

# Striking autapomorphic evolution in *Physotheca* J.J.Engel & Gradst. (Marchantiophyta: Lophocoleaceae) blurred its actual relationships with *Leptoscyphus* Mitt.

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The taxonomic status and phylogenetic position of the monotypic liverwort genus *Physotheca* J.J.Engel & Gradst., including *P. autoica* J.J.Engel & Gradst. endemic to Ecuador, are inferred from phylogenetic analyses of two cpDNA loci. The results indicate that the genus is nested within *Leptoscyphus* and the new combination, *L. autoicus* (J.J.Engel & Gradst.) Vanderp. & Gradst., is made. This indicates, along with an increasing body of evidence, that extreme morphological transformations can obscure the phylogenetic signal present in morphological data.

**Keywords:** *atpB-rbcL*, Ecuador, Liverworts, Molecular phylogeny, Morphology, Páramo, Sexual systems, *trnL*

## Introduction

*Physotheca autoica* J.J.Engel & Gradst. was described as a new liverwort genus and species endemic to Ecuador (Engel & Gradstein, 2003). Its suite of unique character states was thought to warrant inclusion within the new subfamily Physothecoideae (Geocalycaeeae) (Engel & Gradstein, 2003). Geocalycaeeae were subsequently shown to be polyphyletic, and *Physotheca* was resolved as a member of the Lophocoleaceae, within an unsupported clade including two *Leptoscyphus* Mitt. species (Hentschel *et al.*, 2006a). While the combination of undivided, strongly succubously oriented leaves, bifid underleaves, rhizoids restricted to underleaf base, and red-brown coloration of *Physotheca* are indeed reminiscent of *Leptoscyphus*, *Physotheca* differs from any other Lophocoleaceae by several unique features. Unlike the other members of the family, the mode of growth of *Physotheca* is by repeated innovations originating from below the bracts or bracteoles of the gynoecium. As opposed to *Leptoscyphus* species, *Physotheca* is autoecious and abundantly produces inflated, bladder-like perianths that remarkably resemble the

sterile perianths of the completely unrelated genus *Pleurozia* Dumort. *Physotheca*'s 7–8 stratose capsule wall is thicker than in most Lophocoleaceae. Furthermore, the spores of *Physotheca* are larger (31–39 µm in diameter) than those of other Lophocoleaceae, wherein they reach 30 µm at the very most. Finally, while the elaters of other Lophocoleaceae are completely free from the capsule wall, have a persistent wall, and spirals that do not uncoil, the elaters of *Physotheca* are frequently variously uncoiled and their walls are frequently in various stages of collapse, ultimately resulting in the uncoiling of spirals (Engel & Gradstein, 2003).

Several attempts at sequencing *Physotheca* from type material as well as additional collections and DNA extracts used in the Hentschel *et al.* (2006a) study previously failed (Vanderpoorten *et al.*, 2010a). Most recently, two collections made near the type locality could be successfully amplified at two chloroplast loci displaying the appropriate level of variation to discriminate species within *Leptoscyphus* and closely related genera (Devos & Vanderpoorten, 2009). This allows us here to re-assess the taxonomic position and status of *Physotheca* and the taxonomic significance of its unique morphological features.

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## Material and Methods

The two collections of *P. autoica* originated from the same locality in southern Ecuador (Table 1). Two molecular and taxonomic sampling strategies were employed to determine its phylogenetic position and test the status of the genus. First, *trnL* sequences were produced for the two accessions of *P. autoica* using the protocols of DNA extraction, amplification, and sequencing described in Vanderpoorten & Long (2006). These sequences were subsequently compared to those available from GenBank for other genera of Lophocoleaceae, including *Chiloscyphus* Corda as circumscribed by Hentschel *et al.* (2006b, 2007), thus including *Lophocolea* (Dumort.) Dumort. and *Pachyglossa* Herzog & Grolle), *Heteroscyphus* Schiffner, and *Leptoscyphus*, using one accession per species in the latter. Outgroup taxa included members of the Plagiochilaceae (*Plagiochila* (Dumort.) Dumort. and *Pedinophyllopsis* R.M.Schust. & Inoue), Herbertaceae (*Herbertus* Gray), Lepicoleaceae (*Lepicolea* Dumort.), Lepidoziaceae (*Bazzania* Gray and *Lepidozia* (Dumort.) Dumort.) and Trichocoleaceae (*Trichocolea* Dumort.) (see Vanderpoorten & Long, 2006, for further details and GenBank accession numbers), which are closely related to the Lophocoleaceae (Heinrichs *et al.*, 2005), in order to determine the generic position of *Physotheca*. Second, the *atpB-rbcL* intergenic spacer was sequenced for the two accessions of *P. autoica* using the protocols described in Vanderpoorten & Long (2006). The *trnL* and *atpB-rbcL* sequences were then compared to those of the *Leptoscyphus* species included by Devos & Vanderpoorten (2009), each species being represented by 1–5 specimens, to investigate potential closer affinities with the species of that genus. *Leptoscyphus antarcticus* (C.Massal.) Solari and *L. australis* (Gottsche, Lindenb. & Nees) R.M.Schust. were used as outgroups based upon the analyses of Vanderpoorten & Long (2006).

Gaps were inserted where necessary in the alignment to preserve positional homology, and indels were rescored into a separate matrix. Indels were scored for presence–absence, and homologous indels

of different lengths were scored as multistate characters. Gaps were excluded from the analyses.

The two data matrices were submitted to a Maximum Likelihood (ML) analysis after selection of the best-fit model of DNA substitution using the Akaike Information Criterion as implemented by jModeltest (Posada, 2008). Indel evolution was described using a model with a single transition rate among states. The two models describing DNA substitutions and indel variation were simultaneously employed in a partitioned analysis, as implemented by Garli 2.0 (<http://code.google.com/garli/>). Support for branches was assessed after a non-parametric bootstrap analysis with 100 replicates.

## Results

The *trnL* analyses converged towards a single ML tree ( $-\ln L=3422.15$ ). *Leptoscyphus* was resolved as monophyletic with a fairly low 69% bootstrap support and included the two accessions of *Physotheca autoica* (Figure 1). In the combined *trnL+atpB-rbcL* analyses, whose ML tree ( $-\ln L=3773.35$ ) is presented in Figure 2, the two accessions of *P. autoica* were resolved between the clade made up of *Leptoscyphus amphibolius* (Nees) Grolle and *L. infuscatus* (Mitt.) E.W.Jones (= *Leptoscyphus* subg. *Physoscyphus* Grolle), and the large *L. gradsteinii* Vanderp. *et al.* to *L. porphyrius* (Nees) Grolle clade (= *L.* subg. *Leptoscyphus*).

## Discussion

The results confirm previous phylogenetic evidence (Hentschel *et al.*, 2006a) concerning the phylogenetic position of *Physotheca* and indicate that the genus is nested within *Leptoscyphus*. As a consequence, the Physothecoideae must be abandoned as a synonym of Lophocoleaceae (subfam. Leptoscyphoideae), and the new combination, *Leptoscyphus autoicus* (J.J. Engel & Gradst.) Vanderp. & Gradst. *comb. nov.* (*Physotheca autoica* J.J.Engel & Gradst., Taxon 52: 764. 2003), is necessary to accommodate *P. autoica* within *Leptoscyphus*.

The inclusion of *P. autoica* within *Leptoscyphus* indicates that the suite of unique features displayed

**Table 1** Voucher information and GenBank accession numbers for the two accessions of *Physotheca autoica*

	Voucher	Locality	GenBank accession number	
			<i>trnL</i>	<i>atpB-rbcL</i>
<i>P. autoica</i> 1	<i>M. Burghardt &amp; X. Haro-Carrion</i> MB6984 (LG, PC)	Ecuador, Zamora-Chinchipe, Parque Nacional Podocarpus, Estación Científica Fransisco, Camino Antenas, ca 2650 m, shrubby subpáramo, epiphyte on twigs and branches, 26/04/2008	JQ446576	JQ446574
<i>P. autoica</i> 2	<i>M. Burghardt &amp; X. Haro-Carrion</i> MB6973 (LG, PC)	Ecuador, Zamora-Chinchipe, Parque Nacional Podocarpus, Estación Científica Fransisco, Camino Antenas, ca 2650 m, shrubby subpáramo, epiphyte on twigs and branches, 26/04/2008	JQ446577	JQ446575



Figure 1 Most likely tree of the analysis of *trnL* sequences in *Physotheca autoica* and other members of the Lophocoleaceae. Numbers below the branches are non-parametric bootstrap values.

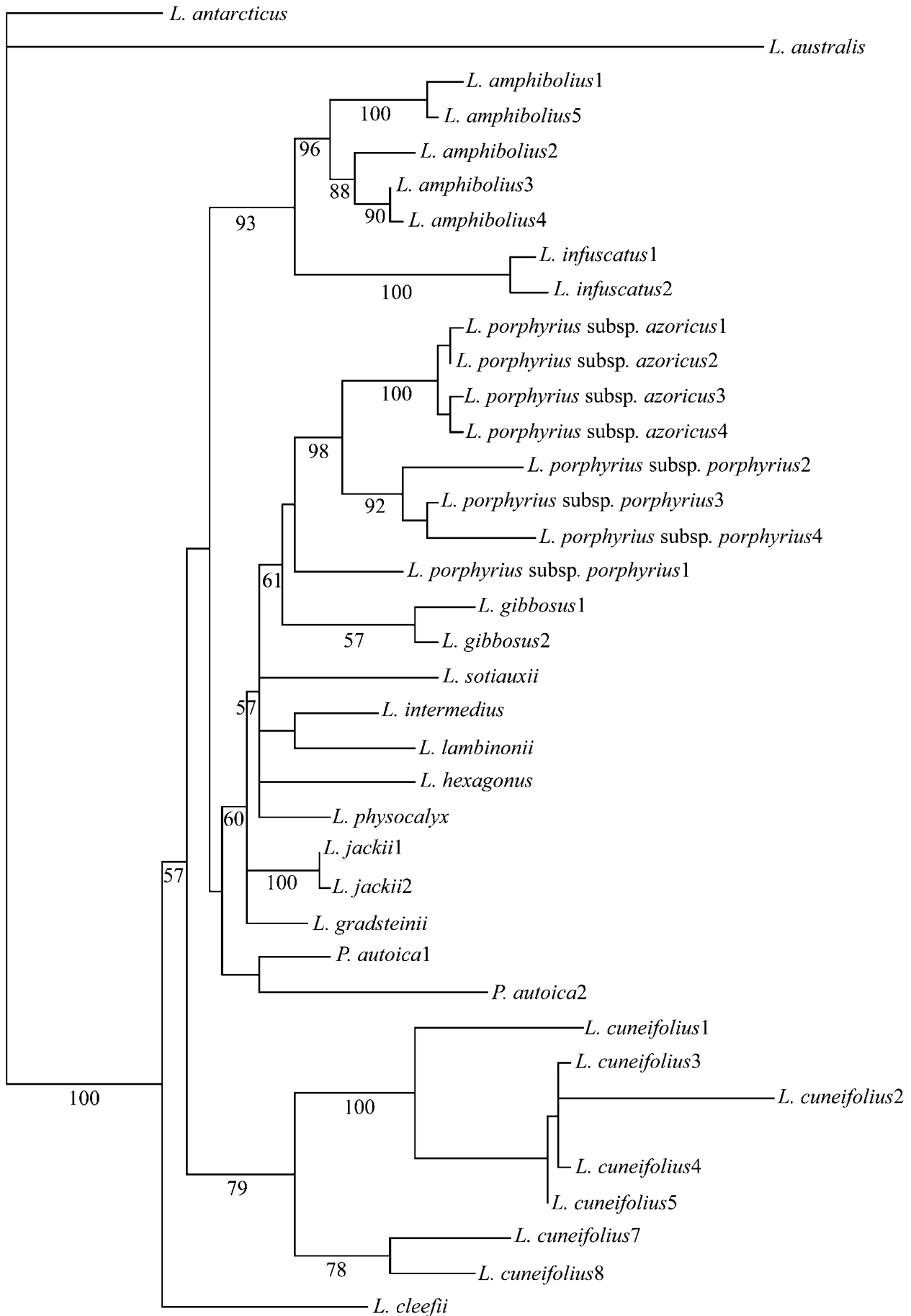


Figure 2 Most likely tree of the analysis of *trnL* and *atpB-rbcL* sequences in *Physotheca autoica* and *Leptoscyphus*. Numbers below the branches are non-parametric bootstrap values.

by the taxon evolved comparatively rapidly from an ancestor displaying the typical *Leptoscyphus* morphology (see the fairly short branch length leading to *P. autoica* in Figure 2 as compared to the ones leading to the different accessions of *L. cuneifolius* (Hook.) Mitt.). In particular, the autoecious condition of *P. autoica* is unique within *Leptoscyphus*. Phylogenetic evidence, however, indicates that shifts in sexual systems can be fairly rapid in bryophytes. Within *Radula* Dumort. for instance, the transition from dioecy to monoecy occurred multiple times independently along the most recent branches of the phylogeny (Devos *et al.*, 2011). These shifts in sexual systems are typically correlated to a suite of morphological transformations (Crawford *et al.*, 2009), which may explain the sudden and substantial morphological transformations observed.

Altogether, these data indicate, along with an increasing body of evidence (see Gradstein *et al.*, 2011, for review), that extreme morphological transformations can obscure the phylogenetic signal present in morphological data. For example, the monospecific liverwort genus *Perssoniella* Herzog, with *P. vitreocincta* Herzog endemic to New Caledonia, was long assigned to its own family Perssoniellaceae because of a series of unique morphological features including its complicate-bilobed leaves with a broad, hyaline border and a very narrow insertion, and its unusual stem anatomy. DNA sequences, however, showed that *Perssoniella* was nested within the large genus *Schistochila* Dumort., family Schistochilaceae (He & Glenny, 2010). Other remarkable cases are the thalloid genera *Metzgeriopsis* K.I. Goebel (Metzgeriopsidaceae) and *Mizutania* Furuki & Iwatsuki (Mizutaniaceae), both from Southeast Asia, which proved to be members of leafy liverwort families, respectively Lejeuneaceae [genus *Cololejeunea* (Spruce) Schiffn.] and Calypogeiaceae (Gradstein *et al.*, 2006; Masuzaki *et al.*, 2010). Examples from Lophocoleaceae include the temperate southern-hemispheric genera *Pachyglossa* Herzog & Grolle, *Amphilophocolea* R.M. Schust., and *Cyanolophocolea* (R.M.Schust.) R.M. Schust., which were shown to belong to *Chiloscyphus* Corda (*Pachyglossa*) and *Heteroscyphus* Schiffn., (*Amphilophocolea*, *Cyanolophocolea*) respectively (Hentschel *et al.*, 2007; Engel *et al.*, 2010; Engel & He, 2010). In Plagiochilaceae, the monospecific neotropical genera *Steeerochila* Inoue, characterized by unusual asexual reproductive devices, and *Szweykowskia* Gradst. & E.Reiner, with bizarre saccate leaves, proved to be members of *Plagiochila* (Dumort.) Dumort. based on molecular data (Heinrichs, 2002). A similar case was recently documented in hornworts, where the monospecific genus *Hattorioceros* (J.Haseg.) J.Haseg. was shown to be nested within *Phaeoceros* Prosk., whereas unique

features among hornworts, including small spores with a canaliculated-striate surface and absence of a triradiate mark, would suggest otherwise (Li *et al.*, 2011).

Substantial taxonomic changes must hence be expected in liverwort taxonomy as the phylogenetic effort increases. In the Neotropics for example, where the highest endemism rate in liverwort genera is observed, most of the endemic genera are stenotypic (1–3 species each) and highly apomorphic (e.g. *Zoopsidella* R.M.Schust., *Pteropsiella* Spruce, *Haesselia* Grolle & Gradst., *Schusterolejeunea* Grolle, *Cephalantholejeunea* R.M.Schust., etc.). These genera, which, for the most part, are assumed to have diverged during the Tertiary owing to the long period (60 million years) of isolation since the opening of the South Atlantic and continuous tectonic activity, are characterized by extreme morphological features (see Vanderpoorten *et al.*, 2010b). It is precisely those stenotypic genera that have recently been shown to be vulnerable to phylogenetic testing since their suites of unique morphologies do not appear to be ancestral but, in contrast, to be recently derived (e.g. Engel *et al.*, 2010; He & Glenny, 2010; Masuzaki *et al.*, 2010; Fuselier *et al.*, 2011). A more complete taxonomic re-assessment of those genera in the context of the ‘Liverwort Tree of Life’ project (<http://www.biology.duke.edu/bryology.LiTol/>) will certainly offer the appropriate framework to propose a revised classification of liverworts and allow a more accurate assessment of character evolution and biogeographic patterns in the group.

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Taxonomic Additions and Changes: *Leptoscyphus autoicus* (J.J.Engel & Gradst.) Vanderp. & Gradst. *comb. nov.* (*Physotheca autoica* J.J.Engel & Gradst.)

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