

# 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 phytogeographic 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 polyphytic. 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., *Triandrophlyllum 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 is 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 austriogenus</i> (Hook. f. & Taylor) J.J. Engel & R.M. Schust.	<i>Pachyglossa austrirena</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.-Stolt., 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. et Taylor) Grolle	Chile, Holz & Franzaring, CH 00-44B (GOET)	AM491290	—
<i>Chiloscyphus itoanu</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 lents</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.-Stolt. & 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-zealandiae</i> (Lehm. & Lindenb.) J.J.Engel & R.M.Schust.	<i>Lophocolea novae-zealandiae</i> (Lehm. & Lindenb.) Nees	Australia, Eggers, AUS 3/81 (JE)	AM282820	—
<i>Chiloscyphus novae-zealandiae</i> var. <i>grandistipulus</i> (Schiffn.) J.J.Engel	<i>Lophocolea novae-zealandiae</i> var. <i>grandistipula</i> (Schiffn.) Váňa	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 trachyopus</i> (Hook. f. & Taylor) Hässel	<i>Clasmatocolea trachyopa</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	—

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			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> (Gottscche) Schiffn.	<i>Heteroscyphus zollingeri</i> (Gottscche) Schiffn.	China, 1998 Koponen et al. 57927	—	AY149879
<i>Hygrolembidium acrocladum</i> (Bergr.) R.M. Schust.	<i>Hygrolembidium acrocladum</i> (Bergr.) 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, Glenny 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 amphibolioides</i> (Nees) Grolle	<i>Leptoscyphus amphibolioides</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 porphyrioides</i> (Nees) Grolle	<i>Leptoscyphus porphyrioides</i> (Nees) Grolle	Ecuador, Schafer-Verwimp (herb. Schafer), 23229/a	—	DQ176707
<i>Leptoscyphus porphyrioides</i> (Nees) Grolle	<i>Leptoscyphus porphyrioides</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 cuspidata</i> (Nees) Limpr.	China, Hunan Province, Sang-Zhi Co., Koponen et al. 48430	—	AY149866
<i>Lophocolea heterophylla</i> (Schrad.) Dumort.	<i>Lophocolea heterophylla</i> (Schrad.) Dumort.	USA, Indiana, MI Sargent's culture collection, #481	—	AF231899
<i>Lophocolea martiana</i> Nees	<i>Cryptolophocolea martiana</i> (Nees) L. Söderstr., Crand.-Stotl. & 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	trnL-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, Nuukso 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 sub trifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	<i>Triandrophyllum sub trifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	Bolivia, Churchill et al. 22800	AJ972455	—
<i>Triandrophyllum sub trifidum</i> (Hook. f. & Taylor) Fulford & Hatcher	<i>Triandrophyllum sub trifidum</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, Glenny 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	CGAAATTGGTAGACGCTGCG	forward	62	Bakalin et al. 2021
<i>trnL</i> -F cpDNA	ATTTGAACCTGGTGACACGAG	reverse	58	Taberlet et al. 1991
<i>trnG</i> -intron cpDNA	ACCCGCATCGTTAGCTTG	forward	56	Pacak and Szwejkowska-Kulinska 2000
<i>trnG</i> -intron cpDNA	GCGGGTATAGTTTAGTGG	reverse	54	Pacak and Szwejkowska-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 500<sup>th</sup> 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 Gen-Bank: 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.

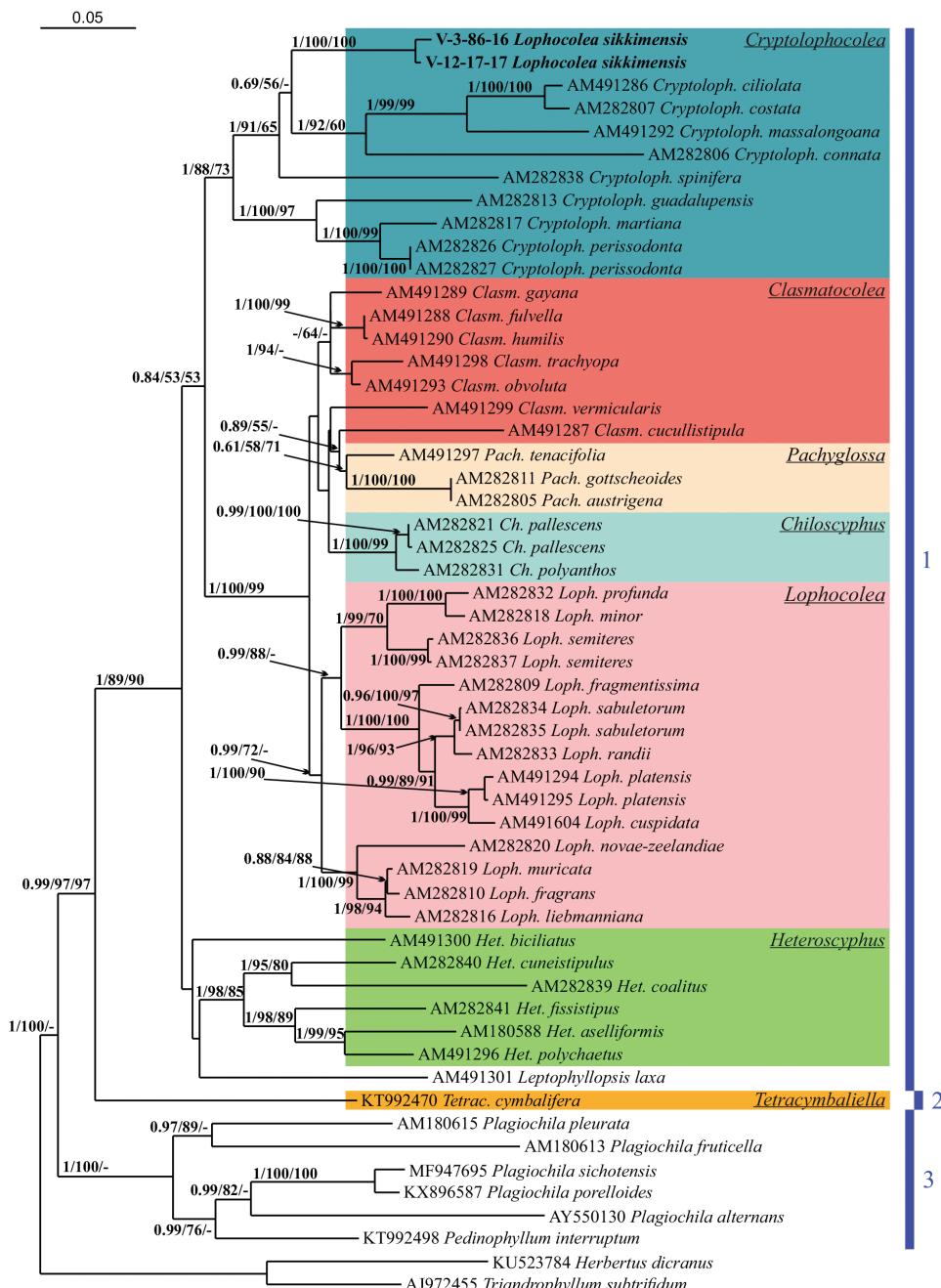
**Table 3.** The characteristics of ITS1–2, and *trnL*-F nucleotide sequence alignments.

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

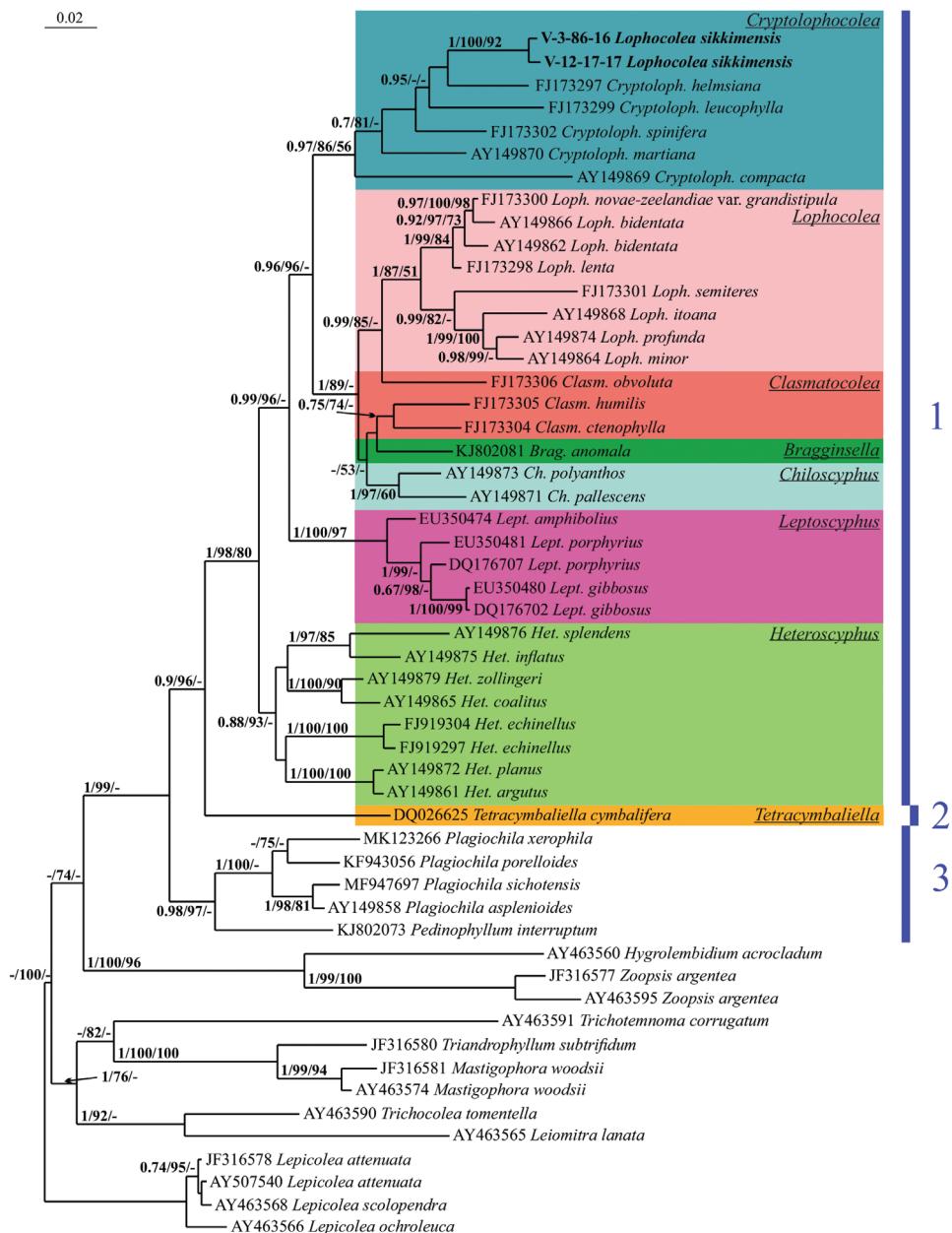
The MP analysis for ITS1–2 yielded a single parsimonious tree with CI = 0.364388 and RI = 0.619946. The ML criterion recovered a bootstrap consensus tree with a log-likelihood = -10978.46. The arithmetic means of the log likelihoods in Bayesian analysis for each sampling run were -11026.6 and -11028.06.

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 1.** Phylogram obtained in a Bayesian analysis for the genus *Cryptolophocolea* and related taxa based on ITS1–2 dataset. The values of Bayesian posterior probabilities and bootstrap support from the MP and ML analyses greater than 0.50 (50%) are indicated. Taxon names and GenBank accession numbers are provided. Newly studied specimens are marked in bold 1 family Lophocoleaceae 2 family Brevianthaceae 3 family Plagiochilaceae.



**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 **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											ITS 1–2, %
0.061	<i>Lophocolea</i> <i>sikkimensis</i> + <i>Cryptolophocolea</i>		—	—	0.164	0.150	0.153	—	0.167	0.156	—	
0	<i>Lophocolea sikkimensis</i>	—		<b>0.128</b>	0.142	0.129	0.130	—	0.154	0.138	0.011	
<b>0.070</b>	<i>Cryptolophocolea</i>	—	<b>0.058</b>		0.168	0.155	0.158	—	0.170	0.161	<b>0.135</b>	
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 infragenetic 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])

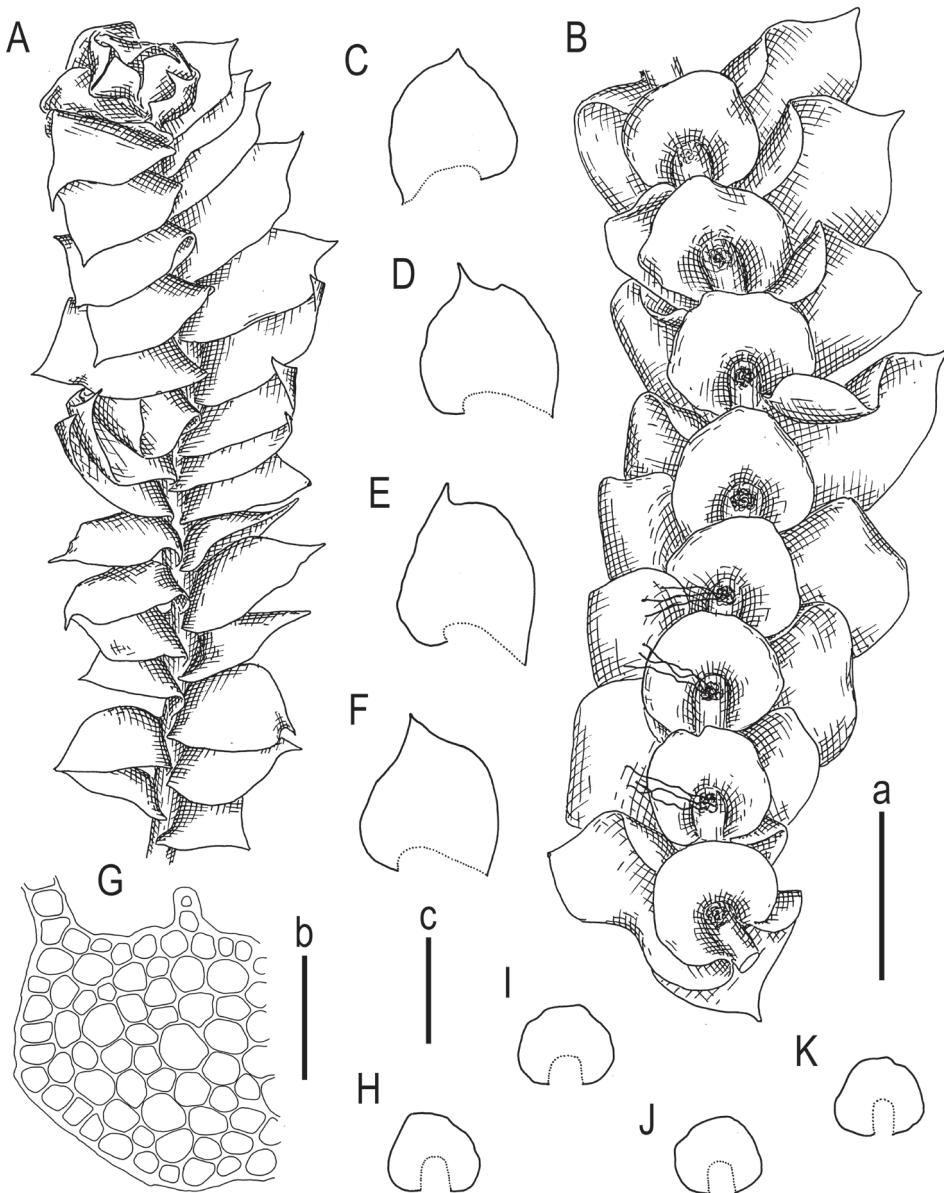
## Discussion

### 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  $\mu$ 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 bicuspitate 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

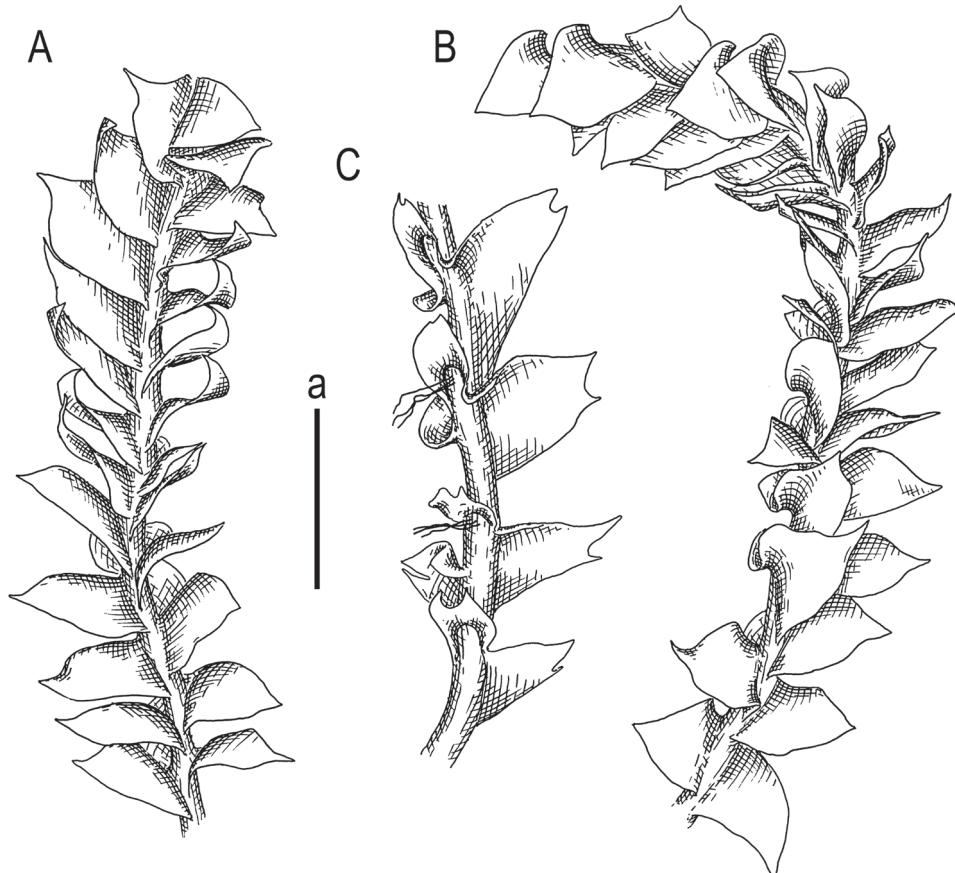
Kitagawa (1974: 35) noted that “Plants occur on various substrata (rocks, rotten logs, humus) but usually do not grow directly on such substrata but creeping larger bryophytes”. In general, the same can be said about the ecological preferences of Vietnamese plants. Meanwhile, it should be noted that 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)

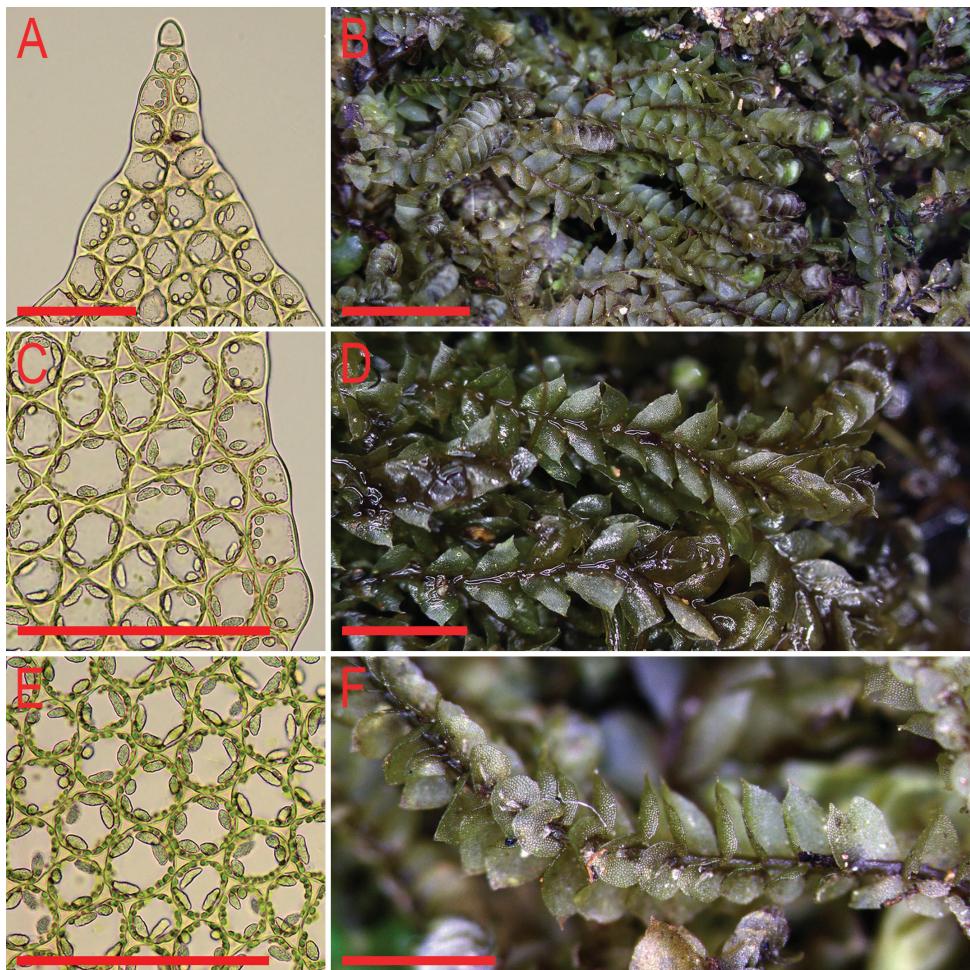
Plants yellowish green, greenish and whitish yellowish to yellowish brownish, sometimes grading to grayish brownish in the herbarium, gentle, very fragile and glistening when dry, forming loose pure patches over other bryophytes or rarely intermixed with *Riccardia* sp., *Herbertus dicranus*, *Plicanthus*, *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 × 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 × 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), 400–600 × 700–850 µm. Midleaf cells subisodiametric, 22–33 µm in diameter or shortly oblong, to 38 µm long, thin-walled, trigones large, mostly triangle to slightly concave or slightly convex, cuticle virtually smooth; cells along leaf margin subisodiametric (subquadrate), 21–25 µm in diameter to slightly elongate along the margin, to 25–27 µm long; oil bodies in the midleaf cells 2–5 per cell, finely granulate, irregularly oblong, ellipsoidal to shortly fusiform, 8–17 × 5–7(–8) µm, 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°18.8'N, 103°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 µm (**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*, *Gymnomitrion 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 chiloscyphoidea* (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. tricorata* (Hässel) Crand.-Stotl. & Stotler.

*Cryptolophocolea connata* (Sw.) L. Söderstr. & Váňa 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. lillienae* (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 & Váňa (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. stephani* (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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