



**Studies on Lophocoleaceae XXII. The systematic position of *Amphilophocolea* R.M.Schust. together with comments on the status of *Tetracymbaliella* Grolle and *Lamellocolea* R.M.Schust.**

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## Abstract

DNA sequence results show that *Amphilophocolea*, a monotypic genus endemic to New Zealand, is nested within *Heteroscyphus*. A morphological examination of specimens that agree with the protologue shows it to be identical to *Heteroscyphus knightii*. *Heteroscyphus cymbaliferus* in the DNA sequence analyses is sister to the clade consisting of the rest of *Heteroscyphus*, and the monophyletic *Chiloscyphus* and *Lamellocolea*, and suggests that the genus *Tetracymbaliella* should be reinstated. *Lamellocolea* is not included in either *Chiloscyphus* or *Heteroscyphus* in the trees derived from sequence data, but its position as sister to *Chiloscyphus* obtained from the Bayesian analysis lacks significant support.

**Key words:** liverworts, Marchantiophyta, *Amphilophocolea*, *Chiloscyphus*, *Heteroscyphus*, Lophocoleaceae, DNA sequence, morphology, phylogeny, taxonomy, classification

## Introduction

The genus *Amphilophocolea* R.M.Schust was described by Schuster (2001), who isolated the genus from other members of Lophocoleaceae on the basis of branching. The genus was described for a single species, *A. sciaphila* R.M.Schust., and based solely on sterile plants. Schuster (2001: 96) remarked that "after study of a bewilderingly large number of taxa I would suggest that instead of the 'traditional' division of lophocoleoids into two genera, *Lophocolea* and *Chiloscyphus*, we recognize four genera on the basis of branching modes" (*Chiloscyphus* Corda, *Stolonivector* J.J.Engel, and the two new genera introduced in that paper, *Cyanolophocolea* R.M.Schust. and *Amphilophocolea* R.M.Schust.). Schuster (2001) argued that all three major branching types (terminal, lateral intercalary, and ventral intercalary) were present throughout Geocalycaceae subfam. *Lophocoleoideae*, but that one of these, the lateral-intercalary type, had been lost in both *Cyanolophocolea* and *Amphilophocolea*. In *Amphilophocolea* the terminal type also had been lost. These losses in branching types identified them as evolutionarily specialized species deserving of generic recognition alongside *Chiloscyphus* and *Stolonivector*. Schuster (2001) also considered whether *Amphilophocolea* might be allied to *Geocalyx*, on the grounds that it has roughened leaf surfaces ("cuticle"), and *Geocalyx* has its leaf surfaces papillose. This would have placed *Amphilophocolea* in Geocalycaceae subfam. *Geocalycoideae*, but gynoecia were needed to confirm the presence of an *Isotachis*-type perigynium. In a discussion of the revised classification of Lophocoleaceae, Schuster (2001: 97) stated that "two criteria stand out: (a) ramification patterns; (b) presence vs. absence of an *Isotachis*-type perigynium," but added that "in the lack of gynoecial data, the status and position of *Amphilophocolea* remains ambiguous." Schuster (2001: 102) in conclusion remarked that "ultimately, the derivative branching pattern—only ventral-

intercalary branches appear to occur—suggests that *Amphilophocolea* should be recognized as an autonomous genus."

We are presented then, with several questions. Is the species deserving of generic rank, i.e., as belonging to an independent genus, *Amphilophocolea*? If not, what is the rightful systematic position of the species? Would additional morphological information, such as the type and position of gametangia, shed light on the systematic placement of *A. sciaphila*? In order to address these questions, we developed a two-pronged approach: a) we conducted a search for gametangia in specimens that we regarded as identical to *A. sciaphila*; and b) we subjected *A. sciaphila* to DNA sequence-based phylogenetic analyses.

## Materials and Methods

### *Taxon sampling for phylogenetic analyses*

Two samples of *Amphilophocolea sciaphila*, recently collected from New Zealand, were included in this study. The remaining ingroup was composed of 15 *Chiloscyphus* species including the generic type species *C. polyanthos*; 11 *Heteroscyphus* species including the generic type *H. aselliformis*; and also one species of the bitypic *Lamellocolea* J.J.Engel of New Zealand, *L. granditexta* (Steph.) J.J.Engel (a second species is now known—Engel & Glenn, in press). The latter has a close affinity to species of *Heteroscyphus* in form of leaves and underleaves, especially to *H. cuneistipulus* (Steph.) Schiffn., but it differs from *Heteroscyphus* in characters of the gynoeceal apparatus and the position of gametangia (Engel 1991). *Lamellocolea* has lamellate perianths and reduced female bracteoles as well as androecia and gynoeceia on main shoots, *Frullania*-type branches or at times on rather short lateral-intercalary branches. *Heteroscyphus* has elamellate perianths, well-developed female bracteoles and androecia and gynoeceia that are always on short, determinate, lateral intercalary branches that never have normal vegetative leaves.

*Plagiochila asplenioides* and *Plagiochilium conjugatum* of the family Plagiochilaceae, sister to Lophocoleaceae (e.g., He-Nygrén et al. 2006; Hentschel et al. 2007), were used as outgroups. In total, 31 taxa were included in the phylogenetic analyses (Table 1). A DNA sequence dataset was obtained from two chloroplast DNA markers, *rbcL* and *trnL-trnF*, for all 31 species, except for an *rbcL* sequence of *Heteroscyphus argutus* and a *trnL-trnF* sequence of *H. aselliformis*, which unfortunately could not be obtained for this study. Novel sequences of *Amphilophocolea sciaphila*, *Heteroscyphus ammophilus*, *H. triacanthus*, and *Lamellocolea granditexta* were generated for both molecular regions. Their sequence accession numbers are in italics in Table 1. Table 1 also provided information of the full species names of the samples used. *Plagiochila asplenioides* was used to root the tree in the phylogenetic analyses.

### *DNA extraction, PCR amplification and sequencing*

DNA was extracted from dry herbarium material, using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). DNA amplification and sequencing were performed using the protocol in He-Nygrén et al. (2004). Of the two molecular regions examined, *rbcL* is a protein-coding gene so that there is no length variation in the sequences obtained. In the present study, the length of *rbcL* sequenced was 1001 bp. The *trnL-trnF* region contains a partial sequence of the leucine transfer RNA<sub>(UAA)</sub>, an intergenic spacer, and a partial sequence of phenylalanine tRNA<sub>(GAA)</sub>. The length of the sequences varied from 406 bp (*Chiloscyphus helmsianus*) to 516 bp (*Heteroscyphus splendens*) largely due to difference in the length of the variable regions of the intron and the intergenic spacer. The alignment of the *trnL-trnF* region was done using MUSCLE (Edgar 2004), and the length of the aligned characters was 534 bp.

### *Phylogenetic analyses*

The dataset of the 31-exemplar *rbcL* and *trnL-trnF* sequence matrix was analyzed using both Bayesian inference and maximum parsimony. In total, 1535 aligned characters were included in the combined dataset. For the Bayesian analyses, using MrBayes version 3.0B4 (Huelsenbeck & Ronquist, 2001; Ronquist, 2004),

the combined data were partitioned into two sets corresponding to the *rbcL* and *trnL–trnF* regions. Three separate runs, each including 10 million generations with four chains and sampling trees and parameters every 100th generation, were completed.

**TABLE 1.** List of taxa and the GenBank accession numbers for *rbcL* and *trnL–trnF* sequences used in the present study. Accession numbers for novel sequences generated in this study are in *Italics*.

<i>Amphilophocolea sciaphila</i> R.M.Schust.	<i>HM439102</i>	<i>HM439107</i>	New Zealand, Glenny 10161
<i>Amphilophocolea sciaphila</i> R.M.Schust.	<i>HM439103</i>	<i>HM439108</i>	New Zealand, Engel & von Konrat 28206
<i>Chiloscyphus cuspidatus</i> (Nees) J.J.Engel & R.M.Schust.	AY149845	AY149866	He-Nygrén & al. 2004
<i>Chiloscyphus helmsianus</i> (Steph.) J.J.Engel & R.M.Schust.	FJ173311	FJ173297	Glenny & al. 2009
<i>Chiloscyphus itoanus</i> (Inoue) J.J.Engel & R.M.Schust.	AY149846	AY149868	He-Nygrén & Piippo 2003
<i>Chiloscyphus japonicus</i> (Steph.) J.J.Engel & R.M.Schust.	AY149847	AY149869	He-Nygrén & Piippo 2003
<i>Chiloscyphus latifolius</i> (Nees) J.J.Engel and R.M.Schust.	AY149842	AY149862	He-Nygrén & Piippo 2003
<i>Chiloscyphus lentus</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	FJ173312	FJ173298	Glenny & al. 2009
<i>Chiloscyphus leucophyllus</i> (Hook.f. & Taylor) Gottsche et al.	FJ173313	FJ173299	Glenny & al. 2009
<i>Chiloscyphus martianus</i> (Nees) J.J.Engel & R.M.Schust.	AY149848	AY149870	He-Nygrén & Piippo 2003
<i>Chiloscyphus minor</i> (Nees) J.J.Engel & R.M.Schust.		AY149843	AY149864 He-Nygrén & Piippo 2003
<i>Chiloscyphus novae-zeelandiae</i> var. <i>grandistipulus</i> (Schiffn.) J.J.Engel	FJ173314	FJ173300	Glenny & al. 2009
<i>Chiloscyphus pallescens</i> (Ehrh. ex Hoffm.) Dumort.	AY149849	AY149871	He-Nygrén & Piippo 2003
<i>Chiloscyphus polyanthos</i> (L.) Corda	AY149851	AY149873	He-Nygrén & Piippo 2003
<i>Chiloscyphus profundus</i> (Nees) J.J.Engel & R.M.Schust.	AY149852	AY149874	He-Nygrén & Piippo 2003
<i>Chiloscyphus semiteres</i> (Lehm.) Lehm. & Lindenb.	FJ173315	FJ173301	Glenny & al. 2009
<i>Chiloscyphus spinifer</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	FJ173316	FJ173302	Glenny & al. 2009
<i>Heteroscyphus echinellus</i> (Lindenb. & Gottsche) J.J.Engel & Xiao L.He	FJ919289	FJ919297	Engel & He 2010
<i>Heteroscyphus argutus</i> (Reinw. et al.) Schiffn.	AY149861		He-Nygrén & Piippo 2003
<i>Heteroscyphus ammophilus</i> (Colenso) R.M.Schust.	<i>HM439104</i>	<i>HM439109</i>	New Zealand, Glenny 10312
<i>Heteroscyphus aselliformis</i> (Reinw. et al.) Schiffn.	AY149841		He-Nygrén & Piippo 2003
<i>Heteroscyphus coalitus</i> (Hook.f.) Schiffn.	AY149844	AY149865	He-Nygrén & Piippo 2003
<i>Heteroscyphus cymbaliferus</i> (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	DQ026592	DQ026625	He-Nygrén & al. 2006
<i>Heteroscyphus inflatus</i> (Steph.) S.C.Srivast. & A.Srivast.	AY149853	AY149875	He-Nygrén & Piippo 2003
<i>Heteroscyphus planus</i> (Mitt.) Schiffn.	AY149850	AY149872	He-Nygrén & Piippo 2003
<i>Heteroscyphus splendens</i> (Lehm. & Lindenb.) Grolle	AY149854	AY149876	He-Nygrén & Piippo 2003
<i>Heteroscyphus triacanthus</i> (Hook.f. & Taylor) Schiffn.	<i>HM439105</i>	<i>HM439110</i>	New Zealand, Glenny 9947
<i>Heteroscyphus zollingeri</i> (Gottsche) Schiffn.	AY149856	AY149879	He-Nygrén & Piippo 2003
<i>Lamellocolea granditexa</i> (Steph.) J.J.Engel	<i>HM439106</i>	<i>HM439111</i>	New Zealand, Glenny 9371
<i>Plagiochila asplenioides</i> (L.) Dumort.	AY149839	AY149858	He-Nygrén & al. 2004
<i>Plagiochilion conjugatum</i> (Hook.) R.M.Schust.	DQ026588	DQ026622	He-Nygrén & al. 2006

Compartments were unlinked to allow parameters to vary independently. The analyses were performed under the GTR+I+G model which was used for each of the two partitions within each data set based on the estimates using MrModeltest v. 2.3 (Nylander 2004, Swofford 2002). All sample points prior to stability were discarded as burn-in values, and the remaining points were used to generate a 50% majority consensus tree.

Maximum parsimony analyses were performed using NONA 2.0 (Goloboff 1998) with the WinClada interface (Nixon 1999). Heuristic maximum parsimony searches were conducted by TBR swapping on Wagner trees generated from 1000 random taxon addition sequences, with 100 trees held in memory for each of the 1000 replicate initiations (using the NONA commands hold/100; mult\*1000). This procedure was repeated 10 times (i.e. 10 000 random initiations). Bootstrap support values for branches were calculated employing 1000 replicates, ten search pseudoreplicates, and one starting tree per pseudoreplicate.

## Results

The datasets generated from the two sequenced regions were initially analyzed separately. Congruence between datasets was evaluated by visual comparison of the topologies and levels of clade support arrived at by the two types of analysis (MP and Bayesian). No incongruence between the loci was observed. Of the 1535 aligned characters, 437 (28%) were variable sites and 264 (17%, of which 14% from the *rbcL* and 20% from the *trnL-trnF*) were parsimony informative (MEGA, Kumar et al. 2008). Bayesian inferences based on the combined data set resolve *Amphilophocolea sciaphila* as nested within a group consisting of all the *Heteroscyphus* species except *H. cymbaliferus*, which was resolved as sister to all the remaining ingroup taxa (Fig. 1). *Amphilophocolea sciaphila* is closely related to *Heteroscyphus ammophilus*, *H. inflatus*, *H. aselliformis* and *H. splendens*. The species of *Heteroscyphus* sampled (excluding *H. cymbaliferus*), *Chiloscyphus*, and *Lamellocolea* were supported as a monophyletic group, but the mutual relationship of these three groups was not fully resolved in the analysis. *Chiloscyphus* and *Heteroscyphus* (excluding *H. cymbaliferus*) were resolved as monophyletic groups with strong nodal supports (posterior probability, PP = 1.0 for both clades).

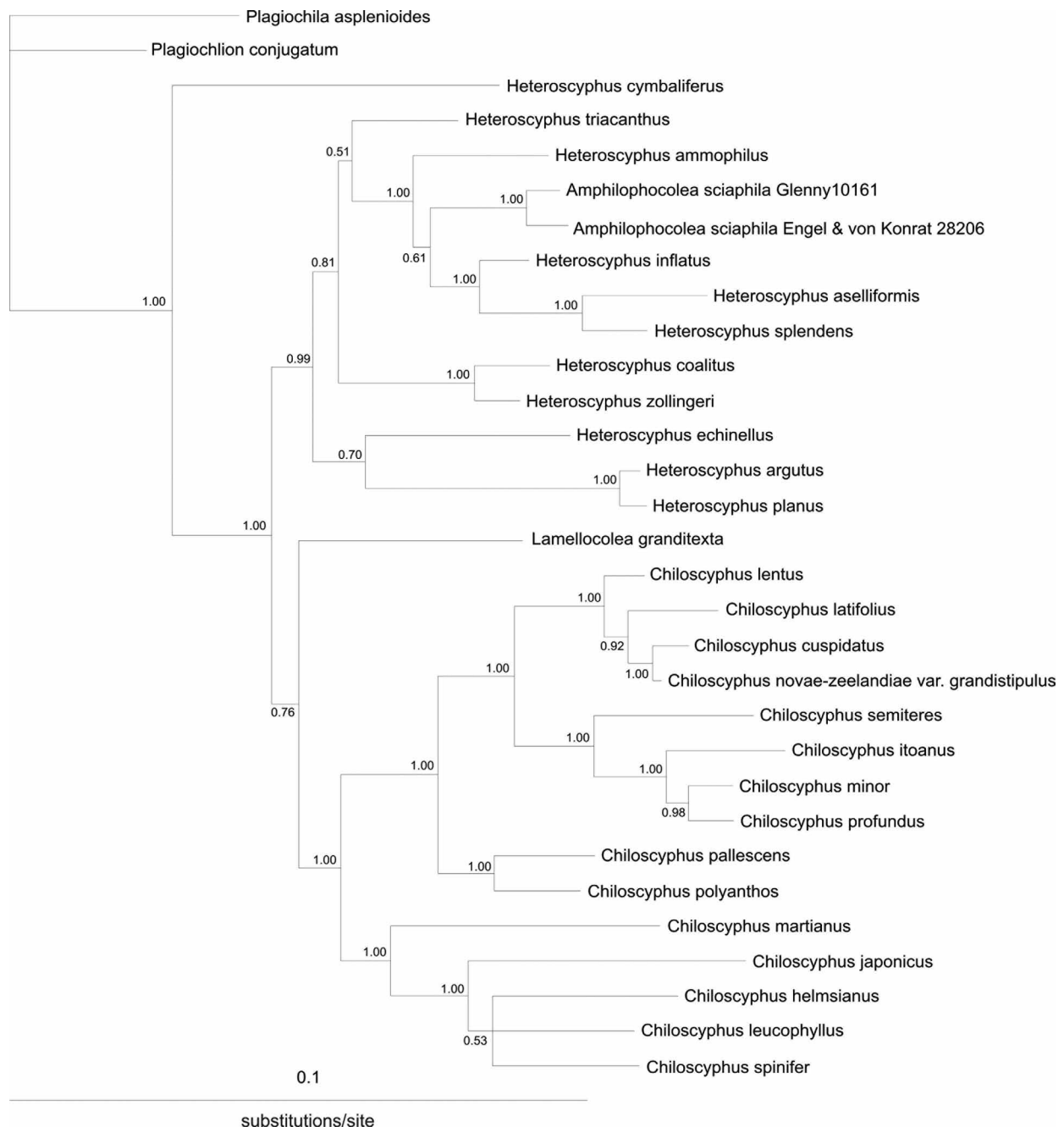
Parsimony analysis identified a single most parsimonious tree, 951 steps long and characterized by a CI and RI of 0.59 and 0.65, respectively. It provided a very similar result as that obtained from the Bayesian analyses (Fig. 2). Both analyses suggested a similar phylogenetic position for *Amphilophocolea sciaphila* and *Lamellocolea granditexta*, and also the same phylogenetic pattern of the subclade grouping of *Heteroscyphus* and *Chiloscyphus*.

## Discussion

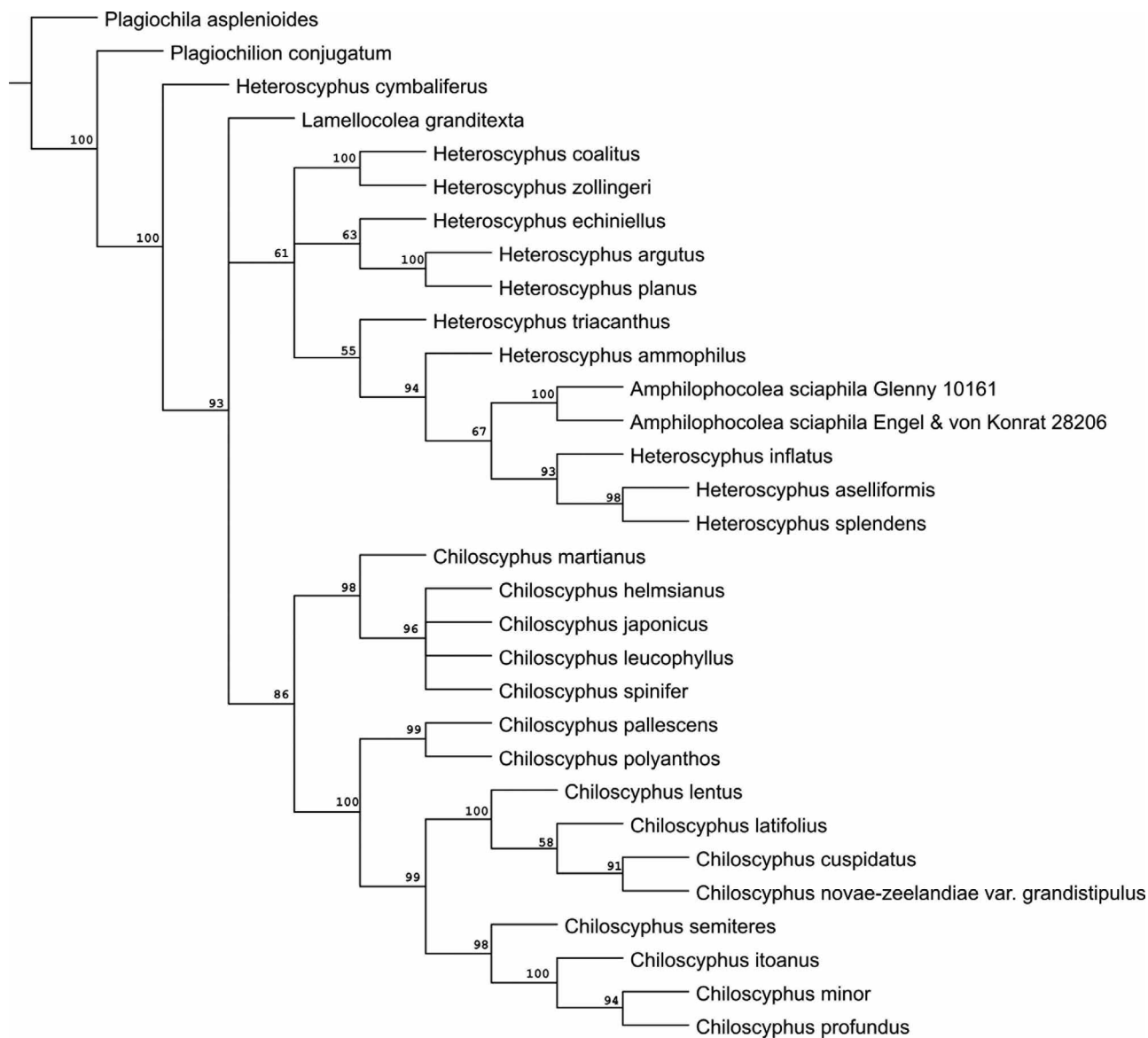
Our present study based on data from the chloroplast *rbcL* and *trnL-trnF* sequences does not recognize the generic status of *Amphilophocolea*. It suggests that *Amphilophocolea* arose from a species of *Heteroscyphus*, and that *Amphilophocolea* belongs to *Heteroscyphus*. Morphological characters that support a common ancestry for *Amphilophocolea* and *Heteroscyphus* are discussed in the section on morphology. The polyphyly of *Heteroscyphus* was accentuated by *H. cymbaliferus* being sister to the remaining ingroup taxa. The phylogenetic position of *Heteroscyphus cymbaliferus* also has been suggested in studies of Glenny et al. (2009) and Engel & He (2010). In the broader-scaled study based on four molecular loci by He-Nygrén et al. (2006), *Heteroscyphus cymbaliferus*, as *Tetracymbaliella cymbalifera*, was resolved as sister to *Brevianthus flavus* of Brevianthaceae and they together formed a sister relationship with Plagiochilaceae.

Our present study and also the studies mentioned above indicate that *Heteroscyphus cymbaliferus* is related rather remotely to species of the other *Heteroscyphus* species sampled and that its assignment to *Tetracymbaliella* indicates more accurately its systematic identity. Morphologically, it has been assumed that ‘the “taxonomic distance” between *Heteroscyphus* and *Tetracymbaliella* is narrow’ (Engel & Schuster 1985,

p. 401). *Tetracymbaliella* differs from *Heteroscyphus* in having leaf and underleaf margins so strongly concave as to form pouches. The genus was described by Grolle (1961b) and three species can be recognized as belonging under Grolle's concept: *Tetracymbaliella cymbalifera* (Hook.f. & Taylor) Grolle and *T. decipens* (Gottsche) Grolle in Australia and New Zealand, and *T. comptonii* (Pearson) Grolle in New Caledonia. Engel and Schuster (1985) treated *Tetracymbaliella* as a subgenus of *Heteroscyphus*, as they were not convinced that the morphological difference of the marginal pouches justified maintaining the genus. The placement of *Tetracymbaliella* as a subgenus within *Heteroscyphus*, however, is not supported by the molecular evidence presented here. In other molecular studies on Lophocoleaceae (e.g., He-Nygrén & Piippo 2003; Hentschel et al. 2006, 2007), *Heteroscyphus* excluding *Tetracymbaliella* has been suggested as a monophyletic group. Confirmation of this result from at least one of the other two species of *Tetracymbaliella* is desirable before formal reinstatement of the genus is made.



**FIGURE 1.** Phylogenetic relationship of *Amphilophocolea sciaphila* based on a combined Bayesian analysis of *rbcL* and *trnL-trnF* sequence datasets from 31 exemplars. A 50% majority-rule consensus tree is presented. Bayesian posterior probabilities  $\geq 0.95$  are indicated. The scale bar represents 0.1 substitutions/site.



**FIGURE 2.** Phylogenetic relationship of *Amphilophocolea sciaphila* based on a combined maximum parsimony analysis of *rbcL* and *trnL-trnF* sequence data sets from 31 exemplars. The tree presented is the most parsimonious tree of 951 steps in length with a CI and RI of 0.59 and 0.65 respectively. Maximum parsimony bootstrap values are given.

Although the present study did not provide support on the systematic relationships of *Lamellocolea*, *Chiloscypus* and *Heteroscyphus*, it shows that *Lamellocolea* is distinct from the other two genera. This result indicates that the morphological characters in leaf and underleaf forms that are shared by *Lamellocolea* and *Heteroscyphus* (see Engel 1991) are likely homoplasious. Other distinct characters, such as the strongly anisophyllous gynoecium and lamellate perianth may truly reflect the systematic identity of *Lamellocolea*.

During the course of study of Australasian Lophocoleaceae by the senior author, it was found that plants with a glaucous surface have both androecia and gynoecia on highly abbreviated ventral-intercalary branches. These plants, on morphological grounds, fit within *Heteroscyphus*, a genus with gametangia usually on short, lateral-intercalary branches, but at times may occur on abbreviated ventral-intercalary branches lacking vegetative leaves, as in, for example, *H. echinellus* (Lindenb. & Gottsche) J.J.Engel & Xiao L.He (Engel and He 2010). The senior author further found that only a single species of *Heteroscyphus* in Australasia has a glaucous surface, and that these plants not only matched those described and illustrated by Schuster (2001) as *Amphilophocolea sciaphila*, but also agreed with *Heteroscyphus knightii* (Steph.) Grolle. That species was

described as *Chiloscyphus knightii* Steph. based on plants from New Zealand, and transferred to *Heteroscyphus* by Grolle (1986). Moreover, *H. knightii* has branching mostly of the ventral-intercalary type, a defining feature of Schuster's genus *Amphilophocolea*.

## Taxonomic treatment

Our study reveals that the affinities of *Amphilophocolea sciaphila* are with *Heteroscyphus*, and that the following new synonymy is required.

### *Heteroscyphus knightii* (Steph.) Grolle

*Chiloscyphus knightii* Steph., Sp. Hepat. 3: 245. 31 Jan. 1908. *Heteroscyphus knightii* (Steph.) Grolle, J. Hattori Bot. Lab. 61: 251. 1986. Type: New Zealand, Knight.

*Chiloscyphus tridentatus* Mitt. in Hooker f., Bot. Antarc. Voy. 3: 228. pl. 179, f. 1. 1859, syn. fide Hodgson (1943). *Heteroscyphus tridentatus* (Mitt.) J.J.Engel & R.M.Schust., Nova Hedwigia 39: 401. 14 Jan. 1985 (1984), non *H. tridentatus* (Sande Lac.) Grolle in Grolle & Piippo, Acta Bot. Fennica 125: 68. 3 April 1984 ≡ *Lophocolea tridentata* Sande Lac., Ann. Mus. Bot. Ludg. Batav. 1: 296. 1864 (Java, New Guinea). Type: Tasmania, Dumont d'Urville, Archer; Grass-tree Hill, Oldfield; St. Patrick's River, Gunn.

*Saccogyna trilobata* Steph., Sp. Hepat. 3: 270. 29 Feb. 1908, syn. fide Hodgson (1958). Type: New Zealand, Colenso.

*Chiloscyphus consistipulus* Steph., Sp. Hepat. 6: 304. 1922, syn. cf. Grolle (1961a). Type: New Zealand, Mickeljohn.

*Amphilophocolea sciaphila* R.M.Schust., Nova Hedwigia 72: 98. f. 1. 2001, **syn. nov.** Holotype: "New Zealand: North Island, E. border of Tongariro Natl. Park: Tree Trunk Gorge; on peaty soil under *Blechnum*, deeply shaded rocks (RMS 95-770)." (herb. R. M.Schuster).

Plants firm, resembling *Bazzania tayloriana* in aspect, loosely creeping to slightly ascending, often forming thin, pure, felt-like, loosely attached, sheet-like mats, occasionally occurring as scattered shoots among other bryophytes, dull and opaque, distinctly glaucous and water repellent, the shoots small to medium, to 2 mm wide, with a distinctive appearance when dry: the dorsal and ventral margins sharply deflexed. Branching infrequent, the branches mostly ventral-intercalary, often in lateral half of underleaf axil; *Frullania*-type branches occasional; stolons lacking. Stems narrow for plant size, the cortex weakly differentiated, in 1 (locally 2) rows of feebly smaller cells, with both cortical and medullary cell walls pale yellow brown and similarly thickened. Rhizoids sparingly developed, hyaline, in tight fascicles from stem at immediate base of underleaves. Leaves subopposite (alternate in suboptimal plants), ± horizontal to sometimes dorsally assurgent, widely spreading, often at 90° to stem, approximate to weakly imbricate, free dorsally, orientation and insertion strongly succubous, the insertion almost longitudinal, forming a ± straight line for its entire length, the lines of insertion rather distant from stem midline dorsally and delimiting a leaf-free strip of 4–6 cells wide; leaves slightly convex, subsymmetrically to at most moderately asymmetrically ovate-subquadrate to oblong ovate; apex subtruncate, variable: (2–)3(–5)-dentate (weaker shoots with bidentate apices moderately common), typically with tooth in ventral half or middle of leaf apex larger, broad-based, and often ± apiculate or at times narrowly rounded at the summit, the apex often with the ventralmost and dorsalmost teeth displaced toward leaf base, the displaced teeth usually broad based, the largest tooth at the leaf apex entire margined, terminating in a single cell (or a pair of laterally juxtaposed cells) or a uniseriate row of 2–3 thick-walled, isodiametric to slightly elongated cells, the terminal cell moderately tapering to a narrowly to broadly rounded summit; apex of suboptimal plants variable: truncate to retuse to 2–3-lobed, with the lobe summit often rounded; dorsal margin broadly arched, typically entire, exceptionally with a small tooth in median portion, short to moderately decurrent; ventral margin ± straight to slightly arched, entire. Cells of median portion of leaf evenly thick walled, the middle lamella distinct, the cells rounded at the angles, trigones absent, median leaf cells 18–25 µm wide, 20–28 µm long; basal cells a little larger but not elongated; surface distinctly glaucous, the surface with a scurfy appearance. Oil-bodies found throughout leaf, large for cell size, hyaline and somewhat glistening or very pale dull gray, 2-4 per median leaf cell, coarsely botryoidal,

the spherules clearly defined and rather uniform in size, the oil-bodies elliptic to (often) irregularly fusiform to subcrescentic, sporadically subtriangular in profile, 6.5–11.1  $\mu\text{m} \times$  3.3–4.6  $\mu\text{m}$ , the globose ones 4.6–5.2  $\mu\text{m}$  in diam. Underleaves 1.4–1.7X the stem width, united to the leaves on both sides, the connate portion 2–5(6) cells wide, the body of the underleaf positioned above the attached pair of leaves, the insertion inverted "U"-shaped, the apex of insertion reaching to about the midpoint of the leaf pair next above; underleaves weakly spreading, distant, with much of the stem exposed below the "U"-shaped insertion at each underleaf base, the underleaves plane to concave (ventral view), oblong-ovate, the lamina  $\pm$  subquadrate to transversely rectangular (e.g., wider than long); apex bifid to 0.5–0.7, the lobes  $\pm$  parallel, narrowly acuminate to subcaudate, entire, ending in a uniseriate row of up to 6 at most moderately elongated, thick-walled cells, the tip cell often capped by a slime papilla; lamina margins on each side with a dentiform to laciniiform process at or a little below the level of the main sinus, the margins otherwise entire or with a small tooth near the base, in suboptimal plants the margins with smaller teeth or completely entire, the sinus at the leaf to underleaf connation broadly angular, at times at 90°, reflexed or not. Asexual reproduction absent.

Plants dioecious. Androecia minute for plant size, short to somewhat long spicate, on reduced, abbreviated, determinate ventral-intercalary branches hidden by lateral leaves above or slightly extending beyond them, the branch origin somewhat variable: often displaced slightly apical of underleaf axil or to ventral-lateral side of stem; bracts cucullate, so much so that a dorsal pocket is not defined, the bracts dorsally assurgent, tightly appressed, decidedly leptodermous, the margins with 2–3 small teeth and copious slime papillae, the bracts monandrous; antheridial stalk biseriate; bracteoles much smaller than underleaves, connate on both sides, without antheridia. Gynoecia on highly abbreviated ventral-intercalary branches; bracts small, the innermost bracts bifid to ca. 0.4, the lobe margins with a few teeth, the lamina margins irregularly denticulate-dentate, the teeth often terminating in a slime papilla. Perianth inflated, somewhat dorsiventrally compressed, the mouth lacinate-lobulate, each narrow lanceolate, tapering to a uniseriate row of 3–6 at most slightly elongated cells, the tip cell tapering to a narrowly rounded summit, the lobes comprised of evenly thick-walled cells.

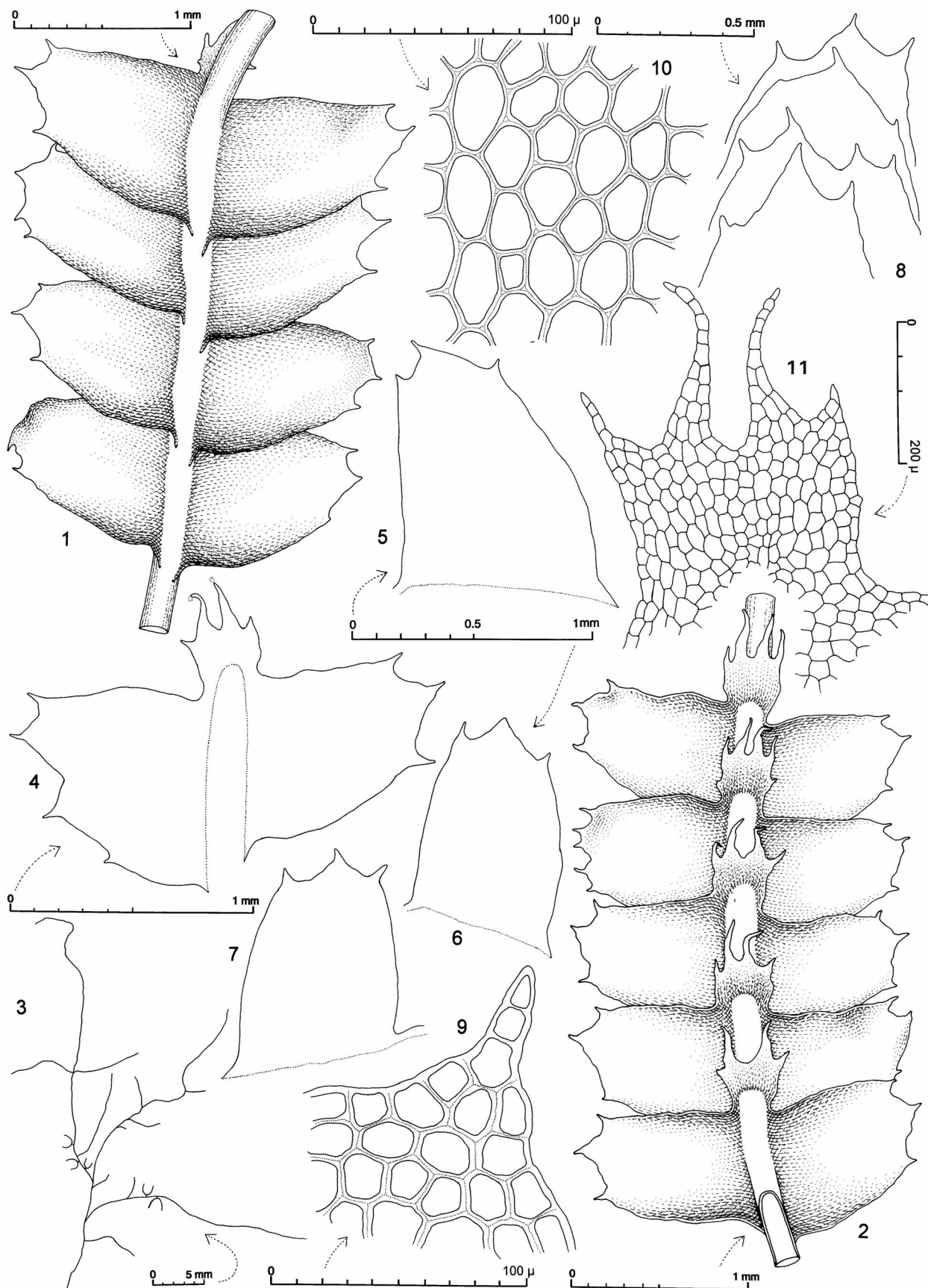
Sporophyte unknown.

**Distribution:**— New Zealand: South Island (220–1500 m), North Island (ca. 700 m [type of *A. sciaphila*]-1240 m); Australia: Tasmania (sea level–1000 m).

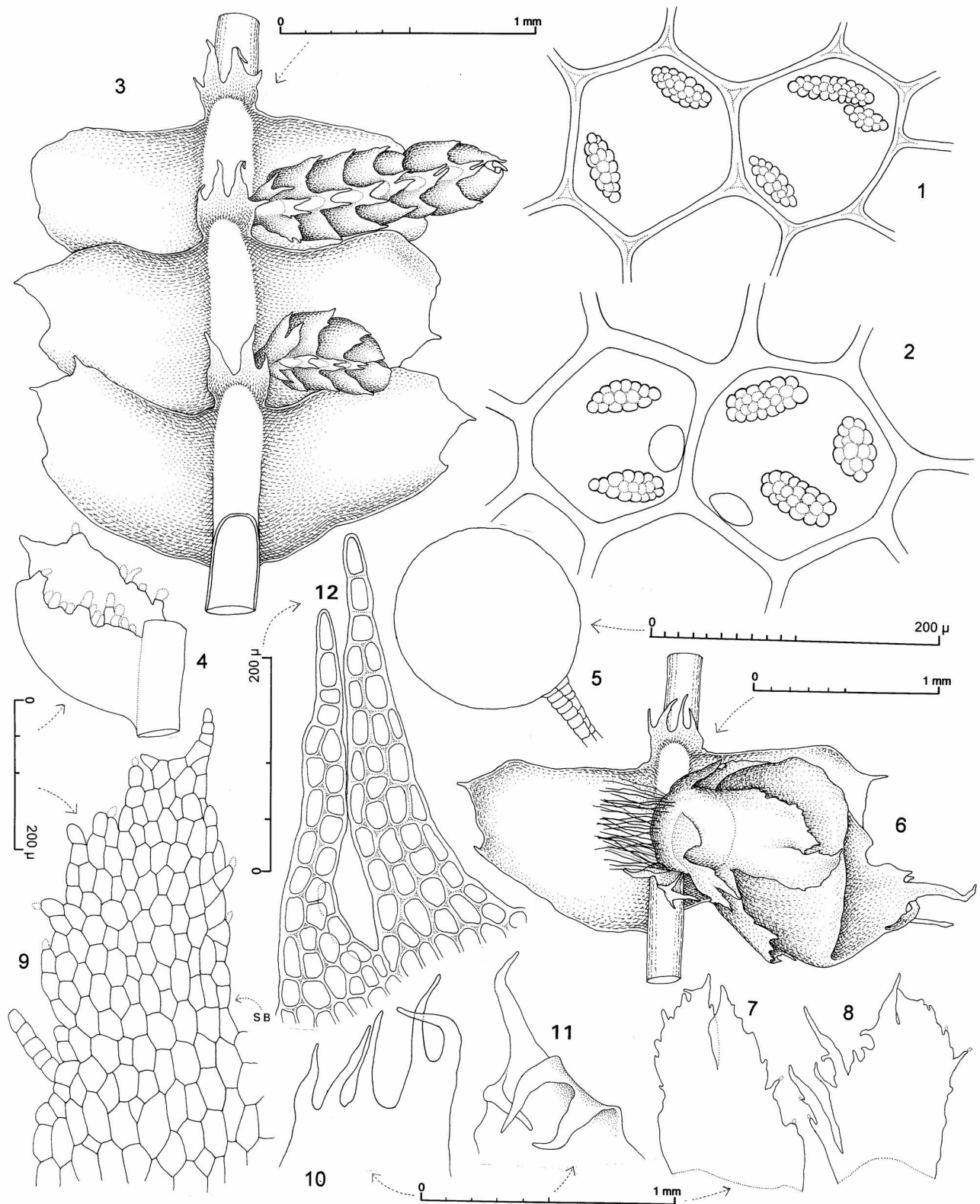
**Comments:**— Suboptimal plants are smaller, alternate-leaved, and have leaf apices that are variable and range from 3-lobed and with lobes that are often rounded at the summit to retuse to bilobed by two rounded lobes (the ventral lobe usually a little larger) to undivided and truncate. Occasional leaves in such populations have sharp lobes, with the middle of the 3 lobes larger as in well-developed phases. Such suboptimal populations have underleaf lamina margins with a small tooth or are altogether entire. The suboptimal phase of the species matches the description and figures of *Amphilophocolea sciaphila* in Schuster (2001, fig. 1). Also, Schuster (2001, p. 98) stated that the underleaves of *A. sciaphila* are conspicuously connate on one side but rarely and feebly so on the other side. The senior author has observed that in species of *Chiloscyphus* and *Heteroscyphus* with leaves connate on both sides, suboptimal plants are typically distinctly connate on the side nearest the adjacent leaf, but on the opposing side are more narrowly and obscurely connate or altogether free. Also, the alternate leaf arrangement and presence of blunt teeth at the leaf apex of *A. sciaphila* coincides with less well-developed plants. The suboptimal phase of the species typically occurs deep in cave-like recesses and plants are notably strongly glaucous. The stronger glaucous condition, along with the morphological features noted for suboptimal plants such as reduced teeth of the leaf apices and underleaf margins, are likely correlated with particularly sheltered, dimly lit niches.

The species may be recognized in the field by the glaucous condition, the small plant stature and the distinctive toothing at the leaf apex. Leaf apices are (2–)3(–5)-dentate with the tooth in the ventral half or the middle of leaf apex typically larger (Fig. 3: 1, 4–6). In three-lobed leaves the teeth on either side of the large, median tooth are each displaced toward the leaf base, but all teeth are typically in the distal 0.2 of the leaf. The degree of development of the glaucous surface seems to be correlated with degrees of exposure to light; particularly strongly glaucous plants typically grow in low-lit niches.





**FIGURE 3.** *Heteroscyphus knightii* (Steph.) Grolle. 1. Sector of leading shoot, dorsal view. 2. Sector of leading shoot, ventral view. 3. Cladograph (U-shaped symbols = gynoecia). 4. Leaf pair and attached underleaf. 5-7. Leaves (all at same scale). 8. Leaf apices. 9. Portion of median lobe of 3-lobed leaf. 10. Median leaf cells. 11. Underleaf. (All from *Engel 13121*, Tasmania, Tasman Peninsula, near Taranna, near junction of Camp Road and Balt Spur Road.)



**FIGURE 4.** *Heteroscyphus knightii* (Steph.) Grolle. 1, 2. Median leaf cells with oil-bodies, above X 1165, below X 1515. 3. Leading shoot with 2 androecia. 4. ♂ Bract. 5. Antheridial stalk. 6. Leading shoot with gynoecium. 7, 8. Innermost ♀ bracts. 9. Portion of lateral half of innermost ♀ bract (the basal sector not shown). 10, 11. Portions of perianth mouth. 12. Lobes of perianth mouth. (Fig. 1, from *Engel 18229*, New Zealand, South Is., Canterbury Prov., Mt. Cook Natl. Park, Glencoe Stream Valley, W of town of Mt. Cook; 2, from *Engel 19484*, Tasmania, Griffiths Creek (tributary of Surprise River), ESE of Mt. Arrowsmith; 3-5, from *Norris 28035*, Tasmania, Cradle Mt. Natl. Park, W shore of Lake St. Clair; 6-12, from *Engel 13121*, Tasmania, Tasman Peninsula, near Taranna, near junction of Camp Road and Balt Spur Road.)

Branches in *H. knightii* are mostly ventral intercalary. Branches are variable in position within the underleaf axil and occur in the middle of the underleaf axil but often are present in various loci in the lateral half of the underleaf axil as well. Terminal, *Frullania*-type branches are occasionally present. Schuster (2001) recorded only ventral-intercalary branches for *Amphilophocolea sciaphila*.

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