm, coriaceous, base rounded, apex acute to obtuse, margin entire, veins 5–6(–7) pairs, abaxially slightly raised, basal vein up to 1/3 the length of the lamina, both surfaces glabrous, the abaxially surface tessellate. Figs axillary on the leafy or leafless branchlets, in pairs or sometimes solitary; peduncle 1–2 mm, basal bracts 3, ca. 1 mm in length, broadly ovate, glabrous; receptacle globose, 8–10 mm in diameter when fresh, greenish to red when mature, densely covered by light-green speckles, glabrous; ostiole ca. 2 mm in diameter; internal hairs absent. Staminate flowers numerous, scattered, sessile; calyces 3–4, light pink, translucent, glabrous, ovate-lanceolate, ca. 2 mm in length; stamens 2–3, slightly shorter than calyx; anther oblong, ca. 1.2 mm in length, not mucronate; filament free, ca. 0.6 mm in length, a few hairs born on the joint of filaments. Gall flowers numerous, sessile; calyx 3–5, light pink, translucent, lanceolate to linear, 2–2.5 mm in length, glabrous; ovary elliptical, base constricted to being gynophore, 0.5–2 mm; style subapical, short, ca. 0.2 mm in length; stigma funnel-form, margin lacerate. Pistillate flowers not seen.

Chinese name. Mo Tuo Rong (墨脱榕).

Etymology. The specific epithet indicates its type locality, Motuo County, Tibet, in China.

Distribution and habitat. Only found in the type locality, i.e. China: Tibet, Linzhi, Motuo County. However, considering that Motuo is close to Assam in India, the new species probably also exists in India. The new species develops very well in the type locality, as it has been recorded in five different villages (Bari, Yarang, Gelin, Deergong and Maniweng). The individuals of the new species are rather abundant

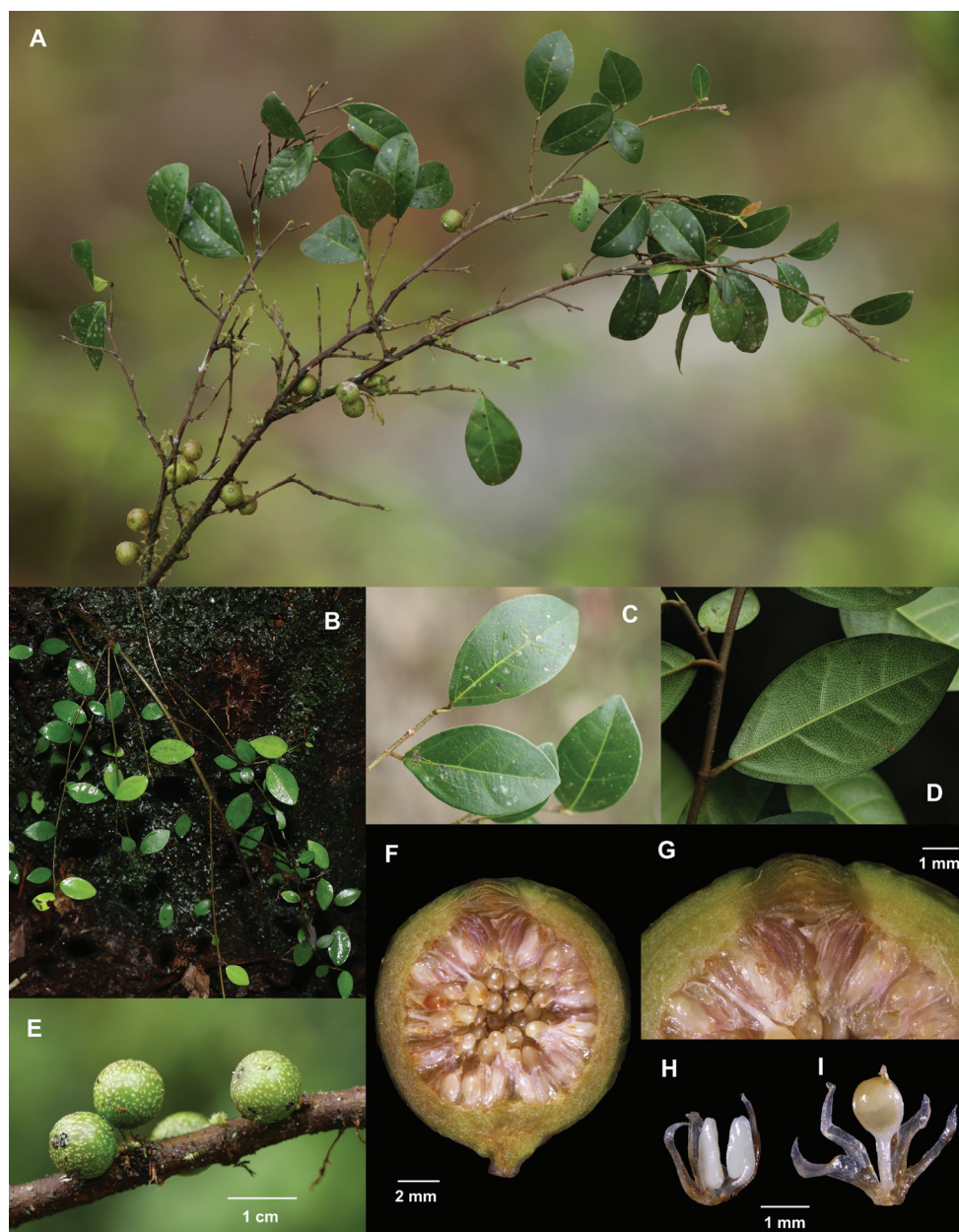


Figure 2. Illustration of *Ficus motuoensis* **A** fruit branch **B** vegetative branch **C** adaxial surface of acrophylls **D** abaxial surface of acrophylls **E** syconia **F** profile of staminate inflorescence **G** ostiole bracts **H** staminate flower **I** gall flower.

without the risk of extinction. The new species grows in the tropical monsoon forest climbing on substrates, such as soils and tree trunks, whereas its fertile branches often break away from the substrate at the time of reproduction. It is located at an altitude of 700–2000 m.

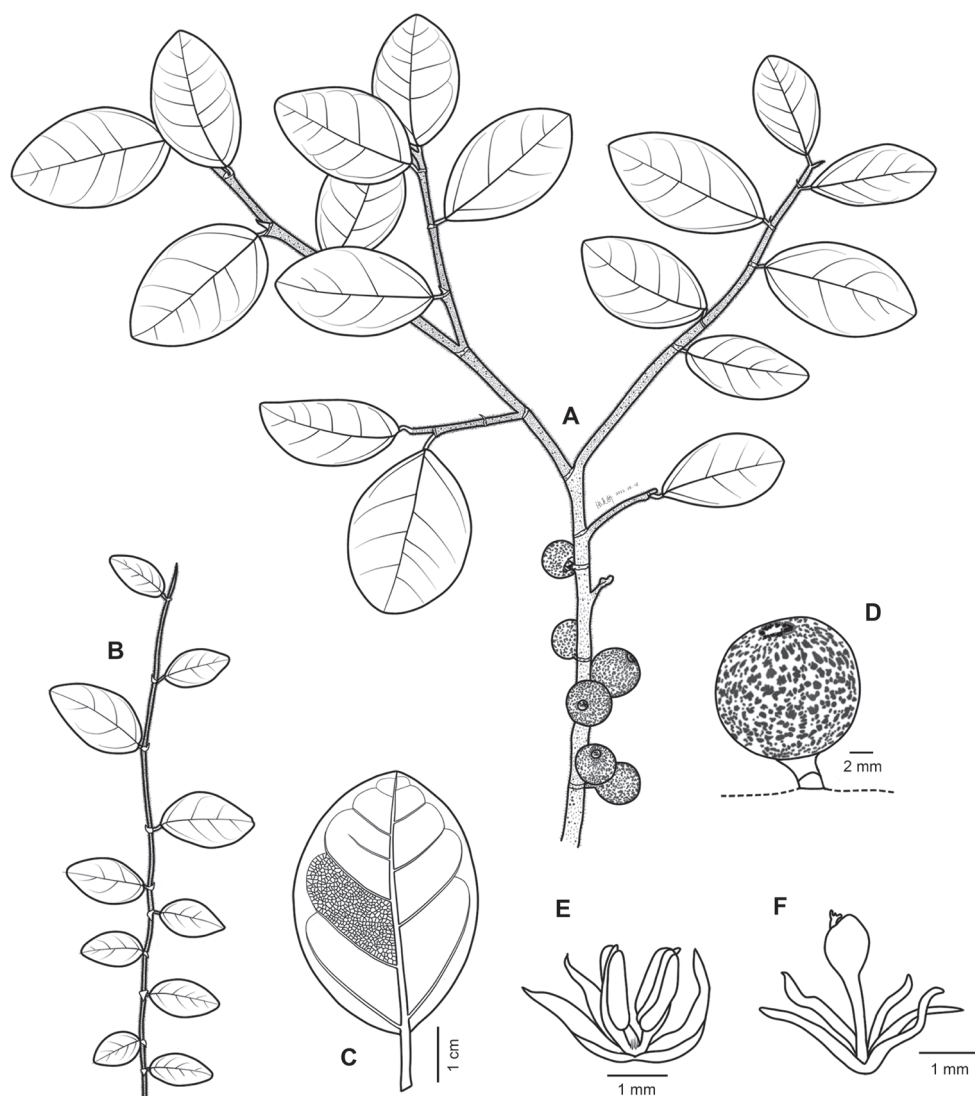


Figure 3. Illustration of *Ficus motuoensis* **A** fruit branch **B** vegetative branch **C** abaxial surface of acro-phylls **D** syconium **E** staminate flower **F** gall flower.

Note. Based on the morphological traits and phylogenetic placement, the new species is related to *F. disticha*, *F. diversiformis* and *F. hederacea*. In the latest division framework of *Ficus*, these three species were assigned to sect. *Apiosycea* (Miq.) Pedern. & Romaniuc (Zhang et al. 2020). However, the taxa in sect. *Apiosycea* are mainly distributed in Malesia (Zhang et al. 2020). In total, six Chinese taxa belong to sect. *Apiosycea*, including *F. hederacea*, *F. laevis*, *F. punctata* Thunb., *F. sagittata* Vahl, *F. trichocarpa* Blume and *F. villosa* Blume, but south China is only the northern limit of their distribution range (Chang et al. 1998; Berg and Corner 2005). Therefore, the new species, which is endemic to southwest China, could be very useful for exploring the biogeography of sect. *Apiosycea*.

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Supplementary material I

Table S1

Authors: Zhen Zhang, Mei-Jiao Zhang, Jian-Hang Zhang, De-Shun Zhang, Hong-Qing Li

Data type: excel file

Explanation note: Detailed information of samples in the study.

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Link: <https://doi.org/10.3897/phytokeys.206.89338.suppl1>

Resurrection of *Drypetes nienkui* (Putranjivaceae), endemic to Hainan, China

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Abstract

Drypetes nienkui (Putranjivaceae), described from Hainan, China, has long been treated as a synonym of *D. indica*. Both species belong to a distinctive group of Asian species characterized by perulate buds that give rise to shoots bearing flowers or inflorescences proximally and leaves distally, 2–3-carpellate ovaries, and elongate styles. However, *D. nienkui* fundamentally differs from *D. indica* in inflorescence architecture and fruiting pedicel length; these or other characters also distinguish *D. nienkui* from the remaining species in this group. *Drypetes nienkui* therefore deserves recognition as a distinct species endemic to Hainan. An expanded description of the species is provided along with a key to the related species.

Keywords

Asia, China, *Drypetes*, Hainan, Putranjivaceae

Introduction

The genus *Drypetes* Vahl (Putranjivaceae Endl., Malpighiales) comprises about 220 species of mostly dioecious trees and shrubs found in the tropics and subtropics worldwide; it is most diverse in Asia, with about 120 species. Thirteen species currently are recognized from China (Qin et al. 2007; Li and Gilbert 2008); of these, nine are reported from Hainan, including the endemic *D. longistipitata* P.T.Li. Among the currently accepted species found in China, there are two, *D. indica* (Müll. Arg.) Pax & K.Hoffm., and *D. longistipitata*, that, along with the Thailand endemic

D. dasycarpa (Airy Shaw) Phuph. & Chayamarit, belong to a group of taxa that are distinctive among Asian *Drypetes* in having prominent buds enclosed in chartaceous scales (perules; Fig. 1A). When the axillary, and sometimes the terminal, buds develop into shoots, the proximal few nodes usually are leafless and produce flowers or inflorescences, while the more distal nodes produce leaves (Fig. 1A). In addition, the pistils, which are 2–3-carpellate, have more or less elongate styles that bear peltate to flabellate stigmas (Fig. 1B); most other *Drypetes* species in the region have 1-carpellate pistils, sessile stigmas, or both. This group of species, which I will refer to as the *D. indica* complex, ranges from Bhutan and northeastern India eastward to Taiwan and southward to Thailand.

Taxonomic concepts within the *Drypetes indica* complex have varied widely. For example, Pax and Hoffmann (1922), the last to review *Drypetes* worldwide, accepted *D. indica* and four other species that I would place in this complex: *D. griffithii* (Hook.f.) Pax & K.Hoffm., *D. hieranensis* (Hayata) Pax & K.Hoffm., *D. karapinensis* (Hayata) Pax & K.Hoffm., and *D. lancifolia* (Hook.f.) Pax & K.Hoffm.. In contrast, Airy Shaw (1972), in a review of Euphorbiaceae s.l. in Thailand, synonymized the latter four species under *D. indica*. He also treated *D. nienkui* Merr. & Chun, a species described from Hainan, China, as a synonym of *D. indica*. He noted that this treatment made for a highly variable species, but felt that specimens from Thailand and Hainan bridged the morphological and geographic gaps between Indian/Burman material and that from Taiwan. Li (1988) expanded Airy Shaw's broad concept of *D. indica* to include *D. longipes* X.H.Song, and this view has been followed in treatments of the genus for China (Li 1988, 1994; Li and Gilbert 2008; Fang et al. 2011). However, Taiwanese botanists have continued to treat the material from there as distinct from *D. indica*, recognizing either both *D. hieranensis* and *D. karapinensis* (Hsieh 1977) or, more recently, synonymizing *D. hieranensis* under *D. karapinensis* (Lu 1986; Hsieh et al. 1993; Boufford et al. 2003). Both *D. dasycarpa* and *D. longistipitata* were described after Airy Shaw (1972) expanded the concept of *D. indica* and have consistently been accepted as distinct taxa, although Airy Shaw (1977) initially described *D. dasycarpa* as a variety of *D. indica*.

Drypetes nienkui was described from a single fruiting specimen (Merrill and Chun 1935). Because an isotype from this gathering is the only specimen of *D. nienkui* at K (pers. obs. and GBIF 2022) and I have seen no other specimens of this species annotated by Airy Shaw, it appears that he (Airy Shaw 1972) only saw this specimen before synonymizing *D. nienkui* under *D. indica*. Having had the opportunity to examine more specimens, including type material, of *D. nienkui* and the other species in the *D. indica* complex from throughout eastern Asia, I believe the broad treatment of *D. indica* pioneered by Airy Shaw (1972) is too inclusive. *Drypetes nienkui*, in particular, is quite distinct, although further species may also deserve recognition. Here I review morphological characters that distinguish *D. nienkui* from *D. indica* and *D. dasycarpa*; I also provide an expanded description of *D. nienkui* and a key to species I currently accept in the *D. indica* complex.

Materials and methods

Morphological characters were examined on specimens held by A, BM, G, IBSC, K, KUN, L, NY, P, and US (herbarium acronyms follow Thiers [continuously updated]), and online images of specimens from AU, CAS, E, HITBC, IBK, MW, NAS, PE, SN, TAI, TI, and TNM available at JSTOR Global Plants (<https://plants.jstor.org/>), the Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>), the Global Biodiversity Information Facility (<https://www.gbif.org/>), and individual herbarium web-sites. Measurements were made using both actual specimens and specimen images containing scale bars.

Results and discussion

Material that Airy Shaw (1972) and others included within *Drypetes indica* exhibits two different inflorescence variants. Specimens from Hainan that have been called *D. indica* or *D. nienkui* have staminate and pistillate flowers borne in cymose inflorescences and pistillate pedicels that are no longer than 12 mm in fruit (Fig. 1C), whereas all the remaining specimens of *D. indica* in the broad sense have fasciculate staminate flowers and solitary pistillate flowers, the pedicels of which become at least 16 mm long in fruit (Fig. 1B); the same is true of *D. longistipitata*. Merrill and Chun (1935) described the infructescences of *D. nienkui* as “depauperate-racemose” (they had only fruiting material), although they actually are cymose. *Drypetes dasycarpa* also has cymose inflorescences and relatively short pistillate pedicels, as was reported by Phuphathanaphong and Chayamarit (2000, 2005). These inflorescence characters were overlooked by Airy Shaw (1972, 1977), perhaps because the specimens of *D. dasycarpa* and *D. nienkui* that he studied at Kew have either only detached fruits or infructescences bearing only a single mature fruit (pers. obs.).

Within the cymose inflorescence group, the specimens sort neatly into two groups based on differences in pubescence, perule size, leaf blade size, staminate pedicel length, and stamen number. The groups correspond to plants from northern and western Thailand (*Drypetes dasycarpa*) and those from Hainan (*D. nienkui*). Within the group with fasciculate staminate flowers and solitary pistillate flowers, it appears that *D. indica* and *D. longistipitata* are distinct, based on differences in leaf blade secondary vein number and prominence, pistillate pedicel length, style length, and drupe size. Specimens of the *D. indica* complex from Taiwan, which have been treated as *D. karapinensis* (of which *D. hieranensis* should be treated as a synonym), differ somewhat from mainland Asian *D. indica* in pubescence, leaf vein number, stamen number, and fruiting pedicel length, but the differences are subtle and overlapping; resolving their taxonomic status is beyond the scope of this paper, and here I treat them both within a broadly defined *D. indica*. Differences among species in the *D. indica* complex are summarized in the key below.

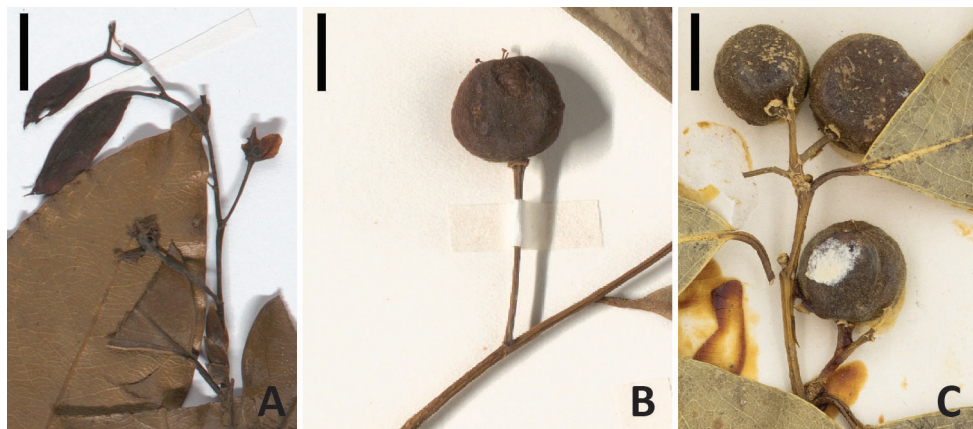


Figure 1. Morphology of the *Drypetes indica* complex **A** *D. indica* (detail of L.2207751), branch tip showing perulate bud below an expanding shoot with proximal flowers and distal leaves **B** *D. indica* (detail of L.2207743) fruit with elongate styles bearing flabellate stigmas **C** *D. nienkui* (detail of NY02684347, holotype) with two cymose infructescences. Scale bars equal 1 cm. Photos **A**, **B** CC 1.0 Naturalis Biodiversity Center **C** CC 4.0 New York Botanical Garden.

Taxonomic treatment

Drypetes nienkui Merr. & Chun, Sunyatsenia 2: 258, fig. 29C, D. 1935.

Type. CHINA. Hainan: Fan Ya, Ng Chi Ling, elev. 1200 m, 8 Nov 1932, *N. K. Chun & C. L. Tso* 44246 (holotype: NY02684347!; isotypes A00055929!, AU042776! (image seen), IBSC0004245!, IBSC0004246!, IBSC0004247!, K000854225!, NAS00417232! (image seen), PE00022638! (image seen), US01269000!).

Description. Trees to 15(–20) m, to 30 dbh, dioecious; branches and branchlets glabrous. **Buds** perulate; perules ovate, 1.5–2 × 1.6–2 mm, apex obtuse to rounded, surfaces glabrous, margins ciliolate. **Leaves:** stipules caducous, not seen; petiole 8–13 mm long, 1.3–1.7 mm diam., canaliculate, glabrous; blade oblong-ovate to oblong-lanceolate, 8–14 × 3–6 cm, chartaceous-coriaceous, base ± asymmetric, broadly cuneate to broadly acute, margins entire, apex acute to gradually short-acuminate, tip obtuse, surfaces glabrous, abaxial light green, somewhat shiny, adaxial olive, shiny; midvein rounded abaxially and adaxially, 2° veins 6–9 per side, slender but not obscure, 3° reticulate, higher order not well differentiated, reticulate, all raised adaxially, areoles 0.6–1 mm diam., freely ending veinlets fairly common. **Staminate inflorescences** cymose, to 2 cm long, 8–10-flowered; peduncle 0.5–0.8 mm diam., glabrous; bracteoles triangular, 0.8–1 mm long, surfaces glabrous, margins ciliate. **Pistillate inflorescences** cymose, 1 cm long (fruiting), 4–7-flowered; peduncle 1.5–1.7 mm diam. (fruiting), glabrous; bracteoles not seen. **Staminate flowers:** pedicels 2–5 mm long, 0.3 mm diam., puberulent; sepals 4, ovate, 1.7–2 × 1.2–1.5 mm, apex rounded, surfaces glabrous, margins ciliate; disc shallowly lobed, glabrous; stamens 8, inserted between disc lobes, filaments 1.5 mm long, terete, glabrous, anthers +/- globose, 0.5 mm

diam., glabrous. **Pistillate flowers** not seen; fruiting pedicels 6–12 mm long, 1.4–1.7 mm diam., puberulent, glabrescent, hairs erect, to 0.1 mm long; ovaries densely hirsute when young. **Drapes** purplish brown, globose or depressed globose, sometimes slightly lobed, 15–18 mm diam., 2–3-locular, surface sparsely hirsute, trichomes whitish, 0.1–0.2 mm long, erect; styles 2–3, 1.5–2 mm long; stigmas +/- flabellate, 0.5 × 0.7 mm; exocarp and mesocarp not differentiated, 0.5 mm thick, leathery or crustaceous, endocarp 0.1 mm thick, cartilaginous. **Seeds** 2–3.

Phenology. *Drypetes nienkui* flowers August–November and fruits November–January.

Distribution and habitat. *Drypetes nienkui* is widespread in the southern half of Hainan, China (see the map for *D. indica* in Fang et al. [2011], as to Hainan only), occurring in forests at 950–2100 m elevation.

Preliminary conservation status. I have been able to locate only 13 gatherings of *Drypetes nienkui*. With the exception of two collections made in 2014 and 2017, all the collections date from between 1932 and 1954, but no data are available regarding population sizes or trends. Applying the criteria of IUCN (2022), *D. nienkui* should receive a preliminary conservation assessment of Data Deficient, although a more complete assessment might show it qualifies as a species of elevated conservation concern.

Key to species in the *Drypetes indica* complex

- 1 Pistillate and staminate inflorescences cymose; fruiting pedicels 5–12 mm long **2**
- Pistillate flowers solitary, staminate inflorescences fascicles (unknown in *D. longistipitata*); fruiting pedicels 16–40 mm long **3**
- 2 Young stems and petioles hairy; perules 5–7 mm long, densely hairy; leaf blades 10–18 cm long; staminate flowers: pedicels 5–10 mm long, disc hairy, stamens 8–12 ***Drypetes dasycarpa***
- Young stems and petioles glabrous; perules 1.5–2 mm long, glabrous except for ciliate margins; leaf blades 8–14 cm long; staminate flowers: pedicels 3–5 mm long, disc glabrous, stamens 8 ***Drypetes nienkui***
- 3 Leaf blade secondary veins 5–8 pairs, prominent; fruiting pedicels 20–40 mm long; styles 1.5–3 mm long; drupes 12–16 mm diam ***Drypetes indica* s.l. (including *D. karapinensis*)**
- Leaf blade secondary veins 4–5(–6) pairs, delicate; fruiting pedicels 16–20 mm long; styles 1 mm long; drupes 10–12 mm diam ***Drypetes longistipitata***

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Appendix I

Specimens examined for comparative morphological studies of the *Drypetes indica* complex. Listed are country, primary collector name and collection number, date of collection, herbarium, and, when available, barcodes (enclosed in square brackets when they do not include the herbarium acronym) or accession numbers (identified by “acc. #”). Specimens that I observed only through online images are marked with an asterisk (*).

***Drypetes nienkui*:** CHINA. *N. K. Chun* 44246, 8-Nov-1932, A00055929, AU042776*, IBSC0004245, IBSC0004246, IBSC0004247, K000854225, NAS00417232*, NY02684347, PE00022638*, US01269000; *Chun W. Y.* 420, 26-Nov-1936, IBSC0311573; *Hainan East Road team* 610, 9-Nov-1954, IBK00165777*, IBSC0311570; *Hainan East Road team* 960, 19-Dec-1954, IBK00165780*, IBK00165784*, IBSC0311574; *Hainan hanging mountain team* 2988, 19-Dec-1954, IBK00165775*, IBSC0311575; *How F. C.* 73525, 29-Aug-1935, AU012778*, IBK00165774*, IBK00165781*, IBSC0311578, NAS00412112*; *How F. C.* 73673, 15-Sep-1935, IBK00165782*, IBSC0311579; *Liu C.* 17CX15977, 10-Sep-2017, KUN1445422*; *Wang C.* 35261, 9-Dec-1933, IBK00165776*, IBSC0311572, NAS00412111*, NY; *Wang C.* 36145, 6-Jan-1934, IBK00165779*, IBSC0311576; *Wang C.* 36238, 9-Jan-1934, IBSC0311577; *Wang C.* 36622, 13-Jan-1934, IBK00165778*, IBSC0311571, SN007954*, NY; *Zhang T.* 14CS8905, 19-Aug-2014, KUN1371389.

***Drypetes dasycarpa*:** THAILAND. *N. Fukuoka* T-62161, 14-Jan-1994, L.2207742; *H. B. G. Garrett* 593, 6-Nov-1930, BM, P05470830*; *A. F. G. Kerr* 4919, 26-Feb-1921, P05563357; *A. F. G. Kerr* 5047, 8-Mar-1921, BM; *H. P. Nooteboom* 807, 21-Jan-1969, K000854258, L.2207753; *T. Yahara* T-50009, 10-Dec-1984, L.2207754.

***Drypetes indica* s.l. (including *D. karpapinensis*):** Bhutan. *A. J. C. Grierson* 1694, 6-Jun-1979, E00310566*. CHINA. *Li Y.-H.* 12533, 28-Jun-1974, HITBC Acc. # 014370*, 081461*. India. *W. Griffith* 4738, K000246683; *J. D. Hooker* s.n., E00310592*, G00318470, G00318473, K000246684, K000246685, K000246686,

K000246687, L.2207755, L.2208087, L.2208088, P05470767, P05470831, P05470832, P05470835, P05470836, P05470837, P05470838. Taiwan. *C.-H. Chen* 505, 1-Apr-1994, TNM [S14022*, S34201*]; *S. T. Chiu* 1657, 31-Mar-1993, TNM [S10365*, S10367*]; *T. I. Chuang* 3117, 10-Jan-1960, TAI Acc. # 067424*, Acc. # 067425*; *S.-W. Chung* 5062, 26-Feb-2002, TNM [S79934*]; *S.-W. Chung* 5072, 26-Feb-2002, TNM [S79933*]; *S.-W. Chung* 5073, 26-Feb-2002, TNM [S79935*]; *Hayata* 45, 23-Apr-1914, A00055901, IBSC0311581, TAI Acc. #01604*, Acc. # 01605*; *H.-Y. Chen* 151, 8-Mar-1998, TNM [S77423*]; *H.-Y. Chen* 163, 8-Mar-1998, TAI Acc. # 251490*; *G.-P. Hsieh* 1383, 1-May-2004, TNM [S108917*]; *Y.-Y. Huang*, 327, 29-Apr-2021, CAS669031*; *V.-P. Leu s.n.*, 6-Apr-1939, TNM [S3042*]; *S.-P. Li* 435, 2-Mar-1995, IBSC0311582, TNM [S172508]; *S.-Y. Lu* 256, 2-Apr-1993, TAI Acc. # 225015*; *S.-Y. Lu* 18279, 6-Feb-1986, L.2207711, MW0756562*, MW0756562*; *S.-Y. Lu* 18606, 11-Mar-1986, L.3799848; *S.-Y. Lu* 18613, 13-Dec-1982, L.2207709; *Ou* 9452, 31-May-1980, TAI Acc. #178887, Acc. # 178895; *S. Sasaki s.n.*, Feb 1912, TI Acc. #01603*; *C. M. Wang* 2126, 25-Feb-1996, CAS669034*; *J.-C. Wang* 8177, 24-Apr-1993, TAI Acc. # 255401; *L. J. Wang s.n.*, 19-Mar-1966, TAI Acc. # 067420*, Acc. # 067421*; *S. Z. Yang* 10703, 10-Apr-1988, IBSC0311565; *S. Z. Yang* 10761, 19-Mar-1989, IBSC0311564; *T. Y. A Yang* 23063, 27-Feb-1997, TNM [S31964*]. Thailand, *A. F. G. Kerr* 12049, 12-Feb-1927, BM, L.2207741, P05470829; *A. F. G. Kerr* 16780, 21-Jan-1929, BM, L.2207744; *A. F. G. Kerr* 17181, 22-Feb-1929, BM, P05563358; *A. F. G. Kerr* 17184, 22-Feb-1929, BM, P05563356; *A. F. G. Kerr* 17991, 7-Jan-1930, L.2207749; *A. F. G. Kerr* 18004, 7-Jan-1930, BM, L.2207748; *A. F. G. Kerr* 18442, 8-Mar-1930, L.2207747; *A. F. G. Kerr* 18448, 5-Mar-1930, BM; *A. F. G. Kerr* 18448A, 9-Mar-1930, BM, L.2207745, P05563359.

***Drypetes longistipitata*:** CHINA. *How F. C.* 70367, 17-Mar-1933, IBK00165773*; *Wei C. F.* 122372, 24-Apr-1959, IBSC0311561; *Xing Z. W.* 6196, 16-May-1995, IBSC0311562, IBSC0311563.

The ECAT dataset: expert-validated distribution data of endemic and sub-endemic trees of Central Africa (Dem. Rep. Congo, Rwanda, Burundi)

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Abstract

In this data paper, we present a specimen-based occurrence dataset compiled in the framework of the Conservation of Endemic Central African Trees (ECAT) project with the aim of producing global conservation assessments for the IUCN Red List. The project targets all tree species endemic or sub-endemic to the Central African region comprising the Democratic Republic of the Congo (DR Congo), Rwanda, and Burundi. The dataset contains 6361 plant collection records with occurrences of 8910 specimens from 337 taxa belonging to 153 genera in 52 families. Many of these tree taxa have restricted geographic ranges and are only known from a small number of herbarium specimens. As assessments for such taxa can be compromised by inadequate data, we transcribed and geo-referenced specimen label information to obtain a more accurate and complete locality dataset. All specimen data were manually cleaned and verified by botanical experts, resulting in improved data quality and consistency.

Keywords

Africa, conservation, data capture, data cleaning, endemics, flora, flowering plants, geographic range, herbarium, IUCN Red List, threatened

Introduction

The alarming rate of biodiversity loss worldwide has increased the need to conduct conservation assessments for the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Rodrigues et al. 2006; Mace et al. 2008). In particular, plants, especially those from species-rich tropical forests, need to be better represented on the IUCN Red List to obtain a representative picture of the state of biodiversity as a whole and to help inform natural resource management and conservation planning (Collen et al. 2008; Stuart et al. 2010). Africa's rainforests, including most in DR Congo, are particularly threatened by climate change and rapid population growth (Réjou-Méchain et al. 2021; Sosef et al. 2021), but a lack of data hampers the preparation of extinction risk assessments (Stévant et al. 2019).

The IUCN Red List assessment procedure uses numerical thresholds within five criteria to classify taxa according to their relative risk of extinction. Criterion B (restricted geographic range) is the most frequently used for plants (Collen et al. 2008; Le Breton et al. 2019), mainly because it does not require population demographic data, which are rarely available for plants and especially for tropical species. Instead,

criterion B allows taxa to be classified as threatened when their geographic range, measured as either the extent of occurrence (EOO) or the area of occupancy (AOO), falls below certain thresholds, provided that at least two out of three additional subcriteria are met concerning its population: (a) severely fragmented or known to exist in no more than a given number of threat-defined locations; (b) continuing decline; or (c) extreme fluctuations (IUCN Standards and Petitions Committee 2019).

The first and perhaps most time-consuming task in preparing conservation assessments under criterion B is to obtain a realistic view of a taxon's past and current distribution. For tropical plants, this is generally derived from herbarium specimens. Although large-scale digitisation programmes have increased the availability of digital biodiversity data, the specimen data are far from complete, up-to-date, accurate, or clean (Graham et al. 2004; Yesson et al. 2007; Nelson and Ellis 2018; Zizka et al. 2020b). On the contrary, limitations in the quantity and quality of plant occurrence data along taxonomic, geographic, and temporal dimensions may hamper their use in research and conservation applications (Meyer et al. 2016). Many specimens in herbaria and databases lack geographic coordinates (Nic Lughadha et al. 2018), requiring additional geo-referencing before calculating range statistics. This is especially relevant for uncommon taxa known from only a few collections, where the inclusion of extra geo-referenced specimen data may cause some parameters used in the assessment process to exceed one of the thresholds, thereby changing the conservation status (Miller et al. 2012). Improving data quality is equally important to obtain the most complete and accurate locality data. Before any spatiotemporal inferences can be drawn from herbarium collections, numerous issues need to be addressed, including conflicting taxonomy, synonymy, misidentifications, imprecise or erroneous coordinates, and duplicate specimens (Soberón and Peterson 2004; Nic Lughadha et al. 2018). As the EOO is affected by geographic outliers, taxonomic and spatial errors could lead to a miscalculation of the extinction risk, especially for threatened taxa (Panter et al. 2020). Several tools and workflows have been developed to implement automated data cleaning, such as Biogeo (Robertson et al. 2016), SpeciesGeoCoder (Töpel et al. 2017), CoordinateCleaner (Zizka et al. 2019), and BDcleaner (Jin and Yang 2020), to mention a few. These tools have proven valuable in detecting and flagging suspect data records that require further inspection but do not provide mechanisms to resolve these issues efficiently. Users may decide to remove suspect records, but this may affect downstream analyses (Maldonado et al. 2015; Zizka et al. 2020a). In our situation, working with poorly-sampled and often range-restricted endemic taxa, correcting such errors is a vital step in ensuring that the assessments use the best available evidence, as advocated by the IUCN (IUCN Standards and Petitions Committee 2019). Also, conservation assessments should be preferably carried out on manually cleaned and expert-validated data (Hjarding et al. 2015; Panter et al. 2020).

Here, we provide a high-quality, expert-validated occurrence dataset compiled by the ECAT project, which is part of the larger Global Tree Assessment (GTA) coordinated by Botanic Gardens Conservation International (BGCI 2021). The ECAT project aimed to prepare global conservation assessments for all trees endemic or sub-endemic

to the region comprising DR Congo, Rwanda, and Burundi, hereafter referred to as Central Africa. It was executed in collaboration with the IUCN SSC Central African and Eastern African Plant Red List Authorities (CARLA and EAPRLA). We summarise how the data was compiled, which included normalisation, harmonisation, aggregation, data transcription, geo-referencing, quality control, data cleaning, and validation by data managers and botanical experts. We conclude by highlighting the taxonomic, spatial, and temporal coverage of the data. The ECAT dataset was not only used to develop or update the conservation assessment of 347 Central African tree taxa, many of which are threatened with extinction, but will also be used in a series of future studies (e.g., studying the effectiveness of the protected area network in the conservation of threatened tree species). Through this project, we hope to support and help guide effective management and conservation strategies to preserve the unique plant diversity of Central Africa.

Project details

Project title

Conservation of Endemic Central African Trees (ECAT) through IUCN Red Listing and Species Distribution Modelling.

Funding

Funding for the ECAT project was provided by the Franklinia Foundation, with a substantial in-kind contribution from Meise Botanic Garden and Missouri Botanical Garden.

Study area

Central Africa, as defined in this study, covers a total of 2.4 million square km, comprising the countries of DR Congo, Rwanda, and Burundi and stretching from a narrow coastal strip at the western border of DR Congo (excluding the Cabinda enclave) to the montane region of the Albertine Rift. The core of this region consists of the Congo Basin, which is the second largest tropical forest area in the world after the Amazon Basin, with much of the area being at low elevation (below 600 m). The natural vegetation of the Congo Basin is classified as Guineo-Congolian rainforest on well-drained sites, with swamp forest on hydromorphic soils (White 1983). In the south-east of DR Congo, at an elevation ranging from ca. 600 m at Lake Upemba to 1750 m in the Hauts-Plateaux, the Zambezian forest-savannah mosaic is dominated by dry tropical woodland (miombo) interspersed with savannah and remnants of dry evergreen woodland (muhulu) (Meerts and Hasson 2016; Pierre Meerts, pers. comm.). Similar dry forests can be found in the northern region of DR Congo, bordering the Central African Republic and South Sudan, forming part of the Guineo-Sudanian

phytoregion (White 1983; Droissart et al. 2018). The Central African region is home to an estimated 11,000 vascular plant species (Sosef 2016), of which more than 1800 are endemic (Sosef et al. 2017).

Methods

Based on data available at Meise Botanic Garden, supplemented with data from the BGCI GlobalTreeSearch (Beech et al. 2017), we compiled a list of 347 tree taxa endemic or sub-endemic to Central Africa, including their geographic distribution and nomenclatural synonyms (totalling 481 names). Accepted names generally follow the African Plant Database (version 3.4.0. 2018), which for Central African taxa draws heavily on the Flore d'Afrique centrale, Flora of Tropical East Africa, and Flora Zambesiaca. We defined a tree as any woody single-stemmed plant at least 3 m tall. Taxa still met this definition if they usually occur as a shrub or a liana and only occasionally in the form of a tree. This list was then verified by the IUCN SSC Global Tree Specialist Group.

We differentiated between endemic and sub-endemic taxa based on their spatial distribution relative to the land borders of DR Congo, Rwanda, and Burundi. We considered 219 taxa as Central African true endemics as their current distribution range is restricted to DR Congo (186 taxa), Rwanda (3), Burundi (2), or a combination of these three countries (28). The remaining 128 taxa from our list were deemed sub-endemic to Central Africa. For 116 of these sub-endemic taxa, all herbarium specimens in our dataset originated from the area delineating DR Congo, Rwanda, and Burundi, extended by a 5-degree buffer zone. For the remaining 12 sub-endemic taxa in our study, most specimens were from Central Africa (70–94%), with only a few collected outside the 5-degree buffer zone (1–23%).

We retrieved the specimen data for these taxa and their synonyms from our institutional collection database (BR; all herbarium acronyms according to Thiers 2018) and supplemented them with data from RAINBIO, a database of tropical African vascular plants distributions (Dauby et al. 2016). RAINBIO contains geo-referenced specimen records from a number of institutional collections with a strong focus on Africa, together with several personal databases collated by individual researchers (for details on the construction of the RAINBIO database and the quality checks performed, see Dauby et al. 2016). Occasionally, missing specimen records were added from online institutional data portals such as TROPICOS and JSTOR-Global Plants, while some verified specimen records were added from other sources such as taxonomic revisions and floras (e.g., Flora of Tropical East Africa, Flora Zambesiaca). The specimen data from all these different sources were thoroughly pre-processed before aggregating them into one comprehensive dataset. For instance, discrepancies in taxon names were resolved and harmonised with respect to synonymy and re-identifications, two-digit country codes were employed in accordance with ISO 3166, collector names were standardised, and collecting dates were converted to YYYY-MM-DD format. The dataset was meticulously reviewed and edited to merge duplicate specimen records

(specimens of the same taxon made by the same collector at the same place and time, usually collected from the same tree). These duplicates often did not have the same quantity and quality of transcribed metadata due to missing information on some specimen labels, incomplete label transcription in the database, or transcription errors. When merging duplicates, we took care to retain all metadata relevant to Red List assessors and to resolve any inconsistencies.

Transcription of specimen labels is often restricted to selected data fields due to resource constraints. As a result, a considerable amount of descriptive information relevant to Red List assessments may be missing from specimen databases. To enrich our data, we transcribed specimen label data focusing mainly on gaps in the locality description, habitat, and elevation. The newly transcribed data allowed us to geo-reference several specimens without coordinates and improve the geo-referencing accuracy of others. Although recent herbarium specimens increasingly contain accurate coordinates captured in real-time using a GPS device, this is not the case for the bulk of the Central African collection at BR that predated GPS devices. It was often possible to infer the geographic coordinates from the transcribed data using historical topographic maps and gazetteers or by checking the collector's itinerary. Specimen records that could not be geo-referenced because the locality description was missing, too vague, or unclear (e.g., illegible handwriting) were removed from the dataset.

The dataset was checked for any spatial errors through an iterative series of inspections. First, we used the R package *CoordinateCleaner* version 2.0–18 (Zizka et al. 2019) to flag records with potentially erroneous coordinates, including those that fall in the ocean or outside the indicated country, those that coincide with country and province centroids, and those with zero latitudes or longitudes. Next, we checked the data for intrinsic consistency in a GIS environment to flag additional problematic records. For example, we verified that the coordinates fell within the province or district as stated on the specimen label and checked the collector's itinerary to evaluate the geo-referencing quality in suspect cases. This approach revealed several spatial errors missed by the automated cleaning. We checked all flagged records one by one and made corrections where appropriate. Common causes of spatial errors were the inversion of latitude and longitude, the lack of a minus sign for south or west, coordinate transformation errors, and typographical errors. Even after extensive data cleaning, some geographic discrepancies remained. Occurrences falling in a country different from the one stated on the specimen label but for which the distance to the land border was less than 5 km were retained in our dataset. We considered this margin acceptable given the uncertainties associated with natural history data, the precision of the locality data, and the spatial precision of the country GIS layers used. Other records whose coordinates were considered incorrect or too imprecise for our purposes (e.g., specimens geo-referenced to the country centroid) were removed from the dataset.

Finally, the expert botanists carrying out the Red List assessments verified all occurrences for each taxon, paying particular attention to spatial outliers that could indicate an error in a specimen's identification or geo-referencing. Verification of taxonomic identification involved physically examining the herbarium specimens or at least

online checking of an image scan where applicable. Not only did the experts detect (and rectify where possible) taxonomic and geographic errors, but they also identified unsuitable records, like those belonging to cultivated specimens or specimens that were locally extinct (e.g., due to habitat loss). Including such records in the calculation of the EOO or number of locations could result in an underestimation of extinction risk (Miller et al. 2012; Nic Lughadha et al. 2018). Records belonging to non-extant populations were flagged in the dataset (not removed) so they would not be used in calculating key geographic range parameters used in the Red List assessment process. Such information is valuable to the assessor to infer a continuing decline as applied in criterion B. Wrongly identified specimens and cultivated specimens were removed from the dataset.

Results

The initial raw dataset contained data from 9956 specimens. The majority of these (83.4%) were deposited in the herbarium of Meise Botanic Garden, underlining its importance for the flora of Central Africa. Other herbaria represented in the dataset are B, BM, BRLU, C, COI, EA, EALA, EPU, FHO, GENT, H, HBG, IEC, IUK, K, KAW, KISA, LBV, LG, LISC, LISU, LSHI, LUKI, LWI, M, MA, MB, MHU, MO, MPU, NDO, NHR, NHT, P, PRE, SRGH, UPS, W, WAG, and YBI.

As part of the data enrichment, we transcribed locality data for 690 specimens, habitat data for 2802 specimens, and elevation data for 3796 specimens (this includes values indicating that information is 'known to be unknown'). One-third of the specimens (33.1%) had no coordinates. During the ECAT project, 2923 specimens were geo-referenced, leaving 372 without spatial data. The new coordinates were derived mainly from maps and gazetteers; only for a small number of them (374) could they be copied from duplicates. After several quality checks on the geo-referencing, we adjusted the coordinates for 1774 specimens. For three-quarters of them, it concerned a relatively minor adjustment moving the occurrence up to 10 km. For the remaining quarter, this exceeded 10 km (up to as much as 3915 km). The taxonomic identification was updated for 509 specimens (changes due to synonymy or misspellings not taken into account). We removed 1046 specimens from the dataset on taxonomic or spatial grounds, leaving 8910 specimens in the cleaned dataset. After merging all duplicate specimens, we obtained a dataset with 6361 geo-referenced plant collection records.

Taxonomic coverage

The ECAT dataset contains distribution data of 337 taxa at specific or infraspecific level (subspecies or variety) belonging to 153 genera in 52 families and 20 orders. The family classification follows APG IV (The Angiosperm Phylogeny Group 2016), except in some cases where the authors thought a more conservative approach to be appropriate (such as still recognising Flacourtiaceae, Sterculiaceae, and Tiliaceae). The

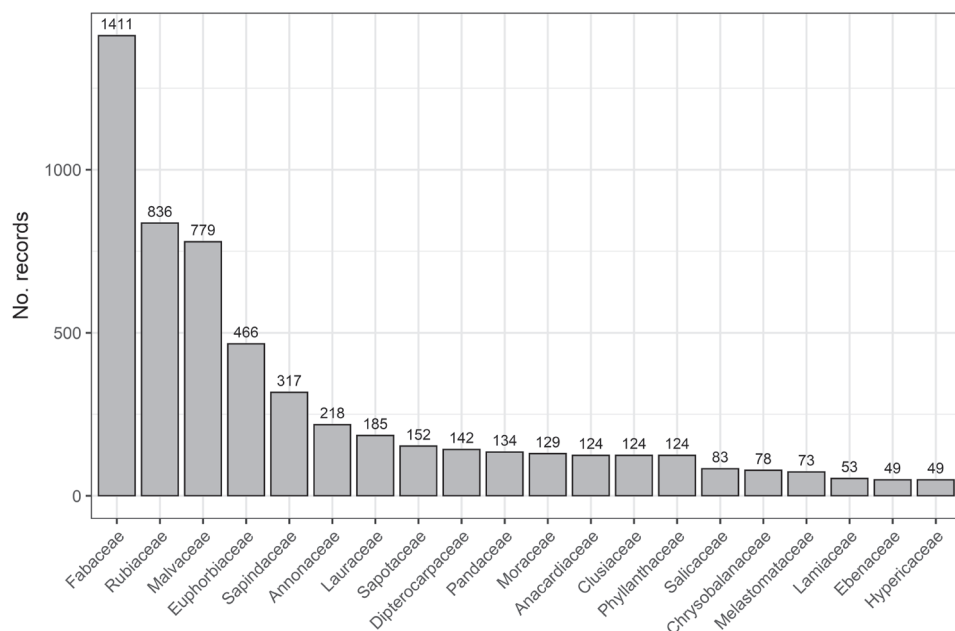


Figure 1. Taxonomic coverage: The top 20 families with the highest number of records of Central African endemic and sub-endemic trees in the ECAT dataset (totalling 6361 records and 337 taxa).

difference in the number of taxa in the dataset (337) compared to our taxon list (347) arose because in the latter several species are present including their infraspecific taxa as separate entries, whereas the corresponding specimens in the dataset were all identified to infraspecific level. The number of records per taxon ranged between 1 and 130 (median: 10; mean: 19 records per taxon). The five most represented families are Fabaceae (22.2%), Rubiaceae (13.1%), Malvaceae (12.2%), Euphorbiaceae (7.3%), and Sapindaceae (5.0%) (Fig. 1).

Taxonomic ranks

Kingdom: Plantae.

Division: Magnoliophyta.

Class: Magnoliopsida.

Order: Apiales, Arecales, Asterales, Boraginales, Brassicales, Celastrales, Ericales, Fabales, Gentianales, Geraniales, Lamiales, Laurales, Magnoliales, Malpighiales, Malvales, Myrtales, Proteales, Rosales, Santalales, Sapindales.

Family: Achariaceae, Anacardiaceae, Annonaceae, Apocynaceae, Araliaceae, Arecaceae, Asteraceae, Bignoniaceae, Boraginaceae, Burseraceae, Capparaceae, Celastraceae, Chrysobalanaceae, Clusiaceae, Combretaceae, Dichapetalaceae, Dipterocarpaceae, Ebenaceae, Euphorbiaceae, Fabaceae, Hypericaceae, Lamiaceae, Lauraceae, Linaceae, Malpighiaceae, Malvaceae, Melastomataceae, Meliaceae, Melianthaceae, Moraceae, Myrsinaceae, Myrtaceae, Octoknemaceae, Pandaceae, Pentaphragaceae,

Phyllanthaceae, Picrodendraceae, Pittosporaceae, Proteaceae, Putranjivaceae, Rhamnaceae, Rhizophoraceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae, Santalaceae, Sapindaceae, Sapotaceae, Scytopetalaceae, Thymelaeaceae, Violaceae.

Common names: flowering plants.

Spatial coverage

General spatial coverage

The occurrence data are relatively well distributed, albeit unevenly over the study area (Fig. 2A). Most records are from DR Congo (83.8%), followed by Rwanda (5.5%), Uganda (2.9%), and Burundi (2.4%), with the remaining records from outside Central Africa (Table 1). Collecting effort has been highest in the following areas: (1) the Greater Virunga Landscape, covering areas around the protected areas of Virunga National Park in DR Congo, Volcanoes National Park in Rwanda, and five national parks (NP) in Uganda (Bwindi Impenetrable NP, Mgahinga Gorilla NP, Queen Elizabeth NP, Rwenzori Mountains NP, and Semliki NP); (2) the Congo-Nile Divide of Rwanda and Burundi, to the east of the Albertine Rift, including Nyungwe NP and Kibira NP; (3) the region covering the eastern part (Kivu) of DR Congo; (4) the UNESCO Biosphere Reserve of Yangambi, situated in the north of DR Congo along the Congo River; (5) the UNESCO Biosphere Reserve of Luki, located in the south-west of DR Congo and about 120 km east of the Atlantic coast; (6) Kinshasa, the national capital of DR Congo; and (7) the area around Mbandaka, the capital of the Équateur province in DR Congo, located near the confluence of the Congo and Ruki Rivers. Logically, the patterns observed for the number of taxa in each grid cell (Fig. 2B) are rather similar since the two are highly correlated (Pearson's $r = 0.810$).

Coordinates

15°49'20"S to 06°31'00"N latitude; 08°48'00"E to 38°30'00"E longitude.

Temporal coverage

The ECAT dataset includes specimens collected between 1882 and 2019, with 206 records not having a date (Fig. 3). The periods of highest collecting activity were the 1930s and 1950s, with an exceptionally high level during the 1956–1960 period. There was reduced survey effort during the two World Wars and since the 1960s following the independence of DR Congo.

Limitations of the data

For a tropical region to be considered reasonably well-known botanically (vascular plants), a rule of thumb is that the minimal level of botanical exploration should be at least 100 specimens per 100 km² (Campbell and Hammond 1989). For Central Africa,

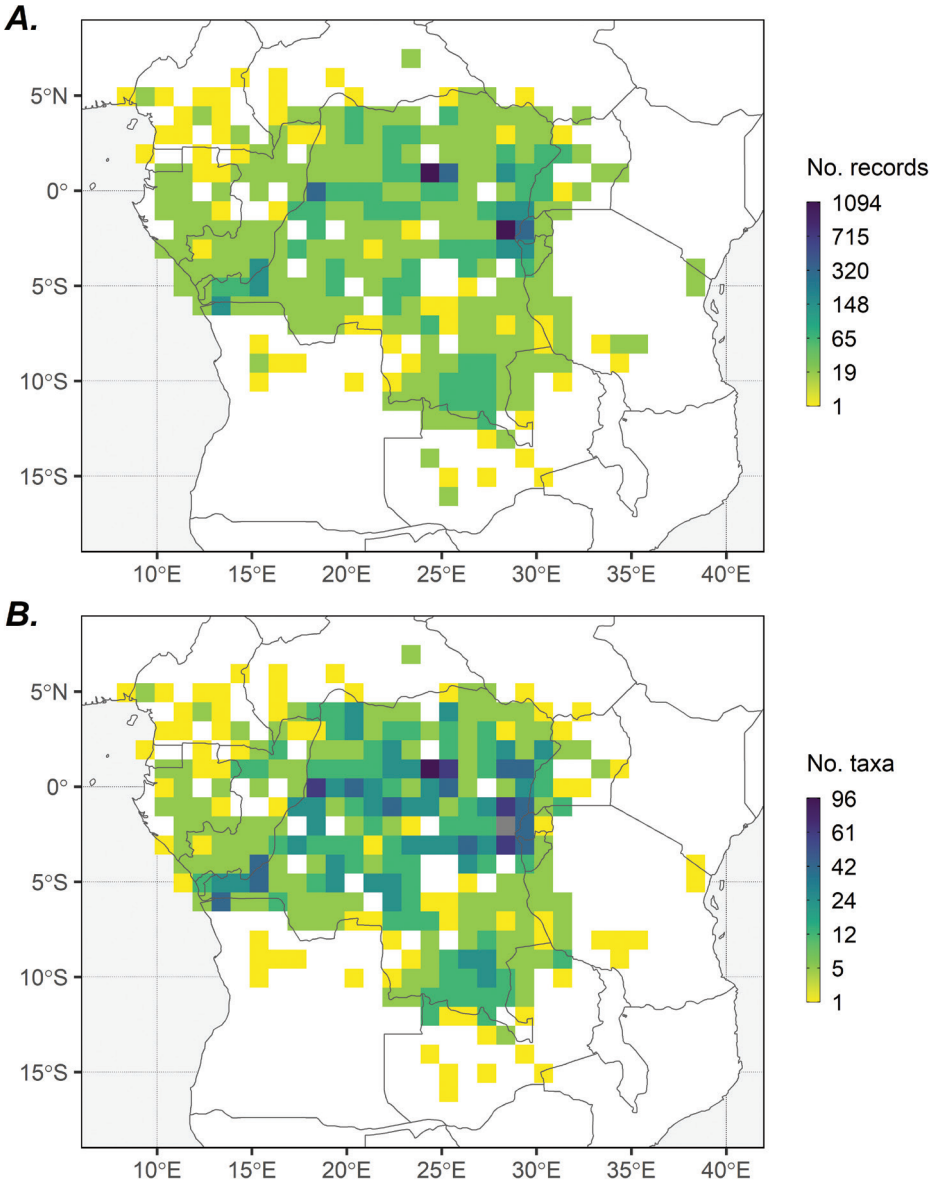
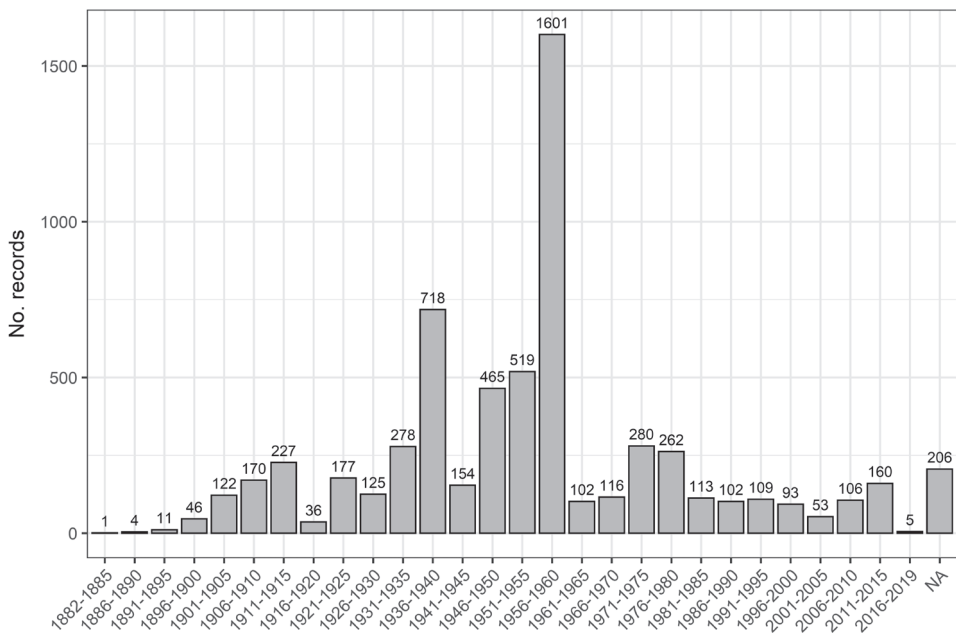


Figure 2. Spatial coverage: One-degree grid maps showing **A** number of records and **B** number of taxa of Central African endemic and sub-endemic trees in the ECAT dataset (totalling 6361 records and 337 taxa).

this means that a minimum of 2.4 million herbarium specimens are needed, or by country, roughly about 2.35 million for DR Congo, 26,000 for Rwanda, and 28,000 for Burundi. The number of realised herbarium collections is estimated at 380,000 for DR Congo, 31,000 for Rwanda, and 37,000 for Burundi (Sosef et al. 2021). These numbers suggest that while Rwanda and Burundi can be regarded as “reasonably well

Table 1. Spatial coverage: Number of specimens (total: 8910) and number of records (total: 3631) per country.

Country	No. specimens	No. records
Democratic Republic of the Congo	7750	5329
Rwanda	413	353
Uganda	186	184
Burundi	172	150
Republic of the Congo	143	123
Zambia	70	66
Gabon	44	41
The United Republic of Tanzania	37	37
Central African Republic	36	31
Cameroon	32	22
Angola	21	20
South Sudan	4	3
Equatorial Guinea (mainland)	2	2
Total	8910	6361

**Figure 3.** Temporal coverage: Number of records per 5-year periods from 1882 to 2019 of Central African endemic and sub-endemic trees in the ECAT dataset (totalling 6361 records and 337 taxa).

known”, the botanical wealth of DR Congo remains “poorly known”. Moreover, the ECAT dataset suffers from the same sampling bias characteristic of many other natural history collections from tropical areas, with specimens collected mainly along roads, near urban populations, and in areas of specific botanical interest, with few collection

records in remote areas (Fig. 2A). With respect to the temporal coverage, most specimens date back to the first half of the 20th century and survey effort has decreased in the last few decades (Fig. 3). The gaps in both spatial and temporal coverage prompt the need for strategic and well-designed field surveys across the region, especially in remote and data-sparse areas with (relatively) intact vegetation and areas where threatened endemics occurred in the past but have not been surveyed for a long time. Such fieldwork is critical to assessing the status of extant populations under changing environmental conditions and identifying priority populations for immediate conservation. New, up-to-date occurrence records are pivotal for updating IUCN Red List assessments, especially for data deficient and threatened taxa, to make informed decisions regarding their conservation and management. Unfortunately, several factors hinder collection activities in Central Africa, including limited funding, inadequate infrastructure, and armed conflicts in DR Congo.

Dataset description

Object name: Darwin Core Archive ECAT: Endemic and sub-endemic Central African Trees.

Character encoding: ISO-8859-1.

Format name: Darwin Core Archive format.

Format version: 1.5.

Distribution: <https://zenodo.org/record/7007770>.

Publication date of data: 2022-08-18.

Licenses of use: Creative Commons Attribution (CC-BY) 4.0 License.

Metadata language: English.

Date of metadata creation: 2022-08-18.

Hierarchy level: Dataset.

Provided fields: language, institutionCode, collectionCode, basisOfRecord, occurrenceID, catalogNumber, recordNumber, recordedBy, georeferenceVerificationStatus, occurrenceStatus, disposition, associatedReferences, otherCatalogNumbers, occurrenceRemarks, materialSampleID, eventDate, year, month, day, habitat, eventRemarks, continent, country, countryCode, stateProvince, locality, verbatimElevation, locationRemarks, decimalLatitude, decimalLongitude, geodeticDatum, coordinateUncertaintyInMeters, verbatimCoordinates, identificationRemarks, scientificName, kingdom, phylum, class, order, family, genus, specificEpithet, infraspecificEpithet, taxonRank, taxonRemarks.

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Lycoris insularis (Amaryllidaceae), a new species from eastern China revealed by morphological and molecular evidence

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Abstract

Lycoris insularis S.Y.Zhang & J.W.Shao, a new fertile diploid species from coastal provinces in eastern China is described. This new species is most similar to *L. sprengeri* in morphology and has been misidentified as the latter for a long time. However, it can be distinguished from the latter by the relatively longer perianth tube (1.5–2.5 cm vs. less than 1.3 cm), a characteristic that was overlooked before. Phylogenetic analysis, based on complete plastid genome, showed that *L. insularis* is not genetically related to *L. sprengeri* in the genus. The former was a sister group of *L. sanguinea*, while the latter was closely related to *L. longituba* and *L. chinensis* and they were respectively located on different clades that were separated at the base of the phylogenetic tree. The chromosome number of *L. insularis* is $2n = 22$. At present, as the new species is relatively widely distributed and the wild population can normally reproduce by seeds, we evaluate it as LC (Least Concern) according to criteria of the IUCN Red List.

Keywords

Lycoris sprengeri, taxonomy, chloroplast genome, cryptic species

Introduction

Lycoris Herb. (Amaryllidaceae) is a typical pan-East Asian genus, currently considered to have about 9 fertile diploid species, mainly distributed in China, Japan and South Korea (Hsu et al. 1994; Ji and Meerow 2000; Kim 2004; Hori et al. 2006; Lu et al. 2020; Zhang et al. 2021; Zhang et al. 2022). China is the centre of the distribution diversity of this genus, with about eight of these fertile diploid species being endemic to this country (Hsu et al. 1994; Lu et al. 2020; Zhang et al. 2021; Liu et al. 2022). Due to the special living habit of flowers and leaves not being able to co-exist, as well as the rich and beautiful flower patterns and colours, *Lycoris* plants are considered to have great horticultural value and have received extensive attention (Hsu et al. 1994; Zhang et al. 2022).

Lycoris sprengeri Comes ex Baker was first described in 1902, being only distributed in China. The type specimen was collected from around Xiangyang City, Hubei Province in Central China (Comes and Baker 1902; Traub and Moldenke 1949; Hsu et al. 1994). Due to extensive investigation of basic plant resources in China, many populations identified as *L. sprengeri* were found and most of them were located in the coastal areas of Zhejiang, Fujian and Jiangsu Provinces. However, there were few collections or photographic records of this species in the central or inland areas near the collection location of the type specimens.

Recently, we collected some populations of *L. sprengeri* from inland areas, including Guangshui City, Hubei Province (near the collection location of the type specimen) and the surrounding areas of Dabie Mountains in Anhui Province. In the field, we noticed that there was a certain difference in morphology between those plants (identified as *L. sprengeri*) occurring inland and those from the coastal area: the flower colour of the former varied from white to dark purple in a population and the length of the perianth tube is short (less than 1.3 cm), while the flower colour of the latter is relatively stable, generally pink and the perianth tube is long (usually about 2.0 cm). After further morphological observations, chloroplast sequence alignment and phylogenetic analysis and checking the related type specimens, we recognised that the plants found in the inland area were the genuine *L. sprengeri*, while those plants collected from the coastal area (identified as *L. sprengeri*) are actually an undescribed new species. Thus, we named it as *Lycoris insularis* S.Y.Zhang & J.W.Shao and describe it here.

Materials and methods

Plant collection and karyotype observation

Bulbs for study and observation were successively collected from the wild in 2016 to 2021. The information for the collection sites is shown in Table 1. Bulbs were cultivated in the Homogeneous Garden in Fengyang County, Chuzhou City, Anhui Province for continuous observation. In June 2022, bulb roots of 3–5 individuals in each *Lycoris insu-*

Table 1. Information of sampled populations and uploaded genomes.

Code	Locations	Altitude	GenBank acc. no
<i>L. sprengeri</i> (inland area)			
01	Yudian Town, Guangshui City, Suizhou City, Hubei Province	278 m	OP034616
02	Qiaotou Town, Mingguang City, Chuzhou City, Anhui Province	91 m	OP034617
03	Yinjian Town, Fengyang County, Chuzhou City, Anhui Province	137 m	OP034618
04	Huangjia Town, Tongcheng City, Anqing City, Anhui Province	122 m	OP034619
05	Pingshan Town, Huaining County, Anqing City, Anhui Province	110 m	OP034620
<i>L. insularis</i> (coastal area)			
01	Damao Island, Dinghai District, Zhoushan City, Zhejiang Province	22 m	OP034614
02	Chunxiao Town, Beilun District, Ningbo City, Zhejiang Province	8 m	OP034615
03	Shanjuan Town, Yixing City, Wuxi City, Jiangsu Province	42 m	ON611639
<i>L. chinensis</i>			
01	Bozhou Town, Xinhuang County, Huaihua City, Hunan Province	542 m	OP034613

laris population were induced by burying them in wet sand and the chromosome number was observed using the methods described by Chen and Li (1985). In August 2022, 50 flowers were randomly selected from each population for dissection and perianth tube lengths were measured. All statistical analyses were performed in SPSS ver. 19.0.

Acquisition, annotation, comparison and phylogenetic analysis of chloroplast genomes

DNA samples were extracted from leaves dried by silica gel according to the modified cetyltrimethylammonium bromide (mCTAB) extraction protocol (Doyle and Doyle 1987; Larridon et al. 2015). After Polymerase Chain Reaction (PCR), we used a NanoDrop 1000 Spectrophotometer and agarose gel electrophoresis to check the DNA quality. DNA library building and Genome Skimming and FastQC were outsourced to the Germplasm Bank of Wild Species in southwest China (China, Kunming), which employed Illumina HiSeq 6000 for analysis. The assembled 3G raw data from Genome Skimming have been analysed, using GetOrganelle v.1.7.1 to obtain the complete chloroplast genome, followed by genome annotation with PGA (Qu et al. 2019; Jin et al. 2020). This study was performed on the nine newly-reported complete chloroplast genomes (Table 1) and 14 complete chloroplast genomes of fertile diploid species were downloaded from NCBI. *Narcissus poeticus* was selected as the outgroup (Könyves et al. 2018). See Fig. 3 for the specific accession number.

All sequences were aligned by MACSE v.2 (Ranwez et al. 2018). Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used to determine the phylogenetic relationships, the best-fit model of DNA substitution being estimated by ModelFinder (Minh et al. 2013; Kalyaanamoorthy et al. 2017). ML analysis was conducted using the GTR + G + I model with 1000 bootstrap replicates by IQtree v.1.6.8 (Nguyen et al. 2015). Bayesian analysis was constructed with eight independent chains for 1,000,000 generations and sampling every 1000 generations by MrBayes v.3.2.6 (Ronquist et al. 2012; Zhang et al. 2022). All phylogenetic analyses were performed in Phylosuite (Zhang et al. 2020).

Results

Morphological comparison and karyotype observation

L. insularis and *L. sprengeri* are very similar in many morphological characteristics, such as the leaves being slightly twisted when young, the apex being lavender and becoming a narrow and a long band when mature; the perianth is mainly pink, the apex is usually pale blue and the perianth lobes are rarely shrivelled, slightly or almost not rolling back. However, after careful observation, we found there are certain differences between them in the perianth tube length and flower colour. In all *L. sprengeri* populations observed so far, the perianth tube is short (0.5–1.3 cm) and the flower colour ranged from nearly white (about 2%) to dark purple (about 2%), although most of the flowers are pale pink. In all populations of *L. insularis*, the perianth tube is relatively long (usually 1.5–2.5 cm) and the flower colours are almost all either deep or shallow pink (Figs 1, 2). The chromosomes numbers of the three *Lycoris insularis* sampled populations (Table 1) were consistently 22 (Fig. 3).

Characteristics of the complete chloroplast genome

The length of complete chloroplast genome of *Lycoris insularis* comprised 158,641–159,121 bp and *L. sprengeri* comprised 158,509–159,348 bp (Fig. 4, Ta-

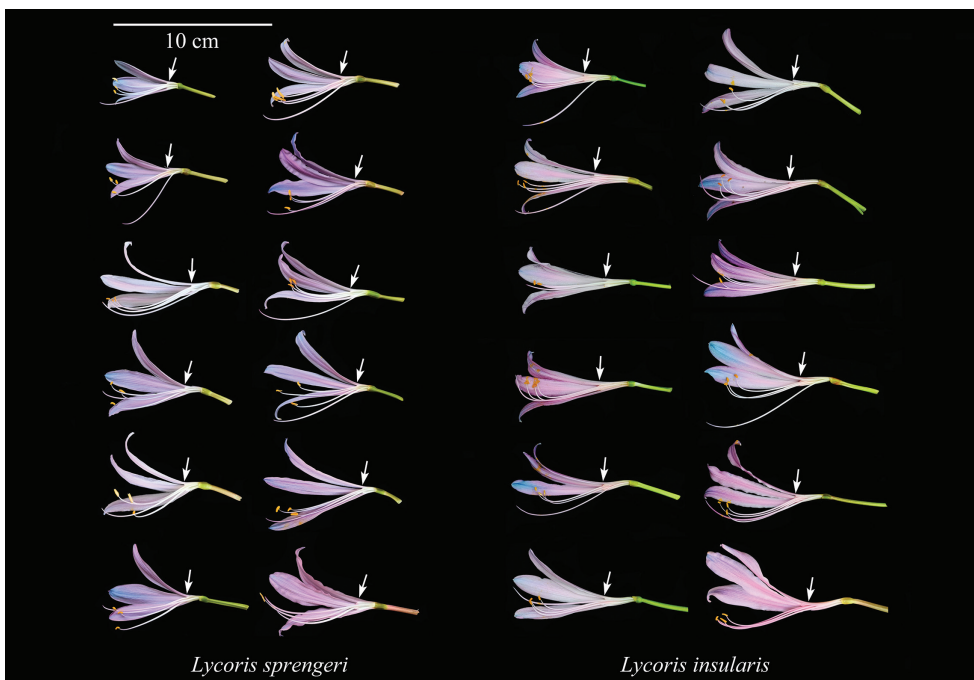


Figure 1. Anatomical comparison of *Lycoris insularis* S.Y. Zhang & J.W.Shao, sp. nov. and *L. sprengeri* Comes ex Baker. The position indicated by the arrow is the top of the perianth tube.

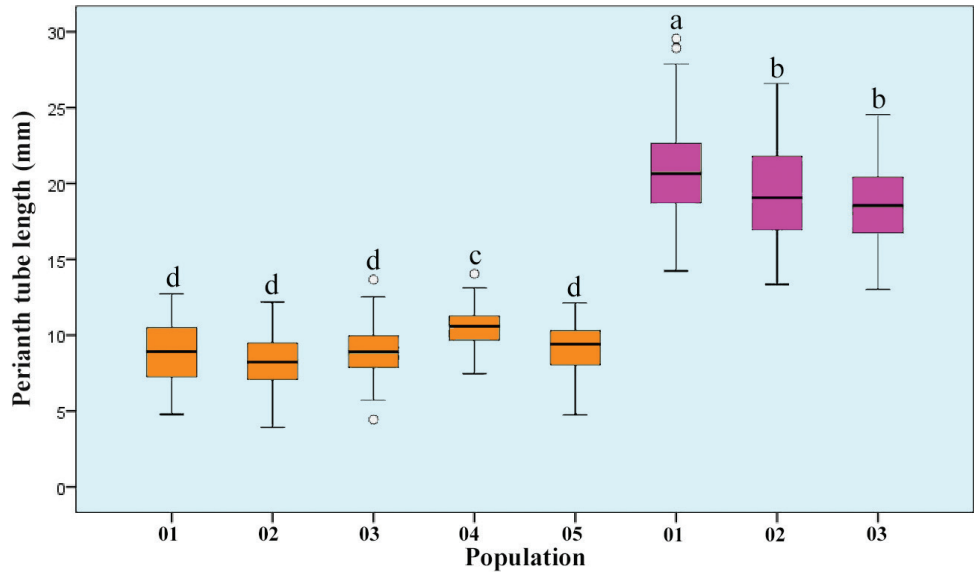


Figure 2. Comparison and variation of perianth tube length of *Lycoris sprengeri* (01–05, filled with orange) and *L. insularis* (01–03, filled with magenta). In the boxplot, the horizontal line shows the median, the bottom and top of the box show the first and third quartiles. Boxplots marked with different letters differ significantly (post hoc test, $P < 0.05$).

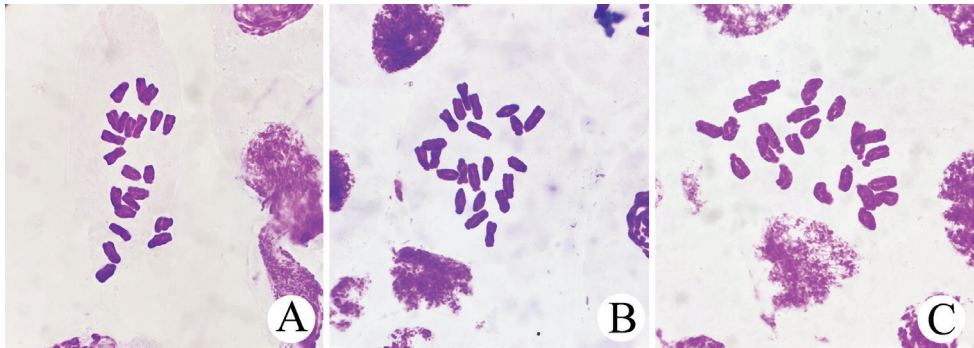


Figure 3. The karyotype of *Lycoris insularis* S.Y. Zhang & J.W.Shao, sp. nov. **A** from the population 01 **B** from the population 02 **C** from the population 03. The information about populations is shown in Table 1.

ble 2). They both possessed typical quadripartite structure: IRa, IRb, LSC and SSC; the characteristics and statistics of the chloroplast genome are summarised in Table 2.

Molecular phylogenetic relationship

The phylogenetic relationship, based on the complete chloroplast genome containing eight species, is shown in Fig. 5. Five individuals, collected from the inland area (*L. sprengeri*) from different geographic populations, formed a monophyletic clade (boot-

Table 2. Basic characteristics of chloroplast genomes of *Lycoris insularis* and *L. sprengeri*.

Characteristic	<i>L. insularis</i>	<i>L. sprengeri</i>
Total length (bp)	158,641–159,121	158,509–159,348
GC%	37.7%–37.8%	37.8%–37.9%
LSC length (bp)	86,488–86,590	86,483–86,941
SSC length (bp)	18,496–18,544	18,469–18,501
IR length (bp)	26,806–27,082	26,764–27,018
Total genes	112	112
Protein-coding genes	78	78
rRNA genes	4	4
tRNA genes	30	30

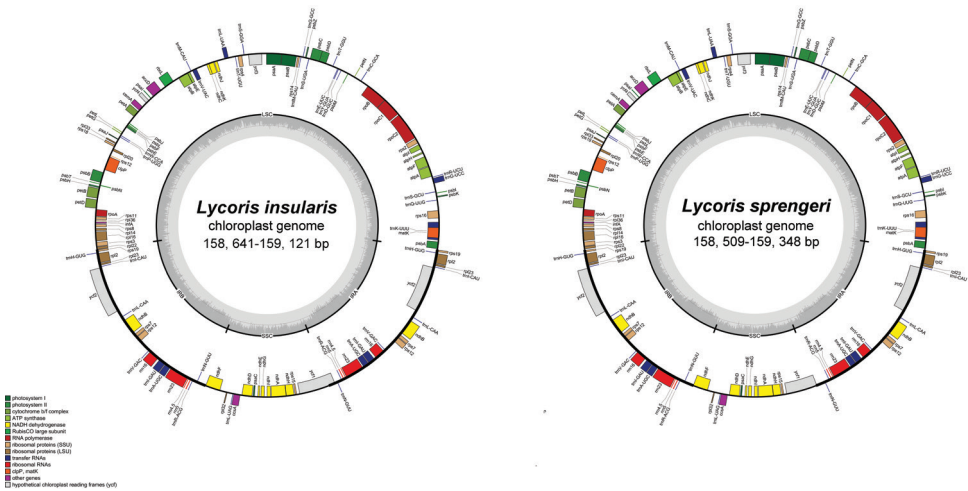


Figure 4. Plastid genome map of *Lycoris insularis* S.Y. Zhang & J.W.Shao, sp. nov. and *L. sprengeri* Comes ex Baker.

strap support (BS) = 100%), which is a sister clade to *L. longituba* + *L. chinensis*. Three individuals, collected from the coastal area (*L. insularis*) and three other sequences online (misidentified as *L. sprengeri*), composed a monophyletic clade (BS = 100%), which is a sister clade to *L. sanguinea*. *L. sprengeri* and *L. insularis*, respectively, are located in different clades and are separated at the base of the phylogenetic tree.

Taxonomic treatment

***Lycoris insularis* S.Y.Zhang & J.W.Shao, sp. nov.**

urn:lsid:ipni.org:names:77305347-1

Fig. 6

Type. CHINA. Zhejiang, Zhoushan City, Dinghai District, Damao Island, 29°56'55.4"N, 122°3'9.46"E, under the broad-leaved forest near the water on the Island, 22 m a.s.l.,

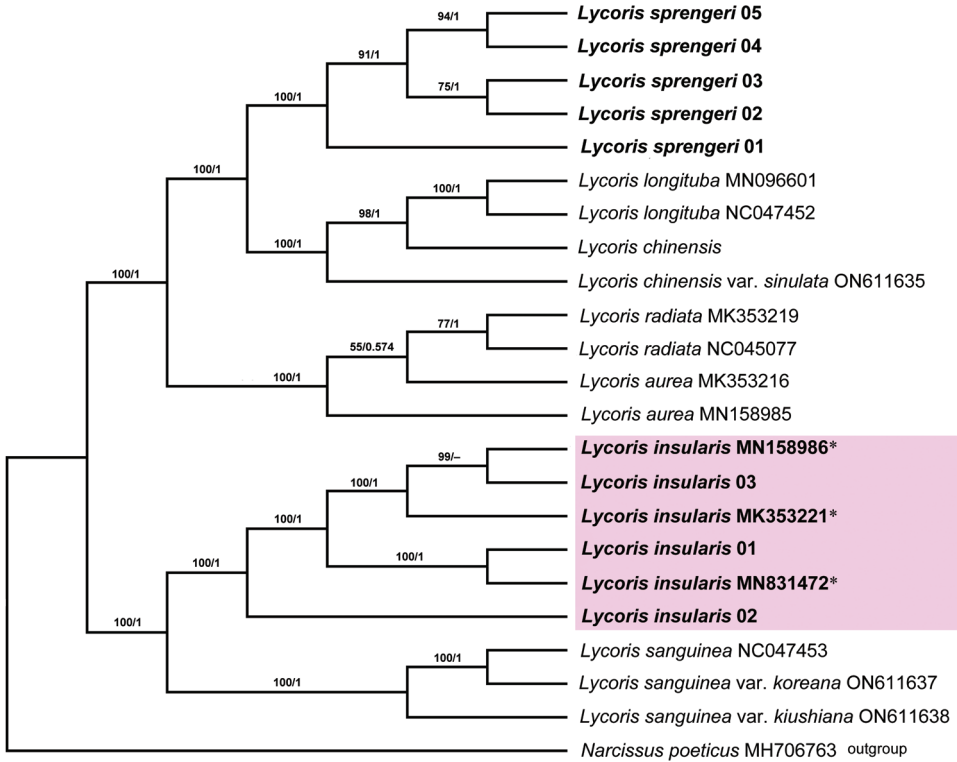


Figure 5. Phylogeny of the fertile diploid *Lycoris* species, based on the complete chloroplast genome. Numbers above branches are Maximum Likelihood bootstrap values (BS)/Bayesian posterior probability (BPP). The species name with accession numbers were download from NCBI. * showing those sequences which were misidentified as *L. sprengeri* in NCBI.

18 August 2019, S.Y. Zhang, ZSY201908001 (holotype: ANUB008515!; isotypes: ANUB008516!, ANUB008517!, CSH0192378!, NPH001410!).

Diagnosis. The new species resembles *Lycoris sprengeri* by young leaves swirling and rising, tepals usually pink with blue apex and tepals length substantially similar, but differs in significantly longer perianth tube (1.5–2.5 cm vs. 0.5–1.3 cm).

Description. Perennial herb. Bulb subglobose or ovate, 3–5 cm in diameter, covered brown epidermis, with fine lines on the epidermis. Leaves linear, often 6–9, blunt apex, appearing in winter or early spring, 40–60 cm long, 6–15 mm wide, pale green, mid-rib slightly sunken, covered with a little white powder. Inflorescence scapose, 40–60 cm high, green or reddish-brown; two spathe bracts, lanceolate, about 3 cm long, 8–12 mm wide; 5–7 flowers per umbels, pedicels 1.5–3 cm long, diameter about 3 mm; flowers usually pink with blue apex, occasionally white, blue or all pink; perianth lobes oblanceolate, 4.5–7 cm long, about 0.9–1.5 cm wide, apex slightly reversed; perianth tubes cream or pink, about 15–25 mm long. Filaments 5–7 cm long, pink, slightly longer than tepals; anther yellow, 3–5 mm long; pistil length 6–10 cm,

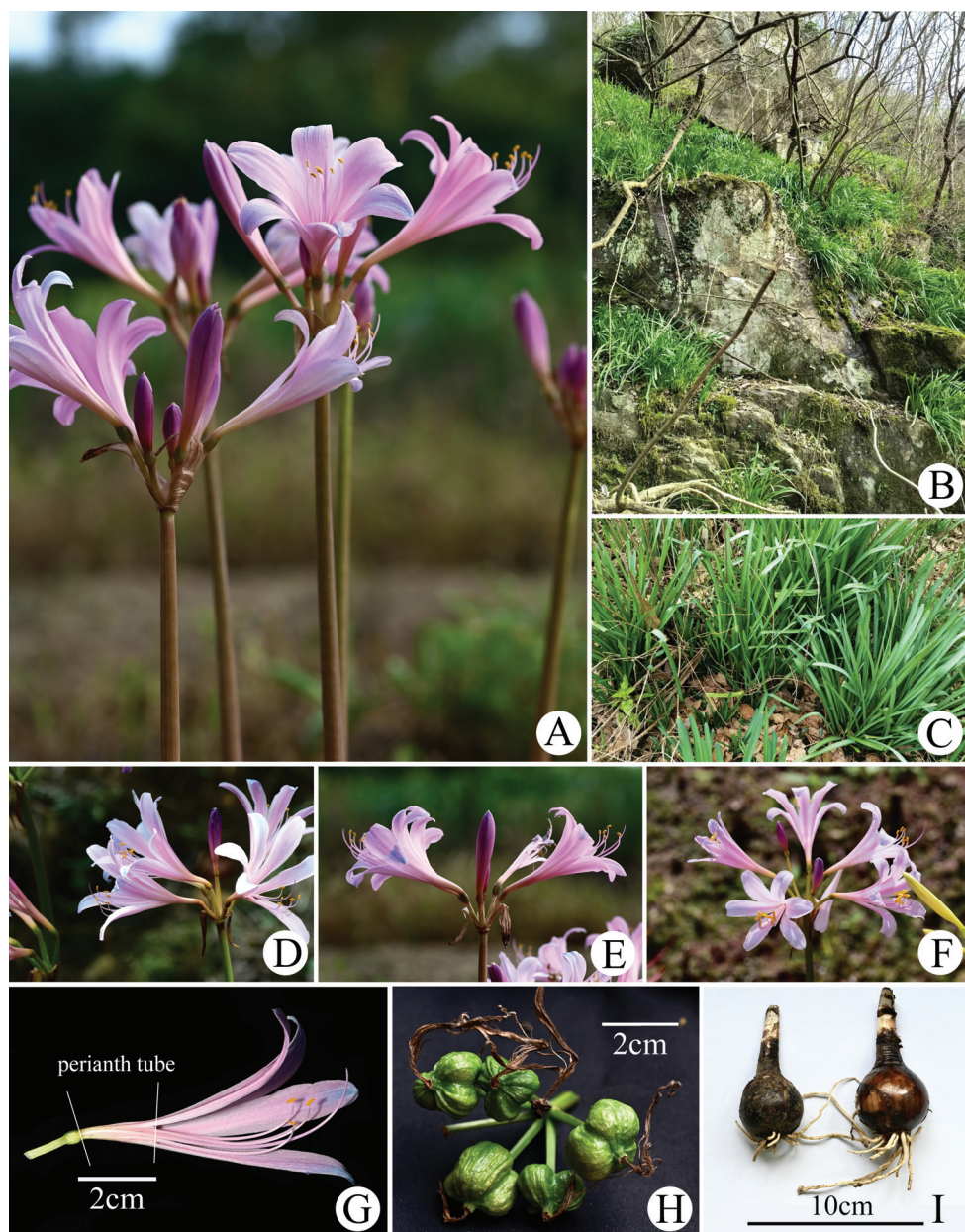


Figure 6. Morphology of *Lycoris insularis* S.Y. Zhang & J.W. Shao, sp. nov. **A** inflorescence **B** habitat **C** plants in leaf stage **D, E, F** different individuals **G** anatomy of the flower **H** fruit **I** bulbs **G** was photographed by Cheng-sheng Li.

pink or with purple apex. Ovary 5 mm in diameter, spherical and green. Capsules three-lobed, green or with light brown when mature; seeds black, spherical, about 7 mm in diameter.

Phenology. Flowering from late July to late September; fruiting in September to October; leaves growing in winter or early spring (December to February of the following year).

Distribution and habitat. Most of the populations grow on the hillsides or island slopes near the sea (the coastal areas of Shanghai City, Zhejiang Province and Fujian Province) and sporadic populations grow around the inland hills and valleys (Fig. 7).

Chinese Name. Hǎi bīn shí suàn (海滨石蒜).

Etymology. Latin *insula*, island, and *-aris*, belonging to; the specific epithet alludes to occurrence of the new species in Damao Island.

Reproduction. This species can reproduce asexually by duplication of bulbs (about three times per two years) and also can sexually reproduce through seeds, taking about five years from seed germination to flowering.

Conservation status. Compared with other species in *Lycoris*, *L. insularis* has a wider distribution area and many populations grow on inaccessible islands. After our field investigation, a large number of bulbs in Yixing and Huzhou were excavated for greening and landscaping, but there are still a large number of *L. insularis* populations in the wild in other areas. Thus, we classified its conservation status as LC (Least Concern), according to the IUCN Red List Criteria (IUCN 2019).

Additional specimen examined (paratypes). China. Jiangsu Province: Yixing City, Shanjuan Cave, 25 Aug 1960, *Wen-zhe Fang* 00110645 (PE); Shanghai City:

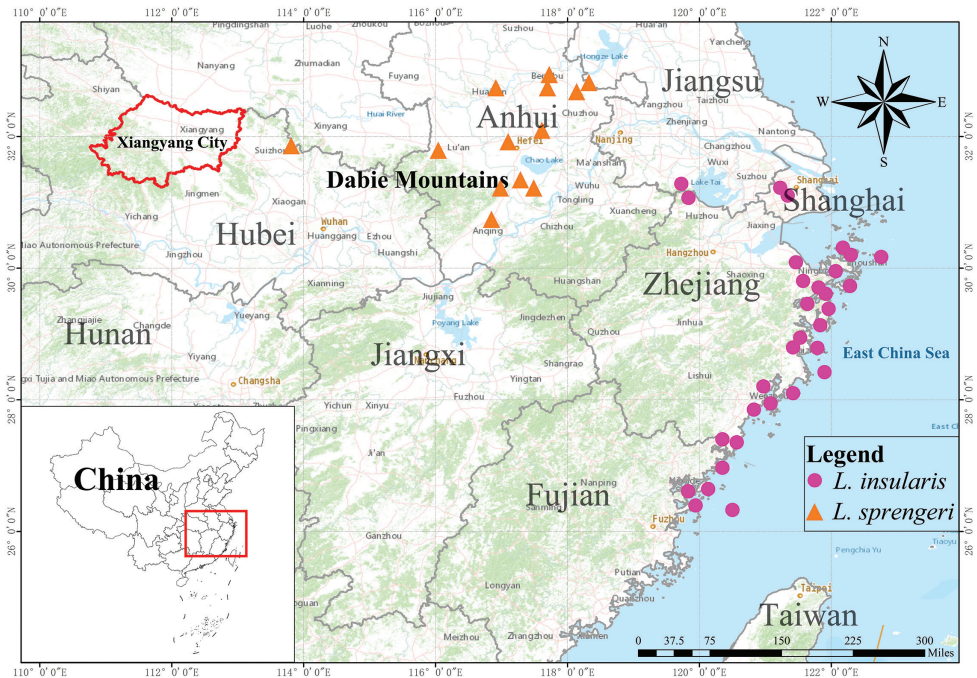


Figure 7. Distribution map of *Lycoris insularis* S.Y. Zhang & J.W. Shao, sp. nov. and *L. sprengeri* Comes ex Baker. Distribution information is based on specimen inspections and actual surveys. Xiangyang City is the origin of the type specimen of *L. sprengeri*.

Songjiang District, Tianma Mountain, 8 Sept 1963, *Guang-jin Fan* 00110641 (PE); Songjiang District, Tianma Mountain, alt. 59 m, 14 Sept 2013, *Ting Zhang* 1393054 (KUN); Songjiang District, Dongshe Mountain, alt. 21 m, 28 Jul 2015, *Bin-jie Ge, Tian Li* CSH0098245 (CSH); Zhejiang Province: Putuo District, Taohua Island, alt. 19 m, 18 Sept 2017, *Yong-jie Guo, Li Huang, Zheng-yu Zuo & Ting Guo* 1446199 (KUN); Jiaojiang County, Jiangshan Island, alt. 3 m, 25 Oct 2016, *Yong-jie Guo, Qiao-rong Zhang, Li Huang, Lian-yi Li, Pei Li, De-ming He, Ying-hong Yang* 1451350 (KUN); Ruian County, Shuangfengshan Island, alt. 46 m, 11 Oct 2017, *Yong-jie Guo, Qiao-rong Zhang, Yun-hua Fang, Xing-xu Sun* 1450112 (KUN); Putuo District, Putuo Mountain, alt. 62 m, 24 Aug 2015, *Bao-cheng Wu* NAS00591943, NAS00591944, NAS00591945 (NAS); Putuo District, Xiaomayi Island, 26 Jul 2011 *Qi Tian, Zheng-wei Wang* CSH0116798 (CSH); Putuo District, Daqing Mountain, alt. 18 m, 21 Sept 2015, *Bin-jie Ge, Xu Yuan* CSH0101312 (CSH); Daishan County, Yushan Village, 21 Sept 2012, *Xi-yang Ye* CSH0032460 (CSH).

Key to the fertile diploid native species of *Lycoris*

- | | | |
|---|--|-------------------------|
| 1 | Leaves appear in autumn (Sept–Oct) | 2 |
| – | Leaves appear in winter or spring (Dec–Feb) | 5 |
| 2 | Leaves apex obtuse | 3 |
| – | Leaves apex acuminate | 4 |
| 3 | Flowers red, stamen 6–8 cm | <i>L. radiata</i> |
| – | Flowers rose-red, stamen 3–3.5 cm | <i>L. wulingensis</i> |
| 4 | Pedicels 15–22 mm, perianth tube length 1.2–1.5 cm | <i>L. aurea</i> |
| – | Pedicels 8–9 mm, perianth tube length 1.5–2 cm | <i>L. traubii</i> |
| 5 | Leaves width 0.5–1.5 cm, apex rose-red when young | 6 |
| – | Leaves width 1.5–3 cm, apex always green | 8 |
| 6 | Flowers orange | <i>L. sanguinea</i> |
| – | Flowers pink, apex usually blue | 7 |
| 7 | Perianth tube length 1.5–2.5 cm | <i>L. insularis</i> |
| – | Perianth tube length 0.5–1.3 cm | <i>L. sprengeri</i> |
| 8 | Flowers actinomorphic, perianth tubes 3–6 cm | <i>L. longituba</i> |
| – | Flowers zygomorphous, perianth tubes 1.5–2.5 cm | 9 |
| 9 | Leaves width ca. 1.5 cm, tepals orange, apex usually red | <i>L. tsinlingensis</i> |
| – | Leaves width 1.5–2.5 cm, tepals yellow to light orange | <i>L. chinensis</i> |

Discussion

Based on our research, especially the phylogenetic tree constructed by the complete chloroplast genome (Fig. 5), it can be clearly seen that those plants (identified as *L. sprengeri*) collected from the inland and coastal areas are two distinct entities (Comes and Baker 1902; Traub and Moldenke 1949). The original description and type specimen photos of *L. sprengeri* (in Kew, *Sprenger* K000901061, <http://specimens.kew.org/>

herbarium/K000901061) both show that it has a short perianth tube (about 0.5 cm), which is consistent with those plants from inland populations collected by the authors. Therefore, we recognised that those inland plants with a short perianth tube (usually less than 1.3 cm) was the genuine *L. sprengeri*, while those coastal plants with a relatively long perianth tube (usually longer than 1.5 cm) is the new species, i.e. *L. insularis*.

The known karyotypes of fertile diploid species in *Lycoris* can be roughly divided into two categories: $2n = 12-16$ (such as *L. traubii*, *L. aurea*, *L. chinensis* and *L. longituba*) or $2n = 22$ (such as *L. sanguinea*, *L. radiata*, *L. wulingensis* and *L. sprengeri*) (Kurita 1986; Hsu et al. 1994; Zhang et al. 2021; Zhang et al. 2022). Other sterile hybrid species are always with odd or allotriploid karyotypes. The chromosome number of *L. insularis* ($2n = 22$) is in line with the premise of being a fertile species. Furthermore, *L. insularis* has a relatively wide distribution area; its wild population can normally seed; and the seeds can develop into new plants at a high rate; thus, it can easily form a large population (more than 5,000 bulbs) and the individuals are scattered (i.e. do not show obvious clustering). All these factors indicate that *L. insularis* is a diploid fertile species and possibly has great value in the breeding and application of *Lycoris*.

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