Systematics and biogeography of selected genera in Lejeuneaceae subfamily Lejeuneoideae

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Abstract

Lejeuneaceae represent the most species-rich epiphytic lineage of leafy liverworts with approximately 1000 species in 68 currently accepted genera. This subcosmopolitan family has its centre of diversity in the humid tropics and constitutes an important component of cryptogamic, especially epiphytic diversity. Lejeuneaceae are characterized by incubous leaves divided into a large dorsal lobe and a small ventral, usually 1-3 toothed lobule which is broadly attached to the lobe along a keel, a hyaline papilla situated near the lobule apex, beaked perianths within each one archegonium, and the occasional occurrence of ocelli. Morphological and molecular studies resolved the family in two subfamilies and four main lineages. However, the systematic position of many taxa and their delimitations are still subject to controversy. To clarify these issues, my study focused on several critical genera using three molecular markers (cp *rbc*L and *trn*L-F, nrITS) and a dense taxonomic sampling. Phylogenetic analyses resolved the relationships within *Diplasiolejeunea* and *Lejeunea*, and clarified the systematic positions of *Microlejeunea, Harpalejeunea, Myriocolea, Sphaerolejeunea,* and *Bromeliophila*.

A molecular phylogenetic study of *Diplasiolejeunea* points to extensive morphological homoplasy but allows to assign three main clades to subgenera. The phylogeny supports a deep split into a Neotropical and a Paleotropical clade, the latter structured into Australasian, Asian and Afromadagascan lineages. The Neotropical clade is further subdivided into two geographically and ecologically separated lineages, the epiphytic montane subgenus *Physolejeunea* and the epiphyllous lowland subgenus *Diplasiolejeunea*. This strong correlation between ecological traits and geographical distribution range could indicate the rise of the Andes' influence in stimulating the diversification of *Physolejeunea*, perhaps by providing new habitats. A few *Diplasiolejeunea* species have intercontinental ranges. Range formation of Cenozoic *Diplasiolejeunea* is obviously a result of rare long distance dispersal, frequent short distance dispersal, extinction, recolonization, and niche conservatism.

Lejeunea is one of the largest genera of Lejeuneaceae. My molecular studies resolved *Lejeunea* in two robust lineages, corresponding to *L*. subg. *Lejeunea* and *L*. subg. *Crossotolejeunea*. Neotropical accessions dominate early diverging lineages of both main clades of *Lejeunea.* This pattern suggests an origin in the Neotropics followed by several colonizations from the Neotropics into the Paleotropics and vice versa. Most Afromadagascan clades are related to Asian clades. Several temperate *Lejeunea* radiations were detected. 82 of the 91 investigated *Lejeunea* species could be identified to species level. 54 species were represented by multiple accessions, of these, 25 para- or polyphyletic, 29 monophyletic. *L.* subg. *Lejeunea* shows a tendency of monoecy whereas *L.* subg. *Crossotolejeunea* shows a tendency of dioecy. Some dioecious as well as some monoecious species have disjunct ranges. Using molecular and morphological evidence, *Harpalejeunea fischeri*, *H. filicuspis*, *H. latitans* and *Pluvianthus squarrosus were transferred* to *Microlejeunea*. Two monotypic genera, rheophytic *Myriocolea* and epiphyllous *Sphaerolejeunea* were transferred to *Colura* and *Lejeunea* respectively. Our studies also confirmed the monophyly of *Bromeliophila* and provided evidence for a sister relationship of *Bromeliophila* and *Prionolejeunea*; this clade is in turn sister to *Cyclolejeunea*. Despite an extensive morphological overlap, *Bromeliophila* is not closely related to *Lejeunea*.

Extended Summary

This dissertation is about the molecular phylogenetic studies of several critical genera within family Lejeuneaceae. In **Extended Summary** chapter, section 1 to section 3 introduces Lejeuneaceae and reviews the state of research that has been done on this family. Section 4 reviews the molecular studies that I have done on this family. Section 5 summarizes results from all my studies and discusses possibilities for future work. The **Appendix** chapter includes all the publications during my studies on Lejeuneaceae.

1. General Introduction

Extant Viridiplantae includes two divisions, Chlorophyta and Streptophyta, the latter encompasses all embryophytes and a paraphyletic assemblage of Charophycean algae. The evolution and diversification of land plants from a group of Charophycean algae about 470-500 million years ago (Wodniok *et al.*, 2011) was a major event in the earth's history, which lead to the terrestrial ecosystem today. Instead of morphologically most complex *Charales* (Karol *et al.*, 2001; Qiu, 2008; Qiu *et al.*, 2006), recent studies resolved the unicellular and filamentous conjugating green algae *Zygnematales* (Timme *et al.*, 2012; Turmel *et al.*, 2007; Wodniok *et al.*, 2011) or a clade containing *Coleochaetales* and *Zygnematales* (Laurin-Lemay *et al.*, 2012; Turmel *et al.*, 2002; Turmel *et al.*, 2006; Wodniok *et al.*, 2011) as the sister group of land plants.

Bryophytes are a collective term for liverworts, mosses, and hornworts and represent the earliest divergences of land plants. Although the precise divergent order of these early lineages has been the subject of debate (Garbary *et al.*, 1993; Goremykin and Hellwig, 2005; Groth-Malonek and Knoop, 2005; Kelch *et al.*, 2004; Nishiyama *et al.*, 2004; Renzaglia *et al.*, 2000), multiple lines of evidence suggest that three bryophyte clades form a paraphyletic grade at the base of embryophytes with liverworts resolved as the earliest divergence and mosses as sister to horworts and polysporangiophytes. Alternative topologies resolving the hornworts as sister to mosses plus polysporangiophytes are less well supported (Chang and Graham, 2011; Qiu, 2008; Qiu *et al.*, 2006). The hypothesis behind this scenario is the pressure towards sporophytes' growing increasing autonomy as the major evolutionary drive, i.e., development of shoot apical meristem, photosynthetic activity, water conducting tissue, and longevity (Ligrone *et al.*, 2012). The three bryophyte clades share a haploid dominant life cycle with unbranched sporophytes growing attached to the gametophytes. Sporophyte development in liverworts depends only on embryonic formative cell division and expansion. Moss sporophytes are mainly developed by a transient basal meristem which, however, acquires indeterminate activity in hornworts and polysporangiophytes (Kato and Akiyama, 2005; Ligrone *et al.*, 2012). Recent functional analysis reveals that KNOX1 genes are regulators of sporophytic (diploid) meristematic genes (Sakakibara *et al.*, 2008; Sano *et al.*, 2005; Singer and Ashton, 2007). KNOX2 genes are regulators maintaining diploid differentiation by suppression of the gametophytic development program (Sakakibara *et al.*, 2013). Reorganization and duplication of KNOX1/KNOX2 could help to establish the multi-cellule diploid generation.

2. Liverworts

Spore and tubular remains with apparent liverwort affinities date back to the Ordovician (ca. 470 Ma) (Wellman *et al.*, 2003). The earliest macrofossil assigned to liverworts is the Lower Devonian (407–411 Ma) *Riccardiothallus devonicus* (Guo *et al.*, 2012), which was recently added to the two other Devonian liverworts, *Metzgeriothallus sharonae* (385-392Ma) (Hernick *et al.*, 2008) and *Pallaviciniites devonicus* (Hueber) R.M.Schust. (Oostendorp, 1987). Different from other bryophytes, liverwort sporophytes mature within its gametophyte confines and lack differentiations of stomata, columella or meristematic tissue. Based on gametophyte growth forms, liverworts are traditionally subdivided into two main groups, the Marchantioids (complex thalloid liverworts) and the Jungermannioids (simple thalloid and leafy liverworts). Current estimates are close to 7500 liverwort species (Von Konrat *et al.*, 2010) in 376 genera and 74 families (Crandall-Stotler and Stotler, 2000), of which at least 85% are leafy Jungermannioids (Schuster, 1984).

During the past decade, molecular studies have greatly improved our understanding of liverwort phylogeny (Crandall-Stotler *et al.*, 2009; Crandall-Stotler *et al.*, 2005; Davis, 2004; Forrest and Crandall-Stotler, 2005; He-Nygren *et al.*, 2006; Heinrichs *et al.*, 2005a; Knoop, 2010; Lewis *et al.*, 1997; Vilnet *et al.*, 2009; Wahrmund *et al.*, 2008). Based on the 18S rRNA gene, Capesius (1995) and Capesius and Bopp (1997) proposed that liverworts are polyphyletic. A 24 cp-rbcL dataset (Lewis *et al.*, 1997) also tended to support this hypothesis albeit with weak support. However, subsequent comprehensive studies (Beckert *et al.*, 1999; Duff and Nickrent, 1999; Nishiyama and Kato, 1999; Samigullin *et al.*, 1998) with dense sampling and multiple genes unanimously supported the monophyly of liverworts. Stech and Frey (2001) divided liverworts into four classes: Jungermanniopsida, Marchantiopsida, Treubiopsida and Blasiopsida, based on *trn*L-F sequences. Heinrichs *et al.* (2005a), He-Nygren *et al.* (2006), and Forrest *et al.* (2006) revealed similar patterns but adopted three classes, Haplomitriopsida, Marchantiopsida and Jungermanniopsida. Meanwhile, the systematic positions of many liverwort groups have been clarified by molecular studies. Forrest and Crandall-Stotler (2004) and Crandall-Stotler *et al.* (2005) resolved a paraphyletic Metzgeriidae and pointed to extensive morphological homoplasy within this subclass. Wheeler (2000) supported the monophyly of subclass Marchantiidae encompassing three orders: *Marchantiales*, *Sphaerocarpales*, *Monocleales*, Boisselier-Dubayle *et al.* (2002) further pointed out that *Sphaerocarpales* and *Monocleales* are nested within *Marchantiales*. Their study on Marchantiidae indicated high degree of incongruence between molecular and morphological evidence, supporting a trend of morphological reduction in Marchantiidae in molecular and combined trees. Long (2006) proposed four new higher taxa for complex thalloid liverworts: families Dumortieraceae and Neohodgsoniaceae, orders *Neohodgsoniales* and *Lunulariales.* Schill *et al.* (2010) divided *Mannia* into the subgenera *Mannia* and *Neesiella*, and synonymized *Asterella* subg. *Graciles* with *Mannia* subg. *Neesiella*. Rubasinghe *et al.* (2011) resolved Cleveaceae in four lineages, corresponding to *Sauteria*, *Peltolepis*, *Clevea* and a narrowly defined *Athalamia*. So far, leafy liverworts were most intensively studied. Many familial and genus level molecular phylogenies have been produced, e.g., Adelanthaceae (Feldberg *et al.*, 2010), *Bryopteris* (Hartmann *et al.*, 2006), *Frullania* (Heinrichs *et al.*, 2012a; Hentschel *et al.*, 2009), *Herbertus* (Feldberg and Heinrichs, 2006), Lejeuneaceae (Sukkharak *et al.*, 2011; Wilson *et al.*, 2004; Wilson *et al.*, 2007a), Lepidoziaceae (Cooper *et al.*, 2012a; Cooper *et al.*, 2011), *Jubula* (Pätsch *et al.*, 2010), *Leptoscyphus* (Devos and Vanderpoorten, 2009), *Lophozia* (Vilnet *et al.*, 2008), *Plagiochila* (Heinrichs *et al.*, 2006), *Porella* (Hentschel *et al.*, 2007b), *Ptilidium* (Kreier *et al.*, 2010), and *Scapania* (Heinrichs *et al.*, 2012c).

Divergence time estimates suggest a Late Ordovician origin of Marchantiophyta, a separation of Haplomitriopsida from the remainder of liverworts in the Early Devonian, a split of Jungermanniopsida and Marchantiopsida in the Late Devonian, and a separation of Metzgeriidae and Jungermanniidae in the Late Carboniferous (Heinrichs *et al.*, 2007). Leafy Jungermanniidae split into Porellales and Jungermanniales in the Early-Middle Permian but many extant genera and families date back only to the Cretaceous and Early Cenozoic. Other dating studies (Cooper *et al.*, 2012b; Hartmann *et al.*, 2006; Wilson *et al.*, 2007b) also support that much of the family level diversity of liverworts was established in the Cretaceous, and much of the genus level diversity in the Cenozoic. Extensive diversification of leafy lineages within the Cenozoic might relate to changes in forest composition. The prosperity of angiosperm-dominated forests and increased forest complexity might provide various new niches for liverwort lineages to diversify (Cooper *et al.*, 2012b; Heinrichs *et al.*, 2007). Based on family level analysis of embryophyte divergence times, Fiz-Palacios *et al.* (2011) proposed that ferns and mosses radiated in the shadow of angiosperms while for liverworts and gymnosperms such patterns were not detected. However, Cooper *et al.* (2012b) doubted this “slowdown” in liverwort diversification patterns because a family level sampling often results in oversampling of deep nodes and strongly biased inferences towards downturns (Cusimano and Renner, 2010).

Liverworts are a group of simple plants with conserved morphological characters and long evolutionary history (Heinrichs *et al.*, 2007; Magill, 2010; Shaw *et al.*, 2011), which is often related to evolutionary stasis and lack of genetic potential (Frahm, 2000 & 2004; Pfeiffer, 2000). Geographic patterns within this lineage are thus sometimes attributed to ancient geological events (Devos and Vanderpoorten, 2009; Schaumann *et al.*, 2003; Stech and Frey, 2004). However, patterns on the first sight congruent with ancient vicariance scenarios may in fact conceal a complex mixture of relictual distributions and more recent speciation and dispersal events (Devos and Vanderpoorten, 2009). Recent molecular dating brings back dispersal hypotheses (Hartmann *et al.*, 2006; Heinrichs *et al.*, 2009) or invokes more complex scenarios for putative Gondwanan disjunctions including Laurasian migration (Davis *et al.*, 2002; Weeks *et al.*, 2005). The spores of many widespread bryophyte species can withstand long periods of desiccation, high levels of UV radiation, and temperature extremes (van Zanten, 1978; Van Zanten and Pócs, 1981). Local and regional dispersal appears to be quite effective in many species, at least in those that regularly produce spores or propagules (Snall *et al.*, 2004; Zartman *et al.*, 2006; Zartman and Nascimento, 2006). Many studies also indicate bryophyte dispersal is sufficient even at continental scales to genetically homogenize plants (Cronberg, 2002; Grundmann *et al.*, 2007; Van der Velde and Bijlsma, 2003; Vanderpoorten *et al.*, 2008). In general, morphologically circumscribed bryophyte species usually have wider distribution ranges than vascular plants (Shaw, 2001). Intercontinental distribution ranges have been proposed for many liverwort species. These hypotheses have partly been confirmed by molecular data, e.g., for *Frullania arecae* and *F. ericoides* (Hentschel *et al.*, 2009), *F. tamarisci* (Heinrichs *et al.*, 2010), *Herbertus juniperoideus* (Feldberg *et al.*, 2007), *Metzgeria pubescens* (Fuselier *et al.*, 2011), *Plagiochila boryana* (Heinrichs et al., 2005b), *P. corrugata* (Heinrichs *et al.*, 2004), *Porella cordaeana* (Heinrichs *et al.*, 2011) and *Porella swartziana* (Hentschel *et al.*, 2007b), as well as *Scapania nemorea*, *S. paludosa*, *S. subalpina* and *S. undulata* (Heinrichs *et al.*, 2012c).

3. Lejeuneaceae

Leafy Jungermanniidae split in two orders assigned to as Porellales and Jungermanniales (Heinrichs *et al.*, 2005a). Jungermanniales contain mainly terrestrial species characterized by often diffusely distributed rhizoids, lateral and ventral branching, unicellular spores and frequent presence of mycorrhiza-like mutualisms. Porellales contain mainly epiphytic species and are characterized by lack of ventral branching and mycorrhiza-like mutualisms, rhizoids in bundles, frequent occurrence of endosporous protonemata, incubous foliation and the development of watersacs (Gradstein *et al.*, 2001; Heinrichs *et al.*, 2005a; Schuster, 1980).

Lejeuneaceae represents the most species-rich epiphytic family of liverworts with some 1000 species in 68 currently accepted genera (Gradstein, 2013). This family has its centre of diversity in the humid tropics and constitutes an important component of cryptogamic, especially epiphytic diversity (Gradstein, 1997; Gradstein *et al.*, 2006). Studies show that up to 70% of liverwort species of tropical lowland forests belong to Lejeuneaceae (Cornelissen and Ter Steege, 1989; Zartman, 2003). This family is characterized by incubous leaves divided into a large dorsal lobe and a small ventral, usually 1-3 toothed lobule which is broadly attached to the lobe along a keel, a hyaline papilla situated near the lobule apex, beaked perianths within each one archegonium, and the occasional occurrence of ocelli.

Spruce (1884) accepted only two genera in Lejeuneaceae, the monospecific genus *Myriocolea* and the speciose genus *Lejeunea* which he classified in 39 subgenera. Subsequent authors classified Lejeuneaceae into two artificial groups, “Holostipae” and “Schizostipae” based on either entire or bifid underleaves (Evans, 1935; Schuster, 1963). Herzog (1957) divided Lejeuneaceae into Lejeuneoideae and Cololejeuneoideae based on the number of underleaves per leaf pair. Mizutani (1961) recognized that “Holostipae” and “Schizostipae” differ constantly by sporophytic characters, and he renamed the two subfamilies Ptychanthoideae (Holostipae) and Lejeuneoideae (Schizostipae). Later, Mizutani (1985) merged Cololejeuneoideae with Lejeuneoideae. Recent morphological and molecular phylogenetic analyses (Gradstein *et al.*, 2003; Heinrichs *et al.*, 2005a; Wilson *et al.*, 2004; Wilson *et al.*, 2007a) recovered a paraphyletic Ptychanthoideae (19 genera) and a monophyletic Lejeuneoideae (49 genera), the latter including three main lineages: Lejeuneeae, Brachiolejeuneeae, and Symbiezidiae. Ahonen and Muona (2003) excluded *Nipponolejeunea* from Lejeuneaceae and moved it to Jubulaceae whereas the two controversial genera *Bryopteris* and *Metzgeriopsis* were proved to be genuine members of this family based on molecular evidence (Wilson *et al.*, 2004). Many genera in Lejeuneaceae were reduced to subgeneric rank or synonymy by molecular studies, e.g., *Metzgeriopsis* (Gradstein *et al.*, 2006), *Cystolejeunea* (Wilson *et al.*, 2004), *Dendrolejeunea* (Sukkharak *et al.*, 2011), *Taxilejeunea* and *Leucolejeunea* (Wilson *et al.*, 2007a), and *Oryzolejeunea* (Ye *et al.*, 2013).

4. Molecular Studies

Subfamily Ptychanthoideae and Lejeuneoideae can be distinguished by a set of morphological characters, i.e., Lejeuneoideae have underleaves either bifid or entire and usually 2-celled ventral merophytes in contrast to entire underleaves and at least 4-celled ventral merophytes in Ptychanthoideae; Lejeuneoideae have an articulate seta (12+4 cell rows), erect capsule valves with 5-6 elaters per valve, capsule valve hinge horseshoe-, butterfly-, or broadly quadrate-shaped, not extending to the valve margin, whereas Ptychanthoideae have a non-articulate seta (16+4 cell rows), suberect to obliquely spreading capsule valves with 6-20 elaters per valve which are also fixed at the surface of the valve, and a rectangular valve hinge extending to the valve margin.

The subfamily Lejeuneoideae includes three tribes, Brachiolejeuneeae (8 genera), Symbiezidieae (1 genus) and Lejeuneeae (40 genera). Lejeuneeae, the largest tribe in Lejeuneaceae, is mainly characterized by usually bifid underleaves (rarely entire or lacking) with bistratose bases, segmented or homogeneous (Massula-type) oil bodies, occasional presence of ocelli, seta of 12 outer rows of cells, and the butterfly - or broadly quadrate-shaped valve hinge (Gradstein, 2013; Wilson *et al.*, 2007a). Based on molecular and morphological evidence, Gradstein (2013) classified Lejeuneeae into eight subtribes, Cheilolejeuneinae (4 genera), Echinolejeuneinae (3 genera), Cyclolejeuneinae (3 genera), Lepidolejeuneinae (2 genera), Lejeuneinae (5 genera), Ceratolejeuneinae (2 genera), Drepanolejeuneinae (2 genera), and Cololejeuneinae (12 genera).

My studies focused on several critical genera in two subtribes, Cololejeuneinae and Lejeuneinae. Previous morphological and molecular studies of Lejeuneaceae (Gradstein *et al.*, 2003; Wilson *et al.*, 2007a) have recovered these two subtribes as robust monophyletic lineages, which contain the majority of the family diversity. Species within these two groups are usually highly specialized to an epiphytic living and grow in extreme habitats, such as leaf surfaces of vascular plants and twigs. Some species in these two groups can even grow in periodically submerged running waters as rheophytes (Gradstein and Reiner-Drehwald, 2007; Heinrichs *et al.*, 2012e; Reiner-Drehwald and Gradstein, 1995). However, few studies (Hartmann *et al.*, 2006) have investigated genus level molecular phylogenies within Lejeuneeae genera. Considering widespread morphological homoplasy within Lejeuneeae and subsequent taxonomic confusions, it is necessary to apply molecular methods to study and test the systematic positions and biogeography of these genera in a phylogenetic context. In the following paragraphs, I will introduce the genera studied in my molecular studies.

4.1. *Diplasiolejeunea*

Diplasiolejeunea is a pantropical genus growing on living leaves, fine twigs, and smooth-barked trunks, usually in well-illuminated sites from sea level to 4150 m. This genus is mainly characterized by one underleaf per leaf in combination with rather large but not sac-like leaf lobules (Zhu and So, 2001). Due to extensive morphological homoplasy, previous morphological systematic studies usually arrived at conflicting results (Grolle, 1966; Pócs, 2006; Pócs and Schäfer-Verwimp, 2006; Schäfer-Verwimp, 2004 & 2006; Schäfer-Verwimp and Reiner-Drehwald, 2009; Tixier, 1985). The uncertainty regarding species circumscription has led to divergent estimates of global diversity, ranging from 35 (Schuster, 1980) to 70 *Diplasiolejeunea* species (Schäfer-Verwimp, 2006). Most *Diplasiolejeunea* species seem to have rather narrow distribution ranges, in contrast to the pantropical ranges of *D. cavifolia* Steph., *D. cobrensis* Steph. and *D. rudolphiana* Steph. (Grolle, 1995; Zhu and So, 2001). Hence, it would be necessary to test the species concept, as well as to carve out the distribution pattern of this genus and explore the possible reasons for its range formation. To this end, my comprehensive study on *Diplasiolejeunea* in **Appendix 1** sampled 122 accessions covering the whole range of the genus, three genomic regions (cp rbcL and trnL-F, nrITS), and six morphological and ecological characters. The recovered topology and ancestral area reconstructions show geographically separated clades as well as ecologically and altitudinally separated clades. My study supports a deep split into a Neotropical and a Paleotropical clade, the latter structured into Australasian, Asian and Afromadacascan lineages. Presented results confirm the ranges of two pantropical species (*D. cavifolia*, *D. rudolphiana*), provide evidence for dispersal from the Neotropics into the Paleotropics, indicate speciation along altitudinal gradients and demonstrate extensive morphological homoplasy. I propose a revised supraspecific classification of *Diplasiolejeunea* into a predominantly Paleotropical subgenus *Physolejeunea* and predominantly Neotropical subgenera *Austrolejeuneopsis* and *Diplasiolejeunea*, the former containing mainly epiphytic species, the latter mainly epiphylls.

4.2. *Lejeunea*

Lejeunea Lib. is one of the most species rich and taxonomically complex genera of Lejeuneaceae. This genus is currently circumscribed by long-inserted leaves, divided or undivided underleaves, leaf lobules with an unreduced first tooth and a marginal hyaline papilla, small, segmented or homogeneous oil bodies, lack of ocelli, lejeuneoid innovations, unwinged female bracts and inflated perianths with 0-5 smooth or toothed wings. *Lejeunea* was first described by Libert (1820) to include only two species: *Cololejeunea calcarea* and *Lejeunea cavifolia* (Grolle, 1971). Adopting a broad genus concept, Gottsche *et al.* (1844-1847) described ca. 300 tropical species of *Lejeunea*. Spruce (1884) further grouped all the Lejeuneaceae elements into two genera, the monospecific genus *Myriocolea* and the speciose genus *Lejeunea*. Later on, Stephani (1898-1925) provided a global treatment of liverworts; his Species Hepaticarum included numerous new species, many of which were known only from single accessions, creating a multitude of synonyms. Most binomials in *Lejeunea* are now assigned to other genera or are reduced to synonyms. Reiner-Drehwald accepted 165 binominals out of 1749 Latin American *Lejeunea* names as effectively belonging to this genus (Reiner-Drehwald, 1999). Revisions and updated checklists of *Lejeunea* for different regions have also been published, i.e., 12 species were recognized for Japan (Mizutani, 1961), 30 species for China (Piippo, 1990), 9 species for Europe (Grolle, 1983), 39 species for Sub-Saharan Africa (Wigginton and Grolle, 1996), 25 species for East African Islands (Grolle, 1995), 16 species for North America (Schuster, 1980), 12 species for southern South America (Solari, 1983), and 41 species for Brazil (Reiner-Drehwald, 2007). However, the exact number of *Lejeunea* species remains unclear due to a lack of comprehensive taxonomic studies (Gradstein *et al.*, 2003) and scarcity of diagnostic morphological characters (Gradstein *et al.*, 2003; Hartmann *et al.*, 2006; Heinrichs *et al.*, 2009; Reiner-Drehwald and Ilkiu-Borges, 2007; Renner *et al.*, 2011; Schuster, 1983; Wiggington, 2004).

Meanwhile, subgenus circumscription is also a major problem in *Lejeunea*. Spruce (1884) described 39 subgenera in *Lejeunea*, of which the majority was later elevated to genus rank by Schiffner (1893) since Spruce's "*Lejeunea*" represents nearly the whole family Lejeuneaceae as it is now understood. Subsequent authors (Gradstein and Reiner-Drehwald, 2007; Grolle, 1984a & 1984b & 1995; Schuster, 1963) added further 13 subgenera to *Lejeunea*. Most of these subgenera, were not accepted by other workers (Reiner-Drehwald, 1999). Recent morphological and molecular studies indicated that many previously defined *Lejeunea* subgenera do not merit subgeneric status and were reduced to synonyms of *Lejeunea*, namely, subg. *Apolejeunea* and *Hygrolejeunea* (Schuster, 1980), *Crossotolejeunea* (Reiner-Drehwald and Goda, 2000), *Inflatolejeunea* and *Macrolejeunea* (Reiner-Drehwald and Schafer-Verwimp, 2008b), and *Taxilejeunea* (Wilson et al., 2007a). Subgenus *Microlejeunea* was treated as an independent genus by many recent authors (Ah-Peng and Bardat, 2011; Bischler *et al.*, 1963; Grolle, 1995). By now, eight *Lejeunea* subgenera are provisionally accepted, *Chaetolejeunea* (Schuster, 1963), *Heterolejeunea* (Grolle, 1995), *Nanolejeunea* (Schuster, 1980), *Neopotamolejeunea* (Gradstein and Reiner-Drehwald, 2007), *Otigoniolejeunea* (Spruce, 1884), *Pleurolejeunea* (Schuster, 1963) and *Sphaerocolea* (Schuster, 1963). Subgenus *Lejeunea* was considered to be a polymorphic group including nine sections (Schuster 1957, 1980). A comprehensive study of *Lejeunea* would hopefully establish the species limits as well as an improved subgenus circumscription.

Compared to three pantropical species in *Diplasiolejeunea*, 25.6% (21/82) of the investigated *Lejeunea* species show disjunct ranges, many of these species span several continents. Molecular phylogenetic studies of this genus and subsequent ancestral area reconstruction would be crucial to recover the distribution pattern of *Lejeunea,* providing insights into the range formation of the genus. In **Appendix 2**, I produced a large *Lejeunea* phylogeny incorporating three genomic regions (nrITS, cp *rbc*L and *trn*L-F) and 332 accessions covering the whole distribution range of the genus. My study recovered two major lineages differing in sexuality tendencies, which were subsequently assigned to two subgenera, subgenus *Lejeunea* and subgenus *Crossotolejeunea*. Nearly half of the species (25/54) with multiple accessions were resolved as para- or polyphyletic. Ancestral area reconstructions pointed to a Neotropical origin of *Lejeunea* and confirmed the previous hypothesis that the tropics are the cradle and museum of Lejeuneaceae diversity.

4.3. *Microlejeunea*

Microlejeunea has been treated either as a genus (Ah-Peng and Bardat, 2011; Bischler *et al.*, 1963; Grolle, 1995) or as a subgenus of *Lejeunea* (Schuster, 1980; Thiers, 1997). Both treatments seem consistent with the available phylogenies (Ahonen and Muona, 2003; Wilson *et al.*, 2004) which placed *Microlejeunea* in a sister relationship to *Lejeunea*. Wilson *et al.* (2007a) also resolved the monospecific genus *Pluvianthus* and *Harpalejeunea* in a sister relationship to *Lejeunea*, whereas this study lacked representatives of *Microlejeunea.* Therefore, the systematic position of *Pluvianthus* is still in need of further investigations with a dense sampling of the related genera.

Morphologically, *Microlejeunea* is characterized by sinuose stems, presence of three medullary cells, perpendicular branching, large lobules being usually longer than wide, dimorphic lobules, small bifid underleaves, bracts being larger than the leaves and often shortly connate at their bases, male bracts being distinctly larger than the leaves (Bischler *et al.*, 1962), a tendency to produce ocelli (Schuster, 1957), and the presence of lejeuneoid subgynoecial innovations (Grolle, 1995). *Pluvianthus* differs from *Lejeunea* by its rather large plant size, utriculiform leaves, laminar elaters, and capsule microanatomy (Schuster and Schäfer-Verwimp, 1995).

Harpalejeunea closely resembles *Microlejeunea* but has underleaves with diverging lobes, those of *Microlejeunea* being forward directed (Grolle and Reiner-Drehwald, 1999). The generic circumscription of *Harpalejeunea* is still subject to controversy. Grolle and Reiner-Drehwald (1999) separated *Harpalejeunea* by its lejeuneoid subgynoecial innovations from *Drepanolejeunea* with pycnolejeuneoid innovations. In contrast, Schuster (1980) considered *Harpalejeunea filicuspis* to be an element of *Drepanolejeunea*, despite the presence of lejeuneoid subgynoecial innovations. In **Appendix 3** I tested the current genus concepts by including representatives of *Harpalejeunea*, *Microlejeunea*, *Pluvianthus*, and *Drepanolejeunea* in a three-marker dataset of Lejeuneeae. My study constantly placed *Pluvianthus* and three *Harpalejeunea* species within the *Microlejeunea* clade. Based on the molecular lineages recovered and a reinterpretation of morphological traits, the above four species were transferred to

Microlejeunea. Microlejeunea and *Harpalejeunea* are accepted at genus level.

4.4. *Myriocolea*

Spruce (1884) accepted only two genera in the Lejeuneaceae, the monotypic *Myriocolea* with its single representative *Myriocolea irrorata* and the speciose genus *Lejeunea* with hundreds of species. This rheophytic genus *Myriocolea* differs from other Lejeuneaceae species by the exclusive presence of *Radula*-type branches, transversely inserted leaves without well delimited lobuli, presence of numerous antheridia per bract, and an extraordinary high number of clustered gynoecia (Gradstein *et al.*, 2004; Thiers, 1983). The systematic position of *Myriocolea* is controversial. Schuster (1963) set up a new subfamily Myriocoleoideae to include *Myriocolea* and *Cladocolea,* a taxon treated as a synonym of Lejeuneoideae by Gradstein (1994). The latter treatment was also supported by cladistic analyses of morphological character states of Lejeuneaceae (Gradstein *et al.*, 2003) where *Myriocolea* was resolved in an unsupported *Cololejeunea-Tuyamaella* clade. This relationship was also recovered by several molecular phylogenies based on one or two molecular markers (Gradstein *et al.*, 2006; Wilson *et al.*, 2004). Phylogenetic analyses of a comprehensive four molecular marker set of Lejeuneaceae consistently located *Myriocolea* in a clade with *Macrocolura* and *Colura* (Wilson *et al.*, 2007a). However, lack of comprehensive sampling of *Colura* (three *Colura* species) hampered the discovery of the true relationships of *Myriocolea*. In **Appendix 4**, I present the results of phylogenetic analyses of a dataset comprising two chloroplast genome regions and one nuclear genome region and a comprehensive taxonomic sampling of *Macrocolura*, *Myriocolea* and *Colura*. Molecular phylogenetic analyses resolved *Myriocolea* within *Colura*.

4.5. *Sphaerolejeunea*

Sphaerolejeunea Herzog was set up for a single species, *S. umbilicata* Herzog and based on a single specimen collected by E.P. Killip in 1922 in the Andes of Colombia (Herzog, 1938). This epiphyllous species stands out by leaf lobes bordered by several rows of hyaline cells, basally leafless gynoecial branches and perianths lacking both a beak and keels. These perianths hardly exceed the surrounding vegetative leaves

(Herzog, 1938) and open in an umbilicus at their dorsal side (Reiner-Drehwald and Drehwald, 2002). In 2001 a second locality of *Sphaerolejeunea umbilicata* was discovered in the Andes of northern Peru (Reiner-Drehwald and Drehwald, 2002). The respective herbarium specimen allowed for a detailed morphological investigation of the species and the proposal of a close relationship of *Sphaerolejeunea* and *Lejeunea* (Reiner-Drehwald and Drehwald, 2002). In the framework of a molecular phylogenetic study of *Lejeunea,* three molecular markers of *Sphaerolejeunea* were sequenced and included in a large sequence alignment of Lejeuneaceae to clarify its systematic position (**Appendix 5)**. *Sphaerolejeunea* was resolved within *Lejeunea* and transferred to this genus, as *Lejeunea drehwaldii* Heinrichs & Schäfer-Verwimp.

4.6. *Bromeliophila*

Bromeliophila R.M. Schust. was established for *Peltolejeunea natans* Steph., a Brazilian endemic, growing exclusively in the tanks of Bromeliaceae (Schuster, 1994), with the lower parts of the shoots often submerged and only the upper parts above the water. Schuster (1994) pointed to close relationships of *Bromeliophila* and *Lejeunea*, a view shared by Gradstein (1997) who considered the generic status to be “critical”. However, Gradstein described a second Neotropical species, *Bromeliophila helenae* Gradst. Earlier, Grolle (1985) transferred *Peltolejeunea natans* to *Lejeunea*, as *Lejeunea natans* (Steph.) Grolle. Recent molecular phylogenetic studies of Lejeuneaceae confirmed a general trend towards the acceptance of larger genera (Humphreys and Linder, 2009), especially merging small or monotypic genera into larger ones. A similar result was also expected for *Bromeliophila* since several authors proposed close relationships (Gradstein, 1997; Schuster, 1994) of *Bromeliophila* and *Lejeunea* based on their extensive morphological overlap or treated the generitype *Peltolejeunea natans* as an element of *Lejeunea* (Grolle, 1985). In **Appendix 6**, the systematic position of *Bromeliophila* is clarified in the framework of a comprehensive molecular phylogenetic-taxonomic study of Lejeuneaceae. This molecular study supports a sister relationship of *Bromeliophila* and *Prionolejeunea*, which is in turn sister to *Cyclolejeunea*.

5. Discussion

Molecular phylogenetic studies in several genera of Lejeuneeae allow deeper insights into this taxonomically complex group. The phylogeny of the pantropical genus *Diplasiolejeunea* shows ecologically as well as geographically separated lineages. Based on the recovered topology and the distribution of subgenus type species, three major clades were formally named, leading to the acceptance of a pantropical subg. *Physolejeunea*, a predominantly neotropical epiphytic subg. *Austrolejeunopsis*, and a predominantly neotropical epiphyllous subg. *Diplasiolejeunea*. Ancestral area reconstructions of *Diplasiolejeunea* support dispersal scenarios in shaping current distribution patterns, which is also corroborated by the *Lejeunea* biogeography reconstruction with 25.6% disjunct species (21/82). The *Lejeunea* phylogeny contradicts with previous subgenus classification and many existing species concepts. Based on the presence of type species in each clade, we assign subgenus *Lejeunea* to the major clade I and subgenus *Crossotolejeunea* to the major clade II. These two major clades differ in sexuality tendencies, clade I shows a tendency of monoecy whereas clade II shows more often dioecy. Nearly half of the species (44.6%) with multiple accessions are para- or polyphyletic, of these, monoecious taxa contribute to 66.7%, diocious taxa take 33.3%. Majority (70.4%) of diocious taxa, in contrast to 42.9% in monecious taxa, form monophyletic lineages. Monecious taxa (27.3%) as well as diocious taxa (23.7%) show disjunct distribution range.

The presented studies clarified the systematic positions of several controversial taxa, based on molecular phylogenies and a reinterpretation of morphological characters. Monotypic *Myriocolea* is transferred to *Colura*, monotypic *Pluvianthus* and three *Harpalejeunea* species are transferred to *Microlejeunea*, monotypic *Sphaerolejeunea* is transferred to *Lejeunea*, adding to the growing evidence that many small genera nest in larger ones. However, *Bromeliophila* breaks the rule. *Bromeliophila* was previously believed to be an element of *Lejeunea* based on its extensive morphological overlap with *Lejeunea* whereas molecular studies unexpectedly placed *Bromeliophila* in a sister relationship to *Prionolejeunea*.

5.1. Biogeography

The clear geographic pattern shown in *Diplasiolejeunea* in **Appendix 1** is surprising with regard to the high dispersal ability of spore plants (van Zanten, 1978; Vanderpoorten *et al.*, 2010; Wolf *et al.*, 2001), and has not yet been documented for any other liverwort genus. *Diplasiolejeunea* splits into pantropical subgenus *Physolejeunea*, neotropical epiphytic subgenus *Austrolejeuneopsis*, and neotropical epiphyllous subgenus *Diplasiolejeunea*. Epiphytic subgenus *Austrolejeuneopsis* occurs in high altitudinal Andean cloud forests whereas epiphyllous subgenus *Diplasiolejeunea* occurs in lowland forests and low montane regions. This strong correlation could point to the rise of Andes' influence on the assembly of *Diplasiolejeunea* diversity, possibly by stimulating the diversification of the subgenus *Austrolejeuneopsis*, perhaps by providing new habitats. The clear geographical pattern shown in *Diplasiolejeunea* could be indicative of Gondwanan vicariance, however, divergence time estimates suggest an origin of *Diplasiolejeunea* in the Oligocene (Wilson *et al.*, 2007b), posterior to the Mesozoic breakup of the Southern landmass. Therefore, a combination of frequent short distance dispersal, occasional long distance dispersal, extinction and recolonization, as well as niche conservatism was invoked to explain the observed pattern.

The biogeographical pattern recovered in *Lejeunea* in **Appendix 2** is not so distinct, compared with that observed in *Diplasiolejeunea*. Both main clades of *Lejeunea* show a more even representation of putative regions of endemism, indicating that long distance dispersal is more frequent in *Lejeunea* than in *Diplasiolejeunea* as long as we assume similar ages for both genera. Both S-diva and Mesquite reconstructions indicated that *Lejeunea* originated and initially diversified in the Neotropics with subsequent dispersal into other tropical as well as temperate regions. A few temperate lineages nested within tropical clades. This pattern appears to be consistent with the role of the tropics as a cradle and museum of diversity, and mirrors observations for the whole family Lejeuneaceae.

In the systematic examination of *Myriocolea*, 17 *Colura* accessions show a geographic structure into Asian-Australasian, Neotropical and African lineages. However, whether this pattern would sustain or not when more accessions are included, is still left to future studies.

5.2. Cryptic speciation

An important question in bryophyte systematics is whether disjunctive geographical distributions, morphological uniformity, and long evolutionary history, reflect evolutionary stasis or if morphological uniformity masks underlying genetic complexity (Shaw *et al.*, 2002). Traditional hypotheses regarding liverworts as unmoving, unchanging sphinxes of the past (Crum, 1972) is frequently challenged. Increasing molecular phylogenetic studies in bryophytes reveal the existence of numerous cryptic biological species that have accumulated genetic divergence without concordant morphological disparities. Morphologically cryptic biological entities have been observed in the genera *Conocephalum* (Odrzykoski and Szweykowski, 1991), *Frullania* (Ramaiya *et al.*, 2010), *Leptoscyphus* (Vanderpoorten *et al.*, 2010), *Marchesinia* (Heinrichs *et al.*, 2009), *Metzgeria* (Fuselier *et al.*, 2009), *Pellia* (Odrzykoski *et al.*, 1996), *Ptilidium* (Kreier *et al.*, 2010), and are also present in *Diplasiolejeunea* and *Lejeunea*. For example, the pantropical species *D. cavifolia* is regarded to be a somewhat variable species (Schäfer-Verwimp and Reiner-Drehwald, 2009) including forms with strongly T-shaped lobule teeth (earlier recognized as *D. matoubae* and *D. zacatepecensis*) as well as forms without such teeth. This species forms a robust lineage with two main clades; however, the different morphotypes are present in both. The two clades occur sympatrically in the West Indies, and can thus neither be separated using geographical nor morphological evidence. Many cryptic speciations occur along altitudinal gradients, i.e., *D. pauckertii, L. reflexistipula. L. reflexistipula* is an Andean species, characterized by entire incubous underleaves and relatively large size. Molecular studies resolved this species into two robust clades, one from lower altitude, the other from higher altitude including two accessions of *L. reflexistipula* var. *costaricensis* and three accessions of *L. reflexistipula*. The two clades cannot be separated by morphological evidence, indicating cryptic speciation along atlitudinal gradients.

5.3. Taxa relocation

Liverworts are a group of simple and primitive plants with a long evolutionary history. Traditional taxonomy on this group usually relied mainly on morphological descriptions and observations. The resulting classification scheme was often problematic due to extensive morphological homoplasy, especially in the family Lejeuneaceae. Recent molecular studies have greatly improved the classification of liverworts. Based on the recovered lineages and reinterpretation of morphological characters, numerous smaller genera were incorporated into larger ones.

My phylogenetic study in **Appendix 3** resolved a monotypic genus *Pluvianthus* and three *Harpalejeunea* species, namely *H. filicuspis*, *H. fischeri*, and *H. latitans* in a clade with several species of *Microlejeunea* including the generitype *M. africana*. Morphologically these species shared with *Mircrolejeunea* three medullary cells, a tendency to produce ocelli and forwardly directed underleaf lobes. Typical *Harpalejeunea* are similar to *Microlejeunea* in many aspects, but differ from *Microlejeunea* by their widely diverging underleaf lobes (Grolle and Reiner-Drehwald, 1999). Hence a transfer of the above three species of *Harpalejeunea* to *Microlejeunea* is justified both by molecular and morphological data.

Molecular studies in **Appendix 4** resolved *Myriocolea irrorata* in the *Colura* sect. *Colura* clade. Morphologically, these two genera share one underleaf per leaf (also present in *Diplasiolejeunea* and *Macrocolura*) instead of one underleaf per leaf pair as presented in the rest Lejeuneaceae (Gradstein *et al.*, 2003). *Myriocolea irrorata* differs from *Colura* by lack of sack-like lobules, whereas a tendency to lose lobules is also seen in *Colura* sect. *Heterophyllum* and *Colura corynephora*. Therefore, *Myriocolea irrorata* is transferred to *Colura*. The rheophytic, nutrient-rich habitat may explain the untypical leaf development of *Colura irrorata* because a structure for water storage or zoophagy is not needed in such an environment. Rheophytes from different taxonomic groups exhibit a parallel development, because running waters and regular flooding shape them into flood resistant plants (Van Steenis, 1981), e.g., *Myriocoleopsis* and *Myriocolea* (Gradstein *et al.*, 2004), *Lejeunea* subg. *Neopotamolejeunea* (Gradstein and Reiner-

Drehwald, 2007), and *Cololejeunea stotleriana* (Gradstein et al., 2011).

Reiner-Drehwald and Drehwald (2002) pointed out that *Sphaerolejeunea* may represent the sister group of *Lejeunea*. Despite many similarities, *Sphaerolejeunea* differs from *Lejeuna* by the development of a second lobule tooth, gynoecial branches without leaves at the base, beakless perianths being umbilicate on the dorsal side and valves with five marginal elaters lacking thickenings. However, the molecular studies in **Appendix 5** indicate that *Sphaerolejeunea* is nested in *Lejeunea* and that its morphological peculiarities represent autapomorphies of a *Lejeunea* species.

Although there is a general trend towards merging smaller genera into larger ones, a few exceptions break the rule. Morphologically, *Bromeliophila* stands out by its 5-keeled perianth with each keel doubled winged, and entire leaf margin in contrast to 2-keeled perianth and denticulate leaf margin in *Prionolejeunea* and *Cyclolejeunea*. Phylogenetic analyses in **Appendix 6** confirmed the monophyly of *Bromeliophila* and provided evidence for a sister relationship of *Bromeliophila* and *Prionolejeunea*; this clade is in turn sister to *Cyclolejeunea*. *Bromeliophila* and *Prionolejeunea* share *Lejeunea* type gynoecial innovations in contrast to pycnolejeunoid gynoecial innovations in *Cyclolejeunea.* Based on the molecular topology and morphological evidence, *Cyclolejeunea* is subdivided in subgenus *Cyclolejeunea* with ocelli and rounded gemmae, monospecific subgenus *Hyalolejeunea* without ocelli, and monospecific subgenus *Nephrolejeunea* with elongate gemmae and ocelli.

5.4. Future Prospects

Previous molecular and morphological studies divided Lejeuneaceae into 2 subfamilies and 4 lineages but deep nodes remained unsupported. Current phylogenetic study of several genera within subfamily Lejeuneoideae clarified their circumscription whereas the inter-relationships among many problematic taxa remains unclear, i.e. *D. pauckertii* group, *D. pellucida-caribea* complex, *L. flava* complex, *L. anisophylla* complex. Future studies incorporating molecular markers (Chang and Graham, 2011; Qiu *et al.*, 2006), cytological studies, spore ultra-structure studies (von Konrat *et al.*, 2012), geometric morphometric analysis (Renner, 2012) and phylogenomic studies (Timme *et* *al.*, 2012) may attain better phylogenetic resolution to these problems.

Recent biogeographical studies (Feldberg *et al.*, 2007; Feldberg *et al.*, 2010; Hartmann *et al.*, 2006; Heinrichs *et al.*, 2006) on leafy liverworts unanimously pointed to dispersal scenarios in shaping current distribution ranges. My studies on *Lejeunea* and *Diplasiolejeunea* also confirm this pattern. However, the discussion of the impact of geological events cannot be detailed without reasonable time constraints. So far, only several dating studies (Cooper *et al.*, 2012b; Hartmann *et al.*, 2006; Heinrichs *et al.*, 2007; Wilson *et al.*, 2007b) have been conducted in leafy liverworts. These studies all point to the origin of many genera of leafy liverworts in the Cretaceous or Cenozoic. Future dating studies incorporating more fossil records and dense taxon sampling will be essential to illustrate the general biogeographical pattern of Lejeuneaceae and the impacts of geological events.

Plant geographical pattern is affected by sexual system, spore dispersal ability and plant ecological fitness. Thus future studies in these areas will help to better illustrate the dynamics of plant genetic structure. To achieve this point, population studies at species level (Bączkiewicz, 2013), spore germination studies, and hybrid speciation studies, would be of prime importance. Hybridization (Shaw, 1998) and polyploidy in bryophytes is drawing increasingly attention (Self, 2002; Shaw and Goffinet, 2000). Hybridization is an important type of evolution (Natcheva and Cronberg, 2004), which might be the reason for the lack of support in some nodes. However, hybridization in leafy liverworts has only been demonstrated in European *Porella* × *baueri*, an allopolyploid derivative of *P. cordaeana* and *P. platyphylla* (Boisselierdubayle and Bischler, 1994). Heinrichs *et al.* (2011) also suggest an ancient hybrid origin of *P. platyphylloidea* from crossings of *P. cordaeana* and *P. platyphylla* s.str., inferred from the incongruent signals of chloroplast and nuclear markers.

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Appendix 1

Tramps, narrow endemics and morphologically cryptic species in the epiphyllous liverwort *Diplasiolejeunea*

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Abstract

Diplasiolejeunea is a pantropical, epiphytic genus of leafy liverworts that occurs from the lowlands to more than 4000 m. Phylogenetic analyses of a molecular dataset consisting of three markers (nuclear ribosomal ITS region, plastidic *trn*L-F region and rbcL gene) and 122 accessions (plus two outgroups, *Colura* and *Cololejeunea*) indicate that the evolutionary diversity of *Diplasiolejeunea* is underestimated by current morphology-based classification. Four morphologically semi-cryptic species have been recovered. The molecular phylogenies support a deep split into a Neotropical and a Paleotropical clade, the latter structured into Australasian, Asian and Afromadacascan lineages. Presented results confirm the ranges of two pantropical species (*D. cavifolia*, *D. rudolphiana*), provide evidence for dispersal from the Neotropics into the Paleotropics, indicate speciation along altitudinal gradients and demonstrate extensive morphological homoplasy. We propose a revised supraspecific classification of *Diplasiolejeunea* into a predominantly Paleotropical subgenus *Physolejeunea* and predominantly Neotropical subgenera *Austrolejeuneopsis* and *Diplasiolejeunea*, the former containing mainly epiphytic species, the latter mainly epiphylls. Several clades are supported by combinations of morphological character states, and could be assigned to sections at some later point. This is the first comprehensive phylogeny of a largely epiphyllous genus of liverworts.

Keywords: classification, dispersal, Lejeuneaceae, phylogenetic biogeography, Porellales,

1. Introduction

Molecular phylogenetic studies have greatly improved our understanding of relationships within liverworts. These studies led to numerous adjustments of family and genus concepts (Crandall-Stotler et al., 2009) and allowed for new insights into their biogeography (Shaw, 2001). Among the most remarkable findings was the evolutionarily dynamic nature of liverworts (Heinrichs et al., 2009a; Vanderpoorten et al., 2010a), rejecting the hypothesis of bryophytes being “unmoving, unchanging sphinxes of the past” (Crum, 1972). However, this dynamic nature of colonization, extinction and local differentiation is often poorly recognizable using morphological evidence alone and thus breakthrough studies required the application of molecular methods ranging from isozyme studies to DNA based studies, the latter using haplotype variation at population level and phylogenetic approaches at deeper taxonomic levels (Odrzykoski and Szweykowski, 1991; Feldberg et al., 2007; Devos and Vanderpoorten, 2009; Forrest et al., 2011; Heinrichs et al., 2011; Laenen et al., 2011). Related studies demonstrated a complex genetic structure of morphologically circumscribed liverwort species and provided evidence for morphologically cryptic or semicryptic speciation (Wachowiak et al., 2007; Fuselier et al., 2009; Heinrichs et al., 2010; Ramaiya et al., 2010; Heinrichs et al., 2011). It is now proven that only species-level phylogenies with extensive population sampling will allow for an approximation of the actual liverwort diversity, and to better understand speciation processes and distribution range formation (Kreier et al., 2010).

Lejeuneaceae, with an estimated number of about 1000 species (Wilson et al., 2007a), represent the most species rich epiphytic lineage of leafy liverworts (Heinrichs et al., 2005a). This family is common throughout the humid tropics, especially in the lowlands were up to 70% of the present liverworts belong to Lejeuneaceae (Cornelissen and Ter Steege, 1989; Zartman, 2003). In epiphyllous communities Lejeuneaceae are even more dominant, totalling to more than 90 % of the present bryophyte species (Pócs, 1996; Sonnleitner et al., 2009; Benavides & Sastre-De Jesús, 2011). Epiphyllous Lejeuneaceae are characterized by a rather small or even minute size, frequent vegetative reproduction by multicellular, disciform gemmae, and are closely attached to the substratum (Gradstein et al., 1997). Due to their diminutive size and reduced morphology, it is far from surprising that they belong to the taxonomically most difficult groups of Lejeuneaceae (Gradstein et al., 2006).

Diplasiolejeunea is a pantropical, partly epiphyllous Lejeuneaceae genus growing on living leaves, fine twigs, and smooth-barked trunks, usually in well-illuminated sites from sea level to 4150m (Gradstein et al., 2001; Schäfer-Verwimp, 2004). This genus is easily recognized by the presence of one underleaf per leaf in combination with rather large but not sac-like leaf lobules (Zhu & So, 2001). *Diplasiolejeunea* species taxonomy has been the subject of several morphological studies, often with controversial results (Grolle, 1966; Tixier, 1985; Schäfer-Verwimp, 2004, 2006; Pócs, 2006; Pócs and Schäfer-Verwimp, 2006; Schäfer-Verwimp and Reiner-Drehwald, 2009). The uncertainty regarding species circumscription has led to divergent estimates of global diversity, ranging from 35 (Schuster, 1980) to 70 *Diplasiolejeunea* species (Schäfer-Verwimp, 2006). Most of these species seem to have rather narrow distribution ranges, in contrast to the pantropical ranges of *D. cavifolia* Steph., *D. cobrensis* Steph. and *D. rudolphiana* Steph. (Grolle, 1995; Zhu & So, 2001).

Up to now, less than 20 % of the Lejeuneaceae species have been included in molecular phylogenetic studies (Ahonen et al., 2002; Hartmann et al., 2006; Wilson et al., 2007a; Heinrichs et al., 2009b; Gradstein et al., 2011; Renner et al., 2011; Sukkharak et al., 2011); hence this family is in need of further investigations. Here we present the first global phylogeny of a partly epiphyllous genus of Lejeuneaceae, *Diplasiolejeunea,* based on 122 accessions that cover the whole range of the genus, and sequences from the nuclear ribosomal internal transcribed spacer region (nrITS), along with the plastid DNA *rbc*L gene, and the *trn*L-F region. We test current species and range concepts by including multiple accessions and examine whether the recovered phylogenetic relationships correspond to/or conflict to morphologically circumscribed taxa. Our data support intercontinental ranges of a few *Diplasiolejeuna* species, presence of several so far unrecognised species, morphologically cryptic speciation, diversification along altitudinal gradients, and geographically separated main clades.

2. Material and methods

2.1 *Taxon sampling and outgroup selection*

Taxa studied, including GenBank accession numbers and voucher details, are listed in Table 1. All voucher specimens were carefully examined and original identifications were corrected when necessary. To identify DNA vouchers we used monographic treatments (e.g., Tixier, 1985; Reyes, 1982; Schäfer-Verwimp et al., 2004, 2006), original descriptions (e.g., Schäfer-Verwimp et al., 2005; Pócs and Schäfer-Verwimp, 2006) and type material preserved in the herbaria EGR, G, GOET, JE, and PC (abbreviations according to Holmgren et al., 1990).

Besides availability of material, ingroup taxa were selected to represent the morphological variation and geographical distribution of *Diplasiolejeunea*. Multiple accessions of several species were used to explore intraspecific genetic variation. Based on the analyses of Gradstein et al. (2006) and Wilson et al. (2007a), *Cololejeunea vitalana* and *Colura acroloba* were designated as outgroup taxa for phylogenetic reconstruction.

2.2 *DNA extraction, PCR amplification and sequencing*

Plant tissue was isolated from herbarium collections. Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: *rbc*L gene and *trn*L-F region from Gradstein et al. (2006), and nrITS1-5.8S-ITS-2 region from Hartmann et al. (2006). Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR. Voucher specimens for all sequences were deposited in EGR, GOET or JE. Three hundred and fourty three sequences were newly generated for this study; 9 sequences were downloaded from Genbank (http://www.ncbi.nlm.nih.gov/genbank/).

2.3 *Phylogenetic analyses*

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall, 1999). Ambiguous positions were excluded from all alignments and lacking data were coded as missing. Some analyses were carried out using a comprehensive alignment including all studied accessions, whereas other analyses used a reduced alignment with one accession per taxon as required for plotting of several morphological characters and ecological preferences (see Table 2). The reduced alignment comprises the accessions with the most complete sequences. The *rbc*L sequence of the *D. plicatiloba* specimen Schäfer-Verwimp 14246 was combined with the *trn*LF and ITS sequences of specimen Pócs 0057/R to get a complete sequence set for the reduced alignment.

Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10

(Swofford, 2000). MP heuristic searches of the comprehensive and the reduced datasets were conducted with the following options implemented: heuristic search mode, 1000 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Non-parametric bootstrapping values (Felsenstein, 1985) were generated as heuristic searches with 1,000 replicates, each with ten random-addition replicates. The number of rearrangements was restricted to ten millions per replicate. Bootstrap percentage values (BPVs) ≥ 70 were regarded as good support (Hillis and Bull, 1983). Where more than one most parsimonious tree was found, trees were summarized in a strict consensus tree.

The three genomic regions and the combined chloroplast DNA dataset vs nrITS dataset were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70% (Mason-Gamer and Kellog, 1996). The trees gave no evidence of incongruence. Hence the datasets were combined. The influence of *D. alata* on the robustness of the reconstructed phylogeny was tested by excluding this species from the analyses. jModeltest 0.1 (Posada, 2008) was used to select a model of evolution for the maximum likelihood (ML) analyses of the large combined dataset. A General Time Reversible (GTR) model (Tavaré, 1986) was chosen with proportion of invariable characters (*I*) and among-site rate heterogeneity modelled as discrete gamma distribution with four rate categories, and its estimated parameters (Γ) (Goldman, 1993). ML trees were generated using the programs PAUP* and GARLI version 0.96 beta (Zwickl, 2006). The PAUP*-Analysis was performed as heuristic search, MULTrees option on, collapse zero length branches off, and TBR branch swapping. All GARLI analyses were performed with the default settings and several times repeated. The default setting of GARLI was also employed to calculate bootstrap values for ML analyses based on 200 bootstrap replicates.

Bayesian inference was implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) on the large combined dataset using a GTR substitution model. A Bayesian search was carried out with four simultaneous Markov chains, ten million generations and sampling every 100th generation. Tracer version 1.3 (Rambaut and Drummond, 2003) was used to examine the parameters and determine the number of trees needed to reach stationarity (burn-in). Bayesian posterior probability (BPP) confidence values were generated from trees saved after this initial burn-in. Values were regarded as significant when BPP ≥ 0.95 (Larget and Simon, 1999).

2.4 *Reconstruction of ancestral areas of distribution, distribution of morphological character states and growth preferences*

Ancestral areas of distribution were reconstructed using MP criteria as implemented in Mesquite ver. 2.72 (Maddison and Maddion, 2004) based on the ML topology of the combined dataset. Data on distribution ranges of the investigated taxa were obtained from the literature, in addition to own observations. Given the wide distribution ranges of some species, the putative distribution range of endemism was coded as covering four possible areas: Neotropics, Afromadagascar, Asia, Australasia. The ranges of the outgroup species were coded as “unknown” because the closest relatives of *Diplasiolejeunea* have not yet been determined (see Wilson et al. 2007a). We plotted several morphological characters (see table 2) on the MP tree of the reduced dataset that are relevant for the systematics of the genus (Schuster, 1970; Tixier, 1985; Schäfer-Verwimp, 2004). We did not map generative structures because of their high infraspecific variation in the current *Diplasiolejeunea* classification. Growth preferences of the investigated characters were mapped based on own observations during fieldwork in all parts of the tropics, and data from the literature. We also mapped the altitude of the DNA vouchers on the ML topology.

3. Results

3.1 *Small combined dataset*

Of a total of 2148 character sites, 467 were parsimony informative, 257 autapomorphic and 1424 constant. The MP analysis resulted in four equally parsimonious trees with a length of 2116 steps, a consistency index (CI) of 0.48 and a retention index (RI) of 0.61. The strict consensus tree is depicted in Fig. 1.

Diplasiolejeunea is split into a well supported (BPV=78) mainly Neotropical clade II, and an unsupported (BPV<50) Paleotropical clade I with the Australasian *D. plicatiloba* (clade I-a) placed sister to a clade that is divided into an Asian (clade 1-b) and an Afromadagascan lineage (clade I-c). Clade I includes the type of *D.* subgen. *Physolejeunea*, *D. plicatiloba*. Clade II splits into an unsupported clade II-a with the type of *D*. subgen. *Austrolejeuneopsis*, *D. alata*, and a robust (BPV=87) clade II-b with the generitype *D. pellucida*. *Diplasiolejeunea alata* forms a long branch in all MP trees (not shown). Exclusion of *D. alata* from the dataset does not lead to changes in the topology but to a BPV of 75 for clade II-a. All investigated morphological characters proved to be homoplasious but several derived clades share certain character state combinations. Clade II-a includes mostly epiphytes wheras the majority of species belonging to clade II-b predominanly occurs as epiphylls.

3.2 *Large combined dataset*

The PAUP* ML analysis of the large dataset led to three nearly identical topologies with -ln=18325.54055, of which one is depicted in Fig. 2. This topology was also found with GARLI and Mrbayes. The MP topology is confirmed with the extended dataset albeit with high BPV and BPP values for both clade I (90/0.99) and clade II (91/1.00). The sister relationship of the Asian and the Afromadagascan clade is well supported with a BPV of 75 but gets no significant support in the Bayesian analysis. Multiple accessions of morphologically circumscribed species form monophyletic lineages with the exception of *D. unidentata* nested in *D. rudolphiana* and *D. caribea* nested in *D. pellucida*. *Diplasiolejeunea pauckertii* and *D. pluridentata* are polyphyletic. Five accessions represent undescribed species.

Pantropical ranges are confirmed for *D. cavifolia* and *D. rudolphiana* although the ancestral area reconstruction indicates a Neotropical origin of these species (Fig. 3). Accessions of *D.* subgen. *Austrolejeuneopsis* were usually collected in high montane regions, in addition to a few accessions from the lowlands or low montane regions (*D. rudolphiana*, *D. unidentata*). Representatives of *D*. subgen. *Diplasiolejeunea* were mostly collected in low montane regions or lowlands.

4. Discussion

4.1. *Supraspecific classification*

Schuster (1970) classified *Diplasiolejeunea* in three subgenera, *Austrolejeuneopsis*, *Physolejeunea* and *Diplasiolejeunea* s.str. based on the absence (*Austrolejeuneopsis*, *Physolejeunea*) or presence of ocelli (*Diplasiolejeunea* s.str.). He set up subgen. *Physolejeunea* to include a single Australasian species with very large leaf lobules, *D. plicatiloba* (as *D. lyratifolia*), and proposed subgen. *Austrolejeuneopsis* - typified with the Neotropical *D. alata* - for a few species with moderately sized, strongly inflated lobules.

This classification is not supported by the molecular phylogenies (Figs. 1 and 2) since the few species without ocelli are diffusely distributed in the molecular topologies (Fig. 1, character 3), and the type species of subgen. *Austrolejeuneopsis* and *Physolejeunea* nested in subgen. *Diplasiolejeunea* sensu Schuster (1970) and Tixier (1985). Our attempt to refine the present classification is hampered by the extensive morphological homoplasy observed in the genus (Fig. 1). However, the recovered phylogenetic hypothesis mirrors geographical differentiation with one mainly Neotropical and one Paleotropical main clade, the latter subdivided in Australasian, Asian and Afromadagascan lineages (Fig. 2). We propose to assign the name *D.* subgen. *Physolejeunea* to the Paleotropical main clade I, and to use the names *D.* subgen. *Austrolejeuneopsis* and *D*. subgen. *Diplasiolejeunea* for the predominantly Neotropical clades IIa and IIb (Fig. 1). These assignments reflect the relationships of the type species of each clade, *D. plicatiloba* nested in clade I, *D. alata* nested in clade IIa, and *D. pellucida* nested in clade IIb. Subgen. *Physolejeunea* achieves good bootstrap support only in the ML analysis; subgen. *Austrolejeuneopsis* is unsupported in both the MP and ML analyses. However, the lack of support for subgen. *Austrolejeuneopsis* is caused by the “long branch” taxon *D. alata*; exclusion of this species leads to a MP bootstrap percentage value of 75 for this clade. The long-branch of *Diplasiolejeunea alata* is not caused by a large inversion or related structural evolution event but appears to be the result of higher mutation rates than average rate of *Diplasiolejeunea*. The long branch has been found in both, the nuclear and the chloroplast datasets (data not shown).

Diplasiolejeunea s.str. includes predominantly epiphyllous species whereas epiphytic occurrences are more typical for subgen. *Austrolejeuneopsis*. The different subclades of clades I, IIa, and IIb differ by certain character state combinations or tendencies, e.g., inflexed leaf margins in combination with strongly inflated lobules and ovate, acute underleaves in clade II-a1 (Fig. 1), and can be assigned to sections *Physolejeunea* (Ia), *Cornutae* (Ib), *Austrolejeuneopsis* (IIa2) and *Diplasiolejeunea* (IIb2). New sections for subclades Ib, IIa3, IIb1 will be established elsewhere.

4.2. *Species classification*

In this study, we explore the support of species that are defined using a morphological–typological approach. We sample multiple accessions of morphologically circumscribed species and explore their consistence with the molecular data using monophyly as the core criterion. The majority of currently accepted species concepts, e.g. the different forms of phylogenetic species concepts and the unified species concept (de Queiroz, 2007), consider reciprocal monophyly as the explicit criterion or a property of species. Incongruence of morphological taxa with DNA sequence based phylogenetic results is considered as evidence for limitations of species concepts relying exclusively on the diagnostics of morphological features. We consider here only three criteria, diagnostic morphology, biogeographic consistency, and reciprocal monophyly, in the absence of studies on the reproductive biology. However, we need to stress out the recovered congruence between phylogenetic hypothesis obtained from DNA sequences of either the chloroplast or the nuclear markers. These results may be valued as indicators of reproductive isolation.

Based on the phylogeny shown in Fig. 2 we propose several refinements of the current *Diplasiolejeunea* classification including recognition of five new species that will be formally described in a separate paper. Only one of the five new species (Fig. 1, spec. nov. V) was tentatively identified as an undescribed entity prior to the molecular study based on its morphological characters. Four of the five new species were placed in currently accepted species prior to our molecular investigation, but in some cases weak morphological disparities were already recognized. New species I was originally identified as *D. pauckertii* but stands out among specimens identified as this species by very densely arranged, squarrose leaves. The new species II and III were identified as *D. cavifolia* but were collected at higher altitudes than *D. cavifolia* s.str. (Fig. 3). Morphologically they differ slightly in underleaf shape, general lack of T-like lobule teeth, gender distribution, and lack of gemmae. One new species (spec. nov. IV) resembles *D. caribea* but stands out by leaf lobes with a broad marginal border consisting of small, subquadrate to (short-)rectangular cells. The study of additional material will likely result in a better understanding of the morphological disparity among these species and allow to identify characters for specimen identification. However, morphology alone was not sufficient to recognize these phenotypes as independent biological entities, accentuating the importance of integrative, molecular-morphological studies to recognize the actual liverwort diversity (Dayrat, 2005, Will et al., 2005 and Heinrichs et al., 2009b).

Our data tend to support narrow species concepts in *Diplasiolejejeunea* but the example *D. inermis* contradicts this finding (Fig. 2). *Diplasiolejeunea inermis* was separated from *D. pellucida* by a free tooth on the margin of the lobule (incurved in *D. pellucida*; Tixier, 1985) and can usually be separated without much doubt. We chose typical forms of both taxa including two specimens from the same locality in Venezuela (Venezuela 1: *D. inermis*, 2: *D. pellucida*). These accessions produced identical sequences and form a robust monophyletic lineage within *D. pellucida* (Fig. 2). We furthermore observed morphological intermediates when checking larger specimen sets. Based on our phylogeny and the observed intermediate forms we regard *D. inermis* as a synonym of *D. pellucida*, and the direction of the outer lobule tooth subject to infraspecific variation. The situation is complicated by the presence of *D. caribea* in the *D. pellucida* clade. This taxon differs from *D. pellucida* by its T-shaped first (median) and a free second (apical) lobule tooth and is possibly a recent derivative of a local *D. pellucida* population. *Diplasiolejeunea malleiformis* closely resembles *D. pellucida* and is often treated as a variety of the latter (Schäfer-Verwimp and Reiner-Drehwald, 2009). However, West Indian accessions of *D. malleiformis* form an independent lineage (Figs. 1, 2). Incongruence of morphological and molecular variation is also seen in the *D. rudolphiana* / *D. unidentata* clade. *Diplasiolejeunea rudolphiana* and *D. unidentata* differ by the length and orientation of the lobule teeth, and sex distribution. However, both accessions of *D. unidentata* from Guadeloupe are nested in *D. rudolphiana* and placed sister to a *D. rudolphiana* accession from the same region. Inclusion of *D. unidentata* samples from other parts of the Neotropics is necessary to ellucidate the status of both taxa.

4.3. *Cryptic speciation*

There is increasing evidence for molecular variation without concordant morphological differentiation in liverworts. Morphologically cryptic biological entities have been observed in the genera *Conocephalum* (Odrzykoski and Szweykowski, 1991), *Frullania* (Ramaiya et. al., 2010), *Leptoscyphus* (Vanderpoorten et al., 2010b), *Marchesinia* (Heinrichs et al., 2009b), *Metzgeria* (Fuselier et al., 2009), *Pellia* (Odrzykoski et al., 1996), *Ptilidium* (Kreier et al., 2010) and others, and are also present in *Diplasiolejeunea*.

Diplasiolejeunea pauckertii is a narrowly circumscribed, montane species characterized by inrolled leaf lobules with obscurely toothed free margins, ocelli and gemmae. However, a few *D. pauckertii* accessions are placed outside the main lineage of this species (Fig. 2). The accessions Ecuador (I) and (II) come from lower altitudes than other *D. pauckertii* accessions of this species (Fig. 3) and could thus be indicative of morphologically cryptic speciation along altitudinal gradients. Another *D. pauckertii* morphotype forms a robust monophyletic lineage with *D. involuta* ssp. *andicola*.

Diplasiolejeunea pluridentata occurs in similar habitats as *D. pauckertii*. Two accessions of this species form a paraphyletic grade within the *D. pauckertii-involuta-erostrata* clade; another accession is placed sister to a clade with *D. pauckertii* and *D. involuta* ssp. *andicola*. It is still unclear if the grade holds when more accessions are included, and complete sequence stretches become available.

The pantropical species *D. cavifolia* is regarded to be a somewhat variable species (Schäfer-Verwimp and Reiner-Drehwald, 2009) including forms with strongly T-shaped lobule teeth (earlier recognized as *D. matoubae* and *D. zacatepecensis*) as well as forms without such teeth. This species forms a robust lineage with two main clades (Fig. 2); however, the different morphotypes are present in both. The two clades occur sympatrically in the West Indies, and can thus neither be separated using geographical nor morphological evidence. We consider *D. cavifolia* to be another example of morphologically cryptic speciation.

4.4. *Biogeography*

4.4.1 Geographical pattern and evolution along altitudinal gradients.

Both the MP and ML analyses (Fig. 1, Fig. 2 and Fig. 3) reveal a clear geographical pattern with a Paleotropical and a Neotropical main lineage. This clear pattern is surprising with regard to the high dispersal ability of spore plants (Wolf et al., 2001, van Zanten, 1978 and Vanderpoorten et al., 2010a), and to our knowlegde has not yet been documented for any other liverwort genus. The observed pattern could be indicative of Gondwanan vicariance, however, divergence time estimates suggest an origin of *Diplasiolejeunea* in the Oligocene (Wilson et al., 2007b), posterior to the Mesozoic breakup of the Southern landmass (McLoughlin, 2001). The main clades of *Diplasiolejeunea* seem to be the result of a few dispersal events in the early history of the genus, and subsequent local diversification. Mapping the altitudinal distribution and growth preferences of the investigated species on the molecular trees (Fig. 1 and Fig. 3) reveals ecological and geographical tendencies for the Neotropical main clades IIa (subgen. *Austrolejeuneopsis*) and IIb (subgen. *Diplasiolejeunea*). Subgenus *Diplasiolejeunea* includes species which grow predominantly on living leaves of angiosperms and ferns rather than on trunks and twigs. This preference for an epiphyllous growth is correlated with an occurrence in the lowlands and lower montane regions; however, a few exceptions prove the rule (*D. pocsii*, *D. spec*. nov. II and III). In contrast, subgen. *Austrolejeuneopsis* includes mostly montane or Andean species with a preference for epiphytic occurrences, in addition to a few epiphytes from lower altitudes. This pattern may sustain the hypothesis of a strong influence of the rise of the Northern and Central Andes on the assembly of this liverwort diversity by triggering the diversification of the Neotropical *D.* subg. *Austrolejeuneopsis* clade, perhaps by providing new habitats. Entrance of lowland and mid-elevation epiphytes into Andean cloud forests and subsequent speciation could explain the observed pattern, and has also been proposed for other neotropical plant groups including the fern *Serpocaulon* (Kreier et al., 2008) and Bromeliaceae (Givnish et al., 2004). In short, the recovered evidence indicates that the same processes influence the diversification of derived liverworts and angiosperms in the Neotropics (Pennington et al., 2010 and Antonelli and Sanmartin, 2011). Similar speculations cannot yet be made for the Paleotropical main clade since the regional sampling is still very limited, especially for continental Africa. Our attempts to extract DNA from numerous accessions from this region were unsuccessful with the exception of *D. villaumei*, of which three Kenyan and one Madagascan accession were studied (Fig. 2). The related sequences are very similar, and provide no evidence for a separation of East African Island and Continental African lineages as seen in *Plagiochila* sect. *Vagae*(Heinrichs et al., 2005b). The East African Islands have been regarded as a centre of diversification of *Diplasiolejeunea* (Tixier, 1985), and it would be worthwhile to carve out the relationships of the island and the mainland populations.

4.4.2 Intercontinental species ranges, dispersal and migration routes

Our study confirms a pantropical range of *D. cavifolia*. The considerable sequence similarities of the accessions forming the pantropical subclade (Fig. 2) indicate recent emergence and fast range expansions or occasional intercontinental gene exchange (Fig. 3). Pantropical ranges of liverworts are rather uncommon, and have otherwise been supported by molecular data only for *Frullania arecae* and *F. ericoides* (Hentschel et al., 2009).

According to morphological evidence (Zhu and So, 2001), *D. rudolphiana* is another pantropical species. We could include only accessions from the Neotropics and Madagascar in our sampling. These accessions form a robust monophyletic lineage together with accessions of *D. unidentata* (Fig. 2). The extensive sequence similarities of our *D. rudolphiana* accession from Guadeloupe and those from Madagascar may be more consistent with the hypothesis of recent long distance dispersal rather than the hypothesis of fragmentation of a broader range triggered by the climate cooling during the Neogene (Zachos et al., 2001). *Diplasiolejeunea cavifolia* and *D. rudolphiana* are nested in the Neotropical *Diplasiolejeunea* clade (Fig. 3), providing evidence for a Neotropical origin of these species and dispersal into the Paleotropics. The same direction of propagation has recently been proposed for several other liverwort species based on molecular phylogenies, e.g., for the tropical American–African *Herbertus juniperoideus* (Feldberg et al., 2007), *Plagiochila boryana* and *P. corrugata* (Heinrichs et al., 2005b). *Diplasiolejeunea cobrensis* is the third pantropical species within this genus, however, we were only able to sequence accessions from Madagascar and Mayotte; an accession from Brazil (Schäfer-Verwimp 8730b) did not yield PCR products. Although it would be desirable to test the range of this species with molecular data, we can tentatively reconstruct an Afromadagascan origin based on its presence in the Afromadagascan *Diplasiolejeunea* clade.

Diplasiolejeunea includes narrow endemics such as the Indonesian *D. ingekarolae* and widespread "tramp" species occurring on one to several continents. A similar situation is present in many spore plant lineages, and has been demonstrated, e.g., for the moss genus *Daltonia* with the pantropical species *D. marginata* and *D. splachnoides* (Yu et al., 2010), and the fern *Nephrolepis* with the pantropical species *N. biserrata* (Hennequin et al., 2010).

Germination experiments provided evidence for a higher survival rate of spores of widespread liverworts under conditions required to be successfully dispersed in the trade wind zones (van Zanten and Gradstein, 1988), even though the physiological characteristics of such spores are still largely unknown (Löbel and Rydin, 2010). Germination experiments have not yet been conducted in *Diplasiolejeunea*, hence we cannot explain the different ranges by characters of the spores. Many liverworts are capable of vegetative reproduction through propagules or unspecific gametophyte fragments. Vegetative reproduction by propagules has generally been accepted as an important dispersal mode for epiphytic bryophytes (van Zanten and Pócs, 1981), at least over short distances (Löbel and Rydin, 2010), and has been documented for the majority of *Diplasiolejeunea* species (Fig. 1, character 8). It is thinkable that these dispersal entities also contribute to the formation of disjunct ranges (Pohjamo et al., 2006). The pantropical species *D. cavifolia* and *D. rudolphiana* seem to have a broader ecological amplitude than other species of this genus, and occur not only in moist forests but also in more mesic woodlands, plantations and orchards (Gradstein et al., 2001). This broad amplitude together with an assumed tolerance of diaspores against drought and frost could explain the success of these species and their pantropical ranges. All three pantropical species of *Diplasiolejeunea* occur at rather low altitudes; however, this is not a general phenomenon for intercontinentally distributed liverworts. *Plagiochila boryana* is an example of a tropical American–African species occurring at high altitudes.

4.4.3 Range formation and diversification

Our study points to geographically restricted clades in *Diplasiolejeunea*, as well as to clades consisting of species groups with similar ecological and altitudinal preferences. This pattern is obviously the result of occasional long distance dispersal, frequent dispersal over short distances, local diversification, and perhaps also niche conservatism. It is also likely that extinction and recolonization processes contributed to the formation of current ranges. This combination of processes obviously shaped many liverwort ranges; examples come from the genera *Bryopteris* (Hartmann et al., 2006), *Herbertus* (Feldberg et al., 2007), *Plagiochila* (Heinrichs et al., 2006), *Syzygiella* (Feldberg et al., 2010) and others.

The observed patterns contradict a general panmixis hypothesis as well as Crum‘s (1972) hypothesis of bryophytes being “unmoving, unchanging sphinxes of the past” (Crum, 1972). Instead, they are commemorative of biogeographical patterns in angiosperms (Renner, 2004 and Bartish et al., 2011), although bryophyte species tend to have larger ranges. However, already Shaw (2001) pointed out that morphological uniformity of bryophytes belies complex underlying genetic and phylogenetic patterns. Since then, numerous molecular studies confirmed this hypothesis (Stech and Wagner, 2005, Grundmann et al., 2007, Shaw et al., 2008, and Ramaiya et al., 2010), accentuating that our understanding of speciation processes in bryophytes is still limited. In this regard, *Diplasiolejeunea* confirms the rule.

4.4.4. Perspectives

The present study is the first comprehensive molecular phylogenetic investigation of a largely epiphyllous genus of liverworts. Despite their diminutiveness and the small size of many vouchers, we were able to include about fifty percent of the expected *Diplasiolejeunea* species diversity (Schäfer-Verwimp, 2006). Hence our topologies allow for a reclassification of the genus, and provide a framework for forthcoming monographic studies. Presently molecular studies of *Diplasiolejeunea* are hampered by the limited number of herbarium specimens suitable for DNA extraction. Future fieldwork will thus be essential to complete the phylogeny of *Diplasiolejeunea*; and to further elucidate the relationships in several critical complexes such as the andine *D. pauckertii* group and the *D. pellucida-caribea* complex.

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Table 1. Taxa used in the present study, including information about the origin of the studied material, voucher information, and the herbarium where the voucher is deposited, as well as GenBank accession numbers. Sequences in bold were obtained from GenBank. Herbarium acronyms follow Holmgren et al. (1990).

Taxon	Origin and altitude	Collector and herbarium	GenBank accession number		
			nrITS	*trn*L-F	*rbc*L
Cololejeunea vitalana	Costa Rica	Schäfer-Verwimp & Holz SV/H-0473/A (GOET)	**DQ987348**	**DQ238573**	**DQ238564**
Colura acroloba	Fiji Isls.	Pócs 02361/BK (EGR)	**DQ987306**	**DQ238586**	**DQ238565**
Diplasiolejeunea alata	Costa Rica, Cartago, 2460 m	Schäfer-Verwimp & Holz SV/H-0422 (GOET)	JQ729355	JQ729579	JQ729471
D. alata	Ecuador (I), Pichincha, 1980 m	Schäfer-Verwimp et al. 24116/A (GOET)	JQ729353	JQ729577	JQ729469
D. alata	Ecuador (II), Zamora-Chinchipe, 2750–2780 m	Schäfer-Verwimp & Preussing 23311 (GOET)	JQ729354	JQ729578	JQ729470
D. alata	Panama, Chiriqui, 1740 m	Schäfer-Verwimp et al. 30956 (GOET)	JQ729352	JQ729576	JQ729468
D. andringitrae	Madagascar (I), Toamasina Prov., 1080–1214 m	Pócs et al. 9890/CT (GOET, Paratype)	JQ729338	JQ729561	JQ729453
D. andringitrae	Madagascar (II), Andringitra Mts., 750–1000 m	Pócs et al. 9472/AC (EGR, Paratype)	JQ729339	JQ729562	JQ729454
D. armatiloba	Guadeloupe, Basse Terre, 950 m	Schäfer-Verwimp & Verwimp 22580/C (GOET)	JQ729388	JQ729613	JQ729505
D. borhidiana	Dominican	Schäfer-Verwimp &	JQ729391	JQ729616	JQ729508

	Republic, Santiago, 930 m	Verwimp 27214/C (GOET)			
D. brunnea	Bolivia, Cochabamba, 195 m	Abrahamczyk 29013 (GOET)	JQ729400	JQ729624	–
D. brunnea	Brazil, Mato Grosso, 320 m	Schäfer-Verwimp 11380 (GOET)	JQ729402	JQ729626	JQ729517
D. brunnea	Dominican Rep., Santiago, 930 m	Schäfer-Verwimp & Verwimp 27206/A (GOET)	JQ729399	JQ729623	JQ729515
D. brunnea	Ecuador (I), Zamora-Chinchipe, 2002 m	Schäfer-Verwimp & Nebel 31968/A (GOET)	JQ729406	JQ729630	JQ729520
D. brunnea	Ecuador (II), Napo, 400–450 m	Schäfer-Verwimp & Nebel 31704 (GOET)	JQ729405	JQ729629	JQ729519
D. brunnea	Ecuador (III), Zamora-Chinchipe, 1860 m	Schäfer-Verwimp & Preussing 23237/A (GOET)	JQ729401	JQ729625	JQ729516
D. caribea	Guadeloupe (I), Basse Terre, 450 m	Schäfer-Verwimp & Verwimp 22287/A (GOET)	JQ729374	JQ729598	JQ729491
D. caribea	Guadeloupe (II), Basse Terre, 600 m	Schäfer-Verwimp & Verwimp 22133/B (GOET)	JQ729378	JQ729602	JQ729495
D. caribea	Guadeloupe (III), Basse Terre, 950 m	Schäfer-Verwimp & Verwimp 22580/A (GOET)	JQ729379	JQ729603	JQ729496
D. caribea	Guadeloupe (IV), Basse Terre, 615 m	Schäfer-Verwimp & Verwimp 22172/A (GOET)	JQ729380	JQ729604	JQ729497
D. cavifolia	Brazil, Santa Catarina, 3 m	Schäfer-Verwimp & Verwimp 13598	JQ729415	JQ729638	–

		(GOET)			
D. cavifolia	Dominica, M. Trois Pitons, 1000 m	Schäfer-Verwimp & Verwimp 17812 (GOET)	JQ729422	JQ729645	–
D. cavifolia	Dominican Rep. (I), Mons. Noel, 955 m	Pócs & Pócs 03145/M (EGR)	JQ729420	JQ729643	JQ729533
D. cavifolia	Dominican Rep. (II), Santiago, 930 m	Schäfer-Verwimp & Verwimp 27214/A (GOET)	JQ729416	JQ729639	JQ729529
D. cavifolia	Ecuador, Tungurahua, 1540 m	Schäfer-Verwimp et al. 24241/B (GOET)	JQ729417	JQ729640	JQ729530
D. cavifolia	Fiji Isls., Central Viti Levu, 800 m	Pócs 08035/H (EGR)	JQ729413	JQ729636	JQ729527
D. cavifolia	Guadeloupe (I), Basse Terre, 780 m	Schäfer-Verwimp & Verwimp 22402/E (GOET)	JQ729421	JQ729644	JQ729534
D. cavifolia	Guadeloupe (II), Basse Terre, 743 m	Schäfer-Verwimp & Verwimp 22137/B (GOET)	JQ729423	JQ729646	JQ729535
D. cavifolia	Guadeloupe (III), Basse Terre, 900 m	Schäfer-Verwimp & Verwimp 22597 (GOET)	JQ729419	JQ729642	JQ729532
D. cavifolia	Indonesia, Sumatra, 1580 m	Schäfer-Verwimp & Verwimp 24923/B (GOET)	JQ729410	JQ729633	JQ729524
D. cavifolia	Madagascar (I), Andasive, 995 m	Pócs & Zsabo 9484/AE (EGR)	JQ729412	JQ729635	JQ729526
D. cavifolia	Madagascar (II), Fianarantsoa Prov., 865–1015 m	Pócs & Pócs 04118/CB (EGR)	JQ729414	JQ729637	JQ729528
D. cavifolia	Malaysia (I),	Schäfer-Verwimp &	JQ729411	JQ729634	JQ729525

	Pahang, 1280 m	Verwimp 18584/B (GOET)			
D. cavifolia	Malaysia (II), Perak, 1340 m	Schäfer-Verwimp & Verwimp 19036/A (GOET)	JQ729418	JQ729641	JQ729531
D. cavifolia	Panama, Prov. Panama, 970 m	Schäfer-Verwimp & Verwimp 30738 (GOET)	JQ729424	JQ729647	–
D. cavifolia	Réunion, Piton de la Fournaise, 1030 m	Schäfer-Verwimp 20385 (GOET)	JQ729407	JQ729631	JQ729521
D. cavifolia	Venezuela (I), Bolivar, 1370 m	Pócs et al. 0225EA (EGR)	JQ729409	JQ729632	JQ729523
D. cavifolia	Venezuela (II), Bolivar, 1370 m	Pócs et al. 0225EA-1 (JE)	JQ729408	–	JQ729522
D. cobrensis	Madagascar, Toamasina, 4 m	Pócs et al. 9882/AE (GOET)	JQ729337	–	JQ729452
D. cobrensis	Mayotte Isl, Poroani, 500 m	Pócs et al. 05092/R (EGR)	JQ729336	JQ729560	JQ729451
D. cornuta	Réunion (I), Cirque de Salazie, 1800 m	Schäfer-Verwimp et al. 20012 (GOET)	JQ729341	JQ729564	JQ729456
D. cornuta	Réunion (II), Gîte de Bélouve, 1520 m	Pócs 08063/E (EGR)	JQ729342	JQ729565	JQ729457
D. cubensis	Guadeloupe (I), Basse Terre, 950 m	Schäfer-Verwimp & Verwimp 22580/B (GOET)	JQ729392	JQ729617	JQ729509
D. cubensis	Guadeloupe (II), Basse Terre, 1100 m	Schäfer-Verwimp & Verwimp 22388/C (GOET)	JQ729393	JQ729618	JQ729510
D. eggersii	Costa Rica, San José, 1600 m	Schäfer-Verwimp & Holz SV/H-0238	JQ729368	JQ729591	JQ729484

		(GOET)			
D. eggersii	Panama, Chiriqui, 1670 m	Schäfer-Verwimp & Verwimp 30933 (GOET)	JQ729367	JQ729590	JQ729483
D. erostrata	Ecuador (I), Loja, 2970 m	Schäfer-Verwimp 31769/B-1 (GOET)	JQ729426	JQ729649	JQ729537
D. erostrata	Ecuador (II), Loja, 2970 m	Schäfer-Verwimp & Nebel 31769 (GOET)	JQ729427	JQ729650	JQ729538
D. erostrata	Ecuador (III), Zamora-Chinchipe, 2740 m	Schäfer-Verwimp 23335 (GOET)	–	JQ729651	JQ729539
D. ingekarolae	Indonesia, Sumatra, 1520 m	Schäfer-Verwimp & Verwimp 24906/B (GOET, isotype)	JQ729335	JQ729559	JQ729450
D. involuta ssp. *andicola*	Ecuador (I), Loja, 3050 m	Schäfer-Verwimp & Preussing 23288 (GOET)	JQ729442	JQ729665	JQ729551
D. involuta ssp. *andicola*	Ecuador (II), Zamora-Chinchipe, 2860 m	Schäfer-Verwimp & Preussing 23508 (GOET)	**DQ987288**	**DQ238582**	**AY548096**
D. involuta ssp. *involuta*	Costa Rica (I), San José, 3400 m	Schäfer-Verwimp & Holz SV/H-0147/A (GOET)	JQ729438	JQ729661	JQ729548
D. involuta ssp. *involuta*	Costa Rica (II), Cartago, 2500 m	Morales & Blanco 24/2 (GOET)	JQ729441	JQ729664	JQ729550
D. involuta ssp. *involuta*	Dominican Rep., Cordillera Central, 2170–2200 m	Schäfer-Verwimp & Verwimp 26680/B (GOET)	JQ729437	JQ729660	JQ729547
D. involuta ssp. *involuta*	Ecuador, Zamora-Chinchipe, 2650 m	Mandl B6 (GOET)	JQ729439	JQ729662	JQ729670
D. involuta ssp. *involuta*	Panama, Bocas del Toro, 3200 m	De Gracia et al. 376 (GOET)	JQ729440	JQ729663	JQ729549

D. johnsonii	Dominican Rep., La Vega, 1945 m	Pócs & Pócs 03150/T (EGR)	JQ729364	JQ729587	JQ729480
D. johnsonii	Ecuador, Zamora-Chinchipe, 1060 m	Schäfer-Verwimp & Preussing 23426/A (GOET)	JQ729363	JQ729586	JQ729479
D. jovet-astiae	Malaysia (I), Pahang, 1640 m	Schäfer-Verwimp & Verwimp 18902/B (GOET)	JQ729672	JQ729556	JQ729447
D. jovet-astiae	Malaysia (II), Pahang, 1300 m	Schäfer-Verwimp & Verwimp 18620/C (GOET)	JQ729333	JQ729557	JQ729448
D. kraussiana	Madagascar, Fianarantsoa Prov., 1700–1750 m	Pócs et al.04115/CA (GOET)	JQ729348	JQ729572	JQ729463
D. leiocarpa	Guadeloupe (I), Basse Terre, 1170 m	Schäfer-Verwimp & Verwimp 22384 (GOET)	JQ729386	JQ729610	–
D. leiocarpa	Guadeloupe (II), Basse Terre, 1160 m	Schäfer-Verwimp & Verwimp 22385 (GOET)	JQ729387	JQ729611	JQ729503
D. leiocarpa	Guadeloupe (III), Basse Terre, 670 m	Schäfer-Verwimp & Verwimp 22143 (GOET)	JQ729674	JQ729612	JQ729504
D. malleiformis	Dominica, 450 m	Schäfer-Verwimp 17784/B (GOET)	JQ729397	JQ729622	–
D. malleiformis	Guadeloupe (I), Basse Terre, 400 m	Schäfer-Verwimp & Verwimp 22496 (GOET)	JQ729394	JQ729619	JQ729511
D. malleiformis	Guadeloupe (II), Basse Terre, 650 m	Schäfer-Verwimp & Verwimp 22300/C (GOET)	JQ729395	JQ729620	JQ729512
D. malleiformis	Guadeloupe (III),	Schäfer-Verwimp &	JQ729396	JQ729621	JQ729513

	Basse Terre, 615 m	Verwimp 22172/B (GOET)			
D. malleiformis	Guadeloupe (IV), Basse Terre, 700 m	Schäfer-Verwimp & Verwimp 22328 (GOET)	JQ729398	–	JQ729514
D. ornata	Madagascar, Tomasina Prov., 220–300 m	Pócs & Szabó 9878/F (GOET)	JQ729340	JQ729563	JQ729455
D. papilionacea	Ecuador, Napo, 1970 m	Schäfer-Verwimp et al. 24212/A (GOET)	JQ729362	JQ729585	JQ729478
D. patelligera	Malaysia, Pahang, 1460 m	Schäfer-Verwimp & Verwimp 18802/A (GOET)	JQ729334	JQ729558	JQ729449
D. pauckertii	Brazil, Rio de Janeiro, 2340 m	Schäfer-Verwimp & Verwimp 15022 (GOET)	JQ729432	JQ729655	JQ729544
D. pauckertii	Costa Rica, Cartago, 2460 m	Schäfer-Verwimp & Holz SV/H-0418 (GOET)	JQ729434	JQ729657	–
D. pauckertii	Ecuador (I), Zamora-Chinchipe, 1880 m	Schäfer-Verwimp & Preussing 23219/B (GOET)	JQ729435	JQ729658	JQ729545
D. pauckertii	Ecuador (II), Zamora-Chinchipe, 1860 m	Schäfer-Verwimp & Preussing 23528/A (GOET)	JQ729436	JQ729659	JQ729546
D. pauckertii	Ecuador (III), Zamora-Chinchipe, 2500 m	Mandl A7 (GOET)	JQ729433	JQ729656	–
D. pauckertii	Ecuador (IV), Carchi, 3700 m	Schäfer-Verwimp et al. 24383/D (GOET)	JQ729428	JQ729669	JQ729540
D. pauckertii	Panama (I), Bocas del Toro, 3335 m	De Gracia et al. 338 (GOET)	JQ729429	JQ729652	JQ729541

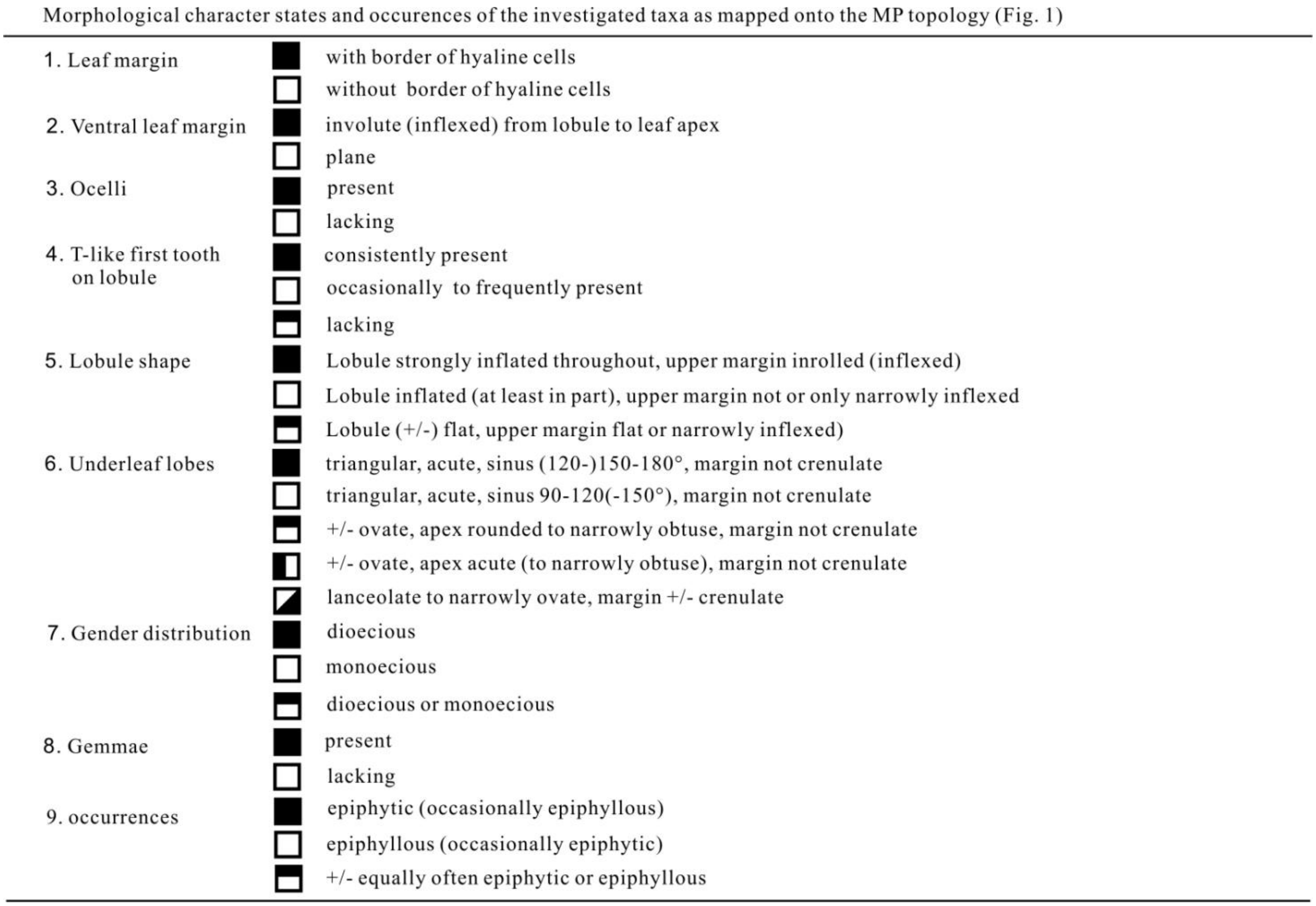
D. pauckertii	Panama (II), Chiriqui, 2600 m	Schäfer-Verwimp & Verwimp 30921 (GOET)	JQ729430	JQ729653	JQ729542
D. pauckertii	Panama (III), Chiriqui, 2600 m	Schäfer-Verwimp & Verwimp 30908 (GOET)	JQ729431	JQ729654	JQ729543
D. pellucida	Brazil, São Paulo, 570 m	Schäfer-Verwimp & Verwimp 11234/A (GOET)	JQ729376	JQ729600	JQ729493
D. pellucida	Ecuador, Pichincha, 1380 m	Schäfer-Verwimp et al. 24134 (GOET)	JQ729383	JQ729607	JQ729500
D. pellucida	French Guiana, Kaw Mts., 300 m	Holz FG00–24 (GOET)	JQ729382	JQ729606	JQ729499
D. pellucida	Panama, Chiriqui, 1670 m	Schäfer-Verwimp & Verwimp 30936/A (GOET)	JQ729384	JQ729608	JQ729501
D. pellucida	Venezuela (I), Bolivar, 1340 m	Pócs et al. 00227/CO (EGR)	JQ729377	JQ729601	JQ729494
D. pellucida	Venezuela (II), Bolivar, 1340 m	Pócs et al. 00227/CG (EGR)	JQ729385	JQ729609	JQ729502
D. plicatiloba	Australia, Tasmania, 750 m	Pócs & Pócs 0057/R (EGR)	JQ729332	JQ729555	–
D. plicatiloba	New Zealand, South Isl., 750–850 m	Schäfer-Verwimp & Verwimp 14246 (GOET)	JQ729671	JQ729554	JQ729446
D. pluridentata	Costa Rica, San José, 3400 m	Schäfer-Verwimp & Holz SV/H-0147/B (GOET)	JQ729445	JQ729668	JQ729553
D. pluridentata	Ecuador, Zamora-Chinchipe, 2220 m	Schäfer-Verwimp & Preussing 23366/A (GOET)	JQ729444	JQ729667	JQ729552
D. pluridentata	Ecuador, Zamora-	Sauer & Gradstein	JQ729443	JQ729666	–

	Chinchipe, 2200 m	MS-E-143 (GOET)			
D. pocsii	Ecuador (I), Zamora-Chinchipe, 2550 m	Schäfer-Verwimp & Nebel 32080 (GOET)	JQ729389	JQ729614	JQ729506
D. pocsii	Ecuador (II), Zamora-Chinchipe, 2780 m	Schäfer-Verwimp & Preussing 23320 (GOET)	JQ729390	JQ729615	JQ729507
D. ranomafanae	Madagascar, Fianarantsoa Prov., 1080–1160 m	Pócs 04129/Y, Paratype (GOET)	JQ729343	JQ729566	JQ729458
D. replicata	Brazil, Rio de Janeiro, 2420 m	Schäfer-Verwimp & Verwimp 15032 (GOET)	JQ729361	JQ729584	JQ729477
D. replicata	Costa Rica, San José, 2100 m	Schäfer-Verwimp & Holz SV/H-0246/C (GOET)	JQ729356	JQ729580	JQ729472
D. replicata	Ecuador (I), Pichincha, 2300 m	Schäfer-Verwimp et al. 24523 (GOET)	JQ729357	JQ729581	JQ729473
D. replicata	Ecuador (II), Zamora-Chinchipe, 2900 m	Schäfer-Verwimp & Preussing 23496/B (GOET)	JQ729360	JQ729583	JQ729476
D. replicata	Panama, Chiriqui, 1880 m	Schäfer-Verwimp & Verwimp 30983 (GOET)	JQ729359	JQ729582	JQ729475
D. replicata	Venezuela, Mérida, 3090–3200 m	Léon & Pócs 9707/BG (EGR)	JQ729358	–	JQ729474
D. rudolphiana	Brazil, São Paulo, 150 m	Lüth 3416 (GOET)	JQ729373	JQ729597	JQ729490
D. rudolphiana	Dominican Rep., Santiago, 930 m	Schäfer-Verwimp & Verwimp 27214/B (GOET)	JQ729371	JQ729595	JQ729488
D. rudolphiana	Guadeloupe,	Schäfer-Verwimp &	JQ729370	JQ729594	JQ729487

	Basse Terre, 180 m	Verwimp 22512/B (GOET)			
D. rudolphiana	Madagascar (I), Toamasina Prov., 10 m	Pócs & Szabo 9887/P (GOET)	–	JQ729592	JQ729485
D. rudolphiana	Madagascar (II), Toamasina Prov., 4 m	Pócs et al. 9882/AE (pp.) (EGR)	JQ729372	JQ729596	JQ729489
D. rudolphiana	Panama, Veraguas, 850 m	Schäfer-Verwimp & Verwimp 30843 (GOET)	JQ729369	JQ729593	JQ729486
D. unidentata	Guadeloupe (I), Basse Terre, 400 m	Schäfer-Verwimp & Verwimp 22500/A (GOET)	JQ729365	JQ729588	JQ729481
D. unidentata	Guadeloupe (II), Basse Terre, 615 m	Schäfer-Verwimp & Verwimp 22168/A (GOET)	JQ729366	JQ729589	JQ729482
D. villaumei	Kenya (I), Coast Prov., 1980–2210 m	Pócs et al. 04042/AP (EGR)	JQ729349	JQ729573	JQ729464
D. villaumei	Kenya (II), Coast Prov., 1980–2210 m	Pócs et al. 04042/Q (EGR)	JQ729350	JQ729575	JQ729466
D. villaumei	Kenya (III) Coast Prov., 1980–2210 m	Pócs et al. 04042/QR (EGR)	JQ729351	–	JQ729467
D. villaumei	Madagascar, Prov. Antananarivo, 1500 m	Pócs et al. 9851/Z (EGR)	–	JQ729574	JQ729465
D. zakiae	Madagascar (I), NE Andasive, 1000 m	Pócs & Szabó 9486/K (GOET)	JQ729345	JQ729568	–

D. zakiae	Madagascar (II), NE Andasive, 1000 m	Vojtkó & Pócs 9486/S (EGR)	JQ729673	JQ729570	JQ729461
D. zakiae	Madagascar (III), Toamasina Prov., 900–970 m	Pócs et al. 9889/BA (GOET)	JQ729344	JQ729567	JQ729459
D. zakiae	Madagascar (IV), NE Andasibe, 995 m	Vojtkó 9484/AA (EGR)	JQ729346	JQ729569	JQ729460
D. zakiae	Madagascar (V), NE Andasibe, 995 m	Pócs & Szabó 9484/AF (EGR)	JQ729347	JQ729571	JQ729462
D. spec. nov. I	Ecuador, Zamora-Chinchipe, 2460 m	Schäfer-Verwimp & Preussing 23476/A (GOET)	JQ729425	JQ729648	JQ729536
D. spec. nov. II	Ecuador, Loja, 2850 m	Schäfer-Verwimp & Nebel 31834/A (GOET)	JQ729404	JQ729628	JQ729518
D. spec. nov. III	Costa Rica, San José, 2330 m	Schäfer-Verwimp & Holz SV/H-0473/B (GOET)	JQ729403	JQ729627	–
D. spec. nov. IV	Ecuador, Zamora-Chinchipe, 2180 m	Schäfer-Verwimp & Preussing 23364/A (GOET)	JQ729381	JQ729605	JQ729498
D. spec. nov. V	Ecuador, Zamora-Chinchipe, 850 m	Schäfer-Verwimp & Nebel 32094 (GOET)			

Table 2. Morphological character states and ecological preferences of the investigated taxa as mapped onto the MP topology (Fig. 1).



Morphological character states and occurences of the investigated taxa as mapped onto the MP topology (Fig. 1)

1. Leaf margin	■	with border of hyaline cells
	□	without border of hyaline cells
2. Ventral leaf margin	■	involute (inflexed) from lobule to leaf apex
	□	plane
3. Ocelli	■	present
	□	lacking
4. T-like first tooth on lobule	■	consistently present
	□	occasionally to frequently present
	⬒	lacking
5. Lobule shape	■	Lobule strongly inflated throughout, upper margin inrolled (inflexed)
	□	Lobule inflated (at least in part), upper margin not or only narrowly inflexed
	⬒	Lobule (+/-) flat, upper margin flat or narrowly inflexed)
6. Underleaf lobes	■	triangular, acute, sinus (120-)150-180°, margin not crenulate
	□	triangular, acute, sinus 90-120(-150°), margin not crenulate
	⬒	+/- ovate, apex rounded to narrowly obtuse, margin not crenulate
	◧	+/- ovate, apex acute (to narrowly obtuse), margin not crenulate
	◪	lanceolate to narrowly ovate, margin +/- crenulate
7. Gender distribution	■	dioecious
	□	monoecious
	⬒	dioecious or monoecious
8. Gemmae	■	present
	□	lacking
9. occurrences	■	epiphytic (occasionally epiphyllous)
	□	epiphyllous (occasionally epiphytic)
	⬒	+/- equally often epiphytic or epiphyllous

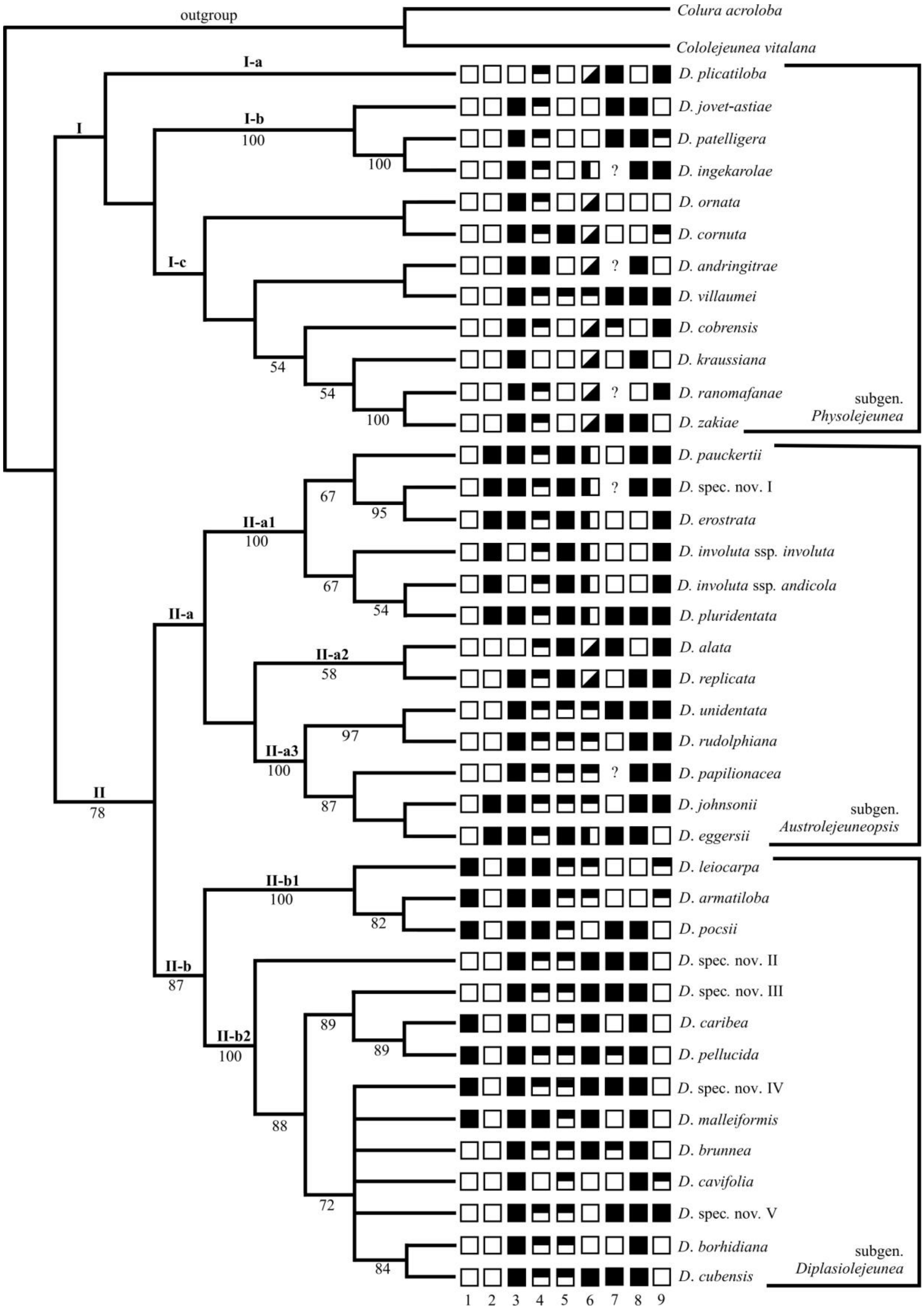


Fig. 1. Rooted strict consensus of 4 most parsimonious trees recovered during heuristic searches of the reduced combined dataset. Bootstrap percentage values >50 are indicated at branches. Morphological character states and ecological preferences (Table2) are mapped on to the tree.

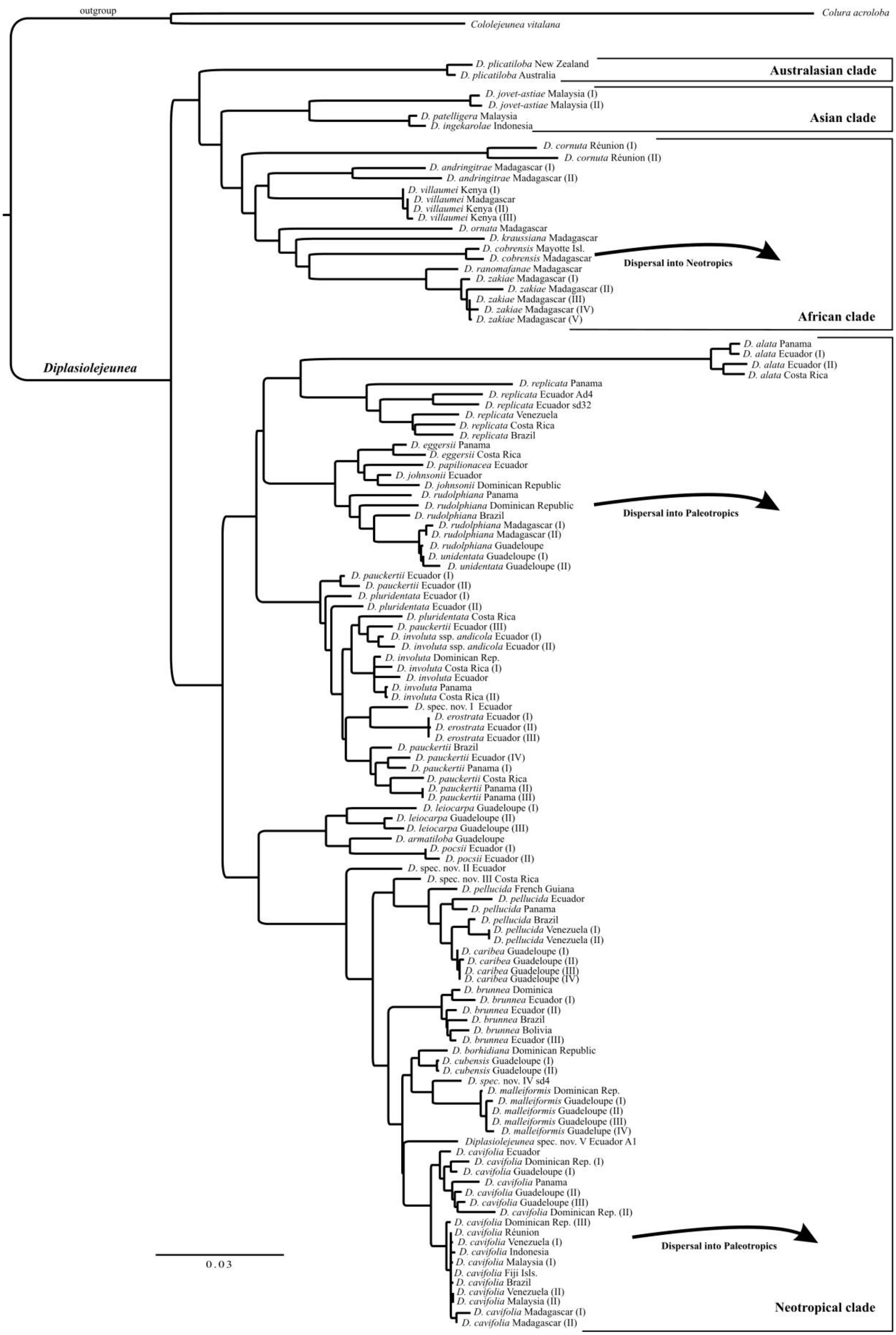


Fig. 2. A most likely phylogram resulting from maximum likelihood analysis of the combined comprehensive dataset. Bootstrap percentage values >50 are indicated at branches. A star indicates Bayesian support of 0.95–1.00.

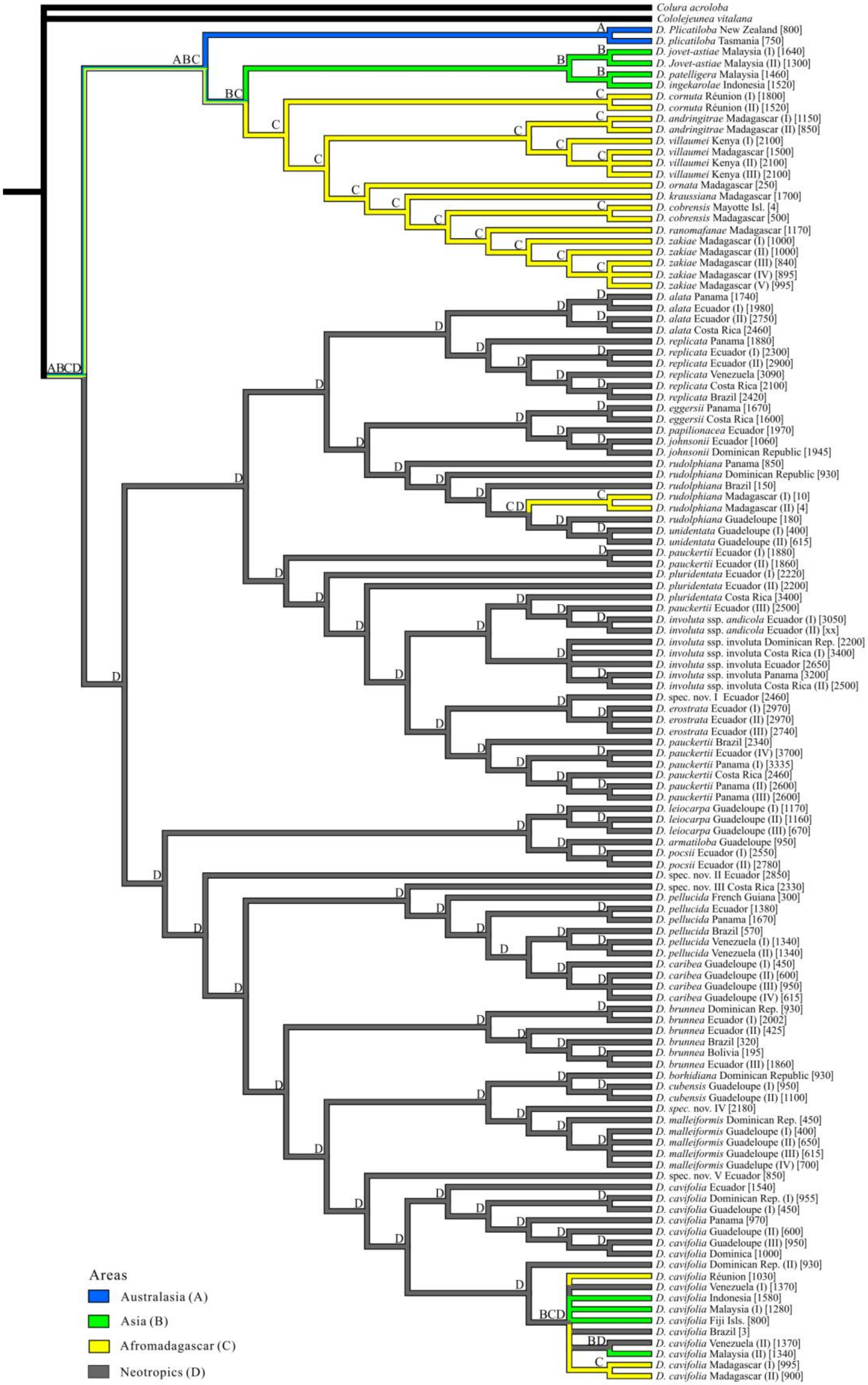


Fig. 3. Maximum likelihood topology of *Diplasiolejeunea*. Branch colors correspond to the most parsimonious reconstruction of distributions of *Diplasiolejeunea* and letters on the node show the reconstructed ancestral distribution. Altitudes of collections sites are indicated in square brackets.

Appendix 2

Molecular phylogeny of the leafy liverwort *Lejeunea* (Porellales): Evidence for a Neotropical origin, uneven distribution of sexual systems and insufficient taxonomy

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Abstract

Background: *Lejeunea* is a largely epiphytic, subcosmopolitan liverwort genus with a complex taxonomic history. Species circumscriptions and their relationships are subject to controversy, biogeographic history and diversification through time are largely unknown.

Methodology and Results: We employed sequences of two chloroplast regions (*trn*L-*trnF*, *rbc*L) and the nuclear ribosomal ITS region of 332 accessions to explore the phylogeny of the *Harpalejeunea-Lejeunea-Microlejeunea* complex. *Lejeunea* forms a well-supported clade that splits into two main lineages corresponding to *L*. subg. *Lejeunea* and *L*. subg. *Crossotolejeunea*. Neotropical accessions dominate early diverging lineages of both main clades of *Lejeunea.* This pattern suggests an origin in the Neotropics followed by several colonizations from the Neotropics into the Paleotropics and vice versa. Most Afromadagascan clades are related to Asian clades. Several temperate *Lejeunea* radiations were detected. Eighty two of the 91 investigated *Lejeunea* species could be identified to species level. Of these 82 species, 54 were represented by multiple accessions (25 para- or polyphyletic, 29 monophyletic). Twenty nine of the 36 investigated species of *L.* subg. *Lejeunea* were monoecious and 7 dioecious. Within *L.* subg. *Crossotolejeunea*, 15 of the 46 investigated species were monoecious and 31 dioecious. Some dioecious as well as some monoecious species have disjunct ranges.

Conclusions/Significance: We present the first global phylogeny of *Lejeunea* and the first example of a Neotropical origin of a Pantropical liverwort genus. Furthermore, we provide evidence for the Neotropics as a cradle of *Lejeunea* lineages and detect post-colonization radiations in Asia, Australasia, Afromadagascar and Europe. Dioecy/monoecy shifts are likely non-randomly distributed. The presented phylogeny points to the need of integrative taxonomical studies to clarify many *Lejeunea* binomials. Most importantly, it provides a framework for future studies on the diversification of this lineage in space and time, especially in the context of sexual systems in Lejeuneaceae.

Key words: biogeography, *Harpalejeunea***,** Lejeuneaceae, *Microlejeunea*, reproductive biology, taxonomy

Introduction

The taxonomic history of the leafy liverwort *Lejeunea* Lib. is best characterized as a story of controversial opinions on species delimitation and assumed relationships. Libert [1] described the genus based on only two species, *Lejeunea calcarea* Lib. [nowadays treated as *Cololejeunea calcarea* (Lib.) Schiffn.] and *Lejeunea serpyllifolia* Lib., the latter being a synonym of *Lejeunea cavifolia* (Ehrh.) Lindb. [2,3]. Soon after Libert's publication, the genus became widely recognized and numerous new species were described [4]. Until the end of the 19th century, the number of *Lejeunea* species exceeded one thousand [5] but early authors applied a wider genus concept than is accepted today. A good example in this regard is the treatment of Spruce [6] who classified *Lejeunea* in 39 subgenera. The majority of these subgenera was elevated to genus rank by Schiffner [7]. Subsequently, further new genera were introduced consisting of former *Lejeunea* species [e.g., 8-10]. As a consequence, *Lejeunea* species sensu Spruce [6] were placed in some 60 different genera [11].

Recent taxonomic and/or molecular phylogenetic studies of Lejeuneaceae led to a considerable reduction of genera [12-18]. This trend becomes particularly apparent in *Lejeunea* since more than a dozen generic names were recently lowered to synonyms of this genus [15,18,19]. *Lejeunea* is currently circumscribed by long-inserted leaves, divided or undivided underleaves, leaf lobules with an unreduced first tooth and a marginal hyaline papilla, small, segmented or homogeneous oil bodies, lack of ocelli, lejeuneoid innovations, unwinged female bracts and inflated perianths with 0-5 smooth or toothed wings [17,20]. *Lejeunea* is recognized for its morphological disparity. Diversification time estimates indicated an origin of *Lejeunea* in the early Cenozoic [21-23]. The genus has its centre of diversity in the humid tropics where the species usually grow as epiphytes on stems, branches, twigs and leaves of a large number of cormophytes but also on rock [24]. Although the vast number of species occur exclusively in tropical climates, the genus is also well represented in temperate regions with a humid climate [25,26].

According to recent estimates the species diversity of *Lejeunea* may exceed three hundred [27], however, the precise number of species is still unclear due to the limited availability of modern revisionary studies [13,28-30]. According to current knowledge, *Lejeunea* includes narrow endemics [29] as well as intercontinentally distributed species such as the subcosmopolitan *Lejeunea flava* (Sw.) Nees [31]. Intercontinental ranges have been accepted for many liverwort species due to an extensive morphological overlap of remote populations and the production of spores and propagules suitable for long-distance dispersal [32-34], although molecular phylogenetic studies incorporating multiple accessions of morphologically-typologically circumscribed liverwort species usually demonstrate a considerable genetic variation and a structure that is related to spatial ranges rather than to morphological disparities [35-40]. These studies also demonstrated the para- or polyphyly of many morphologically circumscribed liverwort species [36,41,42].

The objective of this study is to reconstruct the first comprehensive phylogeny of *Lejeunea* using chloroplast and nuclear DNA markers. This phylogenetic framework is used to reconstruct the origin of the genus and infer evidence, which supports dispersal between the Neotropics and the Paleotropics [40], respectively the hypothesis of a tropical origin of the extant temperate species diversity [22,43]. In addition, we infer the evolution of reproductive systems with the focus on monoicy and dioicy in the evolution of *Lejeunea*. Finally, we test current morphological-typological species concepts by including multiple accessions and examine whether the recovered phylogenetic relationships correspond to/or conflict with morphologically circumscribed taxa.

Results

Phylogeny - Reduced dataset

The reduced dataset comprised one accession per ingroup species (specimens identified only to genus level excluded). Of a total of 2,351 character sites, 725 were parsimony informative, 248 unique to a single accession and 1,378 constant. The maximum parsimony (MP) analysis resulted in 4,578 most parsimonious trees with the features: length of 4,016 steps, consistency index of 0.38, and retention index of 0.69 (Figure 1). Bayesian inference of phylogeny and maximum likelihood (ML) analyses recovered consensus trees respectively optimal trees that were highly similar in their topologies to each other as well as to the MP tree. The four representatives of *Harpalejeunea* (Spruce) Schiffn. formed a clade that was placed sister to a clade comprising two clades of which one included eight *Microlejeunea* Steph. species whereas the other one was composed by 82 *Lejeunea* species. The monophyly of *Lejeunea* achieves bootstrap percentage values (BPVs) of 99 or 100 % and a Bayesian Posterior Probability (BPP) of 1.00 (Figure 1). The *Lejeunea* clade consisted of two main lineages corresponding to *Lejeunea* subg. *Lejeunea* (BPV MP 100 %, ML 100 %, BPP p = 1.00) and *L*. subg. *Crossotolejeunea* Spruce (BPV MP 82 % ML 97 %, BPP p = 1.00). In past classification, the investigated *Lejeunea* species were alternatively placed in 32 different genera, with up to 6 different treatments per species (Figure 1). *Lejeunea* species previously treated as *Taxilejeunea* (Spruce) Schiffn. were diffusely distributed and nested in most *Lejeunea* clades. Elements of *Crossotolejeunea* (Spruce) Schiffn. were found in both main lineages of *Lejeunea*. Twenty nine of the 36 investigated representatives of *Lejeunea* subg. *Lejeunea* were monoicous (81 %) and 7 (19 %) dioicous (Figure 1). Within *Lejeunea* subg. *Crossotolejeunea*, 15 of the 46 investigated species were monoicous (33 %) and 31 dioicous (67 %). Ancestral character reconstruction recovered dioicy as the likely ancestral state of *Lepidolejeunea* R.M.Schust., *Harpalejeunea*, and *Microlejeunea*, whereas the ancestral state of *Lejeunea* was found to be equivocal in maximum parsimony reconstructions. In maximum likelihood reconstructions, dioicy was found to be ancestral with a probability of 0.75 versus a probability of 0.25 for monoicy. The ancestral state of *L*. subg. *Lejeunea* was either resolved as equivocal (50 % of most parsimonious trees) or monoicous (50 % of most parsimonious trees). ML reconstructions recovered a probability of monoicy of 0.71. Similarly, the ancestral state of subg. *Crossotolejeunea* was found to be equivocal in all most parsimonious trees but showed a probability of 0.77 to be dioicous (Table 1).

Phylogeny - Large dataset

The large dataset consisted of 2,351 character sites (909 parsimony informative, 1,212 constant). The MP analysis resulted in more than 350,000 equally parsimonious trees with a length of 6,427 steps, a consistency index of 0.30 and a retention index of 0.83 (not depicted).

The ML phylogeny based on the large dataset is shown in Figure S1. A condensed version without species labeling is depicted in Figure 2. The *Lejeunea* clade was pruned and split in three parts, which are depicted in Figure 3, with BPPs and ML/MP BPVs indicated at branches. The phylogeny was consistent to the topology derived from the reduced dataset albeit without good ML BPV for *Lejeunea* subg. *Crossotolejeunea* (ML BPV = 65 %). Out of the 82 *Lejeunea* species with reliable species identification, 54 were represented by multiple accessions. Twenty five of these 54 *Lejeunea* species were resolved as para- or polyphyletic, whereas 29 were monophyletic. Intercontinental ranges of several *Lejeunea* species were confirmed.

Biogeography

The most parsimonious reconstruction of ancestral areas of distribution based on the large dataset (Figures 2, 3 A-C) indicated a Neotropical origin of *Lejeunea* as well as of its subgenera *Crossotolejeunea* and *Lejeunea*. The S-Diva reconstruction generated from the reduced dataset suggested two scenarios. In scenario one both subgenera originated in the Neotropics, whereas in the other scenario two alternative solutions were found for *L*. subg. *Crossotolejeunea* (Figure 4). In the second scenario, *L*. subg. *Crossotolejeunea* originated in an area comprising the Neotropics but also Europe plus Macaronesia and North Africa. African and Asian accessions were found to be nested in derived lineages. *Lejeunea* subg. *Crossotolejeunea* comprised a species rich radiation in Afro-Madagascar, Africa, and Asia that likely originated from a single colonization of the Paleotropics from the Neotropics. Each four clades of *Lejeunea* were recovered with occurrences in Australasia or North America respectively, five clades with occurrences in Macaronesia and Atlantic Europe, and seven clades with occurrences in temperate/subtropical Asia (Figure 3). The subcosmopolitan *L. flava* complex nested in an African lineage. Accessions from Gough Island were resolved in Neotropical lineages; accessions from Easter Island in tropical Asian clades. The African-Neotropical *L. trinitensis* Lindenb. & Gottsche nested in a Neotropical clade; the Neotropical *L. adpressa* Nees in a clade dominated by Asian accessions. North American accessions of *L. lamacerina* (Steph.) Schiffn. are placed sister to European/Macaronesian accessions.

Discussion

Supraspecific classification

Recent molecular phylogenetic studies identified a monophylum with representatives of *Harpalejeunea*, *Microlejeunea* and *Lejeunea* [17]. Furthermore, a recent report showed that the putatively allied genus *Bromeliophila* R.M.Schust. [20] forms a sister relationship with *Prionolejeunea* (Spruce) Schiffn. rather than nesting in *Lejeunea* [44]. Morphologically, *Lejeunea* differs from the former two genera by a lack of ocelli [the sole representative of *Lejeunea* with ocelli, *L. huctumalcensis* Lindenb. & Gottsche, belongs to another main lineage of Lejeuneaceae (Czumay et al., unpublished)]. The monophyly of *Harpalejeunea*, *Microlejeunea* and *Lejeunea* is confirmed in our study, with *Microlejeunea* placed sister to *Lejeunea*.

Lejeunea has been classified in some 50 subgenera of which 17 are still accepted as part of *Lejeunea*. These subgenera are usually defined by one or a few morphological character states, and their recognition and circumscription is subject to controversy. A good example is *Lejeunea* subg. *Taxilejeunea* which was alternatively treated as separate genus *Taxilejeunea*, and as such accepted by several recent authors [9,12,45,46], although the morphology of both genera is largely overlapping [12]. This situation is reflected in our phylogeny, with *Taxilejeunea* elements in nearly all lineages of *Lejeunea* (Figure 1). The problematic circumscription of *Lejeunea* taxa is also reflected in the alternative placement of the 82 identified species of our study in 32 different genera of Lejeuneaceae (Figure 1), with one species in up to six genera (*Lejeunea apiculata* Sande Lac.). *Lejeunea* splits into two main clades with heterogeneous morphology. One includes the generitype *L. serpyllifolia* (= *L. cavifolia*) and the types of three further subgenera; the other clade comprises types of four different subgenera, the oldest available subgenus name being *L.* subg. *Crossotolejeunea* Spruce (type: *L. boryana* Mont.) (Figure 1). *Lejeunea* subg. *Crossotolejeunea* was proposed for monoicous species with decurved and acuminate leaf apices, and 5-keeled perianths with denticulate and fimbriate keels [6]. A few years later, *Crossotolejeunea* was raised to generic rank [7]. However, *Crossotolejeunea* was synonymized with *Lejeunea* because the diagnostic character combinations were found to be inconsistent among species considered to belong to *Crossotolejeunea* [13]. The polyphyly of *Crossotolejeunea* as circumscribed by Spruce [6] is confirmed in the presented study by recovering *Crossotolejeunea* representatives in both main clades of *Lejeunea* (Figure 1). However, the presence of the type species *L. boryana* in the second main clade allows the assignment of *L*. subg. *Crossotolejeunea*. Incongruence of morphology-based classifications and molecular phylogenies was reported for a rapidly increasing number of genera of liverworts such as *Athalamia* Falc. [47], *Cololejeunea* (Spruce) Schiffn. [48], *Diplasiolejeunea* (Spruce) Schiffn. [40], *Frullania* Raddi [49], *Plagiochila* (Dumort.) Dumort. [50], *Porella* L. [51], *Radula* Dumort. [52], *Scapania* (Dumort.) Dumort. [53], *Syzygiella* Spruce [54], and *Telaranea* Schiffn. [55]. Together, these studies clarified the phylogeny of these liverworts and provided the foundation to introduce new classifications using holophyly as the main criterion [40,52,56-58]. Unfortunately, many of these newly circumscribed taxa lack obvious morphological diagnostic characters hampering assignments of species to these clades using solely morphology.

In this study we propose to assign the two main *Lejeunea* clades to *Lejeunea* subg. *Crossotolejeunea* and *Lejeunea* subg. *Lejeunea* but hesitate to establish further supraspecific entities. In our opinion, it is premature to introduce a comprehensive classification of the two subgenera into sections since our *Lejeunea* sampling is still rather incomplete in the context of taxonomic sampling. In addition, further studies are required to explore the morphological features of species recovered in well supported clades. A good example in this regard is the morphological treatment of *L. pulverulenta* (Gottsche ex Steph.) M.E.Reiner [46]. In this study, *L. pulverulenta* was assumed to be aligned with *L. controversa* Gottsche and *L. cerina* (Lehm. & Lindenb.) Gottsche et al. based on morphological similarities, e.g. the papillose leaf cells with trigones and intermediate cell wall thickenings. A sister relationship of *L. pulverulenta* and *L. controversa* (*L*. subg. *Crossotolejeunea*) is confirmed (Figure 1) but *L. cerina* is found to be nested in *Lejeunea* subg. *Lejeunea* instead of *L*. subg. *Crossotolejeunea*.

The morphology of many *Lejeunea* species has not yet been exhaustively studied and our knowledge is often restricted to descriptions of the gross morphology of the gametophyte. Schuster [9,31] repeatedly pointed to the taxonomical value of character states visible only in living plants, namely the oil bodies, and the sporophytes. Only recently it was shown that the rough surface of *Lejeunea* species is not necessarily caused by papillae but can also result from the production of surface waxes [59]. We need comprehensive morphological datasets of gametophytes and sporophytes besides expansion of molecular datasets to establish a hierarchical classification of *Lejeunea* into subgenera and sections. These data will also demonstrate whether clades share certain morphologies or can only be defined by DNA sequence evidence.

Circumscription of species

The present study addressed the reliability of current morphological-typological species concepts in *Lejeunea* by sampling multiple accessions of several currently accepted species. In the absence of studies on speciation processes and the maintenance of species borders in *Lejeunea*, we consider three criteria - diagnostic morphology, biogeographic consistency, and reciprocal monophyly - as the most reliable procedure to identify putative species [60]. Congruence between the phylogenies derived from either the nuclear or the chloroplast markers is interpreted as evidence for reproductive isolation. Hence we regard incongruence of morphologically circumscribed taxa with molecular phylogenies as evidence for the limitations of our current morphology-based classification. However, integration of molecular and exhaustive morphological data allows often but not always for a reconsideration of morphological features considered to be of diagnostic importance and result in modified species circumscriptions [e.g., 61-64]. These short-term solutions are practical and helpful despite the amount of efforts required. In addition, they may allow to recognize the extent of the failure of current taxonomic practice.

Multiple accessions of 29 *Lejeunea* species formed monophyletic lineages but 25 species proved to be para- or polyphyletic (Figure 3 A-C). The ratio of nearly 50% rejection of currently accepted species is remarkable and requires further using of more comprehensive datasets and analyses. These datasets may expand not only the number of accessions studied per species but also explore the genetic diversity by employing markers that will allow a more comprehensive study of the genotypic distinction such as ISSRs, AFLPs, and SNPs. Exhaustive studies with such marker-systems hold special promises for lineages with a low clade diversity such as the *Lejeunea cavifolia* – *L.*

eckloniana Lindenb. – *L. holtii* Spruce-complex. The high number of non-monophyletic *Lejeunea*-species indicates that our current morphology-based classification does not adequately consider the possible presence of morphologically cryptic or semicryptic entities, and local endemism [38,62,65]. Some studies reported evidence for rather limited morphological variation among *Lejeunea* species and thus morphologically similar plants may be placed in different main clades of *Lejeunea*. A good example is the *Lejeunea tumida* Mitt. complex whose representatives are placed in both main clades of *Lejeunea* although they were earlier treated as a single species [30,42]. This observation is consistent with the results available for other genera of Lejeuneaceae, namely *Marchesinia* Gray [36], *Ptychanthus* Nees [66], *Mastigolejeunea* (Spruce) Schiffn. and *Thysananthus* Lindenb. [67]. All these studies suggest that we currently underestimate Lejeuneaceae species diversity. Examples supporting this notion are reported here with *Lejeunea flava* and *L. laetevirens* Nees & Mont., which may in fact represent complexes including several independent entities. *Lejeunea flava* has been studied exhaustively using morphological evidence and several subspecies or segregates have been proposed [10,31,68,69]. However, we were not able to adopt these taxonomical concepts for our phylogeny (Figure 3 C) although we could recognize some morphological tendencies and found the morphologically well separated species *L. acuta* Mitt. and *L. tuberculosa* Steph. nested in the *L. flava* clade. The *L. laetevirens* complex is similarly problematic since our phylogeny indicates that several still unrecognized entities hide in *L. laetevirens* s.l.: A robust clade with Neotropical and Macaronesian accessions of *L. laetevirens* is placed sister to a Neotropical clade with *L. laetevirens* morphotypes as well as multiple accessions of *L. multidentata* M.E.Reiner & Mustelier and *L. ramulosa* (Herzog) R.M.Schust. The latter two species differ from *L. laetevirens* by dentate or acute leaves. *Lejeunea multidentata* was aligned with *L. boryana* Mont. and *L. controversa* rather than with *L. laetevirens* based on shared dull appearance caused by strongly papillose cells [70,71], however, according to our phylogenies these species are not closely related. An extension of the sampling is necessary to revise the taxonomy of the *L. laetevirens* clade. The same holds true for the polyphyletic *L. anisophylla* Nees & Mont. and several other problematic binomials.

Dispersal biogeography

Liverworts produce spores and small propagules that are capable for distribution through air currents over larger distances [72,73]. However, population studies of liverworts generally show a spatial distribution of genetic diversity that does not correspond to a general panmixis hypothesis [74,75]. Thus, the current distribution of liverworts is not random and biogeographic studies frequently recover conserved biogeographic patterns that can be interpreted by considering the combination of processes such as occasional long distance dispersal, frequent dispersal over short distances, local extinction, and local diversification [76,77]. The reported distribution of *Lejeunea* suggests that this genus is not an exception and that conserved spatial patterns exist. Although the limited availability of lejeuneoid fossils prevents us from a detailed reconstruction of divergence times (the two Miocene fossils *Lejeunea* sp. [78] and *Lejeunea palaeomexicana* Grolle [79] cannot be assigned to any of our *Lejeunea* clades) an early Cenozoic origin of the genus can be assumed based on the existing estimates [21-23]. This time frame provides information about the position of the continents which is important in distinguishing between establishment via long-distance dispersal versus vicariance as the preferred explanation for the observed disjunct ranges. Dispersal over larger distances seems to occur only infrequently in *Lejeunea*, as is indicated by the clear geographical structure of disjunct species as well as multi-species clades. A good example is the *L. lamacerina* clade that splits into a North American and a European/Macaronesian lineage, without any evidence of recent geneflow. The unsatisfactory taxonomy of many other investigated clades hampers similar statements, however, the long branches in many morphologically circumscribed species and their para- or polyphyly provide evidence for local diversification/speciation. Evidence for lacking or restricted geneflow between distant liverwort populations has been demonstrated several times [74,80] and can also be concluded for *Lejeunea*. Local diversification subsequent to successful long-distance dispersal seems to dominate the evolutionary history of *Lejeunea*. Accordingly, the majority of the investigated *Lejeunea* species has regional distribution ranges but about 23 % of the identified species are more widespread and occur in at least two of our ten putative areas of endemism. Examples include the Neotropical-Macaronesian range of *L. laetevirens*, the Neotropical-Asian range of *L. trinitensis* Lindenb. & Gottsche (Figure 3 B) and the

African-Asian range of *L. anisophylla* (Figure 3 C).

Neotropical origin

The early diverging lineages of both main clades of *Lejeunea* occur predominantly in the Neotropics. Thus, our reconstructions revealed a Neotropical origin of *Lejeunea* with subsequent dispersal into other tropical as well as temperate regions. A Neotropical origin has been shown for several lineages of angiosperms, namely Burmanniaceae [81], Burseraceae [82], Gentianaceae [83] and Malpighiaceae [84]. It has also been discussed for the grammitid clades of polygrammoid ferns [85,86] and the Neotropical-African liverwort *Bryopteris* (Nees) Lindenb. [87] but has not yet been proposed for any subcosmopolitan liverwort genus based on molecular data. This is partly caused by the limited access to comprehensive phylogenies of species-rich liverwort genera [40,49,51-54,76,77,88]. The lejeuneoid genus *Diplasiolejeunea* shows a somewhat different pattern with a deep split into a Paleotropical and a Neotropical clade [40], but a few Pantropical species soften this otherwise strict separation by indicating occasional intercontinental dispersal events. In contrast to the pattern in *Diplasiolejeunea* both main clades of *Lejeunea* show a more even representation of putative regions of endemism, indicating that long distance dispersal is more frequent in *Lejeunea* than in *Diplasiolejeunea* as long as we assume similar ages for both genera.

Our topologies point to several dispersal events from the Neotropics into Africa (*L. trinitensis*, *L. phyllobola* Nees & Mont.). This pattern is not uncommon in leafy liverworts and has been recovered for *Herbertus juniperoideus* (Sw.) Grolle [77], *Marchesinia brachiata* (Sw.) Schiffn. [36], *Plagiochila boryana* Steph. [56] and the genus *Bryopteris* [87]. The subcosmopolitan *L. flava* complex appears to have originated in Africa and subsequently colonized large parts of the tropics and adjacent regions, with several dispersal events between the Old and the New World. This pattern of older spatial separations followed by young inter-continental dispersals was reported for a few plants such as the fern genus *Nephrolepis* Schott [89] and the pantropical liverwort *Plagiochila* sect. *Vagae* Lindenb. [56]. Our phylogenies support close relationships of African and Asian *Lejeunea* floras, however, the Neotropical *L. adpressa* is of Paleotropical, most likely Asian, origin (Figure 3 C). *Lejeunea*-accessions from the Polynesian Easter Island are likewise related to Asian clades whereas the *Lejeunea* accessions from Gough Island (Southern Atlantic Ocean) are nested in Neotropical lineages. A South American origin of Gough Island liverworts has already been demonstrated for the genus *Herbertus* [90]. The Macaronesian accessions of *L. laetevirens* are nested in a Neotropical clade, indicating dispersal from the Neotropics into Macaronesia. This pattern seems to be common in leafy liverworts and has also been reconstructed for species of *Plagiochila* [91] and *Leptoscyphus* Mitt. [92].

The tropics as a cradle and museum

Lejeunea has its centre of diversity in the humid lowlands and lower montane sites of the tropics; its diversity in temperate regions is considerably lower. This pattern is consistent with the widely recognized latitudinal biodiversity gradient [43,93-96]. Various hypotheses have been introduced to explain the origin of this gradient (see [43] for review) of which some involve the rather controversial concept of niche conservatism. So far, very little attention has been given to latitudinal biodiversity gradients in seed-free land plants, but is starting to be explored in ferns (see [97]) and here in the liverwort genus *Lejeunea*. In accordance with the general hypothesis of a latitudinal diversity gradient, *Lejeunea* includes only a few temperate lineages, which are in each case nested in tropical clades.

The pattern observed for *Lejeunea* appears to be consistent with the role of the tropics as a cradle and museum of diversity [98-100], and mirrors observations for the whole family Lejeuneaceae [22]. Liverwort families with a centre of diversity in the tropical highlands can show considerably different patterns and may have entered the tropics from temperate regions [76]. Interestingly, temperate species were not always found to possess a tropical sister species but evidence for several radiations in temperate regions were discovered, including two multi-species clades with occurrences in Australasia, one with occurrences in temperate Asia, and two with occurrences in Macaronesia and Atlantic Europe (Figs 3 A-C). The discovery of these clades provides opportunities to test some of the arguments concerning the origin of the latitudinal diversity gradient such as niche conservatism and different speciation rates [97,101]. The recovery of radiations in the temperate climate zones of Australasia resembles the recent report of a New Zealand radiation of grammitid ferns [102]. Grammitid ferns share with *Lejeunea* their origin in tropical regions and their preference to climates with high humidity. These examples may indicate the possibility of high speciation rates in temperate climates caused by ecological opportunities. The observed change in the climatic niche preferences is again consistent with reports in tree ferns growing in the wet temperate climates of Australasia [103].

Sexual systems in a largely epiphytic genus

About two third of liverworts are dioicous [104] whereupon the distribution of dioicous and monoicous species differs from genus to genus. The speciose genus *Plagiochila* is a prime example of a completely dioicous group whereas monoicous species dominate in *Cololejeunea* (Spruce) Schiffn., *Riccia* L. and *Riccardia* Gray [24,105]. The evolution of sexual systems has so far been studied for only two genera of liverworts using a phylogenetic framework: the largely epiphytic leafy liverworts *Radula* Dumort. and *Diplasiolejeunea* [40,106]. Only 16 of the ca. 200 *Radula* species are monoicous whereas monoicy and dioicy is more evenly distributed in *Diplasiolejeunea*. Single monoicous species of *Radula* were resolved in several otherwise dioicous clades, a similar supposedly random pattern was observed in *Diplasiolejeunea*. Monoicy in *Radula* was also not correlated with obligate epiphytism but occurred in facultative epiphytic lineages [106].

In *Lejeunea* we observed an uneven distribution of sexual systems (Figure 1). *Lejeunea* subg. *Lejeunea* is dominated by monoicous species whereas dioicous species dominate in *L*. subg. *Crossotolejeunea*. Similarly to the situation in *Radula*, some monoicous species clustered in clades dominated by dioicous species, in particular in *L*. subg. *Crossotolejeunea.* However, monoicous species are the most frequent in *L*. subg. *Lejeunea* and our character reconstruction (Table 1) recovered some indications for the transition from dioicy to monoicy in the early diversification of the genus. We also found evidence for a rather frequent change of the reproductive system during the history of the genus with a minimum number of character state changes: five times in *L*. subg. *Lejeunea* and nine times in *L*. subg. *Crossotolejeunea.*

Monoicous species are potentially capable to produce sporophytes through selffertilization. On one hand this may allow a more frequent establishment of new populations via long distance dispersal, but on the other hand this may result in invariable genotypes, accumulation of genetic load, and limited adaptation to new environments [105]. However, dioicy is not necessarily a barrier to regular sporophyte development. Many *Plagiochila* species frequently produce sporophytes as do at least some dioicous species of *Frullania* and *Porella* [50,105]. Thus, future studies need to explore the accumulation of genetic load, effective population size, and the temporal stability of habitats as factors that shape the evolution of reproductive systems in *Lejeunea*.

According to existing data, both dioicous and monoicous *Lejeunea* species are able to form disjunct ranges. However, disjunctions over large distances might not necessarily be the result of spore dispersal but could also be caused by vegetative reproduction through propagules. Vegetative reproduction plays an important role in the range formation of liverworts and enhances the chances of establishing in a new environment, especially for dioicous species. A dioicous long-distance disperser is trapped in a very small area unless it is able to colonize its new environment through vegetative distribution. Accordingly the likelihood of the arrival of spores of the other sex clearly increases with range expansion through vegetative distribution. However, *Lejeunea* includes only few species that frequently produce propagules [107], despite wide species distribution ranges. A further aspect may be variation in the extinction risks caused by the different sexual systems but very little evidence exists to evaluate this factor.

Schuster [31] emphasizes the importance of monoicy for species colonizing unstable epiphytic habitats but many *Lejeunea* species are dioicous. This trend is even more evident in the sister genus *Microlejeunea* which is nearly completely dioicous [24], despite its general preference for epiphytic habitats. The same applies to *Radula.* Devos et al. [106] speculate that dioicous epiphytes often distribute vegetatively, not only through specialised propagules but also through unspecialized gametophyte fragments, and that they are often not strictly depending on epiphytic environments. Kraichak [108] reinforces this argument by demonstrating a correlation of reproduction through asexual propagules and an epiphyllous mode of life in Lejeuneaceae.

Currently the importance of monoicy for an epiphytic mode of life and long distance dispersal is rather unclear since the available studies point to more complex interrelationships. Future studies should not only focus on an extension of the phylogenetic sampling and improvements of the underlying taxonomy but also on the ecological ranges of disjunct liverworts. Intercontinentally distributed *Diplasiolejeunea* species have broader ecological amplitudes compared to geographically more restricted species [40], allowing for the colonization of a larger number of environments and enhancing the chance of a permanent establishment. We also need comprehensive studies on the resistance of spores and vegetative propagules of liverworts against drought and frost and the ability of sporophyte production under suboptimal climatic conditions.

Perspectives

Lejeunea is a prime example to illustrate the current state of affairs in liverwort classification. After three centuries of morphology-based research a plethora of taxa have been proposed in this genus, of which only a small part has been included in modern revisions, reflecting the limited number of liverwort specialists dealing with these taxonomically difficult plants. Our molecular data add to growing evidence that not all biologically relevant entities can be detected using solely morphology, and that the acceptance of a considerable intraspecific morphological variation may lead to an underestimation of the actual number of biological species [109,110]. Thus, concepts considering cryptic and semi-cryptic species may provide more realistic estimates than the current practice. Based on our topology it is possible to identify species complexes that are not yet properly understood and that need to be studied using extended datasets. We urgently need molecular studies incorporating numerous accessions of morphologically circumscribed species from throughout their range. Only combined molecular-morphological studies will allow to understand range formation and to establish more natural species circumscriptions [111]. These studies will also facilitate estimates of the real number of biological species of liverworts. It is not unlikely that a portion of these species will not exhibit morphological disparities or can at best been identified using statistical methods and larger series of reference specimens [112]. In such a situation, reference sequences (= DNA barcodes) are the most promising approach to obtain reliable identifications of these plants [113]. However, the establishment of the DNA barcodes needs to go hand-in-hand with critical taxonomic revision of species-rich genera like *Lejeunea*. The reported phylogeny provides the framework enabling the design and management of these studies because the major task of taxonomic revisions can be separated in groups of species belonging to the same clade.

Materials and Methods

Taxon sampling and outgroup selection

Taxa studied, including GenBank accession numbers and voucher details, are listed in Table S1. Ingroup taxa were selected according to availability and to represent the morphological variation and geographical distribution of *Lejeunea*. Representatives of the sister genera *Harpalejeunea* and *Microlejeunea* [17] were included to test the *Lejeunea* genus concept. Multiple accessions of several species were used to explore intraspecific genetic variation. Representatives of *Lepidolejeunea* were selected as outgroup species based on the analyses of [14] and [17]. Altogether 332 accessions from the herbaria AK, DUKE, EGR, GOET, JE, L, or NSW were used for this study.

DNA extraction, PCR amplification and sequencing

Upper parts of a few gametophytes were isolated from herbarium specimens. Total genomic DNA was extracted using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: *rbc*L gene and *trn*L-F region from [114], and nrITS1-5.8S-ITS-2 region from [87]. Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing primers were those used for PCR. Newly generated sequences were assembled and edited using SeqAssem [115]. Seven hundred and nineteen sequences were newly generated for this study; 175 sequences were downloaded from Genbank.

Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 [116] resulting in a *rbc*L alignment with 895 positions, *trn*L-F 441 and an nrITS alignment with 1,015 putatively homologous sites. Ambiguous positions were excluded from all alignments and lacking data were coded as missing. Two datasets were compiled and analysed separately: dataset 1 (= large dataset) included all studied accessions, whereas dataset 2 (= reduced dataset) included only one accession per identified ingroup species. Accessions identified only to genus level were excluded from dataset 2. Phylogenetic trees based on the reduced dataset were used to visualize the current supraspecific classification of *Lejeunea* and to explore the evolution of monoicy/dioicy.

Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10 [117]. MP heuristic searches of the comprehensive and the reduced datasets were conducted with the following options implemented: heuristic search mode, 1,000 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Non-parametric bootstrapping values [118] were generated as heuristic searches with 1,000 replicates, each with ten random-addition replicates. The number of rearrangements was restricted to ten millions per replicate. Bootstrap percentage values (BPV) ≥ 70 were regarded as good support [119]. Where more than one most parsimonious tree was found, trees were summarized as strict consensus tree(s). The three genomic regions and the combined chloroplast DNA dataset vs nrITS dataset were first analysed separately to check for topological incongruence. The consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70 % [120]. The trees gave no evidence of incongruence. Accordingly, the datasets were combined.

The program jModeltest 0.1.1 [121] was used to select a best-fit model of sequence evolution for the maximum likelihood (ML) analyses of the each genomic region, using the Akaike information criterion. The following models were chosen for the respective data divisions: (*rbc*L) TPM1uf+I+G; (*trn*L-F) TVM+G and (nrITS) TIM3+G. A partitioned ML bootstrap analysis was conducted using the program Garli 2.0 [122]. The analysis was run until five million generations were completed without significant improvement (ln *L* increase of 0.01) to the topology. Node support was evaluated through 200 bootstrap replicates in which each repetition terminated after 100,000 generations were completed without topological improvements.

Bayesian inference was implemented in the program MrBayes 3.2 [123] allowing different models for each partition. Bayesian searches were carried out with four simultaneous Markov chains, ten million generations, and sampling every 1000th generation. The first 25% of trees were discarded as burn-in. Bayesian posterior probability (BPP) confidence values were generated from trees saved after this initial burn-in. Values were regarded as significant when BPP ≥ 0.95 [124].

Ancestral areas of distribution

Data on distribution ranges of the investigated taxa were obtained from the literature. Given the wide distribution ranges of some species, the putative distribution range of endemism was coded as covering ten possible areas: Neotropics, North America, Southern South America, Europe with North Atlantic Islands (e.g. Macaronesia) and North Africa (Africa north of the Sahara), Afro-Madagascar (sub-Saharan Africa, Madagascar, Mascarenes, Seychelles, and São Tomé), continental Asia (comprising temperate and subtropical regions), tropical Asia (including Melanesia and tropical Australia), temperate Australia and New Zealand, Tristan da Cunha Islands and Easter Island. Ancestral areas of distribution were reconstructed using two different approaches. The first approach was based on the large dataset and considers the presence of several unidentified species with unclear distribution ranges. To overcome this problem, the putative region of endemism (= the ten regions mentioned above, see also Fig. 2) of every accession was coded rather than the species range. Subsequently we reconstructed ancestral areas of distribution using MP criteria as implemented in Mesquite ver. 2.75 [125] based on the ML topology.

In the second approach we used dataset 2 including each one accession per identified species and a coding of the complete species range. Ancestral areas of distribution were reconstructed using S-DIVA [126] as implemented in RASP 2.0 based on 7,500 Bayesian trees from the reduced dataset.

Evolution of reproductive systems

The occurrence of dioicous/monoicous reproductive systems was scored by evaluating the information provided in the literature for each species included in dataset 2 [13,28,31,45,68,127-137]. In case both character states were indicated (*L. hibernica* Grolle, [131]), the species was scored as monoicous. These efforts resulted into a matrix of two character states without any polymorphic or unknown character states. To explore the evolution of this character, we used the results of the MP analyses of the reduced dataset. Maximum parsimony character reconstructions were carried out using Mesquite 2.75. The character states were plotted over all most parsimonious trees recovered in the MP analysis of the reduced dataset. Nodes absent from some of these trees were ignored. In addition, we carried out maximum likelihood analyses using the MK model [138] and the strict consensus tree obtained from the most parsimonious tree set.

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Table 1. Ancestral character reconstruction of dioecious/monoecious reproductive systems. The reconstruction is based on the reduced dataset using Maximum Parsimony (MP) and Maximum Likelihood (ML).

	MP Dioecy	MP Monoecy	ML Dioecy	ML Monoecy
Lepidolejeunea	yes	No	0.93	0.07
Harpalejeunea	yes	No	0.98	0.02
Microlejeunea	yes	No	0.93	0.07
Lejeunea	equivocal	equivocal	0.75	0.75
L. subg. *Lejeunea*	equivocal (50%) no (50%)	equivocal (50%) yes (50%)	0.29	0.71
L. subg. *Crossotolejeunea*	equivocal	equivocal	0.77	0.23
clade *L. lamacerina-L. grossitexta*	no	yes	0.01	0.99
clade *L. hibernica-L. oracola*	no	yes	0.29	0.71
clade *L. pallescens- L. topoensis*	no	yes	0.24	0.76
clade *L. catinulifera-L. oracola*	yes	No	0.75	0.25
clade *L. amaniensis-L. oracola*	no	yes	0.10	0.90
clade *L. asperrima-L. controversa*	equivocal	equivocal	0.40	0.60
clade *L. trinitensis-L. adpressa*	yes	No	0.92	0.08
clade *L. trinitensis-L. ruthei*	yes	No	0.96	0.04
clade *L.capensis-L. adpressa*	yes	No	0.97	0.03

Table S1. Taxa used in the present study. Information about the origin of the studied material, vouchers, as well as GenBank accession numbers is included. New sequences in bold face.

Taxon	*Origin*	*Collector*	*GenBank Accession No.*		
			rbcL	trnL-F	nrITS
Harpalejeunea. grandis Grolle & M.E.Reiner	Colombia	Cleef 6450 (GOET)	KC313144	KC313184	KC313106
H. grandistipula R.M.Schust.	Ecuador	Schäfer-Verwimp et al. 24163/B (GOET)	KC313145	KC313185	KC313107
H. marginalis (Hook.f. & Taylor) Steph.	Chile	Schäfer-Verwimp & Verwimp 8082 (GOET)	KC313147	KC313187	KC313109
H. molleri (Steph.) Grolle	Azores	Schäfer-Verwimp & Verwimp 29334 (GOET)	KC313148	KC313188	KC313110
Lejeunea acuta Mitt.	Comoros	Pócs et al. 9276/CX (EGR)	**KF556383**	**KF556133**	**KF556601**
L. acuta	Kenya	Chuah-Petiot Mb 22 (JE)	**KF556384**	**KF556134**	**KF555917**
L. adpressa Nees	Dominican Rep. (I)	Schäfer-Verwimp & Verwimp 26931/B (GOET)	**KF556386**	**KF556136**	**KF555919**
L. adpressa	Dominican Rep. (II)	Schäfer-Verwimp & Verwimp 27215/F (GOET)	**KF556387**	**KF556137**	**KF555920**
L. adpressa	Dominican Rep. (III)	Pócs & Pócs 03156/F (EGR)	**KF556385**	**KF556135**	**KF555918**
L. alata Gottsche	Malaysia	Schäfer-Verwimp & Verwimp 18912 (GOET)	-----------	**KF556140**	**KF555922**
L. amaniensis E.W.Jones	Ethiopia	Hylander 4589 (EGR)	**KF556393**	**KF556144**	**KF555925**
L. amaniensis	Kenya (I)	Malombe 5012/cau.x2 (EGR)	**KF556390**	**KF556141**	**KF555923**
L. amaniensis	Kenya (II)	Malombe & Chituyi 5006/Si.1aS5 (EGR)	**KF556392**	**KF556143**	**KF556603**
L. amaniensis	Kenya (III)	Malombe & Soita 5008/K.1cS5 (EGR)	**KF556391**	**KF556142**	**KF555924**
L. anisophylla Nees & Mont.	China	Koponen et al. 56008 (H)	AY125943	AY144484	AY125348
L. anisophylla	Fiji Isls.	Pócs & Pócs 03307/O (EGR)	**KF556397**	**KF556149**	**KF555929**
L. anisophylla	Indonesia	Schäfer-Verwimp & Verwimp 21131 (GOET)	**KF556398**	**KF556150**	**KF555930**
L. anisophylla	Japan	Yamaguchi 24461 (GOET)	**KF556400**	**KF556152**	**KF555931**

L. anisophylla	Kingdom of Tonga	Schäfer 18341 (GOET)	**KF556394**	**KF556145**	**KF555926**
L. anisophylla	Equatorial Guinea	Müller B312/B (EGR)	**KF556399**	**KF556151**	**KF556605**
L. anisophylla	São Tomé and Príncipe (I)	Shevock & Daniel 34368 (EGR)	**KF556401**	**KF556153**	**KF556606**
L. anisophylla	São Tomé and Príncipe (II)	Pócs & Pócs 34749 (EGR)	**KF556395**	**KF556146**	**KF555927**
L. anisophylla	Vietnam (I)	Pócs 02103/V (EGR)	**KF556396**	**KF556148**	**KF556604**
L. anisophylla	Vietnam (II)	Pócs 02105/J (EGR)	**KF556529**	**KF556303**	**KF556629**
L. anisophylla	Vietnam (III)	Pócs 02103/C (EGR)	-----------	**KF556147**	**KF555928**
L. apiculata Sande Lac.	Indonesia	Schäfer-Verwimp & Verwimp 20985 (GOET)	-----------	**KF556154**	**KF555932**
L. aquatica Horik.	Japan	Higuchi 1021 (JE)	-----------	**KF556155**	**KF555933**
L. asperrima Spruce	Panama	Schäfer-Verwimp & Verwimp 30817 (GOET)	**KF556402**	**KF556157**	**KF555935**
L. asperula (Steph.) Mizut.	Papua New Guinea	Streimann 40815 (JE)	-----------	**KF556156**	**KF555934**
L. bermudiana (A.Evans) R.M.Schust.	USA	Shaw 14939 (DUKE)	**KF556403**	**KF556158**	**KF555936**
L. boryana Mont.	Ecuador	Schäfer-Verwimp & Nebel 33218 (GOET)	**KF556404**	-----------	**KF555937**
L. boryana	French Guiana (I)	Hartmann et al. 04-054 (GOET)	-----------	**KF556160**	-----------
L. boryana	French Guiana (II)	Holz FG 00-0103 (GOET)	**KF556405**	**KF556159**	**KF555938**
L. cancellata Nees & Mont.	Costa Rica (I)	Schäfer-Verwimp & Holz SV/H-0507/C (GOET)	**KF556409**	**KF556164**	**KF555942**
L. cancellata	Costa Rica (II)	Schäfer-Verwimp & Holz SV/H-0374/A (GOET)	**KF556410**	**KF556165**	**KF556607**
L. cancellata	Dominican Rep.	Schäfer-Verwimp & Verwimp 27005 (GOET)	**KF556411**	**KF556166**	**KF556608**
L. cancellata	Panama	Schäfer-Verwimp & Verwimp 30850 (GOET)	**KF556408**	**KF556163**	**KF555941**
L. capensis Gottsche	Brazil	Schäfer-Verwimp & Verwimp 15057 (GOET)	-----------	**KF556167**	**KF555943**
L. catinulifera Spruce	Ecuador	Gradstein & Mandl 10141 (GOET)	DQ983688	DQ987411	DQ987307
L. catinulifera	Ecuador	Wilson et al. 04-01 (GOET)	DQ983687	DQ987432	DQ987328

L. catinulifera	Ecuador	Schäfer-Verwimp et al. 24259/A (GOET)	**KF556413**	-----------	**KF555945**
L. catinulifera	Ecuador	Schäfer-Verwimp et al. 24188 (GOET)	**KF556412**	**KF556168**	**KF555944**
L. catinulifera	Ecuador	Schäfer-Verwimp et al. 24248 (GOET)	**KF556414**	**KF556169**	**KF555946**
L. cavifolia (Ehrh.) Lindb.	Belgium	Heinrichs et al. 3816 (GOET)	**KF556419**	**KF556174**	**KF555950**
L. cavifolia	Finland	Ahonen s.n. (H)	AY125945	AY144486	AY125347
L. cavifolia	France	Schäfer-Verwimp & Verwimp 27247 (GOET)	**KF556421**	**KF556176**	**KF555952**
L. cavifolia	Georgia	Hentschel Bryo 04382 (JE)	**KF556418**	**KF556173**	**KF555949**
L. cavifolia	Germany (I)	Heinrichs 3695 (GOET)	AY548102	DQ238581	DQ987259
L. cavifolia	Germany (II)	Schäfer-Verwimp & Verwimp 28787 (GOET)	**KF556420**	**KF556175**	**KF555951**
L. cavifolia	Germany (III)	Schäfer-Verwimp & Verwimp 28806 (GOET)	**KF556415**	**KF556170**	**KF555947**
L. cavifolia	Greece (I)	Düll 1./10.5.2004 (JE)	**KF556417**	**KF556172**	**KF555948**
L. cavifolia	Greece (II)	Schäfer-Verwimp & Verwimp 15888 (GOET)	**KF556416**	**KF556171**	-----------
L. cerina (Lehm. & Lindenb.) Gottsche, Lindenb. & Nees	Costa Rica	Schäfer-Verwimp & Holz SV/H-0471 (GOET)	**KF556425**	**KF556180**	**KF555955**
L. cerina	Dominican Rep.	Schäfer-Verwimp & Verwimp 26978 (GOET)	-----------	**KF556182**	**KF555957**
L. cerina	Ecuador (I)	Schäfer-Verwimp & Nebel 32122 (GOET)	**KF556423**	**KF556178**	**KF555954**
L. cerina	Ecuador (II)	Wilson et al 04-02 (GOET)	DQ983686	DQ987433	DQ987329
L. cerina	Ecuador (III)	Wilson et al 04-13 (GOET)	DQ983689	DQ987441	DQ987339
L. cerina	Ecuador (IV)	Schäfer-Verwimp & Nebel 31942 (GOET)	**KF556422**	**KF556177**	**KF555953**
L. cerina	Ecuador (V)	Nöske & Holz 165 (GOET)	**KF556424**	**KF556179**	**KF556609**
L. cerina	Guadeloupe	Schäfer-Verwimp & Verwimp 22473 (GOET)	**KF556426**	**KF556181**	**KF555956**
L. cocoes Mitt.	Indonesia	Schäfer-Verwimp & Verwimp 21050 (GOET)	**KF556430**	**KF556186**	**KF555961**

L. cocoes	Solomon Isls.	Seaward 108088 (JE)	-----------	**KF556187**	**KF555962**
L. colensoana (Steph.) M.A.M.Renner	New Zealand (I)	Renner 300101 (AK)	-----------	JF308572	JF308543
L. colensoana	New Zealand (II)	Renner 300127a (AK)	-----------	JF308576	JF308547
L. colensoana	New Zealand (III)	Renner 300044 (AK)	-----------	JF308565	JF308536
L. colensoana	New Zealand (IV)	Renner 300130 (AK)	-----------	JF308577	JF308548
L. colensoana	New Zealand (V)	Renner 300028 (AK)	-----------	JF308562	JF308533
L. colensoana	New Zealand (VI)	Renner 300140 (AK)	-----------	JF308578	JF308549
L. colensoana	New Zealand (VII)	Renner 300104 (AK)	-----------	JF308573	JF308544
L. colensoana	New Zealand (VIII)	Renner 300103 (AK)	-----------	JF308574	JF308545
L. colensoana	New Zealand (IX)	Renner 300127 (AK)	-----------	JF308575	JF308546
L. colensoana	New Zealand (X)	Renner 300039 (AK)	-----------	JF308564	JF308535
L. colensoana	New Zealand (XI)	Renner 300030 (AK)	-----------	JF308563	JF308534
L. controversa Gottsche	Bolivia	Gradstein 7189 (JE)	-----------	**KF556190**	**KF556610**
L. controversa	Brazil	Vital 8794 (GOET)	**KF556431**	**KF556188**	**KF555963**
L. controversa	French Guiana (I)	Hartmann et al. 04-033 (GOET)	**KF556432**	**KF556189**	**KF555964**
L. controversa	French Guiana (II)	Hartmann et al. 04-092 (GOET)	**KF556434**	**KF556192**	**KF555965**
L. controversa	Guadeloupe	Schäfer-Verwimp & Verwimp 22196 (GOET)	**KF556433**	**KF556191**	-----------
L. cristulata (Steph.) M.E.Reiner & Goda	Brazil	Giancotti 17 (JE)	-----------	**KF556193**	**KF555966**
L. cuculliflora (Steph.) Mizut.	Fiji Isls. (I)	Pócs & Pócs 03286/L (EGR)	-----------	**KF556359**	**KF556110**
L. cuculliflora	Fiji Isls. (II)	Pócs & Pócs 03286/D (EGR)	**KF556435**	**KF556194**	**KF556611**
L. cuculliflora	Fiji Isls. (III)	Pócs & Pócs 03274/BL (EGR)	**KF556577**	**KF556358**	**KF556109**
L. curviloba Steph.	Bhutan	Long 10611 (JE)	-----------	**KF556195**	**KF555967**
L. cf. *curviloba*	Indonesia	Gradstein 10300 (GOET)	**KF556427**	**KF556183**	**KF555958**

L. debilis (Lehm. & Lindenb.) Nees & Mont.	Costa Rica (I)	Dauphin 1793 (GOET)	**KF556436**	**KF556196**	**KF555968**
L. debilis	Costa Rica (II)	Biasi et al. 21 (GOET)	**KF556438**	**KF556198**	**KF555970**
L. debilis	Costa Rica (III), La Gamba	Schluder 7 (GOET)	**KF556437**	**KF556197**	**KF555969**
L. deplanata Nees	Dominican Rep., San José de Ocoa	Schäfer-Verwimp & Verwimp 26636 (GOET)	**KF556442**	**KF556202**	**KF555974**
L. deplanata	Ecuador (I), Imbabura	Schäfer-Verwimp et al. 24260/A (GOET)	**KF556440**	**KF556200**	**KF555972**
L. deplanata	Ecuador (II), Pichincha	Schäfer-Verwimp et al. 24252/A (GOET)	**KF556441**	**KF556201**	**KF555973**
L. deplanata	Ecuador (III), Pichincha	Schäfer-Verwimp et al. 24502/C (GOET)	**KF556439**	**KF556199**	**KF555971**
L. discreta Lindenb.	Australia	Thiers & Halling 2219 (L)	**KF556444**	**KF556206**	**KF555977**
L. discreta	China	Zhu 89038 (JE)	-----------	**KF556204**	**KF555976**
L. discreta	Fiji Isls.	Pócs & Pócs 03289/CA (EGR)	**KF556443**	**KF556203**	**KF555975**
L. discreta	Indonesia	Gradstein 12032 (GOET)	-----------	**KF556205**	-----------
L. drehwaldii Heinrichs & Schäf.-Verw.	Peru	Drehwald 4384 (JE)	**KF556445**	**KF556207**	**KF555978**
L. drummondii Taylor	Australia (I)	Streimann 16698 (JE)	-----------	-----------	**KF555979**
L. drummondii	Australia (II)	Pamt 38AL (JE)	-----------	**KF556208**	**KF555980**
L. drummondii	Australia (III)	Jarman 91/4 (JE)	-----------	**KF556209**	**KF555981**
L. drummondii	Australia (IV)	Renner 872058 (NSW)	-----------	JF308584	JF308555
L. eckloniana Lindenb.	Azores	Schäfer-Verwimp & Verwimp 29528 (GOET)	**KF556448**	**KF556212**	**KF555984**
L. eckloniana	La Palma	Schäfer- Verwimp 24788 (GOET)	DQ983690	DQ987457	DQ987357
L. eckloniana	Madeira (I)	Stech 04-271 (L)	**KF556446**	**KF556210**	**KF555982**
L. eckloniana	Madeira (I)	Stech 04-433 (L)	**KF556447**	**KF556211**	**KF555983**
L. epiphylla Colenso	New Zealand	Renner 300056 (AK)	-----------	JF308568	JF308539
L. exilis (Reinw., Blume & Nees) Grolle	Australia	Renner 872056 (NSW)	-----------	JF308583	JF308554
L. exilis	Indonesia (I)	Schäfer-Verwimp & Verwimp 25231 (GOET)	**KF556449**	**KF556213**	**KF555985**

L. exilis	Indonesia (II)	Schäfer-Verwimp & Verwimp 24853 (GOET)	**KF556451**	**KF556215**	**KF555987**
L. exilis	Indonesia (III)	Schäfer-Verwimp & Verwimp 21080/B (GOET)	**KF556450**	**KF556214**	**KF555986**
L. exilis	Malaysia	Gradstein 10336 (GOET)	DQ983691	DQ987472	-----------
L. flava (Sw.) Nees	Brazil (I)	Gradstein (GOET)	**KF556480**	**KF556244**	**KF556010**
L. flava	Brazil (II)	Gradstein s.n. (GOET)	DQ983692	DQ987413	DQ987309
L. flava	La Palma	Schäfer-Verwimp & Verwimp 24780 (GOET)	DQ983693	-----------	DQ987363
L. flava	Tenerife	Drehwald & Drehwald 4121 (GOET)	**KF556478**	**KF556242**	**KF556008**
L. flava	Dominican Rep. (I)	Schäfer-Verwimp & Verwimp 26855/B (GOET)	**KF556479**	**KF556243**	**KF556009**
L. flava	Dominican Rep. (II)	Schäfer-Verwimp & Verwimp 27043 (GOET)	**KF556452**	**KF556216**	**KF556612**
L. flava	Easter Island	Ireland & Bellolio 30111 (JE)	**KF556469**	**KF556234**	**KF556001**
L. flava	Ecuador (I)	Schäfer-Verwimp & Preussing 23435/A (GOET)	**KF556454**	**KF556218**	**KF556613**
L. flava	Ecuador (II)	Schäfer-Verwimp & Preussing 23203/B (GOET)	**KF556471**	**KF556236**	**KF556003**
L. flava	Fiji Isls.	Pócs & Pócs 03269/F (EGR)	**KF556463**	**KF556227**	**KF555996**
L. flava	Gough Island	Gremmen 2005-0794 (EGR)	**KF556484**	**KF556248**	**KF556013**
L. flava	Guadeloupe	Schäfer-Verwimp & Verwimp 22495 (GOET)	**KF556473**	**KF556237**	**KF556005**
L. flava	India	Schäfer-Verwimp & Verwimp 28396 (GOET)	**KF556462**	**KF556226**	**KF555995**
L. flava	Indonesia (I)	Schäfer-Verwimp & Verwimp 25243/C (GOET)	**KF556453**	**KF556217**	**KF555988**
L. flava	Indonesia (II)	Schäfer-Verwimp & Verwimp 21078/A (GOET)	**KF556466**	**KF556230**	**KF555998**
L. flava	Indonesia (III)	Gradstein 12037 (GOET)	**KF556481**	**KF556245**	**KF556619**
L. flava	Indonesia (IV)	Schäfer-Verwimp & Verwimp 24907 (GOET)	**KF556467**	**KF556231**	**KF556616**

L. flava	Indonesia (V)	Schäfer-Verwimp & Verwimp 24825 (GOET)	**KF556460**	**KF556224**	**KF555993**
L. flava	Indonesia (VI)	Schäfer-Verwimp & Verwimp 24806/B (GOET)	**KF556455**	**KF556219**	**KF555989**
L. flava	Indonesia (VII)	Schäfer-Verwimp & Verwimp 24901/A (GOET)	-----------	**KF556232**	**KF555999**
L. flava	Kenya (I)	Chuah et al. 03017/H (EGR)	**KF556566**	**KF556347**	-----------
L. flava	Kenya (II)	Pócs & Pócs 04011/AY (EGR)	-----------	**KF556250**	**KF556620**
L. flava	Kenya (III)	Pócs & Pócs 04013/M (EGR)	**KF556586**	**KF556368**	**KF556120**
L. flava	Kenya (VI)	Pócs & Pócs 04026/X (EGR)	**KF556587**	**KF556369**	**KF556121**
L. flava	Kenya (V)	Pócs & Pócs 04026/X (EGR)	**KF556588**	**KF556370**	**KF556635**
L. flava	Madeira (I)	Drehwald 3722 (GOET)	**KF556472**	**-----------**	**KF556004**
L. flava	Madeira (II)	Stech 09-425 (L)	**KF556474**	**KF556238**	**KF556006**
L. flava	Malaysia (I)	Schäfer-Verwimp & Verwimp 18541/C (GOET)	**KF556459**	**KF556223**	**KF555992**
L. flava	Malaysia (II)	Schäfer-Verwimp & Verwimp 18861/B (GOET)	**KF556458**	**KF556222**	**KF556614**
L. flava	Malaysia (III)	Schäfer-Verwimp & Verwimp 18528/A (GOET)	**KF556461**	**KF556225**	**KF555994**
L. flava	Nepal	Long 17308 (JE)	**KF556470**	**KF556235**	**KF556002**
L. flava	New Zealand, North Island	Braggins 90/30 (GOET)	**KF556475**	**KF556239**	**KF556617**
L. flava	Panama	Schäfer-Verwimp & Verwimp 30873 (GOET)	**KF556482**	**KF556246**	**KF556011**
L. flava	Réunion (I)	Gradstein 12013 (GOET)	**KF556465**	**KF556229**	**KF555997**
L. flava	Réunion (II)	Pócs 9501/J (EGR)	**KF556487**	**KF556252**	**KF556016**
L. flava	Réunion (III)	Pócs 08068/Z (EGR)	**KF556486**	**KF556251**	**KF556015**
L. flava	São Tomé and Príncipe	Shevock 34217 (EGR)	**KF556464**	**KF556228**	**KF556615**
L. flava	South Africa	Arts RSA 25/LL (JE)	-----------	**KF556233**	**KF556000**
L. flava	Sri Lanka (I)	Schäfer-Verwimp & Verwimp 15761 (GOET)	**KF556428**	**KF556184**	**KF555959**

L. flava	Sri Lanka (II)	Schäfer-Verwimp & Verwimp 5631 (GOET)	**KF556456**	**KF556220**	**KF555990**
L. flava	USA (I)	Majestyk & Wilbur 9979 (DUKE)	**KF556476**	**KF556240**	**KF556618**
L. flava	USA (II)	Nelson et al. 18549 (DUKE)	**KF556477**	**KF556241**	**KF556007**
L. grossitexta (Steph.) M.E.Reiner & Goda	Brazil, Paraná	Schäfer-Verwimp & Verwimp 10920 (GOET)	**KF556490**	**KF556255**	**KF556019**
L. grossitexta	Ecuador (I)	Schäfer-Verwimp & Nebel 31985 (GOET)	**KF556489**	**KF556254**	**KF556018**
L. grossitexta	Ecuador (II)	Schäfer-Verwimp & Nebel 32151/A (GOET)	**KF556488**	**KF556253**	**KF556017**
L. grossitexta	Panama	Schäfer-Verwimp & Verwimp 31000 (GOET)	**KF556491**	**KF556256**	**KF556020**
L. helmsiana Steph.	New Zealand (I)	Renner 300050 (AK)	-----------	JF308567	JF308538
L. helmsiana	New Zealand (II)	Renner 300069a (AK)	-----------	JF308570	JF308541
L. helmsiana	New Zealand (III)	Renner 300069 (AK)	-----------	JF308569	JF308540
L. hibernica Grolle	Ireland	Long 11743 (JE)	-----------	**KF556257**	**KF556021**
L. holtii Spruce	Madeira	Drehwald & Drehwald 3719 (GOET)	**KF556492**	**KF556258**	**KF556022**
L. intricata J.B.Jack & Steph.	Ecuador	Schäfer-Verwimp & Nebel 33217 (GOET)	-----------	-----------	**KF556023**
L. isocalycina (Nees.) Steph.	Bolivia	Gradstein 7492 (GOET)	**KF556495**	**KF556261**	**KF556026**
L. isocalycina	Brazil (I)	Costa & Gradstein 3720 (GOET)	**KF556496**	**KF556262**	**KF556027**
L. isocalycina	Brazil (II)	Costa & Gradstein 3864 (GOET)	**KF556493**	**KF556259**	**KF556024**
L. isocalycina	Brazil (III)	Costa & Gradstein 3863 (GOET)	**KF556494**	**KF556260**	**KF556025**
L. isophylla E.W.Jones	Madagascar	Lübenau 21 (EGR)	**KF556497**	**KF556263**	**KF556028**
L. japonica Mitt.	Japan (I)	Mizutani 15618 (L)	**KF556499**	**KF556265**	**KF556030**
L. japonica	Japan (II)	Mizutani 14074 (L)	**KF556498**	**KF556264**	**KF556029**
L. japonica	Russia	Bakalin HRE 63 (GOET)	**KF556500**	**KF556266**	**KF556031**
L. laeta (Lehm. & Lindenb.) Gottsche, Lindenb. & Nees	Ecuador	Schäfer-Verwimp et al. 24412 (GOET)	**KF556501**	**KF556267**	**KF556032**

L. laetevirens Nees & Mont.	Argentina	Reiner MER 985 (JE)	-----------	**KF556278**	**KF556040**
L. laetevirens	Bolivia	Linneo et al. 533 (MO)	**KF556505**	**KF556271**	**KF556034**
L. laetevirens	Costa Rica (I)	Schäfer-Verwimp & Holz SV/H-0315/A (GOET)	-----------	**KF556274**	**KF556623**
L. laetevirens	Costa Rica (II)	Schäfer-Verwimp & Holz SV/H-0406 (GOET)	**KF556507**	**KF556273**	**KF556036**
L. laetevirens	Dominican Rep. (I)	Schäfer-Verwimp & Verwimp 27079 (GOET)	**KF556508**	**KF556275**	**KF556037**
L. laetevirens	Dominican Rep. (II)	Schäfer-Verwimp & Verwimp 27049 (GOET)	**KF556502**	**KF556268**	**KF556033**
L. laetevirens	Dominican Rep. (III)	Schäfer-Verwimp & Verwimp 27009/A (GOET)	**KF556503**	**KF556269**	**KF556621**
L. laetevirens	Dominican Rep. (IV)	Schäfer-Verwimp & Verwimp 27166 (GOET)	**KF556509**	**KF556276**	**KF556038**
L. laetevirens	Ecuador	Schäfer-Verwimp & Nebel 31905/A (GOET)	**KF556504**	**KF556270**	**KF556622**
L. laetevirens	French Guiana	Holz FG00-311 (GOET)	-----------	**KF556277**	**KF556039**
L. laetevirens	Madeira (I)	Schäfer-Verwimp & Verwimp 26013A (GOET)	**KF556406**	**KF556161**	**KF555939**
L. laetevirens	Madeira (II)	Schäfer-Verwimp & Verwimp 26013B (GOET)	**KF556407**	**KF556162**	**KF555940**
L. laetevirens	Mexico	Gradstein & Velasquez s.n. (GOET)	**KF556506**	**KF556272**	**KF556035**
L. lamacerina (Steph.) Schiffn.	Azores (I)	Schäfer-Verwimp & Verwimp 29394 (GOET)	**KF556510**	**KF556279**	**KF556041**
L. lamacerina	Azores (II)	Schäfer-Verwimp & Verwimp 29325/A (GOET)	**KF556513**	**KF556282**	**KF556044**
L. lamacerina	Canada	Schofield & Schofield 95548 (DUKE)	**KF556514**	**KF556283**	**KF556045**
L. lamacerina	Ireland	Long 11625 (JE)	-----------	**KF556286**	**KF556047**
L. lamacerina	Madeira (I)	Stech 04-297 (L)	**KF556512**	**KF556281**	**KF556043**
L. lamacerina	Madeira (II)	Stech 04-361 (L)	**KF556511**	**KF556280**	**KF556042**
L. lamacerina	USA (I)	Risk 13222 (DUKE)	**KF556515**	**KF556284**	**KF556624**

L. lamacerina	USA (II)	Shaw 10368 (DUKE)	**KF556516**	**KF556285**	**KF556046**
L. lomana E.W.Jones	Réunion (I)	Pócs 08068/P (EGR)	**KF556389**	**KF556139**	**KF556602**
L. lomana	Réunion (II)	Pócs 08064/L (EGR)	**KF556388**	**KF556138**	**KF555921**
L. lumbricoides (Nees) Nees	Indonesia	Gradstein & Ariyanti 11028 (GOET)	-----------	**KF556287**	**KF556048**
L. micholitzii Mizut.	Fiji Isls. (I)	Pócs & Pócs 03288/DC (EGR)	**KF556517**	**KF556288**	**KF556049**
L. micholitzii	Fiji Isls. (II)	Pócs & Pócs 03309/L (EGR)	**KF556518**	**KF556289**	**KF556625**
L. micholitzii	Indonesia	Schäfer-Verwimp & Verwimp 24923/E (GOET)	**KF556519**	**KF556290**	**KF556050**
L. microloba Taylor	Fiji Isls. (I)	Pócs & Pócs 03280/CC (EGR)	-----------	**KF556292**	**KF556626**
L. microloba	Fiji Isls. (II)	Pócs 08013/Y (EGR)	**KF556520**	**KF556291**	**KF556051**
L. microloba	Fiji Isls. (III)	Pócs & Pócs 03279/CK (EGR)	**KF556521**	**KF556293**	**KF556627**
L. mimula Hürl.	Fiji Isls. (I)	Pócs 08013/M (EGR)	**KF556522**	**KF556294**	**KF556628**
L. mimula	Fiji Isls. (II)	Pócs 08034/E (EGR)	**KF556523**	**KF556295**	**KF556052**
L. mimula	Indonesia (I)	Schäfer-Verwimp & Verwimp 16973 (GOET)	**KF556524**	**KF556296**	**KF556053**
L. mimula	Indonesia (II)	Schäfer-Verwimp 20930 (GOET)	AY548104	DQ238580	DQ987261
L. minutiloba A.Evans	Easter Island	Ireland & Bellolio 30138 (JE)	**KF556525**	**KF556297**	**KF556054**
L. monimiae (Steph.) Steph.	Ecuador	Schäfer-Verwimp & Preussing 23226/A (GOET)	**KF556526**	**KF556298**	**KF556055**
L. multidentata M.E.Reiner & Mustelier	Dominican Rep. (I)	Pócs & Pócs 03157/A (EGR)	**KF556528**	**KF556300**	**KF556057**
L. multidentata	Dominican Rep. (II)	Pócs & Pócs 03157/A (EGR)	**KF556527**	**KF556299**	**KF556056**
L. neelgherriana Gottsche	Japan	Higuchi BSE 1295 (L)	-----------	**KF556301**	**KF556058**
L. nepalensis (Steph.) H.A.Mill., Bonner & Bischl.	Nepal	Long 17250 (JE)	-----------	**KF556302**	**KF556059**
L. obscura Mitt.	Malaysia	Schäfer-Verwimp & Verwimp 18745/B (GOET)	-----------	**KF556304**	**KF556060**
L. obscura	Indonesia	Schäfer-Verwimp & Verwimp 16737 (GOET)	**KF556530**	-----------	**KF556630**

L. obtusangula	Bolivia (I)	Gradstein 9948 (GOET)	DQ983731	DQ987428	DQ987324
L. obtusangula Spruce	Bolivia (II)	Krömer 869 (GOET)	**KF556532**	**KF556307**	**KF556063**
L. obtusangula	Bolivia (III)	Krömer 1061 (GOET)	-----------	**KF556306**	**KF556062**
L. obtusangula	French Guiana	Holz FG 00-291B (GOET)	**KF556531**	**KF556305**	**KF556061**
L. oligoclada Spruce	Brazil (I)	Schäfer-Verwimp & Verwimp 13590 (GOET)	**KF556533**	**KF556308**	**KF556064**
L. oligoclada	Brazil (II)	Schäfer-Verwimp & Verwimp 10560 (GOET)	**KF556534**	**KF556310**	-----------
L. oligoclada	Brazil (III)	Schäfer-Verwimp & Verwimp 11780 (GOET)	-----------	**KF556309**	-----------
L. oracola M.A.M.Renner	New Zealand (I)	Renner 299972 (AK)	-----------	JF308557	JF308528
L. oracola	New Zealand (II)	Renner 300010 (AK)	-----------	JF308560	JF308531
L. oracola	New Zealand (III)	Renner 300078 (AK)	-----------	JF308571	JF308542
L. oracola	New Zealand (IV)	Renner 300012 (AK)	-----------	JF308561	JF308532
L. oracola	New Zealand (V)	Renner 300003 (AK)	-----------	JF308559	JF308530
L. osculatiana De Not.	Costa Rica	Schäfer-Verwimp & Holz SV/H-0496/B (GOET)	**KF556539**	**KF556315**	**KF556068**
L. osculatiana	Ecuador	Schäfer-Verwimp et al. 24526 (GOET)	**KF556535**	**KF556311**	**KF556065**
L. osculatiana	Panama	Schäfer-Verwimp & Verwimp 30958 (GOET)	**KF556538**	**KF556314**	**KF556631**
L. osculatiana	Venezuela	Drehwald & Reiner-Drehwald 40081 (GOET)	**KF556537**	**KF556313**	**KF556067**
L. pallescens Mitt.	Ecuador (I)	Schäfer-Verwimp & Nebel 32731 (GOET)	**KF556540**	-----------	**KF556069**
L. pallescens	Ecuador (II)	Schäfer-Verwimp & Nebel 31824 (GOET)	**KF556541**	**KF556316**	**KF556070**
L. parva (S.Hatt.) Mizut.	Japan (I)	Mizutani 16204 (L)	**KF556543**	**KF556319**	**KF556073**
L. parva	Japan (II)	Inoue 959 (JE)	-----------	**KF556317**	**KF556071**
L. parva	Japan (III)	Mizutani 15293 (L)	**KF556542**	**KF556318**	**KF556072**
L. patriciae Schäf.-Verw.	Malaysia	Schäfer-Verwimp & Verwimp 18583 (GOET)	-----------	**KF556320**	**KF556074**

L. paucidentata (Steph.) Grolle	Cuba	Pócs & Caluff 9199/CL (JE)	-----------	**KF556321**	**KF556075**
L. paucidentata	Dominica	Schäfer- Verwimp 17737 (GOET)	DQ983695	-----------	DQ987345
L. phyllobola Nees & Mont.	Ecuador	Noeske et al. 204 (GOET)	**KF556600**	**KF556322**	**KF556076**
L. phyllobola	Kenya	Pócs & Pócs 04009/B (EGR)	**KF556544**	**KF556323**	**KF556632**
L. pterigonia (Lehm. & Lindenb.) Schiffn.	Bolivia (I)	Churchill et al. 21851 (GOET)	**KF556546**	**KF556325**	**KF556078**
L. pterigonia	Bolivia (II)	Gradstein 9963 (GOET)	**KF556548**	**KF556327**	**KF556080**
L. pterigonia (Lehm. & Lindenb.) Mont.	Bolivia (III)	Gradstein 9964 (GOET)	DQ983732	DQ987429	DQ987325
L. pterigonia	Bolivia (IV)	Churchill et al. 23467 (GOET)	**KF556547**	**KF556326**	**KF556079**
L. pterigonia	Brazil	Costa & Gradstein 3867 (GOET)	**KF556545**	**KF556324**	**KF556077**
L. pterigonia	Ecuador	Nöske 164 (GOET)	**KF556549**	**KF556328**	**KF556081**
L. puiggariana Steph.	Dominican Rep. (I)	Schäfer-Verwimp & Verwimp 27016/A (GOET)	**KF556550**	**KF556329**	**KF556082**
L. puiggariana	Dominican Rep. (II)	Schäfer-Verwimp & Verwimp 27215/A (GOET)	**KF556551**	**KF556330**	**KF556083**
L. pulverulenta (Gottsche ex Steph.) M.E.Reiner	Bolivia	Reiner-Drehwald & Drehwald 4517 (GOET)	**KF556552**	**KF556331**	**KF556084**
L. ramosossima Steph.	São Tomé and Príncipe (I)	Shevock 34551 (EGR)	-----------	**KF556334**	**KF556087**
L. ramosissima	São Tomé and Príncipe (II)	Shevock 34348A (EGR)	**KF556554**	**KF556333**	**KF556086**
L. ramosissima	São Tomé and Príncipe (III)	Shevock 34451 (EGR)	**KF556553**	**KF556332**	**KF556085**
L. ramulosa (Herzog) R.M.Schust.	Costa Rica	Schäfer-Verwimp & Holz SV/H-0229/A (GOET)	**KF556555**	**KF556335**	**KF556088**
L. ramulosa	Dominican Rep.	Schäfer-Verwimp & Verwimp 26954 (GOET)	-----------	**KF556337**	**KF556633**
L. ramulosa	Ecuador (I)	Wilson et al. 04-24 (GOET)	**KF556556**	**KF556336**	**KF556089**
L. ramulosa	Ecuador (II)	Schäfer-Verwimp et al. 24208 (GOET)	**KF556557**	**KF556338**	**KF556090**
L. reflexistipula (Lehm. & Lindenb.) Gottsche, Lindenb. & Nees	Brazil	Schäfer-Verwimp & Verwimp 12482 (GOET)	**KF556558**	**KF556339**	**KF556091**

L. reflexistipula	Ecuador (I)	Schäfer-Verwimp & Nebel 31707 (GOET)	**KF556560**	**KF556341**	**KF556093**
L. reflexistipula	Ecuador (II)	Schäfer-Verwimp et al. 24215/A (GOET)	**KF556563**	**KF556344**	**KF556096**
L. reflexistipula	Ecuador (III)	Schäfer-Verwimp & Nebel 33162 (GOET)	**KF556559**	**KF556340**	**KF556092**
L. reflexistipula	Ecuador (IV)	Schäfer-Verwimp & Nebel 32032 (GOET)	**KF556561**	**KF556342**	**KF556094**
L. reflexistipula	Peru	Drehwald 10046 (GOET)	**KF556562**	**KF556343**	**KF556095**
L. reflexistipula var. *costaricensis* (Steph.) M.E.Reiner	Costa Rica	Schäfer-Verwimp & Holz SV/H-0434 (GOET)	**KF556565**	**KF556346**	**KF556098**
L. reflexistipula var. *costaricensis*	Panama	Schäfer-Verwimp & Verwimp 30930 (GOET)	**KF556564**	**KF556345**	**KF556097**
L. rhigophila M.A.M.Renner	New Zealand	Renner 300147 (AK)	-----------	JF308579	JF308550
L. rhigophila	New Zealand	Renner 300044a (AK)	-----------	JF308566	JF308537
L. rhigophila	New Zealand	Renner 300149 (AK)	-----------	JF308580	JF308551
L. rotundifolia Mitt.	Costa Rica, Cartago	Schäfer-Verwimp & Holz SV/H-0378 (GOET)	**KF556567**	**KF556348**	**KF556099**
L. rotundifolia	Ecuador	Gradstein 10172 (GOET)	DQ983734	DQ987410	DQ987304
L. rotundifolia	Panama, Chiriqui	Schäfer-Verwimp & Verwimp 31029 (GOET)	**KF556568**	**KF556349**	**KF556100**
L. ruthii (A.Evans) R.M.Schust.	USA (I), North Carolina	Duell 1411 p.p. (JE)	-----------	**KF556350**	**KF556101**
L. ruthii	USA (II), Tennessee	Zartman 681 (DUKE)	**KF556569**	-----------	**KF556634**
L. sordida (Nees) Nees	Fiji Isls. (I)	Pócs & Pócs 03300/AP (EGR)	**KF556570**	**KF556351**	**KF556102**
L. sordida	Fiji Isls. (II)	Pócs & Pócs 03305/J (EGR)	**KF556572**	**KF556353**	**KF556104**
L. sordida	Fiji Isls. (III)	Pócs & Pócs 03305/J (EGR)	**KF556571**	**KF556352**	**KF556103**
L. sordida	Indonesia	Sporn 101 (GOET)	**KF556574**	**KF556355**	**KF556106**
L. sordida	Japan	Yamaguchi 29848 (GOET)	**KF556575**	**KF556356**	**KF556107**
L. sordida	Papua New Guinea	Streimann 41611 (JE)	**KF556573**	**KF556354**	**KF556110**
L. sporadica Besch. & Spruce	Panama	Schäfer-Verwimp & Verwimp 31033 (GOET)	**KF556583**	-----------	**KF556117**

L. subspathulata Spruce	Colombia	Gradstein 8991 (GOET)	**KF556584**	**KF556366**	**KF556118**
L. subspathulata	Dominica	Gradstein 6643 (GOET)	**KF556585**	**KF556367**	**KF556119**
L. tapajosensis Spruce	Ecuador	Nöske et al. 204 (GOET)	**KF556589**	**KF556371**	**KF556122**
L. tasmanica Gottsche	New Zealand	Renner 872054 (NSW)	-----------	JF308581	JF308552
L. topoensis Gradst. & M.E.Reiner	Ecuador (I)	Gradstein & Jost 10163 (GOET)	**KF556590**	**KF556372**	**KF556123**
L. topoensis	Ecuador (II)	Gradstein & Jost 10063 (GOET)	DQ983712	DQ987416	DQ987312
L. topoensis	Ecuador (III)	Gradstein & Jost 10063a (GOET)	**KF556591**	**KF556373**	**KF556124**
L. topoensis	Ecuador (IV)	Wilson et al 04-04 (GOET)	DQ983733	DQ987435	DQ987331
L. trinitensis Lindenb. & Gottsche	Bolivia	Linneo et al. 82 (GOET)	**KF556593**	**KF556375**	**KF556126**
L. trinitensis	Brazil	Vital 10.168 (JE)	**KF556594**	**KF556376**	**KF556127**
L. trinitensis	Mayotte	Pócs & Pócs 05097/C (EGR)	**KF556592**	**KF556374**	**KF556125**
L. tuberculosa Steph.	São Tomé and Príncipe (I)	Pócs & Pócs 34690 (EGR)	**KF556595**	**KF556377**	**KF556128**
L. tuberculosa	São Tomé and Príncipe (II)	Shevock 34776 (EGR)	**KF556483**	**KF556247**	**KF556012**
L. tuberculosa	Thailand	Schäfer-Verwimp & Verwimp 23880 (GOET)	**KF556457**	**KF556221**	**KF555991**
L. tumida Mitt.	New Zealand (I)	Renner 299949 (AK)	-----------	JF308556	JF308527
L. tumida	New Zealand (II)	Renner 300002 (AK)	-----------	JF308558	JF308529
L. umbilicata (Nees) Nees et al.	Indonesia (I)	Gradstein 12076 (GOET)	**KF556598**	**KF556380**	**KF556131**
L. umbilicata	Indonesia (II)	Gradstein 51 (GOET)	**KF556599**	**KF556381**	**KF556132**
L. umbilicata	Indonesia (III)	Schäfer-Verwimp & Verwimp 20794/B (GOET)	**KF556596**	**KF556378**	**KF556129**
L. umbilicata	Indonesia (IV)	Schäfer-Verwimp & Verwimp 16954 (GOET)	**KF556597**	**KF556379**	**KF556130**
L. wallichiana Gottsche	Nepal	Long 16716 (JE)	-----------	**KF556382**	-----------
L. sp. I	Ecuador	Schäfer-Verwimp & Preussing 23533 (GOET)	**KF556536**	**KF556312**	**KF556066**
L. sp. II	Bolivia	Fuentes & Aldana 6473 (GOET)	**KF556429**	**KF556185**	**KF555960**

L. sp. III	Gough Island	Gremmen 2000-0075 (EGR)	**KF556579**	**KF556361**	**KF556112**
L. sp. IV	Fiji Isls.	Pócs 08029/O (EGR)	**KF556580**	**KF556362**	**KF556113**
L. sp. V	Madagascar	Geissler 3498 (EGR)	**KF556578**	**KF556360**	**KF556111**
L. sp. VI	Panama	Schäfer-Verwimp & Verwimp 30834 (GOET)	**KF556582**	**KF556365**	**KF556116**
L. sp. VII	Nepal	Pölt H3071 (JE)	-----------	**KF556363**	**KF556114**
L. sp.VIII	Ethiopia	Hylander 5564 (EGR)	**KF556576**	**KF556357**	**KF556108**
L. sp. IX	São Tomé and Príncipe	Shevock et al. 34316 (EGR)	**KF556485**	**KF556249**	**KF556014**
L. sp. X	Madagascar	Pócs et al. 90100/C (JE)	**KF556581**	**KF556364**	**KF556115**
Lepidolejeunea bidentula (Steph.) R.M.Schust.	China	Koponen et al. 51525 (H)	AY125936	AY144476	AY125340
Lep. eluta (Nees) R.M.Schust.	Bolivia (I)	Churchill & Vasquez 2180 (GOET)	AY548066	DQ238579	DQ987266
Lep. eluta	Bolivia (II)	Drehwald 4833 (GOET)	DQ983696	DQ987379	DQ987257
Lep. integristipula (J.B.Jack & Steph.) R.M.Schust.	Fiji Isls.	Pócs 03307/AC (GOET)	DQ983697	DQ987417	DQ987313
Microlejeunea. africana Steph.	Madagascar	Lübenau 2 (JE)	KC313149	KC313189	KC313111
M. africana	São Tomé and Príncipe (I)	Shevock 34576A (GOET)	KC313150	KC313190	KC313112
M. Africana	São Tomé and Príncipe (II)	Shevock 34576B (GOET)	KC313151	KC313191	KC313113
M. capillaris (Gottsche) Steph.	Costa Rica	Schäfer-Verwimp & Holz SV/H-0489/B (JE)	KC313152	KC313192	KC313114
M. colombiana Bischl.	Dominican Rep.	Schäfer-Verwimp & Verwimp 26614/A (JE)	KC313153	KC313193	KC313079
M. filicuspis (Steph.) Heinrichs, Schäf.-Verw., Pócs & S.Dong	Fiji Isls. (I)	Pócs & Pócs 03306/R (EGR)	KC313137	KC313177	KC313099
M. filicuspis	Fiji Isls. (II)	Pócs & Pócs 03304/A (EGR)	KC313138	KC313178	KC313100
M. filicuspis	Thailand	Pócs & Pócs 07006/A (EGR)	KC313139	KC313179	KC313101
M. fischeri (Tixier) Heinrichs, Schäf.-Verw., Pócs & S.Dong	Uganda (I)	Pócs & Lye 97141/T (EGR)	KC313140	KC313180	KC313102

M. fischeri	Uganda (II)	Pócs & Lye 97142/AM (EGR)	KC313141	KC313181	KC313103
M. fischeri	Uganda (III)	Pócs & Lye 97142/BB (EGR)	KC313142	KC313182	KC313104
M. fischeri	Uganda (IV)	Pócs & Lye 97142/BQ (EGR)	KC313143	KC313183	KC313105
M. latitans (Hook.f. & Taylor) Heinrichs, Schäf.-Verw., Pócs & S.Dong	New Zealand	Schäfer-Verwimp & Verwimp 13869 (JE)	KC313146	KC313186	KC313108
M. sp.	Thailand	Schäfer-Verwimp & Verwimp 16293 (GOET)	-----------	KC313196	KC313117
M. squarrosa (Steph.), Heinrichs, Schäf.-Verw., Pócs & S.Dong	Brazil (I)	Schäfer-Verwimp & Verwimp 14780 (JE)	KC313157	KC313197	KC313118
M. squarrosa	Brazil (II)	Schäfer-Verwimp & Verwimp 14638 (JE)	KC313158	KC313198	KC313119
M. squarrosa	Brazil (III)	Schäfer-Verwimp & Verwimp 13376 (GOET)	DQ983720	DQ987446	DQ987344
M. ulicina (Taylor) Steph.	La Palma (I)	Schäfer-Verwimp & Verwimp 24800 (GOET)	KC313154	KC313194	KC313115
M. ulicina	La Palma (II)	Schäfer-Verwimp & Verwimp 24666 (GOET)	KC313155	KC313195	KC313116

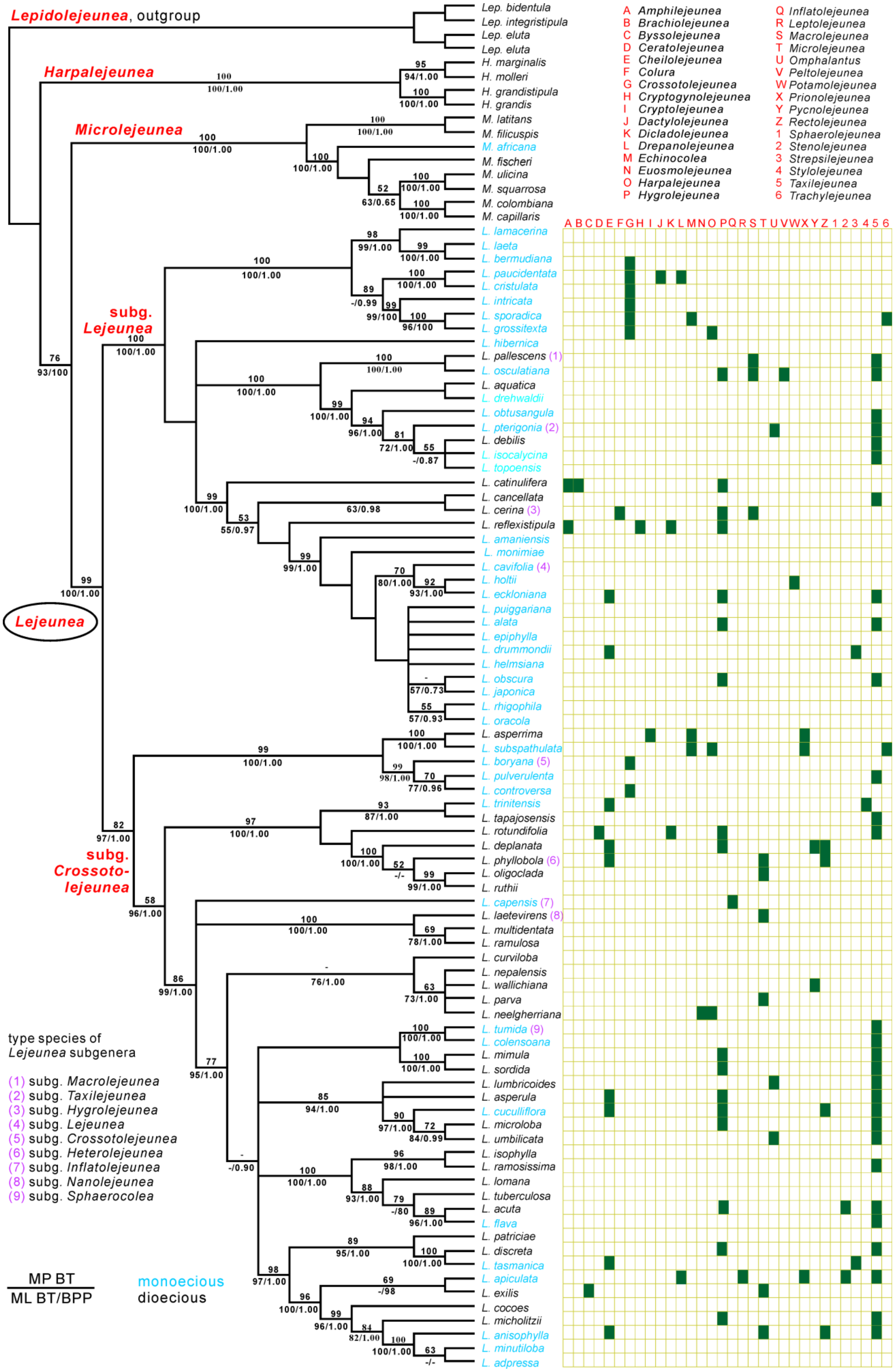

Figure 1.

Strict consensus of 4578 equally parsimonious trees derived from the small dataset including each one accession per species. MP and ML bootstrap percentage values and Bayesian Posterior Probabilities are indicated at branches. Monoecious species are given in blue, dioecious species in black. Type species of subgenera of *Lejeunea* are marked and alternative genus assignments of *Lejeunea* species shown.

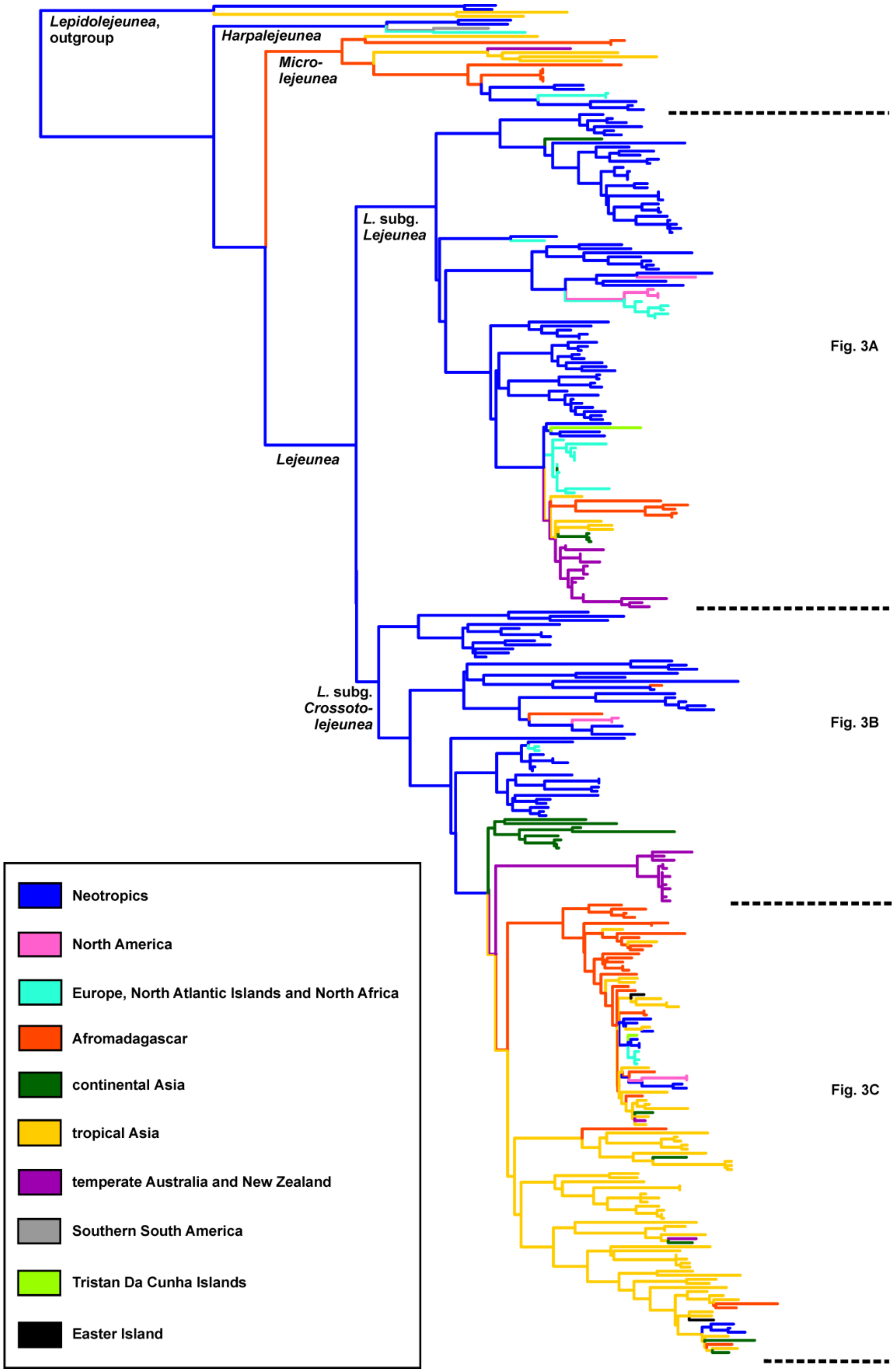

Figure 2.

Condensed Maximum Likelihood phylogeny of the *Harpalejeunea-Lejeunea-Microlejeunea* clade. Branch colors correspond to the most parsimonious reconstruction of ancestral areas of distribution and provide evidence for a Neotropical origin of *Lejeunea*.

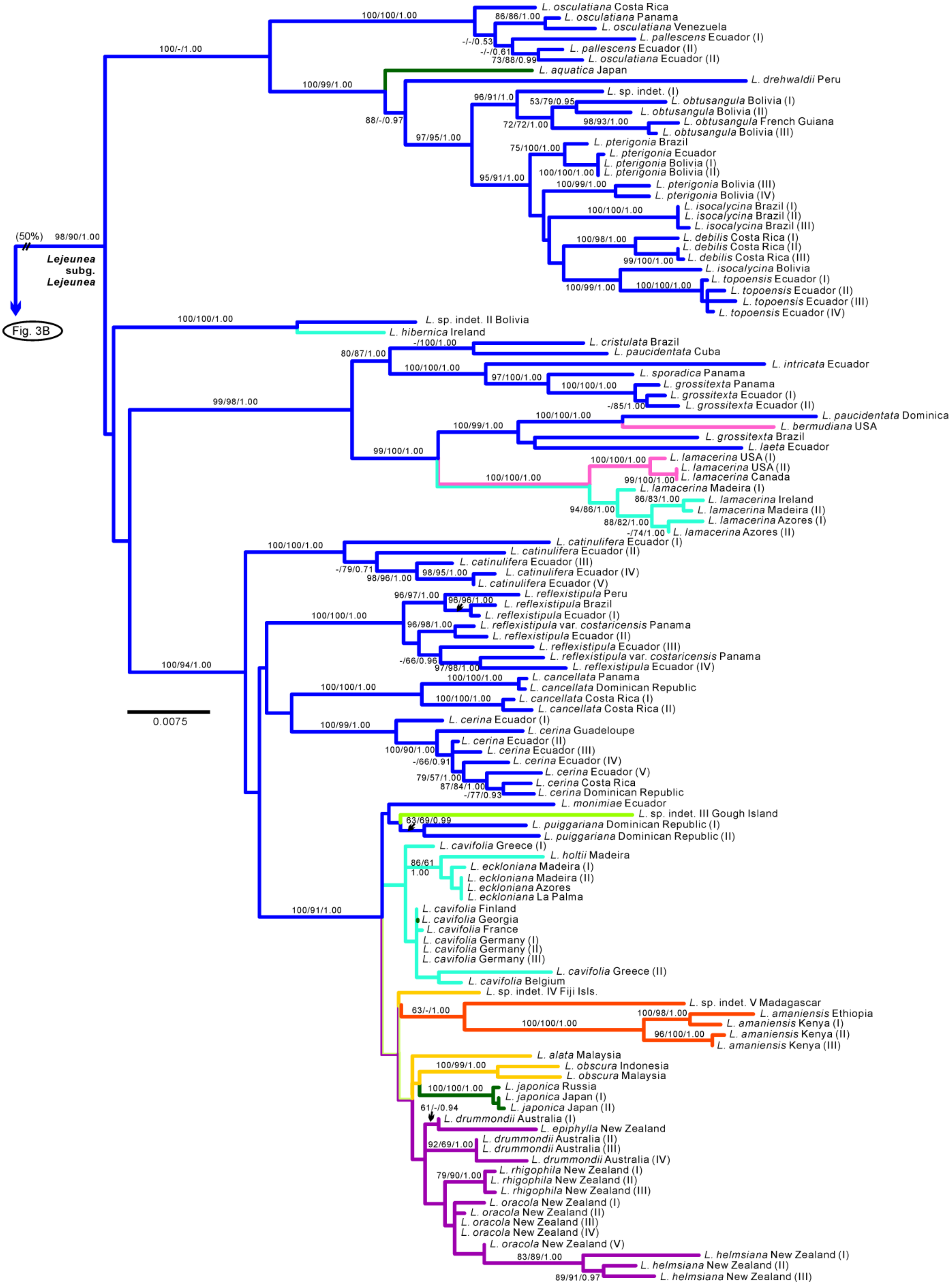

Figure 3 A.

Pruned *Lejeunea* clade from Fig. 2. Fifty nine *Lejeunea* species are represented by multiple accessions, 29 of these are monophyletic, 25 para- or polyphyletic.

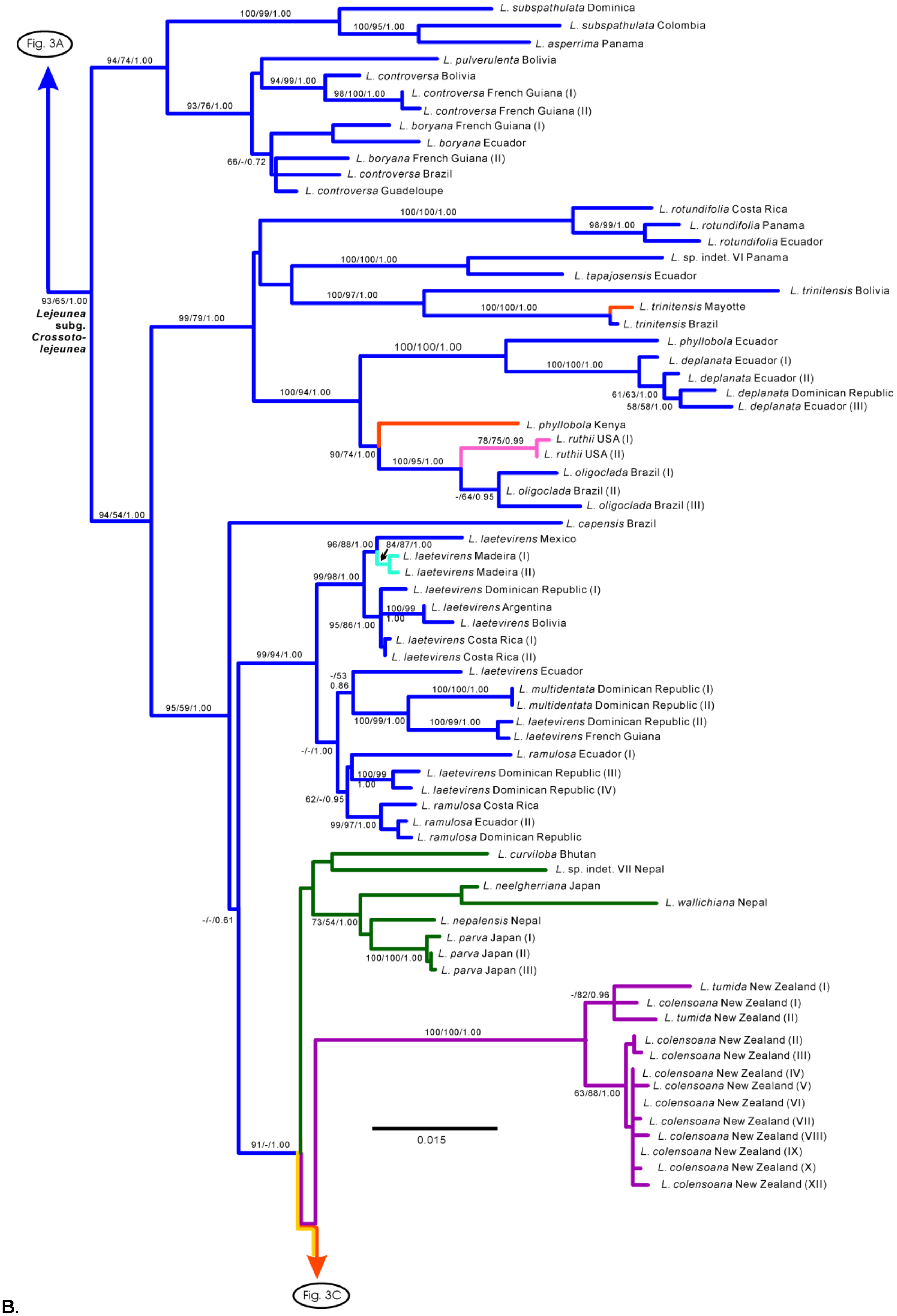

Figure 3 B.

Pruned *Lejeunea* clade from Fig. 2. Fifty nine *Lejeunea* species are represented by multiple accessions, 29 of these are monophyletic, 25 para- or polyphyletic.

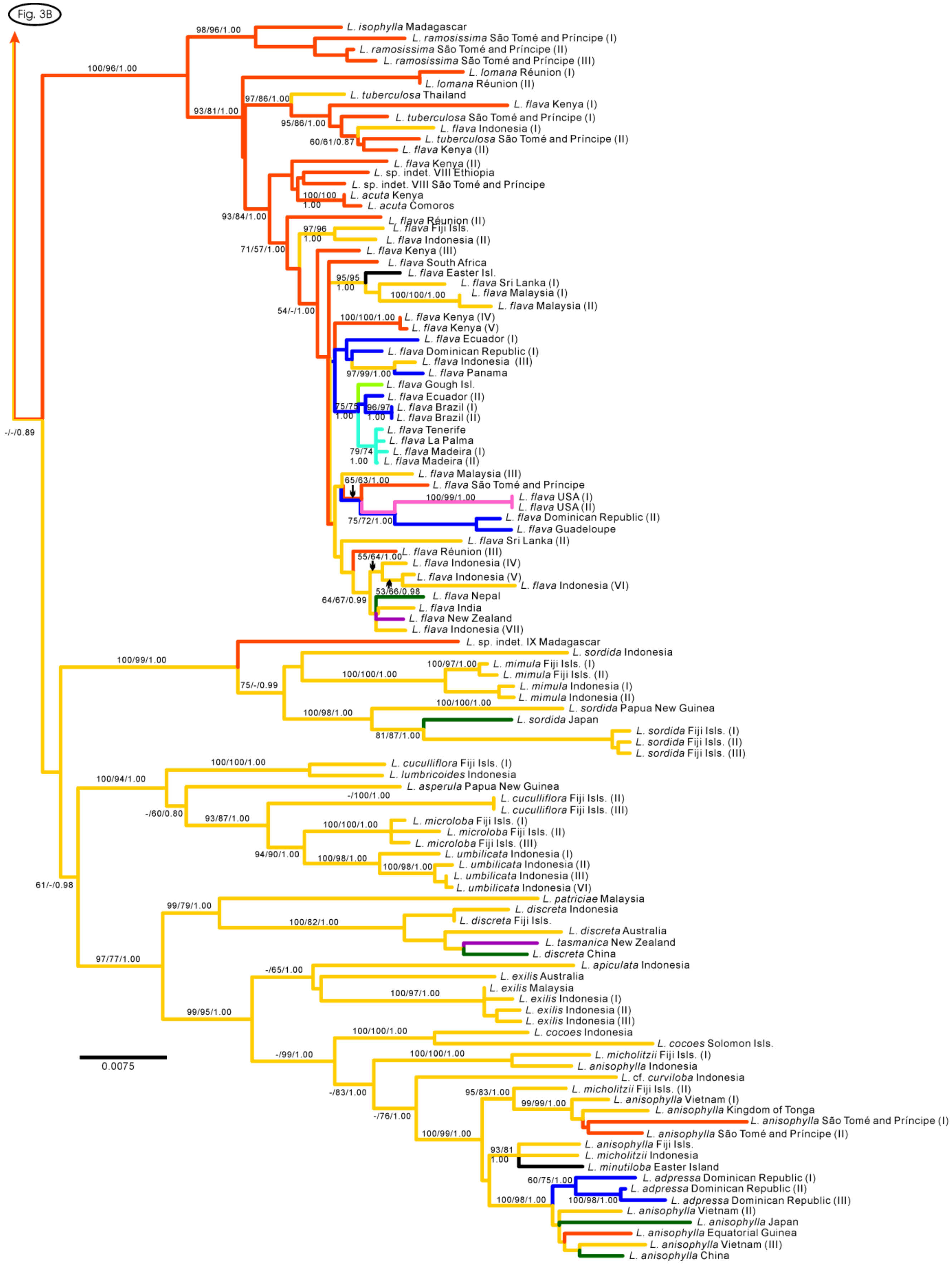

Figure 3 C.

Pruned *Lejeunea* clade from Fig. 2. Fifty nine *Lejeunea* species are represented by multiple accessions, 29 of these are monophyletic, 25 para- or polyphyletic.

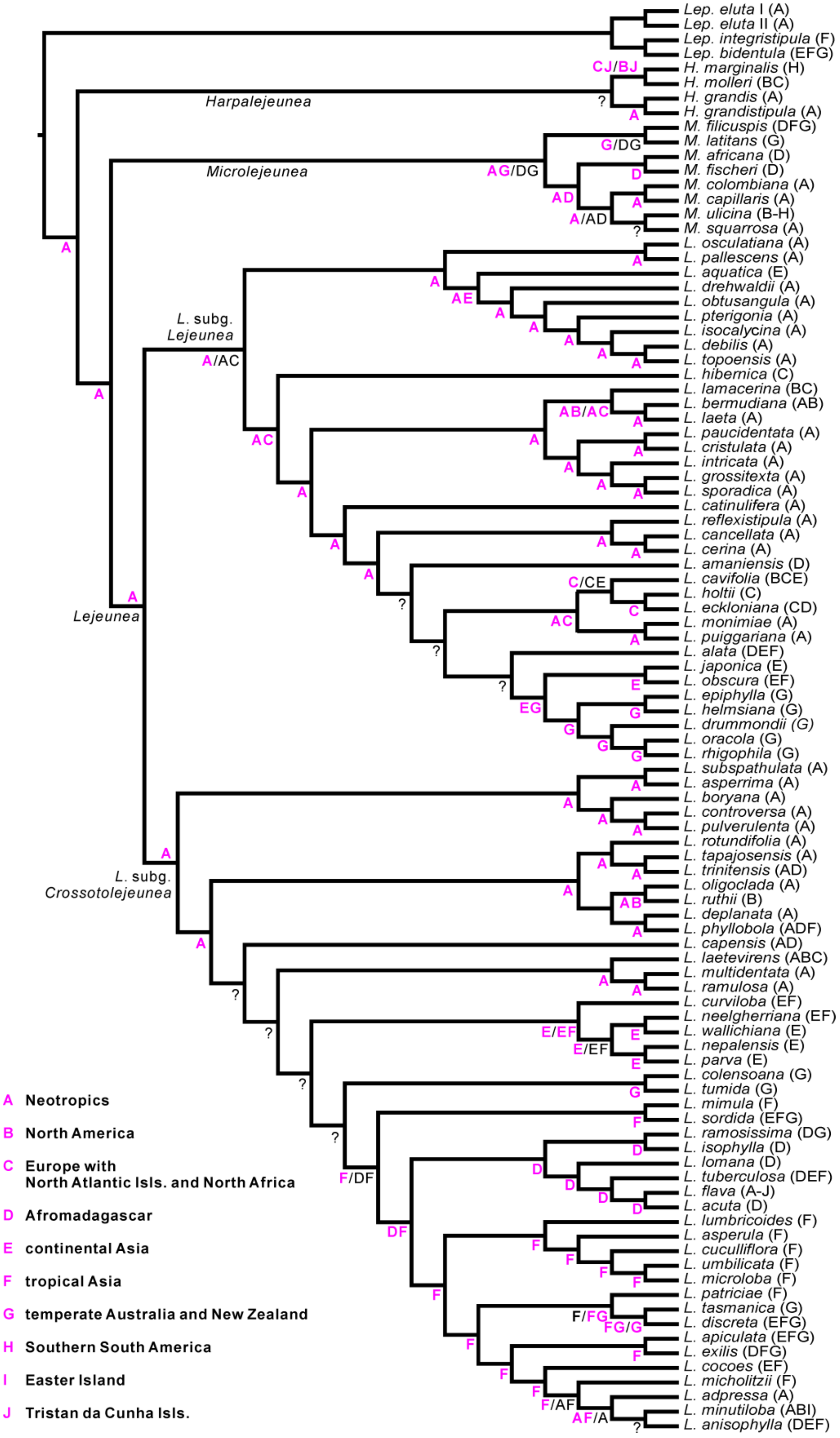

Figure 4.

Ancestral areas of distribution reconstructed using S-DIVA based on 7500 Bayesian trees from the reduced dataset. The distribution of each species is given in brackets according to the ancestral areas of distribution scheme. Putative ancestral areas of distribution are shown at nodes, in case of alternative results the less likely solution is given in black. Question marks indicate ambiguities [more than two alternative proposals]. The reconstruction points to a Neotropical origin of *Lejeunea*

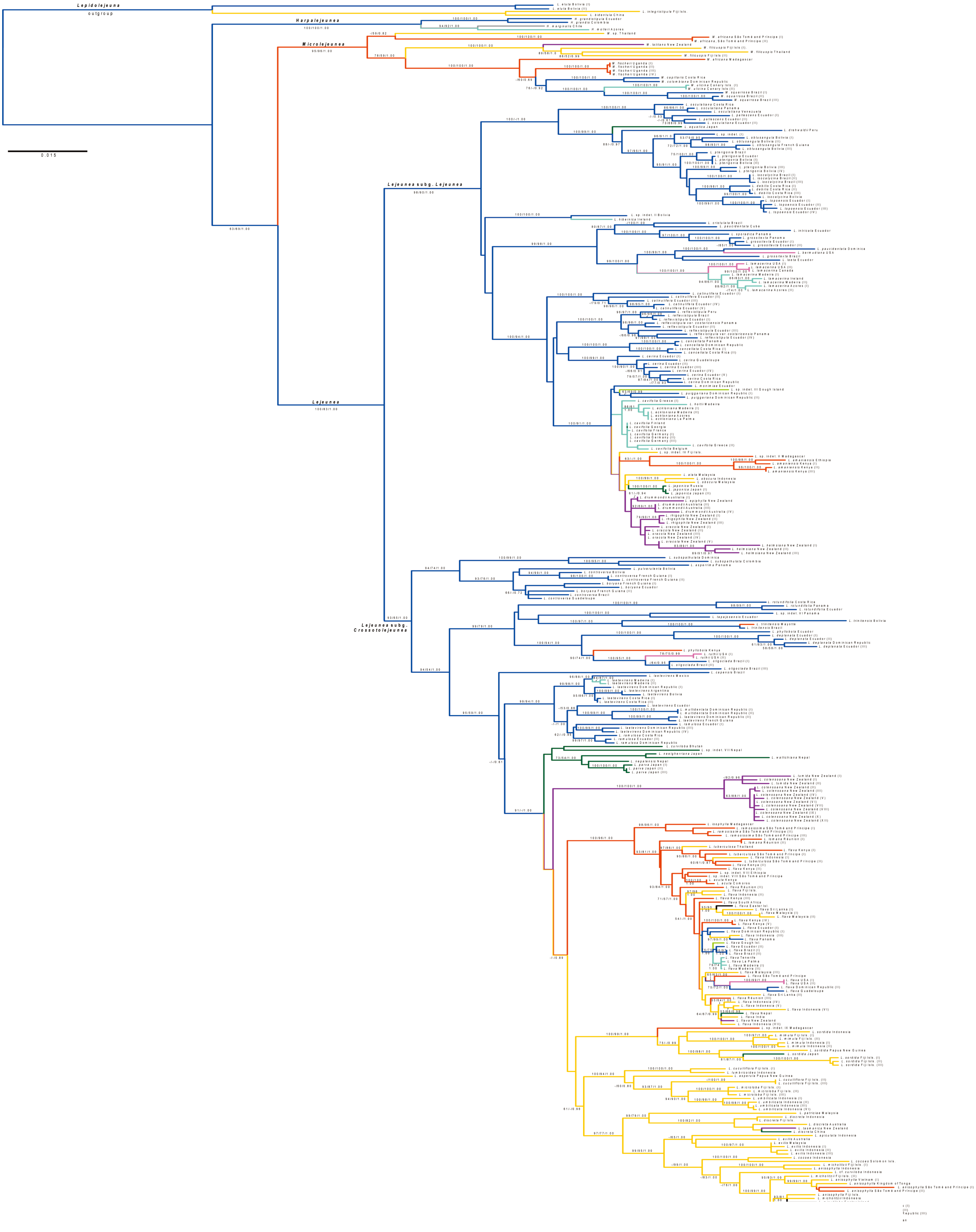

Figure S1. Maximum Likelihood phylogeny of the *Harpalejeunea-Lejeunea-Microlejeunea* clade. ML and MP bootstrap percentage values as well as Bayesian Posterior Probabilities are indicated at branches. Branch colors correspond to the most parsimonious reconstruction of ancestral areas of distribution

Appendix 3

Size doesn´t matter – recircumscription of *Microlejeunea* (Lejeuneaceae, Porellales) based on molecular and morphological evidence

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Abstract

Phylogenetic analyses of a three marker dataset of Lejeuneaceae (chloroplast genome *rbc*L gene and *trn*L-F region, and nuclear ribosomal ITS1-5.8S-ITS2 region) resolve the "*Lejeunea* complex" in three main lineages assigned here to *Harpalejeunea*, *Lejeunea* and *Microlejeunea*. The taxa *Harpalejeunea fischeri*, *H. filicuspis*, *H. latitans* and *Pluvianthus squarrosus* are nested in a clade with several representatives of *Microlejeunea* including the generitype *M. africana*, and are transferred to the latter genus. *Harpalejeunea* and *Microlejeunea* differ from *Lejeunea* by the presence of ocelli. *Harpalejeunea* has diverging, blunt underleaf lobes in contrast to the forward directed, blunt to acute underleaf lobes of *Microlejeunea*. Morphologically similar accessions of *Microlejeunea* form independent lineages. *Drepanolejeunea vandenberghenii* is newly reported for Madagascar, Malawi and Réunion.

Key words: cryptic speciation, *Drepanolejeunea, Harpalejeunea,* Jungermanniopsida, *Lejeunea*, liverwort, *Pluvianthus*, taxonomy

Introduction

Lejeunea Libert (1820: 372) is a species rich and taxonomically complex genus of Lejeuneeae with some 1700 species described worldwide (Reiner-Drehwald 1999). The high number of *Lejeunea* species is due to the wide genus concept of early authors including Gottsche et al. (1844-1847) and Spruce (1884). Later authors placed *Lejeunea* elements sensu Spruce in more than 60 different genera (Gradstein et al. 2004), leading to tentative estimates of species numbers "over 100"; the exact number being unclear due to a lack of comprehensive taxonomic studies (Gradstein & Pinheiro da Costa 2003). Molecular phylogenetic studies resolved several of the segregate genera as nested within *Lejeunea*, e.g. *Taxilejeunea* (Spruce 1884: 212) Schiffner (1893: 125), *Macrolejeunea* (Spruce 1884: 224) Schiffner (1893: 118) (Wilson et al. 2007) and *Sphaerolejeunea* Herzog (1938: 88), results that support a wider genus concept (Heinrichs et al. 2012a). Currently *Lejeunea* is considered to be a morphologically heterogeneous genus with unclear boundaries (Gradstein et al. 2001).

Microlejeunea Stephani (1888: 61) has been treated either as a genus (Bischler et al. 1963, Grolle 1995, Ah-Peng & Bardat 2011) or as a subgenus of *Lejeunea* (Schuster 1980, Thiers 1997). It differs from *Lejeunea*s.str. by sinuose stems, presence of three medullary cells, perpendicular branching, large lobules being usually longer than wide, small underleaves, bracts being larger than the leaves and often shortly connate at their bases, male bracts being distinctly larger than the leaves (Bischler et al. 1962), a tendency to produce ocelli (Schuster 1957), and the presence of lejeuneoid subgynoecial innovations (Grolle 1995). In some molecular phylogenetic analyses of Lejeuneaceae, *Microlejeunea* was placed sister to *Lejeunea* (Ahonen et al. 2003, Wilson et al. 2004); hence both treatments are consistent with the available phylogenies. Another comprehensive molecular phylogeny of Lejeuneaceae (Wilson et al. 2007) resolved the monospecific genus *Pluvianthus* Schuster & Schäfer-Verwimp in Schuster (1994: 213) and *Harpalejeunea* (Spruce 1884: 164) Schiffner (1893: 126) in a sister relationship to *Lejeunea*, however, this study lacked representatives of *Microlejeunea. Pluvianthus* resembles the "*Lejeunea* complex" (*Lejeunea*, *Harpalejeunea* and *Microlejeunea*) in many respects but differs by its utriculiform leaves, laminar elaters, and capsule microanatomy (Schuster & Schäfer-Verwimp 1995). *Harpalejeunea* closely resembles *Microlejeunea* but has underleaves with diverging lobes, those of *Microlejeunea* being forward directed (Grolle & Reiner-Drehwald 1999). The generic circumscription of *Harpalejeunea* is still subject to controversy. Grolle & Reiner-Drehwald (1995) separate *Harpalejeunea* by its lejeuneoid subgynoecial innovations from *Drepanolejeunea* Spruce (1884: 186) Schiffner (1893: 126) with pycnolejeuneoid innovations. In contrast, Schuster (1980) considered *Harpalejeunea filicuspis* (Stephani 1913b: 344) Mizutani (1973: 197) to be an element of *Drepanolejeunea*, despite the presence of lejeuneoid subgynoecial innovations.

In the present study we test the current genus concepts by including representatives of *Harpalejeunea*, *Microlejeunea*, and *Pluvianthus* in a three-marker dataset of Lejeuneeae. Based on the outcome of our phylogenetic analyses we accept the genera *Microlejeunea* and *Harpalejeunea* but transfer several representatives of *Harpalejeunea* and *Pluvianthus* to *Microlejeunea*.

Materials and methods

Taxa studied, DNA extraction, PCR amplification and sequencing:—Plant tissue was isolated from herbarium collections of specimens assigned to the Lejeuneeae genera *Drepanolejeunea*, *Harpalejeunea*, *Microlejeunea* and *Pluvianthus* (Table 1). Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: chloroplast genome *rbc*L gene and *trn*L-F region from Gradstein et al. (2006), and nuclear ribosomal ITS1-5.8S-ITS-2 region from Hartmann et al. (2006). Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR. Newly generated sequences were assembled and edited using SeqAssem (Hepperle 2004).

We compiled a Lejeuneeae ingroup dataset based on Wilson et al. (2007) using our new sequences as well as Genbank sequences (http://www.ncbi.nlm.nih.gov/genbank/). Thirteen representatives of Brachiolejeuneeae and Ptychanthoideae were chosen as outgroup according to the topologies presented in Wilson (2007). The related sequences came also from Genbank (Table 1).

Phylogenetic analyses:—All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall 1999). Ambiguous positions were excluded from the alignments. Missing sequence stretches were coded as unknown. Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10 (Swofford 2000). MP heuristic searches were conducted with the following options implemented: heuristic search mode, 1.000 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Where more than one most parsimonious tree was found, trees were summarized in a strict consensus tree.

Non-parametric bootstrapping values (Felsenstein 1985) were generated as heuristic searches with 1.000 replicates, each with ten random-addition replicates. Rearrangements were restricted to 10,000,000 per replicate. Bootstrap percentage values (BPV) ≥ 70 were regarded as good support (Hillis and Bull 1983). The individual marker sets and the combined chloroplast DNA dataset vs nrITS dataset were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70% (Mason-Gamer and Kellog 1996). The trees gave no evidence of incongruence. Hence the datasets were combined.

jModeltest 0.1 (Posada 2008) was used to select models of evolution for maximum likelihood (ML) analyses of the three molecular markers. A TVM + Γ model for the *rbcL*-partition and GTR + I + Γ models for the *trn*L-F partition and the nrITS partition were implemented in the program GARLI version 2.0 (Zwickl, 2006). Subsequently the datasets were combined and ML trees were generated. All GARLI analyses were performed with the default settings and several times repeated. The default setting of GARLI was also employed to calculate bootstrap values for ML analyses based on 200 bootstrap replicates.

Distribution of ocelli:—Ocelli are modified leaf cells containing only a single large oil body (Suire 1999). Their distribution was reconstructed using MP criteria as implemented in Mesquite ver. 2.72 (Maddison and Maddion, 2004) based on the ML topology. Data on the presence or absence of ocelli were obtained from the literature, in addition to our own observations.

Results

Of the 2,064 investigated sequence characters, 758 were parsimonious informative and 190 were unique to a single specimen (see Table 2 for character state distributions within the single markers). The MP analysis yielded five equally parsimonious trees with a length of 5,487 steps, a consistency index (CI) of 0.30 and a retention index (RI) of 0.66. The strict consensus is depicted in Figure 1. Representatives of *Harpalejeunea*, *Lejeunea* and *Microlejeunea* ("*Lejeunea* complex") formed a polytomous lineage with a BPV of 100. A *Harpalejeunea* clade with the species *H. grandistipula* Schuster (1999: 290), *H. grandis* Grolle & Reiner-Drehwald (1999: 32), *H. marginalis* (Hooker & Taylor

1845: 91) Stephani (1913a: 271) and *H. molleri* (Stephani 1887: 3) Grolle (1989: 89) achieved a BPV of 100. *Harpalejeunea filicuspis*, *H. fischeri* (Tixier 1995: 29), *H. latitans* (Hooker & Taylor 1844: 399) Grolle (1980: 239) and *Pluvianthus squarrosus* (Stephani 1896: 130) Schuster & Schäfer-Verwimp in Schuster (1994: 213) were nested in a clade with several species of *Microlejeunea* including the generitype *M. africana* Stephani (1888: 61). This clade had a BPV of 95. Multiple accessions of *Harpalejeunea filicuspis*, *H. fischeri*, *Drepanolejeunea anoplantha* (Spruce 1884: 189) Stephani (1913b: 325)*, D. vandenberghenii* Buchbender & Fischer (2004: 273), *D. vesiculosa* (Mitten 1861: 116) Stephani (1913b: 356) and *Pluvianthus squarrosus* formed monophyletic lineages. Representatives of *Microlejeunea africana* from Principe Island and Madagascar were placed in separate clades. *Drepanolejeunea* was monophyletic with a BPV of 56. It consisted of two main lineages, each with a BPV of 100. *Drepanolejeunea* was placed in a clade with *Cololejeunea* (Spruce 1884: 291) Schiffner (1893: 121), *Colura* (Dumortier 1831: 32) Dumortier (1835: 12), *Diplasiolejeunea* (Spruce 1884: 301) Schiffner (1893: 121), *Macrocolura* Schuster (1994: 233), *Myriocoleopsis* Schiffner (1944: 234), and *Siphonolejeunea* Herzog in Skottsberg (1942: 744).

The ML tree (Figure 2, ln = -29005,9392) resembled the MP strict consensus tree. The *Harpalejeunea* clade was placed sister to the clade with *Microlejeunea*, *Pluvianthus* and several *Harpalejeunea* species. This sister relationship had a BPV of 66. The *Cololejeunea-Colura-Diplasiolejeunea-Drepanolejeunea-Macrocolura-Myriocoleopsis-Siphonolejeunea* clade achieved a BPV of 83. In our reconstruction ocelli evolved twice in Lejeuneeae and were lost twice in the main “ocelli clade” with a BPV of 99. MP character reconstruction suggested the occurrence of ocelli as a putative apomorphy of derived Lejeuneeae and a loss of this character in the clade comprising *Lejeunea* s.str.

Discussion and taxonomic treatment

Circumscription of genera:— Arguably the *Lejeunea* complex belongs to the most difficult groups of liverworts in terms of genus circumscription. As a consequence of Spruce’s (1884) wide genus concept, numerous heterogeneous elements were placed in *Lejeunea*, and classified into 39 subgenera (Spruce 1884). Most of these subgenera were elevated to genus rank by Schiffner (1893). Later authors established additional genera that often included only one or a few species, e.g., *Amblyolejeunea* Jovet-Ast (1948: 24), *Bromeliophila* Schuster (1994: 226), *Dactylolejeunea* Schuster (1971: 341), and *Metalejeunea* Grolle (1995: 17). Many of these genera were shown to nest within larger genera, and were treated as synonyms (Wilson et al. 2007, Ye & Zhu 2010, Heinrichs et al. 2012b). Recently, a considerable number of these genera were transferred to *Lejeunea* (Heinrichs et al. 2012a). These changes resulted in a rather heteromorphic genus *Lejeunea* including species with different size, leaf and underleaf shape, and different perianth forms (e.g., Reiner-Drehwald & Goda 2000, Ilkiu-Borges 2005, Reiner-Drehwald 2005, Reiner-Drehwald & Schäfer-Verwimp 2008). With a single exception, these species lack ocelli (Reiner-Drehwald & Ilkiu-Borges 2007). In contrast, the *Harpalejeunea-Microlejeunea-Pluvianthus* clade consistently possesses ocelli (Grolle 1995, Schuster & Schäfer-Verwimp 1995, Grolle & Reiner-Drehwald 1999). *Harpalejeunea* is characterized by leaves with basal or suprabasal ocelli, diverging underleaf lobes with blunt, obtuse to rounded apices, and lejeuneoid gynoecial innovations. We already sequenced about 60 accessions of *Harpalejeunea* which fall into the *Harpalejeunea* clade of Figures 1 and 2, however, as a consequence of the unclear species taxonomy (Grolle & Reiner-Drehwald 1999) we included only a few of our *Harpalejeunea* accessions in the present study. Species delimitation and phylogeny of *Harpalejeunea* will be addressed in separate studies.

A few *Harpalejeunea* species, namely *H. filicuspis*, *H. fischeri*, and *H. latitans* were resolved in a clade with several species of *Microlejeunea* including the generitype *M. africana*. These species lack the typical *Harpalejeunea* underleaves; instead they possess underleaves with forward directed lobes (e.g., Mizutani 1973, Figure IV: 12-13; Grolle 1980, Figure 2: f-i; Buchbender & Fischer 2004, Figures 6 and 7). *Microlejeunea* resembles *Harpalejeunea* in many aspects but has the above type of underleaves (Grolle & Reiner-Drehwald 1999). Hence a transfer of the above three species of *Harpalejeunea* to *Microlejeunea* is justified both by molecular and morphological data, and formalized below. Already Schuster (1980: 1177) doubted the systematic position of *H. filicuspis* and considered a placement in *Drepanolejeunea*, as was first proposed by Stephani (1913b: 344). *Drepanolejeunea*, however, has pycnolejeuneoid innovations, not the lejeuneoid ones of the *Lejeunea* complex (Mizutani 1973, Grolle 1980). The difficulties in separating *Harpalejeunea filicuspis* from *Drepanolejeunea* were also evident in our DNA vouchers. All four Indonesian *Drepanolejeunea vesiculosa* accessions were initially identified as *Harpalejeunea filicuspis*.

Pluvianthus forms a derived clade within the *Microlejeunea* lineage. This genus shares with *Microlejeunea* the shape of the underleaves, the presence of three medullary cells and the tendency to produce ocelli (Schuster & Schäfer-Verwimp 1995). Otherwise it is quite different; its sole species *P. squarrosus* differs from other representatives of the *Microlejeunea* clade (Figures 1, 2) by its larger size and robust habit, and larger leaf lobes (see Schuster & Schäfer-Verwimp 1995 for a detailed morphological treatment). However, we support monophyletic genus concepts (Humphreys & Linder 2009) and treat *Pluvianthus* as a synonym of *Microlejeunea*.

Key for identification of the genera of the *Lejeunea* complex

1. Plants lacking ocelli..*Lejeunea**
– Plants with ocelli..2
2. Underleaves ovate, with forward-directed, blunt to acute lobes separated by a narrow sinus ....*Microlejeunea*
– Underleaves obtrapezoid, with diverging, blunt (obtuse to rounded) lobes separated by a wide sinus...*Harpalejeunea*

*the sole representative of the genus with ocelli, *Lejeunea huctumalcensis* Lindenberg & Gottsche in Gottsche et al. (1847: 762) has not yet been included in a molecular phylogenetic study.

Although our treatment leads to a morphologically somewhat unstable *Microlejeunea* concept, the genus can be separated from other genera of the *Lejeunea* complex by the shape of the underleaves and the presence of ocelli, which, however, are best seen in living or freshly collected material. At first glance, the new circumscription may be somewhat irritating, however, numerous genera of liverworts show an even broader morphological variation, e.g., *Chiloscyphus* Corda (1829: 651), *Lejeunea*, *Leptoscyphus* Mitten (1851: 358), *Frullania* Raddi (1818: 9) or *Plagiochila* (Dumortier 1831: 42) Dumortier (1835: 14). Our study supports a general trend towards broader genus concepts in Lejeuneaceae (Heinrichs et al. 2012a, b), which still belong to the poorly studied liverwort families in terms of molecular investigation. Follow-up studies with a denser taxon sampling are needed to scrutinize the new genus concepts.

Distribution of ocelli:—Ocelli are widespread in Lejeuneaceae and occur also in Frullaniaceae (Gradstein et al. 2003). According to our reconstruction (Figure 2), ocelli evolved twice in Lejeuneeae. However, the position of *Pycnolejeunea* (Spruce 1884: 246) Schiffner (1893: 124) is unsecured and lacks bootstrap support. Ocelli were lost twice in the main "ocelli-clade". The pattern will likely be somewhat more complex when ocellate *Cheilolejeunea* (Spruce 1884: 251) Schiffner (1893: 124) (Bastos 2012) and *Cololejeunea* species (Zhu & So 1999) will be included in the dataset.

The presence or absence of ocelli allows for a recognition of two main clades of the "*Lejeunea* complex", *Lejeunea* (without ocelli) and *Microlejeunea* plus *Harpalejeunea* (with ocelli). He & Piippo (1999) and Gradstein et al. (2003) distinguished different types of ocelli based on their distribution in leaves and underleaves and their arrangement; those of *Harpalejeunea* are colorless and form a short row near the leaf base (Gradstein et al. 2001). The 1-3(-6) ocelli of *Microlejeunea* are usually positioned near the leaf base. *Pluvianthus* (*Microlejeunea*) *squarrosus* includes highland forms with leaves with 0-2(4) basal ocelli and forms from lower elevations having up to 16 ocelli in 2–3 rows at the leaf bases, in addition to up to 70 ocelli in the perianth (Schuster & Schäfer-Verwimp 1995). A larger sampling is necessary to evaluate whether the distribution of ocelli in *Pluvianthus squarrosus* is correlated with molecular variation.

Species classification and extensions of range:—Multiple accessions of *Pluvianthus squarrosus* and several *Drepanolejeunea* species form robust monophyletic lineages, indicating congruence of molecular and morphological species concepts (Figs 1, 2). Our study also provides evidence for a larger African range of *Drepanolejeunea vandenberghenii*, a recently described species that is so far known only from Rwanda (Buchbender & Fischer 2004). Several accessions from Madagascar, Malawi and Réunion formed a monophyletic lineage with a *D. vandenberghenii* paratype from Rwanda, and were also in good accordance with the detailed morphological description of Buchbender & Fischer (2004).

Buchbender & Fischer (2004) already pointed to morphological similarities of *D. vandenberghenii* and *D. vesiculosa*. This observation is confirmed in our study where both species form a robust sister relationship. In the present study we adopt a wide species concept for *D. vesiculosa* [incl. *D. physaefolia* (Gottsche 1882: 357) Stephani (1913b: 324); Tixier (1995)]; however, the considerable sequence variation within the *D. vesiculosa*clade may indicate that several independent biological entities are at hand.

In contrast to the situation in *Drepanolejeunea*, not all morphologically circumscribed *Microlejeunea*species were consistent with the relationships supported by the genotypic evidence. The *Microlejeunea africana* specimens from Principe Island—the type locality—and an accession from Madagascar formed independent lineages although they were morphologically very similar. The *Microlejeunea* sp. accession from Thailand was originally identified as *M. ulicina* (Taylor 1844: 115) Stephani (1890: 88), however, *M. ulicina* accessions from the Canary Islands were only loosely related to this accession. Morphologically cryptic or nearly cryptic speciation is a common phenomenon in liverworts (e.g., Odrzykoski & Szweykowski 1991, Wachowiak et al. 2009, Feldberg et al. 2010, Heinrichs et al. 2010, 2011, Kreier et al. 2010, Renner et al. 2011, Dong et al. 2012) and not unexpected in the morphogically strongly reduced genus *Microlejeunea*. A much denser sampling is required to arrive at a more natural classification of the genus, however, only few specimens are available which are suitable for molecular investigation. *Microlejeunea* species often grow intermingled with other bryophytes, and often only in very small cushions. On the other hand it would be worthwile to investigate intercontinentally distributed taxa such as *M. ulicina* for the presence of geographical clades and other morphologically cryptic lineages. It needs to be stressed that *M. africana* has already been treated as a subspecies of *M. ulicina* based on a broad morphological overlap (Vanden Berghen 1972). Grolle (1995) pointed out that the distinction between the two taxa is merely based on geographical patterns and needs clarification; however, the uniform morphology belies a considerable molecular variation.

Taxonomic treatment

Microlejeunea Stephani (1888: 61)
= *Pluvianthus* Schuster & Schäfer-Verwimp in Schuster (1994: 213), *syn. nov.*

Microlejeunea squarrosa (Steph.) Heinrichs, Schäf.-Verw., Pócs & S. Dong, *comb. nov.*
Basionym: *Strepsilejeunea squarrosa* Steph., Hedwigia 35: 130, 1896.
= *Pluvianthus squarrosus* (Steph.) R.M. Schust. & Schäf-Verw., J. Hattori Bot. Lab. 75: 213, 1994.

Microlejeunea filicuspis (Steph.) Heinrichs, Schäf.-Verw., Pócs & S. Dong, *comb. nov.*
Basionym: *Drepanolejeunea filicuspis* Steph., Spec. Hepat. V: 344, 1913.
= *Harpalejeunea filicuspis* (Steph.) Mizut., J. Hattori Bot. Lab. 37: 197, 1973.

Microlejeunea fischeri (Tixier) Heinrichs, Schäf.-Verw., Pócs & S. Dong, *comb. nov.*
Basionym: *Harpalejeunea fischeri* Tixier, Trop. Bryol. 11: 29, 1995.

Microlejeunea latitans (Hook. f. & Taylor) Heinrichs, Schäf.-Verw., Pócs & S. Dong, *comb. nov.*
Basionym: *Jungermannia latitans* Hook. f. & Taylor, London J. Bot. 3: 399, 1844.
= *Lejeunea latitans* (Hook. f. & Taylor) Gottsche, Lindenb. & Nees, Syn. Hepat. 345, 1845
= *Drepanolejeunea latitans* (Hook. f. & Taylor) Steph., Hedwigia 29: 72, 1890.
= *Harpalejeunea latitans* (Hook. f. & Taylor) Grolle, J. Hattori Bot. Lab. 47: 239, 1980.

Perspectives

Our study is a step forward towards a monophyletic genus concept for the *Lejeunea* complex. However, several putatively related genera such as *Bromeliophila* and *Metalejeunea* need to be included in follow-up studies. Our previous attempts to extract DNA from herbarium specimens of these genera were not successful but fresh material may allow to produce sequences of these taxa.

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TABLE 1. Taxa used in the present study, including information about the origin of the studied material, voucher information, as well as GenBank accession numbers. New sequences in bold face.

Taxon	Voucher	*rbc*L	*trn*L-F	nrITS
Acanthocoleus javanicus (Steph.) Kruijt	Bali, Schäfer-Verwimp & Verwimp 20817 (GOET)	DQ983648	DQ987398	DQ987291
Acrolejeunea fertilis (Reinw. et al.) Schiffn.	Bali, Schäfer-Verwimp & Verwimp 17009(GOET)	AY684929	DQ987391	DQ987281
Anoplolejeunea conferta (C.F.W. Meissn. ex Spreng.) A.Evans	Ecuador, Wilson et al. 04-08 (GOET)	DQ983653	DQ987438	DQ987335
Archilejeunea fuscescens (Hampe ex Lehm.) Fulf.	Costa Rica, Bernecker 97 – 53 (GOET)	DQ983655	DQ987384	DQ987267
Aureolejeunea aurifera R.M.Schust.	Costa Rica, Holz CR00-696 (GOET)	AY548082	DQ238569	DQ987272
Aureolejeunea fulva R.M.Schust.	Brazil, Costa & Gradstein 3849 (GOET)	AY548070	--------------	DQ987269
Aureolejeunea quinquecarinata R.M.Schust.	Ecuador, Schäfer-Verwimp & Preußing 23299/A (GOET)	DQ983658	DQ987450	DQ987350
Bryopteris diffusa (Sw.) Nees	Bolivia, Acebey & Villavicencio 855 (GOET)	AY548085	AM237147	AM237095

Ceratolejeunea cornuta (Lindenb.) Steph.	Bolivia, Drehwald 4739 (GOET)	AY548088	DQ238570	DQ987257
Ceratolejeunea cf. *guianensis* (Nees & Mont.) Steph.	Ecuador, Wilson et al. 04-15 (GOET)	DQ983662	DQ987442	DQ987340
Cheilolejeunea acutangula (Nees) Grolle	Mexico, Gradstein & Velasquez s.n. (GOET)	DQ983664	DQ987386	DQ987270
Cheilolejeunea beyrichii (Lindenb.) E.Reiner	Honduras, Allen 17393 (GOET)	AY548091	DQ987387	DQ987271
Cheilolejeunea clypeata (Schwein.) W. Ye & R.L. Zhu	USA, Davis s.n. (GOET)	DQ983699	DQ987426	DQ987322
Cheilolejeunea revoluta (Herz.) Gradst. & Grolle	Costa Rica, Dauphin 1990 (GOET)	DQ983667	DQ987454	DQ987354
Cheilolejeunea rigidula (Mont.) R.M.Schust.	Suriname, Munoz 98-62 (GOET)	DQ983668	DQ987453	DQ987353
Cheilolejeunea xanthocarpa (Lehm. & Lindenb.) Malombe	Brazil, Costa & Gradstein 3839 (GOET)	-------------	DQ987470	DQ987371
Cololejeunea laevigata (Mitt.) Tilden	New Zealand, von Konrat & Herangi 503 (F)	DQ238563	DQ238571	DQ987349
Cololejeunea metzgeriopsis (K.I.Goebel) Gradst. et. al.	Malaysia, Gradstein et al. 10436 (GOET)	DQ238567	DQ242521	DQ987319
Cololejeunea obliqua (Nees & Mont.) Schiffn.	Ecuador, Wilson et al. 04/11 (GOET)	DQ983669	-------------	DQ987337

Cololejeunea peculiaris (Herz.) Benedix	Malaysia, Schäfer-Verwimp & Verwimp 18861/A (GOET)	AY548095	DQ238572	DQ987280
Cololejeunea vitalana Tixier	Costa Rica, Schäfer-Verwimp & Holz SV/H-0473/A (GOET)	DQ238564	DQ238573	DQ987348
Colura acroloba (Mont. ex. Steph.) Ast	Fiji, Pócs 03261/BK (GOET)	DQ238565	DQ238586	DQ987306
Colura cylindrica Herzog	Guadeloupe, Schäfer-Verwimp & Verwimp 22154/B (JE)	JX470969	JX470980	JX470992
Colura irrorata (Spruce) Heinrichs, Y. Yu, Schäf.-Verw. & Pócs	Ecuador, Gradstein et al. 10033 (GOET)	AY548073	DQ238584	DQ987279
Colura ornithocephala Herzog	Ecuador, Schäfer-Verwimp & Nebel 32854 (JE)	JX470974	JX470985	JX470997
Colura tenuicornis A. Evans) Steph.	Dominican Republic, Schäfer-Verwimp & Verwimp 27039 (JE)	JX470967	JX470978	JX470990
Cyclolejeunea peruviana (Lehm. & Lindenb.) A. Evans	Colombia, Gradstein 8546 (GOET)	DQ983672	DQ987383	DQ987265
Diplasiolejeunea cavifolia Steph.	Malaysia, Schäfer-Verwimp & Verwimp 19036/A (GOET)	JQ729531	JQ729641	JQ729418
Diplasiolejeunea pauckertii Steph.	Panama, De Gracia et al. 338 (GOET)	JQ729541	JQ729652	JQ729429

Diplasiolejeunea pellucida (C.F.W. Meissn. ex Spreng.) Schiffner	Ecuador, Schäfer-Verwimp et al. 24134 (GOET)	JQ729500	JQ729607	JQ729383
Diplasiolejeunea unidentata (Lehm. & Lindenb.) Steph.	Guadeloupe, Schäfer-Verwimp & Verwimp 22500/A (GOET)	JQ729481	JQ729588	JQ729365
Drepanolejeunea anoplantha (Spruce) Steph.	Dominican Republic, Schäfer-Verwimp & Verwimp 27059 (JE)	KC313120	KC313159	KC313080
Drepanolejeunea anoplantha	Ecuador, Wilson et al. 04-22 (GOET)	DQ983677	--------------	DQ987372
Drepanolejeunea biocellata A. Evans	Ecuador, Gradstein 10053 (GOET)	AY548097	DQ238578	DQ987276
Drepanolejeunea granatensis (J.B. Jack & Steph.) Bischl.	Ecuador, Schäfer-Verwimp et al. 24383/B (JE)	KC313121	KC313160	KC313081
Drepanolejeunea hamatifolia (Hook.) Steph.	Azores, Schäfer-Verwimp & Verwimp 29482/A (JE)	--------------	KC313161	KC313082
Drepanolejeunea inchoata (C.F.W. Meissn.) Steph.	Ecuador, Gradstein 10169 (GOET)	KC313122	KC313162	KC313083
Drepanolejeunea mosenii (Steph.) Bischl.	Dominican Republic, Schäfer-Verwimp & Verwimp 27063 (JE)	KC313123	--------------	KC313084
Drepanolejeunea navicularis Steph.	Ecuador, Schäfer-Verwimp & Nebel 31772/A (JE)	KC313124	KC313163	KC313085

Drepanolejeunea vandenberghenii Buchbender & Eb. Fisch.	Madagascar, Pócs & Szabó 9778/AB (EGR)	KC313125	KC313164	KC313086
Drepanolejeunea vandenberghenii	Malawi, Pócs 9184/D (EGR)	KC313126	KC313165	KC313087
Drepanolejeunea vandenberghenii	Réunion (I), Pócs 08071/L (EGR)	KC313127	KC313166	KC313088
Drepanolejeunea vandenberghenii	Réunion (II), Pócs 08071/H (EGR)	KC313128	KC313167	KC313089
Drepanolejeunea vandenberghenii	Rwanda, Pócs 6249 (Paratype, EGR)	--------------	KC313168	KC313090
Drepanolejeunea vesiculosa (Mitt.) Steph.	Australia (I), Pócs & Streimann 99109/T (EGR)	KC313129	KC313169	KC313091
Drepanolejeunea vesiculosa	Australia (II), Pócs & Streimann 99122 (EGR)	AY302449	--------------	AY257496
Drepanolejeunea vesiculosa	Indonesia (I), Gradstein 12052 (GOET)	KC313130	KC313170	KC313092
Drepanolejeunea vesiculosa	Indonesia (II), Gradstein 10331 (GOET)	KC313131	KC313171	KC313093
Drepanolejeunea vesiculosa	Indonesia (III), Gradstein 12026 (GOET)	KC313132	KC313172	KC313094
Drepanolejeunea vesiculosa	Indonesia (IV), Gradstein 12014 (GOET)	KC313133	KC313173	KC313095
Drepanolejeunea	Madagascar, Pócs &	KC313134	KC313174	KC313096

vesiculosa	Szabó 9882/G (EGR)			
Drepanolejeunea vesiculosa	Malaysia, Gradstein et al. 10372 (GOET)	DQ983679	DQ987421	DQ987317
Echinolejeunea papillata (Mitt.) R.M.Schust.	New Zealand (I), Schäfer-Verwimp & Verwimp 14195/A (JE)	KC313135	KC313175	KC313097
Echinolejeunea papillata	New Zealand (II), Schäfer-Verwimp & Verwimp 13967 (JE)	KC313136	KC313176	KC313098
Evansiolejeunea roccatii Vanden Berghen	Rwanda, Fischer RWA-1120 (GOET)	DQ983680	DQ987427	DQ987323
Frullanoides corticalis (Lehm. & Lindenb.) van Slageren	French Guiana, Hartmann 04-080 (GOET)	DQ983681	AM237196	AM237143
Fulfordianthus evansii (Fulford) Gradst.	Costa Rica, Dauphin s.n. (GOET)	DQ983683	AM237197	AM237144
Harpalejeunea filicuspis (Steph.) Mizut.	Fiji Isls. (I), Pócs & Pócs 03306/R (EGR)	KC313137	KC313177	KC313099
Harpalejeunea filicuspis	Fiji Isls. (II), Pócs & Pócs 03304/A (EGR)	KC313138	KC313178	KC313100
Harpalejeunea filicuspis	Thailand, Pócs & Pócs 07006/A (EGR)	KC313139	KC313179	KC313101
Harpalejeunea fischeri Tixier	Uganda (I), Pócs & Lye 97141/T (EGR)	KC313140	KC313180	KC313102
Harpalejeunea fischeri	Uganda (II), Pócs & Lye 97142/AM (EGR)	KC313141	KC313181	KC313103

Harpalejeunea fischeri	Uganda (III), Pócs & Lye 97142/BB (EGR)	KC313142	KC313182	KC313104
Harpalejeunea fischeri	Uganda (IV), Pócs & Lye 97142/BQ (EGR)	KC313143	KC313183	KC313105
Harpalejeunea grandis Grolle & M. Reiner	Colombia, Cleef 6450 (Paratype, GOET)	KC313144	KC313184	KC313106
Harpalejeunea grandistipula R.M.Schust.	Ecuador, Schäfer-Verwimp et al. 24163/B (JE)	KC313145	KC313185	KC313107
Harpalejeunea latitans (Hook. F. & Tayl.) Grolle	New Zealand, Schäfer-Verwimp & Verwimp 13869 (JE)	KC313146	KC313186	KC313108
Harpalejeunea marginalis (Hook. f & Tayl.) Steph.	Chile, Schäfer-Verwimp & Verwimp 8082 (JE)	KC313147	KC313187	KC313109
Harpalejeunea molleri (Hook. f. & Tayl.) Grolle	Azores, Schäfer-Verwimp & Verwimp 29334 (JE)	KC313148	KC313188	KC313110
Lejeunea cf. *asthenica* Spruce	Bolivia, Gradstein 9948 (GOET)	DQ983731	DQ987428	DQ987324
Lejeunea cancellata Nees & Mont. ex Mont.	Ecuador, Wilson et al. 04-02 (GOET)	DQ983686	DQ987433	DQ987329
Lejeunea catinulifera Spruce	Ecuador, Wilson et al. 04-01 (GOET)	DQ983687	DQ987432	DQ987328
Lejeunea cavifolia (Ehrh.) Lindb.	Germany, Heinrichs 3695 (GOET)	AY548102	DQ238581	DQ987259

Lejeunea cerina (Lehm. & Lindeb.) Gottsche, Lindenb. & Nees	Ecuador, Wilson et al. 04-13 (GOET)	DQ983689	DQ987441	DQ987339
Lejeunea drehwaldii Heinrichs & Schäf-Verw.	Peru, Drehwald 10014 (JE)	HE995781	HE995782	HE995783
Lejeunea flava (Sw.) Nees	Brazil, Gradstein s.n. (GOET)	DQ983692	DQ987413	DQ987309
Lejeunea cf. *isocalycina* (Nees) Spruce	Ecuador, Wilson et al. 04-04 (GOET)	DQ983733	DQ987435	DQ987331
Lejeunea laetevirens Nees & Mont.	Dominica, Schäfer-Verwimp & Verwimp 17899 (GOET)	AY548103	DQ987402	DQ987296
Lejeunea lamacerina (Steph.) Schiffn.	Canary Islands, Schäfer-Verwimp & Verwimp 24616 (GOET)	DQ983694	--------------	DQ987358
Lejeunea mimula Hürl.	Bali, Schäfer-Verwimp & Verwimp 20930 (GOET)	AY548104	DQ238580	DQ987261
Lejeunea pallescens Mitt.	Ecuador, Schäfer-Verwimp & Preußing 23533 (GOET)	AY548068	DQ238576	DQ987292
Lejeunea paucidentata (Steph.) Grolle	Dominica, Schäfer-Verwimp & Verwimp 17737 (GOET)	DQ983695	DQ987447	DQ987345
Lejeunea cf. *pterigonia* (Lehm. & Lindenb.) Mont.	Bolivia, Gradstein 9964 (GOET)	DQ983732	DQ987429	DQ987325
Lejeunea sp. I	Ecuador, Gradstein & Jost	DQ983712	DQ987416	DQ987312

	10063 (GOET)			
Lejeunea sp. II	Ecuador, Gradstein 10172 (GOET)	DQ983734	DQ987410	DQ987304
Lepidolejeunea bidentula (J.B. Jack & Steph.) R.M. Schust.	China, Koponen et al. 51525 (H)	AY125936	AY144476	AY125340
Lepidolejeunea eluta (Nees) R.M.Schust.	Bolivia, Drehwald 4833 (GOET)	DQ983696	DQ987379	DQ987257
Lepidolejeunea integristipula (Jack & Steph.) R.M.Schust.	Fiji, Pócs 03307/AC (GOET)	DQ983697	DQ987417	DQ987313
Lopholejeunea eulopha (Tayl.) Schiffn.	Australia, Pócs & Streimann 9987/H1 (GOET)	AY548067	DQ987381	DQ987262
Luteolejeunea herzogii (Buchloh) Piippo	Costa Rica, Schäfer-Verwimp & Holz 0294/B (GOET)	DQ983706	DQ987467	DQ987368
Microlejeunea africana Steph.	Madagascar, Lübenau 2 (JE)	KC313149	KC313189	KC313111
Microlejeunea africana	São Tomé and Principe (I), Shevock 34576 A (GOET)	KC313150	KC313190	KC313112
Microlejeunea africana	São Tomé and Principe (II), Shevock 34576 B (GOET)	KC313151	KC313191	KC313113
Microlejeunea capillaris (Gottsche) Steph.	Costa Rica, Schäfer-Verwimp & Holz SV/H-	KC313152	KC313192	KC313114

	0489/B (JE)			
Microlejeunea colombiana Bischl.	Dominican Republic, Schäfer-Verwimp & Verwimp 26614/A (JE)	KC313153	KC313193	KC313079
Microlejeunea ulicina (Tayl.) Steph.	Canary Isls., Schäfer-Verwimp & Verwimp 24800 (GOET)	KC313154	KC313194	KC313115
Microlejeunea ulicina	Canary Isls., Schäfer-Verwimp & Verwimp 24666 (JE)	KC313155	KC313195	KC313116
Microlejeunea sp.	Thailand, Schäfer-Verwimp & Verwimp 16293 (JE)	KC313156	KC313196	KC313117
Macrocolura sagittistipula (Spruce) R.M.Schust.	Dominica, Schäfer-Verwimp & Verwimp 18014 (GOET)	DQ983707	DQ987466	DQ987367
Marchesinia robusta (Mitt.) Schiffn.	Ecuador, Wilson et al. 04-05 (GOET)	DQ983710	DQ987436	DQ987332
Mastigolejeunea auriculata (Wilson & W.J.Hooker) Schiffn.	Bolivia, Churchill 21275 (GOET)	AY548070	DQ987385	DQ987268
Myriocoleopsis gymnocolea (Steph.) M.E. Reiner & Gradst.	Ecuador, Gradstein et al. 10020 (GOET)	DQ238568	DQ238583	DQ987277
Neurolejeunea breutelii (Gottsche) A.Evans	Brazil, Schäfer-Verwimp & Verwimp 14740 (GOET)	DQ983714	DQ987405	DQ987299
Omphalanthus filiformis	Ecuador, Schäfer-	DQ983716	DQ987393	DQ987283

Nees	Verwimp & Preußing 23543 (GOET)			
Omphalanthus ovalis (Lindenb. & Gottsche) Gradst.	Ecuador, Wilson et al. 04-07 (GOET)	DQ983717	--------------	DQ987334
Physantholejeunea portoricensis (Hampe & Gottsche) R.M.Schust.	Guadeloupe, Schäfer-Verwimp & Verwimp 22615 (GOET)	DQ983719	DQ987448	DQ987346
Pluvianthus squarrosus (Steph.) R.M.Sch*ust. & Schäf.-Verw.*	Brazil (I), Schäfer-Verwimp & Verwimp14780 (JE)	KC313157	KC313197	KC313118
Pluvianthus squarrosus	Brazil (II), Schäfer-Verwimp & Verwimp 14638 (JE)	KC313158	KC313198	KC313119
Pluvianthus squarrosus	Brazil (III), Schäfer-Verwimp & Verwimp 13376 (GOET)	DQ983720	DQ987446	DQ987344
Ptychanthus striatus (Lehm. & Lindenb.) Nees	Java, Gradstein 10215 (GOET)	DQ983721	DQ987406	DQ987300
Pycnolejeunea densistipula (Lehm. & Lindenb.) Steph.	Ecuador, Schäfer-Verwimp & Preußing 23368 (GOET)	AY548075	DQ987400	DQ987294
Rectolejeunea berteroana (Gottsche) A. Evans	Guadeloupe, Schäfer-Verwimp & Verwimp 22245/A (GOET)	DQ983724	DQ987444	DQ987342
Schiffneriolejeunea nymannii (Steph.)	Malaysia, Gradstein et al. 10321 (GORT)	DQ983725	DQ987424	DQ987320

Gradst. & Terken				
Siphonolejeunea elegantissima (Steph.) Grolle	Australia, Pócs & Brown 0026/AA (E)	DQ983726	DQ987452	DQ987352
Spruceanthus thozetianus (Gottsche & F. v. Müll.) B. Thiers	Australia, Pócs 01107/M (GOET)	AM384877	DQ987460	DQ987362
Thysananthus spathulistipus (Reinw. et al.) Lindenb.	Bali, Schäfer-Verwimp & Verwimp 20790 (GOET)	DQ983739	DQ987392	DQ987282
Xylolejeunea crenata (Nees & Mont.) X.-L. He & Grolle	Brazil, Schäfer-Verwimp & Verwimp 11225 (GOET)	DQ983740	DQ987443	DQ987341

TABLE 2. Distribution of constant and phylogenetically informative sites for aligned positions of the three genomic regions.

	***rbc*L**	***trn*L-F**	**ITS1-5.8S-ITS2**	**Total**
Number of sites in matrix	914	433	717	2,064
constant	634	202	280	1,116
autapomorphic	80	55	55	190
parsimony informative	200	176	382	758

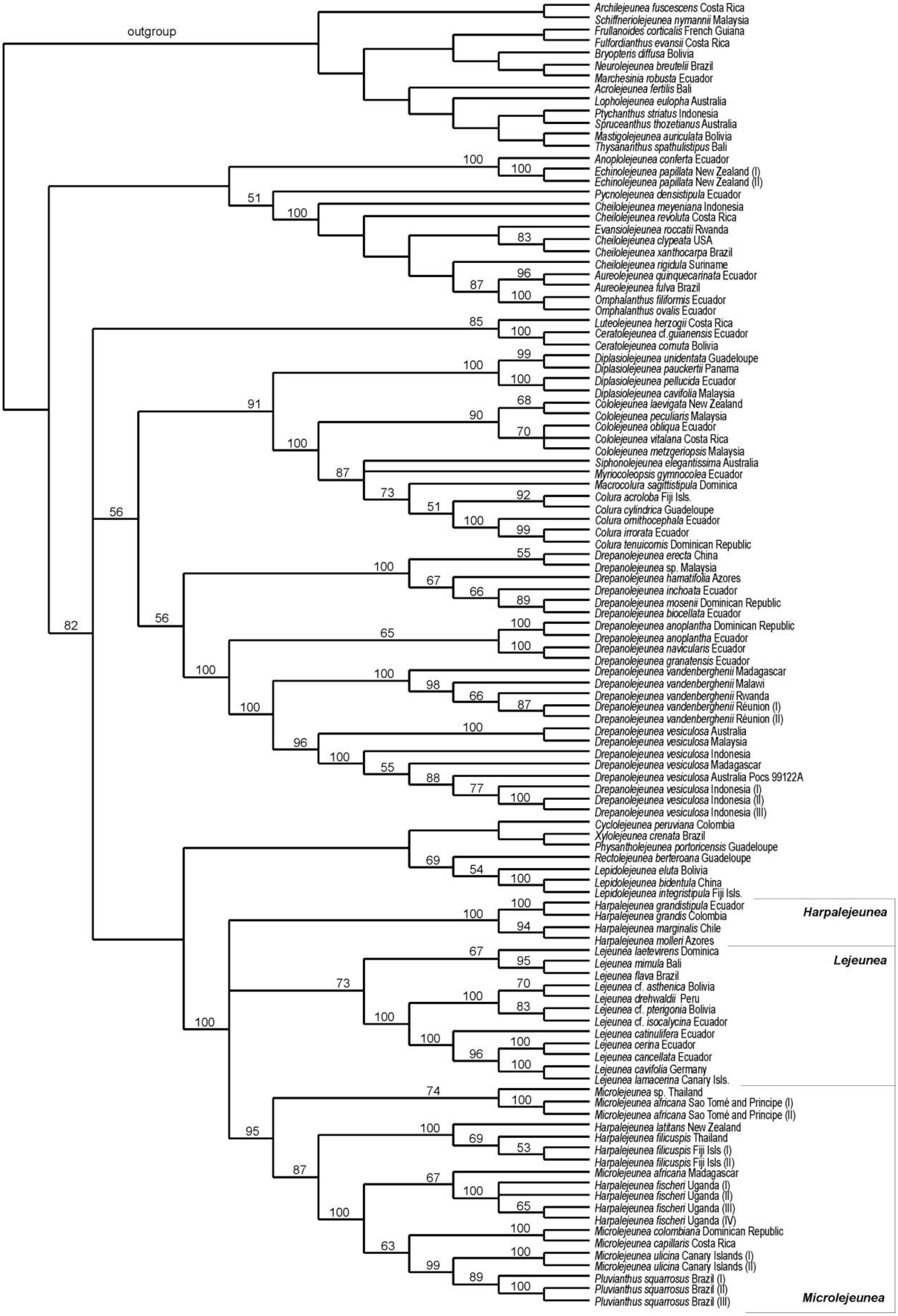

FIGURE 1. Strict consensus of 5 equally parsimonious trees based on the combined nrITS- chloroplast DNA *rbc*L—*trn*L-F dataset with bootstrap percentage values ≥50 at branches.

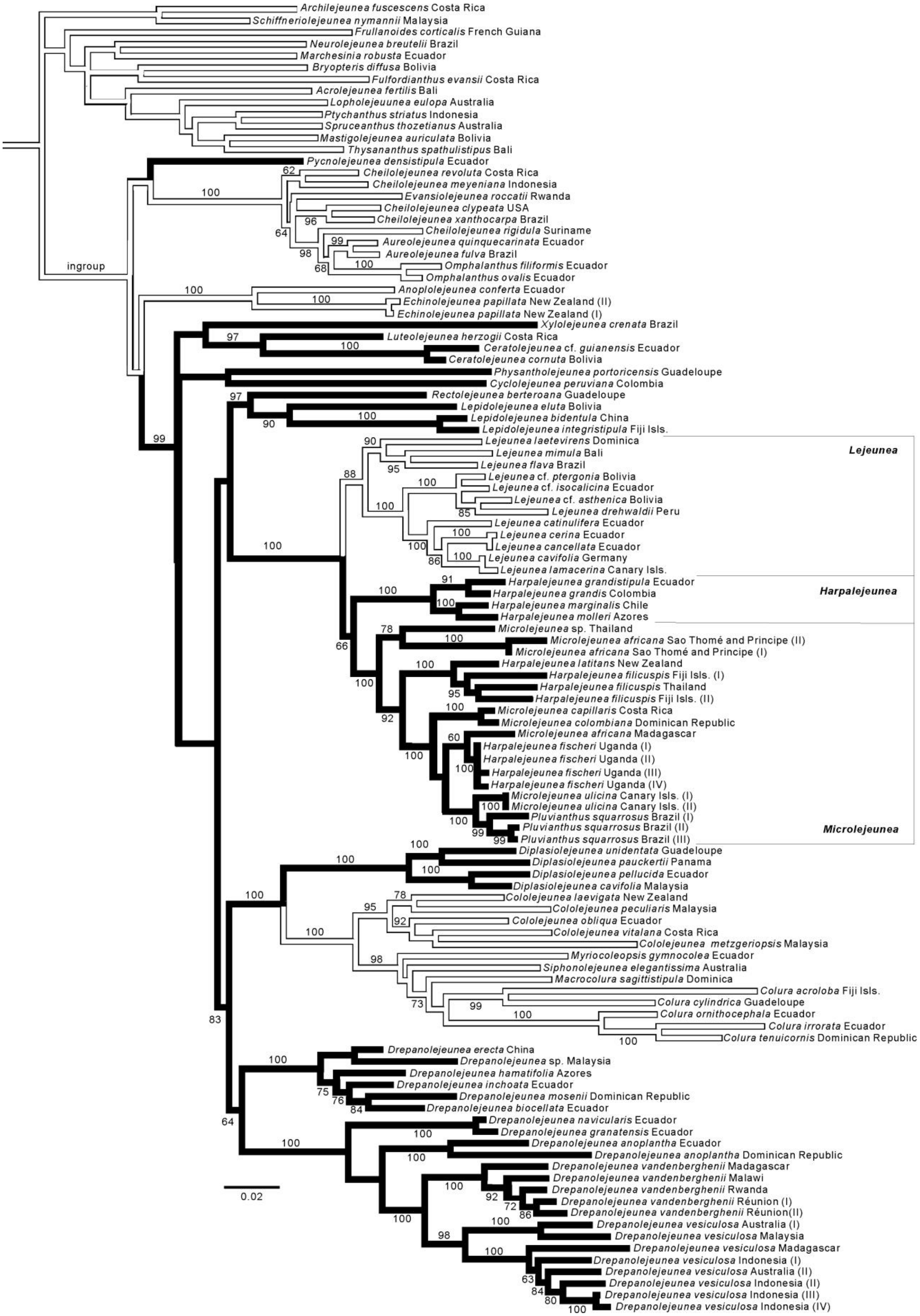

FIGURE 2. Phylogram generated in a maximum likelihood analysis of the combined dataset with bootstrap percentage values ≥50 indicated at branches. Black lines: ocelli present, white lines: ocelli lacking.

Appendix 4

Molecular study of *Myriocolea*: A 150-year old mystery solved: Transfer of the rheophytic endemic liverwort *Myriocolea irrorata* to *Colura*

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Kathrin Feldberg, Jörn Hentschel, Alexander R. Schmidt, Harald Schneider.

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Abstract

Myriocolea irrorata is an endemic rheophytic liverwort known from a few localities in the Eastern Andes of Ecuador. Morphologically it belongs to the Cololejeunea-Tuyamaella clade of Lejeuneaceae, however, due to its exclusively Radula-type branching, transversely inserted, hollow leaves, large size, and an extremely high number of clustered gynoecia it has often been regarded as an isolated element of this group. Phylogenetic analyses of a molecular dataset consisting of three markers (nuclear ribosomal ITS region, plastidic *trn*L-F region and *rbc*L gene) and 20 accessions resolved Myriocolea in one of the main clades of Colura, sister to the generitype Colura calyptrifolia. Based on the molecular topology and a reinterpretation of morphological traits, Myriocolea irrorata is transferred to Colura, as Colura irrorata. The example Myriocolea/Colura adds to growing evidence that rheophytic liverworts may develop unusual morphologies that hamper their classification using exclusively morphology.

Key words: Lejeuneaceae, liverwort, Porellales, taxonomy

Introduction

In 1857, the English botanist Richard Spruce collected a previously unknown rheophytic liverwort along the Río Topo of the Eastern Andes of Ecuador. Describing it in a new genus of Lejeuneaceae, *Myriocolea* Spruce (1884: 305), he stated that “anything more alien from the aspect of a *Lejeunea* cannot well be imagined” (Spruce 1884: 307). Using a very wide genus concept for *Lejeunea* Libert (1820: 372), Spruce only accepted two genera in Lejeuneaceae, *Lejeunea* with several hundreds of species, and the monospecific *Myriocolea* with its single representative *Myriocolea irrorata* Spruce (1884: 305). This species differs from all other Lejeuneaceae by the exclusive presence of Radula-type branches, transversely inserted leaves without well delimited lobuli, presence of numerous antheridia per bract, and an extraordinary high number of clustered gynoecia (Thiers 1983, Gradstein et al. 2004). By its up to ca. 5 cm long, stiff, protruding leafy shoots it is easily recognizable in the field, however, was not redetected until 2002. It grows on twigs of shrubs occurring along the Topo and Zuñac rivers, especially on the periodically submerged riverbanks (Gradstein & Nöske 2002, Gradstein et al. 2004).

The systematic position of the monospecific genus *Myriocolea* has been subject to controversy. Schuster (1963a: 93) set up a new subfamily Myriocoleoideae to include *Myriocolea* and *Cladocolea* Schuster (1963a: 155), hom. illeg. [= *Schusterolejeunea* Grolle (1980: 105)], a taxon treated as a synonym of Lejeuneoideae by Gradstein (1994). The latter treatment was also supported by cladistic analyses of morphological character states of Lejeuneaceae (Gradstein et al. 2003) where *Myriocolea* was resolved in an unsupported *Cololejeunea-Tuyamaella* clade. This relationship was also recovered by several molecular phylogenies based on one or two molecular markers (Wilson et al. 2004; Gradstein et al. 2006). Phylogenetic analyses of a comprehensive four molecular marker set of Lejeuneaceae consistently located *Myriocolea* in a clade with *Macrocolura* Schuster (1994: 233) and *Colura* (Dumortier 1831: 32) Dumortier (1835: 12) (Wilson et al. 2007a). However, the lack of taxonomic sampling of these two genera hampered the discovery of the true relationships of *Myriocolea*. Addition of further *Colura* sequences to the published ones pointed to a possible close relationship of the genera *Colura* and *Myriocolea* (Yu et al., unpublished). Here we present the results of phylogenetic analyses of a dataset comprising two chloroplast genome regions and one nuclear genome region and a comprehensive taxonomic sampling of *Macrocolura*, *Myriocolea* and 15 accessions of *Colura*.

Materials and Methods

Taxa studied, including GenBank accession numbers and voucher details, are listed in Table 1. Besides availability of material, ingroup taxa were selected to represent the morphological variation and geographical distribution of *Colura*. Multiple accessions of several species were used to explore intraspecific genetic variation. Based on the analyses of Wilson et al. (2007a), *Cololejeunea laevigata* (Mitten 1855: 157) Schuster (1963b: 241) and *Cololejeunea vitalana* Tixier (1995: 230) were designated as outgroup taxa for phylogenetic reconstruction. The ingroup includes accessions of *Colura* and *Macrocolura*.

DNA extraction, PCR amplification and sequencing

Plant tissue was isolated from herbarium collections housed at the herbaria EGR, GOET and JE. Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: *rbc*L gene and *trn*L-F region from Gradstein et al. (2006), and nrITS1-5.8S-ITS-2 region from Hartmann et al. (2006). Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR. Thirty six sequences were newly generated for this study and 21 sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall 1999). Ambiguous positions were excluded from all alignments. Maximum parsimony (MP) and maximum likelihood (ML) analyses were carried out with PAUP* version 4.0b10 (Swofford 2000). MP heuristic searches were conducted with the following options implemented: heuristic search mode, 1,000 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Non-parametric bootstrapping values (Felsenstein 1985) were generated as heuristic searches with 1,000 replicates, each with ten random-addition replicates. Bootstrap percentage values (BPV) ≥ 70 were regarded as good support (Hillis & Bull 1983). The individual marker sets and the combined chloroplast DNA dataset vs. nrITS dataset were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70% (Mason-Gamer & Kellogg 1996). The trees gave no evidence of incongruence. Hence the datasets were combined.

jModeltest 0.1 (Posada 2008) was used to select a model of evolution for ML analyses of the combined dataset. A General Time Reversible (GTR) model (Tavaré 1986) was chosen with among-site rate heterogeneity modelled as discrete gamma distribution with four rate categories, and its estimated parameters (Γ) (Goldman 1993). The ML-Analysis was performed as heuristic search, MULTrees option on, collapse zero length branches off, and TBR branch swapping. The confidence of branching was assessed using 300 non-parametric bootstrap resamplings generated as heuristic searches.

Results

Of the 2,321 investigated molecular characters, 468 were parsimony informative and 166 autapomorphic (see Table 2 for character state distributions within the single markers). The MP analysis resulted in a single tree of 304 steps with a consistency index of 0.696 and a retention index of 0.774 (not depicted). The ML analysis likewise resulted in a single tree (Figure 1) that confirmed the MP topology. *Macrocolura* was placed sister to the well supported *Colura*-clade. *Colura* comprised two main clades with ML/MP bootstrap percentage values of 99 and 100 respectively. One main clade included only Neotropical accessions of *Colura* [*Colura calyptrifolia* (Hooker 1813: pl. 43) Dumortier (1835: 12), *C. ornithocephala* Herzog (1952a: 107)*, C. rhynchophora* Jovet-Ast (1948: 27), *C. tenuicornis* (Evans 1900: 455) Stephani (1916: 942)] as well as the monospecific genus *Myriocolea*. The generitype of *Colura*, *C. calyptrifolia*, was placed sister to a clade with two accessions of *Myriocolea*. This sister relationship achieved a ML bootstrap percentage value of 100 and a MP value of 99. The second main clade of *Colura* separated into a robust Asian-Australasian clade comprising *C. inflata* Goebel (1928: 11) and *C. acroloba* (Stephani 1890: 97) Jovet-Ast (1953: 297) and a clade with Australasian [*C. imperfecta* Stephani (1916: 938)], African [*C. digitalis* (Mitten 1886: 325) Stephani (1916: 931)] and tropical American accessions [*C. cylindrica* Herzog (1952a: 106), *C. tortifolia* (Montagne 1843: 265) Trevisan de Saint-Léon (1877: 402)]. The African accession was placed sister to the Neotropical clade in a robust sister relationship. Multiple accessions of several morphologically circumscribed species formedrobust monophyletic lineages with bootstrap percentage values between 93 and 100.

Based on the molecular topology and adopting a monophyly approach we transfer *Myriocolea irrorata* to *Colura*:

Colura (Dumortier 1831: 32) Dumortier (1835: 12)
= *Myriocolea* Spruce (1884: 305)**, *syn. nov.***

***Colura irrorata* (Spruce)** Heinrichs, Y.Yu, Schäf.-Verw. & Pócs, ***comb. nov*.**
Basionym: *Myriocolea irrorata* Spruce (1884: 305).
Type:— ECUADOR, Tungurahua, Río Topo, Spruce s.n. (isotype, G).

Discussion

Classification: General trends

Morphology based classification systems often include small or even monospecific genera. However, modern broad scaled taxonomical studies tend to the acceptance of larger genera and incorporation of many small taxonomic entities, especially when molecular data are included and a monophyly approach is adopted (Humphreys & Linder 2009). Although molecular studies may lead to the division of large genera such as *Jungermannia* Linnaeus (1753: 1131) (Hentschel et al. 2007b), there is a general trend towards larger genus concepts. Phylogenetic analyses repeatedly demonstrated that the character states separating small genera may in fact represent autapomorphies of taxa nested in larger genera (e.g., Heinrichs et al. 2003). Small genera of liverworts that have recently been synonymized with larger genera based on phylogenetic evidence and reinterpretation of morphological character states include *Rhodoplagiochila* Schuster (1978: 247), *Steereochila* Inoue (1987: 279), *Szweykowskia* Gradstein & Reiner-Drehwald (1995: 33) [= *Plagiochila* (Dumortier 1831: 42) Dumortier (1835: 14), Heinrichs et al. 2004], *Metzgeriopsis* Goebel (1888: 54) [= *Cololejeunea* (Spruce 1884: 291) Schiffner (1893: 121), Gradstein et al. 2006], *Pachyglossa* Herzog & Grolle (1959: 150) [= *Chiloscyphus* Corda (1829: 651), Hentschel et al. 2007a] and *Perssoniella* Herzog (1952b: 268) [= *Schistochila* Dumortier (1835: 15), He & Glenny 2010]. The transfer of *Myriocolea* to *Colura* thus confirms a general trend in plant systematics.

Colura and *Myriocolea*

Colura is a large pantropical genus comprising about 70 species (Jovet-Ast 1953, Gradstein et al. 2001, Grolle & Zhu 2002) of which eleven species were investigated in our study. Species of *Colura* are typically recognized by their leaf morphology: the presence of lobules forming an apical sac with an aperture mechanism consisting of a valve and a hinge. This sac varies greatly in size and shape, and may function for water retention and in some species also for zoophagy (Barthlott et al. 2000). *Colura* species grow in well-illuminated sites from the lowlands to about 4,000 m above sea level, usually as epiphytes on twigs and trunks or on leaves, as epiphylls.

At first glance, the position of *Myriocolea irrorata* in the *Colura* sect. *Colura* clade is surprising. However, Thiers (1983) points out that the unique aspect of *Myriocolea irrorata* results from an exaggeration of conditions found elsewhere in the Lejeuneaceae whereas an exclusively Radula-type branching is otherwise unknown in Lejeuneaceae. However, Radula-type branches in combination with Lejeunea-type branches occur, e.g., in several species of *Lejeunea* (Schuster 1994, Reiner-Drehwald 2000a, 2005).

Morphological support for the synonymy of *Myriocolea* and *Colura* comes from the number of underleaves. Usually, Lejeuneaceae have one underleaf per leaf pair, however, a few exceptions prove the rule (Gradstein et al. 2003). The presence of one underleaf per leaf characterizes *Colura*, *Diplasiolejeunea* (Spruce 1884: 301) Schiffner (1893: 121), *Macrocolura* and *Myriocolea*, the latter being here identified as an element of *Colura*. *Colura* (*Myriocolea*) *irrorata* does not produce well-developed lobules; however, the ventral part of the hollow leaves may be interpreted as an incompletely inrolled, large lobule. Other members of *Colura* sect. *Colura* are characterized by a lobule consisting of a sac terminated by a long, conico-cylindric, elongate horn (Grolle & Zhu 2002). *Colura irrorata* is not the only species of *Colura* without a well-developed lobule. In *Colura* sect. *Heterophyllum* Jovet-Ast (1983: 213) there is also a tendency to lose the lobule, which is very much reduced or completely missing in *Colura corynephora* (Nees, Lindenberg & Gottsche in Meyen 1843: 474) Trevisan de Saint-Léon (1877: 402), a phenomenon that parallels the situation in *Colura irrorata*. This fact also raises the classification problem of *Calatholejeunea* Goebel (1928: 8), which generally resembles *Colura* by its pendular segmentation and *Colura irrorata* by its transversely inserted, hollow leaves with unsharply defined lobules. *Calatholejeunea* was morphologically compared to *Colura* by Mizutani (1984) but its molecular phylogeny has not yet been studied.

Colura irrorata is a rheophilous liverwort growing on twigs of shrubs on the periodically inundated riverbanks of the Río Topo. The rheophytic, nutrient-rich habitat may explain the untypical leaf development of *Colura irrorata* because a structure for water storage or zoophagy is not needed in such an environment. Rheophytes from different taxonomic groups exhibit a parallel development, because running waters and regular flooding shape them into flood resistant plants (Van Steenis 1981). Accordingly, *Colura irrorata* shares its long, robust stems, pinnate branching, and the presence of numerous small gametoecial branches with other rheophytic Lejeuneaceae, namely *Myriocoleopsis* Schiffner (1944: 234), *Lejeunea* subg. *Neopotamolejeunea* (Reiner-Drehwald 2000b: 449) Gradstein & Reiner-Drehwald (2007: 484) (Wilson et al. 2007a) and *Cololejeunea stotleriana* (Gradstein et al. 2011: 13). Non-rheophytic *Colura* species have a smaller size, grow usually attached to the substratum, have sac-like lobules, a lower number of gametoecia, and less robust stems. However, the phylogenetic distance between *Colura irrorata* and other members of *C*. sect. *Colura* is low (Fig. 1), indicating that the rheophytic species *C*. *irrorata* originated in rather recent times. This scenario is also supported by the derived position of *C. irrorata*. It is likely that the Ecuadorian endemic *C. irrorata* evolved from a local population of a species close to *C. calyptrifolia* and *C. tenuicornis*, and that the morphological rearrangements of the gametophyte took place in a short period of time. A rapid reorganization of gametophytical traits has also been demonstrated for some epiphytic representatives of *Plagiochila* sect. *Hylacoetes* Carl (1931: 50) (Heinrichs et al. 2003), providing some evidence that an occurrence in extreme habitats may occasionally lead to considerable changes in morphology. The molecular control procedures of such rapid rearrangements and their contribution to plant evolution are still incompletely understood (Stern 2000, Carroll 2008, Theissen 2009, Frankel et al. 2011). However, rheophytic plants appear to be an excellent group on which to study the impact of selection on the establishment of rapid growth habit changes. Research on Japanese occurrences of the terrestrial fern *Osmunda japonica* Thunberg (1780: 209) and the rheophytic *O. lancea* Thunberg (1784: 330) showed that habitat conditions may lead to dramatic changes of the leaf morphology in sister taxa that otherwise share more or less the same genetic information (Imaichi & Kato 1992; Yatabe et al. 2009).

Species concepts and supraspecific classification of *Colura*

Colura species have so far been described using a morphological-typological approach. Multiple accessions of several morphologically circumscribed species form monophyletic lineages (*C. acroloba*, *C. cylindrica*, *C. imperfecta*, *C. irrorata*, *C. ornithocephala*, *C. tortifolia*, Fig. 1), indicating congruence of morphological and phylogenetic species concepts.

Grolle & Zhu (2002) provided the most recent classification of *Colura*. They split *Colura* into two subgenera and six sections, and accepted the monospecific genus *Macrocolura* based on its asymmetrically shaped, only weakly bifid underleaves with rhizoid fascicles originating from their central part. *Colura*, in contrast, has symmetrical, deeply bifid underleaves with rhizoid fascicles originating at the base of the underleaves. Earlier, *Macrocolura* was treated as *Colura* sect. *Lingua* Jovet-Ast ex Thiers (1987: 177). In our study, *Macrocolura* is placed sister to *Colura*, hence both treatments appear consistent with the recovered phylogeny. Based on the molecular topology and the morphological disparities, we tentatively accept *Macrocolura* as a genus. This taxonomic position, however, should be scrutinized by an extension of the *Colura* taxon sampling.

The classification of *Colura* into subg. *Colura* with hinged valves and subg. *Glotta* Grolle & Zhu (2002: 187) having valves without a distinct hinge (Grolle & Zhu 2002), is not confirmed in our study because *C*. subg. *Glotta* (represented in our study by *C.* sect. *Heterophyllum*) nests in *C*. subg. *Colura* (Fig. 1). In total, we included members of four out of six *Colura* sections in our sampling. Sections *Colura* (following inclusion of *Myriocolea*), *Harmophyllum* Grolle (1965: 44) and *Oidocorys* Jovet-Ast ex Grolle (1969: 140) were resolved as monophyletic entities. *Colura* sect. *Heterophyllum* is paraphyletic because *C.* sect. *Harmophyllum* nests in it. Grolle & Zhu (2002) included species with rounded valves in *Colura* sect. *Heterophyllum*, however, according to our topology this section should be split in two entities, of which the *C. imperfect* clade represents *C*. sect. *Heterophyllum* s. str. (Grolle & Zhu 2002). Our sampling, however, is too sparse to reinterpret morphological traits in *C*. sect. *Heterophyllum* s. l. The formal denomination of the *C. acroloba*-*C. inflata* clade should thus await a more comprehensive study, which should also consider representatives of *C.*sect. *Gamolepis* Jovet-Ast (1983: 207) and *Glotta* Grolle & Zhu (2002: 187).

Biogeography

A phylogenetic analysis of the largely epiphyllous genus *Diplasiolejeunea* revealed a remarkably clear geographical structure with several Neotropical and Paleotropical lineages (Dong et al. 2012). A similar structure is seen in the epiphytic-epiphyllous genus *Colura*, containing Asian-Australasian, Neotropical and African lineages (Fig. 1). The occurrence of a relatively high number of *Colura* species on isolated and young oceanic islands such as Fiji (Pócs & Eggers 2007) is best explained by long-distance dispersal through propagules; however, the clear geographical pattern within the genus seems to indicate that long distance dispersal occurs only occasionally. The range of the Cenozoic genus *Colura* (Wilson et al. 2007b) is possibly the result of rare long distance dispersal, frequent short-distance dispersal, local diversification, extinction and recolonization, processes that likely shaped the ranges of numerous other genera of leafy liverworts (Heinrichs et al. 2009). A more detailed biogeographic reconstruction needs a considerable extension of the taxon sampling and inclusion of multiple accessions of species with broad ranges.

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TABLE 1. Taxa used in the present study, including information about the origin of the studied material, voucher information, and the herbarium where the voucher is deposited, as well as GenBank accession numbers. Sequences in bold were obtained from GenBank.

Taxon	**Origin**	**Collector and herbarium**		**Genbank Accession number**	
			*rbc*L	*trn*L-*trn*F	nrITS
Cololejeunea laevigata (Mitt.) R.M. Schust.	New Zealand	von Konrat 81-503 (GOET)	**DQ238563**	**DQ238571**	**DQ987349**
Cololejeunea vitalana Tixier	Costa Rica	Schäfer- Verwimp & Holz SV/H-0473/A (GOET)	**DQ238564**	**DQ238573**	**DQ987348**
Colura acroloba (Mont. ex Steph.) Jovet-Ast	Fiji Isls.	Pócs 03261/BK (GOET)	**DQ238565**	**DQ238586**	**DQ987306**
Colura acroloba	Malaysia	Schäfer-Verwimp & Verwimp 18860/A (JE)	JX470966	JX470977	JX470989
Colura calyptrifolia (Hook.) Dumort.	Dominican Republic	Schäfer-Verwimp & Verwimp 26843 (JE)	JX470970	JX470981	JX470993
Colura cylindrica Herzog	Guadeloupe	Schäfer-Verwimp & Verwimp 22154/B	JX470969	JX470980	JX470992

		(JE)			
Colura cylindrica	Panama	Schäfer-Verwimp & Verwimp 30741 (JE)	JX470973	JX470984	JX470996
Colura digitalis (Mitt.) Steph.	La Réunion	Schäfer-Verwimp & Verwimp 20134 (JE)	-	JX470988	JX471000
Colura imperfecta Steph.	Fiji Isls.	Pócs 03261/BA (GOET)	**DQ238566**	**DQ238585**	**DQ987305**
Colura imperfecta	Papua New Guinea	Streimann 41383/a (JE)	JX470971	JX470982	JX470994
Colura inflata K.I. Goebel	Malaysia	Schäfer-Verwimp & Verwimp 19010/A (JE)	JX470968	JX470979	JX470991
Colura ornithocephala Herzog	Ecuador, Azuay	Schäfer-Verwimp & Nebel 32854 (JE)	JX470974	JX470985	JX470997
Colura ornithocephala	Ecuador, Carchi	Schäfer-Verwimp et al. 24391/A (JE)	JX470975	JX470986	JX470998
Colura rhynchophora Jovet-Ast	Dominica	Schäfer-Verwimp & Verwimp 17755 (JE)	JX470976	JX470987	JX470999
Colura tenuicornis (A. Evans) Steph.	Dominican Republic	Schäfer-Verwimp & Verwimp 27039 (JE)	JX470967	JX470978	JX470990

Colura tortifolia (Nees & Mont.) Trevis.	Ecuador	Wilson et al. 04-12 (GOET)	**DQ983671**	**DQ987440**	**DQ987338**
Colura tortifolia	Guadeloupe	Schäfer-Verwimp & Verwimp 22441/C (JE)	JX470972	JX470983	JX470995
Macrocolura sagittistipula (Spruce) R.M. Schust.	Dominica	Schäfer-Verwimp & Verwimp 18014 (GOET)	**DQ983707**	**DQ987466**	**DQ987367**
Myriocolea irrorata Spruce	Ecuador	Gradstein 10033 (GOET)	**AY548072**	**DQ238584**	**DQ987279**
Myriocolea irrorata	Ecuador	Heinrichs et al. 4497	-	-	JX471001

TABLE 2. Distribution of constant and phylogenetically informative sites for aligned positions of the three genomic regions.

	*rbc*L	*trn*L-F	ITS1-5.8S-ITS2	Total
Number of sites in matrix	935	431	955	2,321
constant	823	324	540	1,687
autapomorphic	42	34	90	166
parsimony informative	70	73	325	468

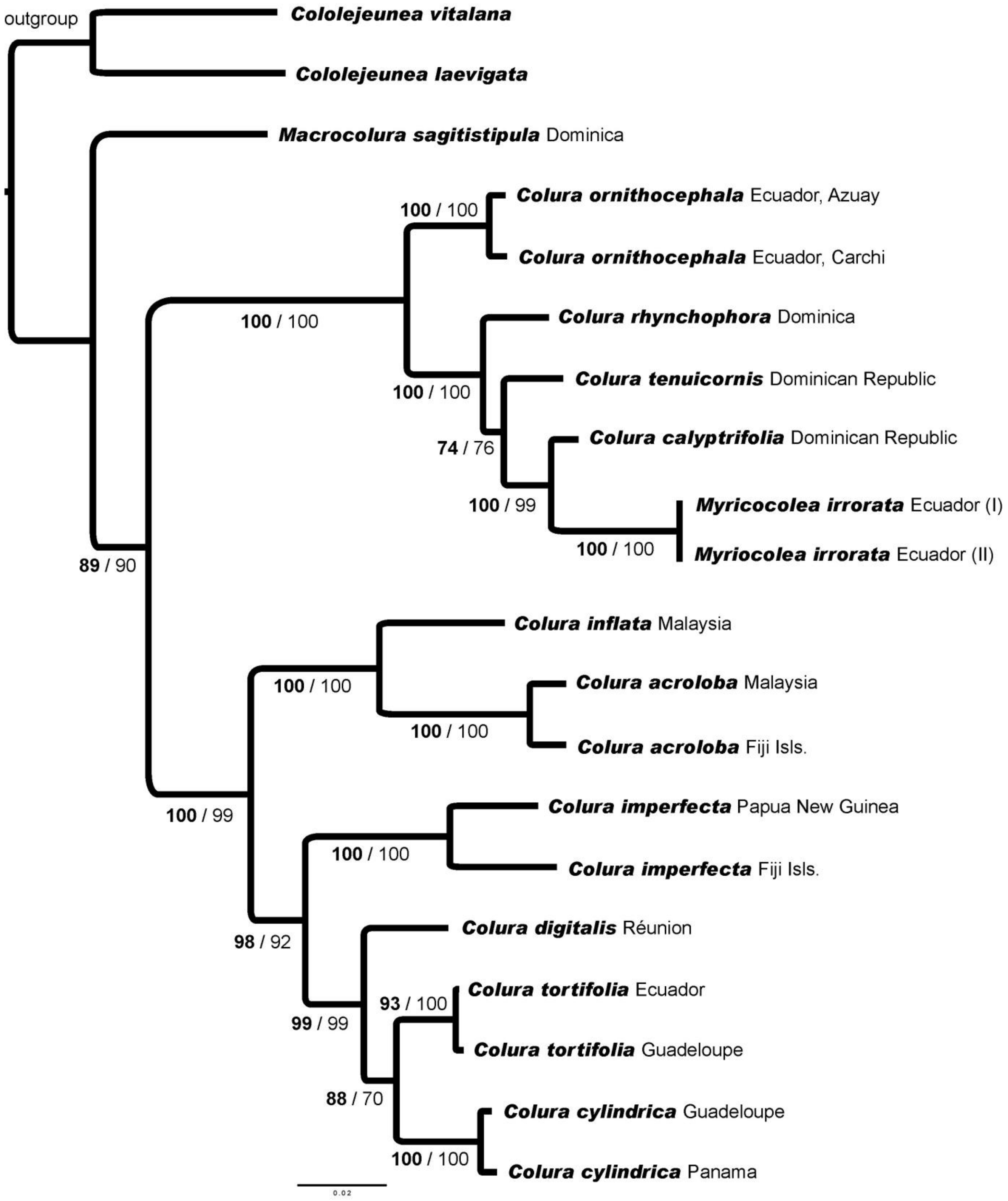

FIGURE 1. Single most likely tree (ln= -9,559.44384) derived from a cp DNA *rbc*L-*trn*L-F – nrITS dataset. ML bootstrap values (bold) and MP bootstrap values (not bold) are indicated at branches. Two accessions of *Myriocolea irrorata* are nested in a robust subclade of *Colura*, and are placed sister to the *Colura*-generitype, *C. calyptrifolia*.

Appendix 5

Sphaerolejeunea (Lejeuneaceae, Porellales) is a synonym of *Lejeunea*

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Abstract

Phylogenetic analyses of a three marker dataset of Lejeuneaceae (chloroplast genome *rbc*L gene and *trn*L-F region, and nuclear ribosomal ITS1-5.8S-ITS-2 region) show the presence of the monospecific genus *Sphaerolejeunea* in a robust subclade of *Lejeunea*. We treat *Sphaerolejeunea* as a synonym of *Lejeunea*, and propose a new name for *Sphaerolejeunea umbilicata*, *Lejeunea drehwaldii*.

Key words: Jungermanniopsida, liverwort, Neotropics, taxonomy

Introduction

Spruce (1884) in his outstanding treatment of Lejeuneaceae accepted only two genera, *Myriocolea* Spruce (1884: 305) and *Lejeunea* Libert (1820: 372), the latter subdivided in 39 subgenera. Most of them were elevated to genus rank by Schiffner (1893), and additional genera were newly established (e.g., Piippo 1986, Schuster 1994, Reiner-Drehwald 2000), leading to the recognition of about 90 genera in Lejeuneaceae (Gradstein et al. 2003). More than sixty of them include species placed in *Lejeunea* by Spruce (Gradstein et al. 2004).

However, after an era in which numerous genera were separated from *Lejeunea*, the recent trend goes to a wider genus concept. Several genera were recently synoymized with *Lejeunea*, namely *Crossotolejeunea*(Spruce 1884: 161) Schiffner (1893: 127) and *Dactylolejeunea* Schuster (1971: 341) (Reiner-Drehwald & Goda 2000), *Amphilejeunea* Schuster (1978: 431) and *Cryptogynolejeunea* Schuster (1994: 215) (Reiner-Drehwald 2005a), *Dicladolejeunea* Schuster (1994: 220) (Reiner-Drehwald 2005b), *Echinocolea* Schuster (1963: 125) (Ilkiu-Borges 2005), *Neopotamolejeunea* Reiner-Drehwald (2000: 449) (Gradstein & Reiner-Drehwald 2007), *Inflatolejeunea* Arnell (1953: 173), *Macrolejeunea* (Spruce) Schiffner in Engler & Prantl (1893: 118) (Reiner-Drehwald & Schäfer-Verwimp 2008), and *Amblyolejeunea* Jovet-Ast (1948: 24) (Zhu & Cheng 2008).

Sphaerolejeunea Herzog (1938: 88) was set up for a single species, *S. umbilicata* Herzog (1938: 88) and based on a single specimen collected by E.P. Killip in 1922 in the

Andes of Colombia. This epiphyllous species stands out by leaf lobes bordered by several rows of hyaline cells, basally leafless gynoecial branches and perianths lacking both a beak and keels. These perianths hardly exceed the surrounding vegetative leaves (Herzog 1938) and open in an umbilicus at their dorsal side (Reiner-Drehwald & Drehwald 2002).

In 2001 U. Drehwald discovered a second locality of *Sphaerolejeunea umbilicata* in the Andes of northern Peru. The related herbarium specimen allowed for a detailed morphological investigation of the species and the proposal of a sister relationship of *Sphaerolejeunea* and *Lejeunea* (Reiner-Drehwald & Drehwald 2002).

In the framework of a molecular phylogenetic study of *Lejeunea* (Dong et al. in prep.) we sequenced three molecular markers of *Sphaerolejeunea*, and included the sequences in a large alignment of Lejeuneaceae. Our phylogenetic analyses show that *Sphaerolejeunea* forms a lineage within *Lejeunea*. Accordingly, we transfer *Sphaerolejeunea umbilicata* to *Lejeunea*, and propose a nomen novum since the taxon *Lejeunea umbilicata*(Nees 1830: 42) Nees, Lindenberg & Gottsche in Meyen (1843: 472) blocks a new combination.

Materials and methods

Taxa studied, DNA extraction, PCR amplification and sequencing

Plant tissue was isolated from a herbarium collection of *Sphaerolejeunea umbilicata* (Peru, Drehwald 10014, JE). Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: chloroplast genome *rbc*L gene and *trn*L-F region from Gradstein et al. (2006), and nuclear ribosomal ITS1-5.8S-ITS-2 region from Hartmann et al. (2006). Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR.

First we compared the new *Sphaerolejeunea* sequences with GenBank sequences using the BLASTN program (Altschul et al. 1990). These BLAST searches pointed to close relationships of *Sphaerolejeunea* and Lejeuneeae. Accordingly we compiled a Lejeuneeae ingroup dataset based on Wilson et al. (2007) using Genbank sequences (http://www.ncbi.nlm.nih.gov/genbank/). Fourteen representatives of Brachiolejeuneeae and Ptychanthoideae were chosen as outgroup. The related sequences came also from Genbank (Table 1).

Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall 1999). Ambiguous positions were excluded from all alignments. Lacking parts of *rbc*L-sequences were coded as “N” (A, C, G or T), lacking parts of other markers as unknown sequence stretches. Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10 (Swofford 2000). MP heuristic searches were conducted with the following options implemented: heuristic search mode, 500 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Where more than one most parsimonious tree was found, trees were summarized in a strict consensus tree.

Non-parametric bootstrapping values (Felsenstein 1985) were generated as heuristic searches with 500 replicates, each with ten random-addition replicates. Bootstrap percentage values (BPV) ≥ 70 were regarded as good support (Hillis and Bull 1983). The individual marker sets and the combined chloroplast DNA dataset vs nrITS dataset were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70% (Mason-Gamer and Kellog 1996). The trees gave no evidence of incongruence. Hence the datasets were combined.

jModeltest 0.1 (Posada 2008) was used to select models of evolution for maximum likelihood (ML) analyses of the three molecular markers. In all cases, a General Time

Reversible (GTR) model (Tavaré 1986) was chosen with proportion of invariable characters (I) and among-site rate heterogeneity modelled as discrete gamma distribution with four rate categories, and its estimated parameters (Γ) (Goldman 1993). Models for the *rbcL*-partition, the *trn*L-F partition and the nrITS partition were implemented in the program GARLI version 2.0 (Zwickl, 2006). Subsequently the datasets were combined and ML trees were generated. All GARLI analyses were performed with the default settings and several times repeated. The default setting of GARLI was also employed to calculate bootstrap values for ML analyses based on 200 bootstrap replicates.

Results

Seven hundred and thirteen of the 2086 investigated characters were parsimony informative, 251 variable but parsimony uninformative, and 1122 constant. The MP analysis resulted in 45 equally parsimonious trees with a length of 4852 steps, a consistency index (CI) of 0.34 and a retention index (RI) of 0.58. The strict consensus tree is depicted in Figure 1. Most deep nodes did not get BPVs. A derived clade with *Harpalejeunea grandistipula* Schuster (1999: 290), *Pluvianthus squarrosus* (Stephani 1896: 130) Schuster & Schäfer-Verwimp in Schuster (1994: 213) and 15 representatives of *Lejeunea* had a BPV of 100, the *Lejeunea*clade a BPV of 81. *Spaerolejeunea umbilicata* was nested in a robust subclade of *Lejeunea* that achieved a BPV of 100.

The Garli analysis resulted in a ML topology (ln=-24516.7446, Figure 2) that resembles the MP strict consensus tree. Again *Sphaerolejeunea* is nested in a derived subclade of *Lejeunea* with a BPV of 100.

Discussion and taxonomic treatment

Reiner-Drehwald and Drehwald (2002) in their detailed morphological treatment of *Sphaerolejeunea* listed numerous analogies with *Lejeunea* including the presence of shoots with a hyalodermis, two cells wide ventral merophytes, proximal hyaline papillae, lejeuneoid innovations, inflated perianths without keels, capsule wall apices with a quadrate cell in the outer layer, and “butterfly-shaped” cell structures in the basal half of the capsule walls. The above authors however pointed out that *Sphaerolejeunea* differs from *Lejeuna*by the development of a second lobule tooth, leaves bordered by hyaline cells [but also present in *Neopotamolejeunea*, another synonym of *Lejeunea* (Reiner-Drehwald 1999, Gradstein & Reiner-Drehwald 2007)], beakless perianths being umbilicate on the dorsal side and valves with five marginal elaters lacking thickenings. Taking into account the morphological similarities Reiner-Drehwald & Drehwald (2002) suggested that *Sphaerolejeunea* may represent the sister group of *Lejeunea*. However, our phylogenies (Figures 1, 2) indicate that *Sphaerolejeunea* is nested in *Lejeunea* and that its morphological peculiarities represent autapomorphies of a *Lejeunea* species rather than of its sister lineage.

We therefore lower *Sphaerolejeunea* to a synonym of *Lejeunea*:

Lejeunea Libert (1820: 372)
= *Sphaerolejeunea* Herzog (1938: 88), *syn. nov*.

The taxon *Lejeunea umbilicata* (Nees 1830: 42) Nees, Lindenberg & Gottsche in Meyen (1843: 472) blocks a transfer of *Sphaerolejeunea umbilicata* to *Lejeunea*. Accordingly, we propose a new name, as follows:

Lejeunea drehwaldii Heinrichs & Schäf.-Verw., **nom. nov.** Replaced name:-*Sphaerolejeunea umbilicata*Herzog, *Annales Bryologici* 11: 88, 1938 (Herzog 1938). Holotype:-COLOMBIA, Cauca, Micay Valley, 1400–1800 m, 1922, Killip 7750 p.p. (JE). Blocking name:-*Lejeunea umbilicata* (Nees) Nees, Lindenberg & Gottsche in Meyen 472, 1843 (Meyen 1843).

Etymology: We dedicate the new name to the bryologist and lichenologist Uwe Drehwald, Göttingen, who collected the DNA voucher of *Sphaerolejeunea* on which our study was based.

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TABLE 1. Taxa used in the present study, including information about the origin of the studied material, voucher information, as well as GenBank accession numbers.

Taxon	Voucher	*rbc*L	*trn*L-F	nrITS
Acanthocoleus javanicus (Steph.) Kruijt	Bali, Schäfer-Verwimp 20817	DQ983648	DQ987398	DQ987291
Acrolejeunea fertilis (Reinw. et al.) Schiffn.	Bali, Schäfer-Verwimp 17009	AY684929	DQ987391	DQ987281
Anoplolejeunea conferta (C.F.W. Meissn. ex Spreng.) A.Evans	Ecuador, Wilson et al. 04–08	DQ983653	DQ987438	DQ987335
Archilejeunea fuscescens (Hampe ex Lehm.) Fulf.	Costa Rica, Bernecker 97–53	DQ983655	DQ987384	DQ987267
Aureolejeunea aurifera R.M.Schust.	Costa Rica, Holz CR00-696	AY548082	DQ238569	DQ987272
Aureolejeunea fulva R.M.Schust.	Brazil, Costa & Gradstein 3849	AY548070	--------------	DQ987269
Aureolejeunea quinquecarinata R.M.Schust.	Ecuador, Schäfer-Verwimp 23299/A	DQ983658	DQ987450	DQ987350
Bryopteris diffusa (Sw.) Nees	Bolivia, Acebey & Villavicencio 855	AY548085	AM237147	AM237095
Ceratolejeunea cornuta (Lindenb.) Steph.	Bolivia, Drehwald 4739	AY548088	DQ238570	DQ987257
Ceratolejeunea cf.	Ecuador, Wilson et al. 04-	DQ983662	DQ987442	DQ987340

guianensis	15			
Cheilolejeunea acutangula (Nees) Grolle	Mexico, Gradstein & Velasquez s.n.	DQ983664	DQ987386	DQ987270
Lejeunea lamacerina (Steph.) Schiffn.	Canary Islands, Schäfer-Verwimp 24616	DQ983694	--------------	DQ987358
Lejeunea mimula Hürl.	Bali, Schäfer-Verwimp 20930	AY548104	DQ238580	DQ987261
Lejeunea pallescens Mitt.	Ecuador, Schäfer-Verwimp 23533	AY548068	DQ238576	DQ987292
Lejeunea paucidentata (Steph.) Grolle	Dominica, Schäfer-Verwimp 17737	DQ983695	DQ987447	DQ987345
Lejeunea cf. pterigonia (Lehm. & Lindenb.) Mont.	Bolivia, Gradstein 9964	DQ983732	DQ987429	DQ987325
Lejeunea sp. I	Ecuador, Gradstein & Jost 10063	DQ983712	DQ987416	DQ987312
Lejeunea sp. II	Ecuador, Gradstein 10172	DQ983734	DQ987410	DQ987304
Lepidolejeunea bidentula (J.B. Jack & Steph.) R.M. Schust.	China, Koponen et al. 51525	AY125936	AY144476	AY125340
Lepidolejeunea eluta (Nees) R.M.Schust.	Bolivia, Drehwald 4833	DQ983696	DQ987379	DQ987257
Lepidolejeunea integristipula (Jack & Steph.) R.M.Schust.	Fiji, Pócs 03307/AC	DQ983697	DQ987417	DQ987313
Lopholejeunea eulopha (Tayl.) Schiffn.	Australia, Pócs & Streimann 9987/H1	AY548067	DQ987381	DQ987262

Luteolejeunea herzogii (Buchloh) Piippo	Costa Rica, Schäfer-Verwimp & Holz 0294/B	DQ983706	DQ987467	DQ987368
Macrocolura sagittistipula (Spruce) R.M.Schust.	Dominica, Schäfer-Verwimp 18014	DQ983707	DQ987466	DQ987367
Marchesinia robusta (Mitt.) Schiffn.	Ecuador, Wilson et al. 04-05	DQ983710	DQ987436	DQ987332
Mastigolejeunea auriculata (Wilson & W.J.Hooker) Schiffn.	Bolivia, Churchill 21275	AY548070	DQ987385	DQ987268
Myriocoleopsis gymnocolea (Steph.) M.E. Reiner & Gradst.	Ecuador, Gradstein et al. 10020	DQ238568	DQ238583	DQ987277
Neurolejeunea breutelii (Gottsche) A.Evans	Brazil, Schäfer-Verwimp 14740	DQ983714	DQ987405	DQ987299
Omphalanthus filiformis Nees	Ecuador, Schäfer-Verwimp 23543	DQ983716	DQ987393	DQ987283
Omphalanthus ovalis (Lindenb. & Gottsche) Gradst.	Ecuador, Wilson et al. 04-07	DQ983717	--------------	DQ987334
Physantholejeunea portoricensis (Hampe & Gottsche) R.M.Schust.	Guadeloupe, Schäfer-Verwimp 22615	DQ983719	DQ987448	DQ987346
Pluvianthus squarrosus (Steph.) R.M.Schust. & Schäfer-Verwimp	Brazil, Schäfer-Verwimp 13376	DQ983720	DQ987446	DQ987344
Ptychanthus striatus (Lehm.	Java, Gradstein 10215	DQ983721	DQ987406	DQ987300

& Lindenb.) Nees				
Pycnolejeunea densistipula (Lehm. & Lindenb.) Steph.	Ecuador, Schäfer-Verwimp 23368	AY548075	DQ987400	DQ987294
Rectolejeunea berteroana (Gottsche) A. Evans	Guadeloupe, Schäfer-Verwimp 22245/A	DQ983724	DQ987444	DQ987342
Schiffneriolejeunea nymannii (Steph.) Gradst. & Terken	Malaysia, Gradstein et al. 10321	DQ983725	DQ987424	DQ987320
Siphonolejeunea elegantissima (Steph.) Grolle	Australia, Pócs & Brown 0026/AA	DQ983726	DQ987452	DQ987352
Sphaerolejeunea umbilicata Herzog	Ecuador, Drehwald 10014	HE995781	HE995782	HE995783
Spruceanthus thozetianus (Gottsche & F. v. Müll.) B. Thiers	Australia, Pócs 01107/M	AM384877	DQ987460	DQ987362
Thysananthus spathulistipus (Reinw. et al.) Lindenb.	Bali, Schäfer-Verwimp 20790	DQ983739	DQ987392	DQ987282
Xylolejeunea crenata (Nees & Mont.) X.-L. He & Grolle	Brazil, Schäfer-Verwimp 11225	DQ983740	DQ987443	DQ987341

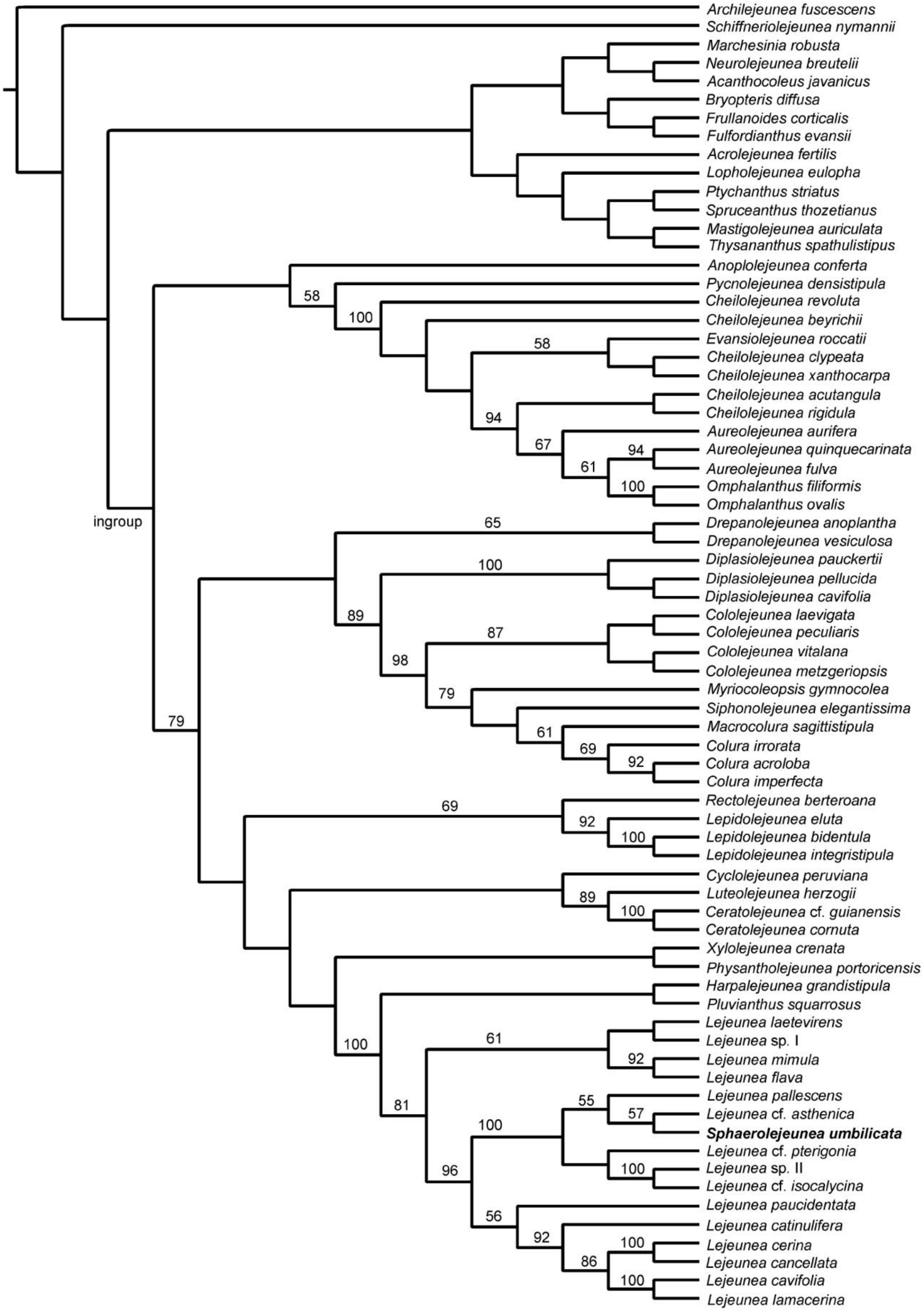

FIGURE 1. Strict consensus of 45 equally parsimonious trees based on the combined nrITS- chloroplast DNA *rbc*L – *trn*L-F dataset with bootstrap percentage values at branches. *Sphaerolejeunea* is nested in a robust subclade of *Lejeunea*.

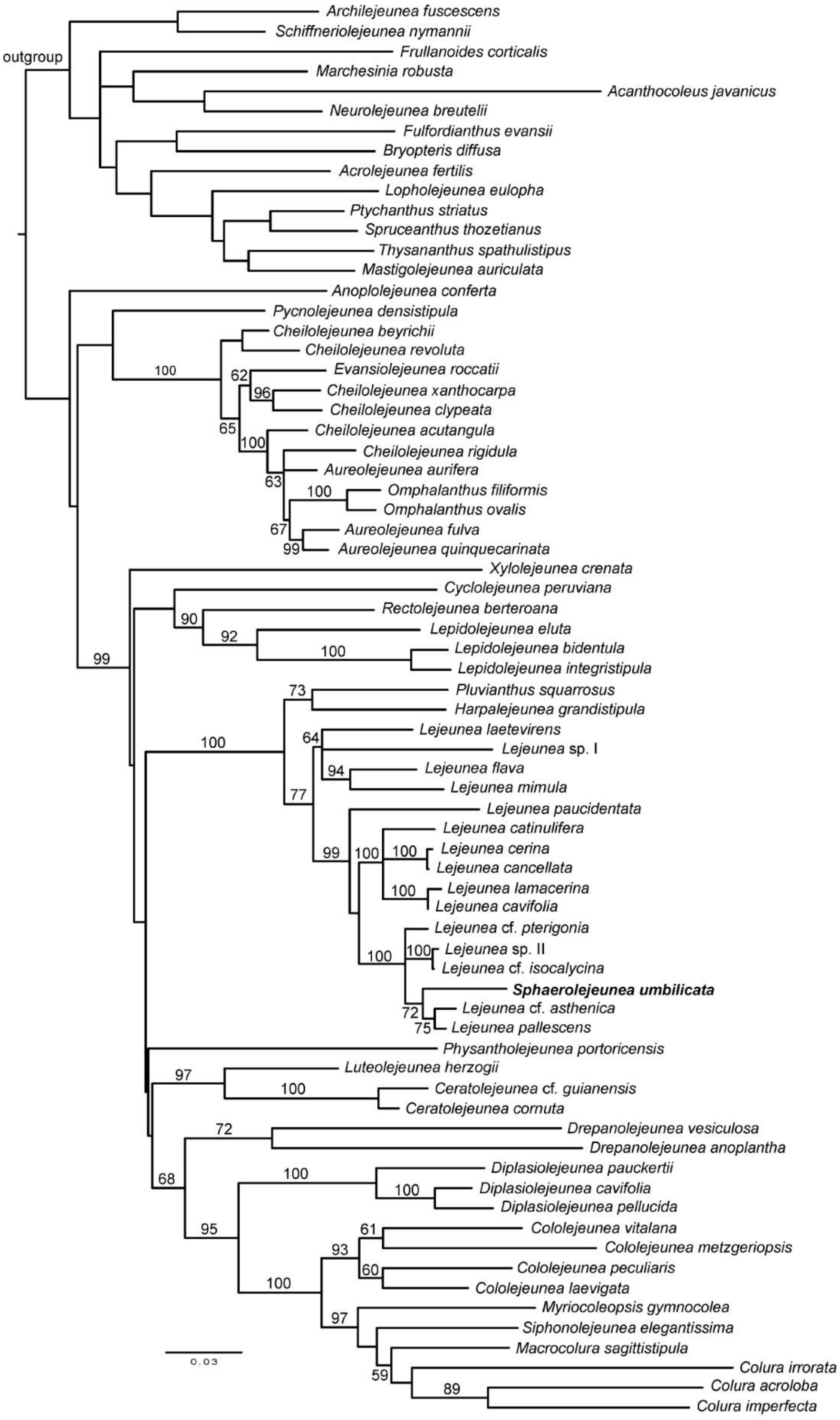

FIGURE 2. Phylogram generated in a maximum likelihood analysis of the combined dataset. Bootstrap values are indicated at branches.

Appendix 6

The Bromeliaceae tank dweller *Bromeliophila* (Lejeuneaceae, Porellales) is a member of the *Cyclolejeunea-Prionolejeunea* clade

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Abstract

The Neotropical genus *Bromeliophila* includes two species that grow exclusively in the water-filled leaf axils of Bromeliaceae. Phylogenetic analyses of a three marker dataset (chloroplast genome *rbc*L gene and *trn*L-F region, and nuclear ribosomal ITS1-5.8S-ITS-2 region) confirmed the monophyly of *Bromeliophila* and provided evidence for a sister relationship of *Bromeliophila* and *Prionolejeunea*; this clade is in turn sister to *Cyclolejeunea*. Despite remarkable morphological similarities, *Bromeliophila* is not closely related to *Lejeunea* and its close relatives *Harpalejeunea* and *Microlejeunea*. Based on the molecular topology and morphological evidence, *Cyclolejeunea* is subdivided in subgenus *Cyclolejeunea* and the monospecific subgenera *Hyalolejeunea* and *Nephrolejeunea*. Genetic relationships among multiple accessions of the same species support currently accepted morphological–typological species concepts.

Keywords: Classification, Epiphyte, Epiphylly, Jungermanniopsida, Liverwort

Introduction

Lejeuneaceae are very abundant in the humid tropics, making up a large part of the local epiphytic, and especially epiphyllous, liverwort diversity (Wilson et al. *2007*). With some 1,000 species in 68 currently accepted genera, they are the largest family of liverworts (Gradstein et al. *2003*, *2013*). Generic limits in Lejeuneaceae, however, often remain controversial (Schuster *1994*).

Recent molecular phylogenetic studies of Lejeuneaceae have led to a considerable reduction of generic names and the establishment of morphologically variable, species-rich taxonomic units (Gradstein et al. *2006*; Wilson et al. *2007*; Heinrichs et al.*2012a*, *b*; Dong et al. *2013*; Ye et al. *2013*). This trend is rather consistent with the trend towards the acceptance of larger genera in all lineages of land plants (Humphreys and Linder *2009*). A good example in this regard is *Lejeunea* Lib. whose current synonymy includes more than a dozen generic names (Heinrichs et al. *2012a*; Ye et al. *2013*).

Bromeliophila R.M. Schust. was set up for *Peltolejeunea natans* Steph., a Brazilian endemic, growing exclusively in the tanks of Bromeliaceae (Schuster *1994*). The lower parts of the gametophytic shoots are often submerged and only the upper parts are located above the water. Schuster (*1994*) considered *Bromeliophila* and *Lejeunea* to be closely related, a view reiterated by Gradstein (*1997*), who deemed the generic status “critical”. However, a second Neotropical species, *Bromeliophila helenae* Gradst., was introduced without further confirmation of the generic classification. The morphological similarities between *Bromeliophila* and *Lejeunea* are illustrated by the classification of Grolle (*1985*), who transferred *Peltolejeunea natans* to *Lejeunea*, renaming it to *Lejeunea natans* (Steph.) Grolle.

In the framework of a comprehensive molecular phylogenetic–taxonomic study of Lejeuneaceae (Hartmann et al. *2006*; Wilson et al. *2007*; Dong et al. *2012*, *2013*; Heinrichs et al. *2009*, *2012a*, *b*; Yu et al. *2013*) we sequenced chloroplast and nuclear markers of both species of *Bromeliophila*. Here, we aim to confirm the monophyly of *Bromeliophila* and provide evidence for a close relationship of *Bromeliophila* with *Prionolejeunea* (Spruce) Schiffn. rather than with *Lejeunea*.

Materials and methods

Taxa studied, DNA extraction, PCR amplification and sequencing

Plant tissue was isolated from herbarium collections of the two known species of *Bromeliophila*, six species of *Cyclolejeunea* A. Evans, and twelve species of *Prionolejeunea* (Table 1). Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Stratec Molecular, Berlin, Germany) prior to amplification. Protocols for PCR were carried out as described in previous publications: chloroplast genome *rbc*L gene and *trn*L-F region from Gradstein et al. (*2006*), and nuclear ribosomal ITS1-5.8S-ITS2 region from Hartmann et al. (*2006*). Bidirectional sequences were generated using a MegaBACE 1,000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR. Newly generated sequences were assembled and edited using SeqAssem (Hepperle *2004*).

We compared our new sequences with GenBank sequences using the BLASTN program (Altschul et al. *1990*). The BLASTN searches confirmed an affiliation of the studied taxa to Lejeuneeae. Accordingly, we compiled a Lejeuneeae ingroup dataset based on Dong et al. (*2013*) and Wilson et al. (*2007*) using our new sequences as well as Genbank sequences (http://www.ncbi.nlm.nih.gov/genbank/). Two representatives of Ptychanthoideae were chosen as outgroup according to the topologies presented in Wilson et al. (*2007*). The related sequences were also obtained from Genbank (Table 1). Phylogenetic analyses of the Lejeuneeae dataset identified the closest relatives of *Bromeliophila*. Based on the outcome of these analyses, we assembled a second dataset consisting of accessions of *Bromeliophila*, *Cyclolejeunea*, and *Prionolejeunea* as the ingroup and three species of *Lepidolejeunea* R.M. Schust. as the outgroup. Whenever possible we included multiple accessions of species to explore infraspecific genetic variation and to examine whether our phylogeny corresponds to morphologically circumscribed entities.

Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall *1999*). Ambiguous positions were excluded from the alignments. Missing sequence stretches were coded as unknown. Bayesian inference of phylogeny was carried out with MrBayes 3.2.1 (Huelsenbeck and Ronquist *2001*) on XSEDE via the Cipres Science Gateway (http://www.phylo.org/sub_sections/portal/). Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10 (Swofford *2000*). MP heuristic searches were conducted with the following options implemented: heuristic search mode, 1,000 random-addition-sequence replicates, tree bisection-reconnection branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Where more than one most parsimonious tree was found, they were summarized in a strict consensus tree.

Non-parametric bootstrapping values (Felsenstein *1985*) were generated as heuristic searches with 1,000 replicates, each with ten random-addition replicates. Rearrangements were restricted to one million per replicate. Bootstrap percentage values (BPV) ≥70 % were regarded as good support (Hillis and Bull *1993*). The individual marker sets and the combined chloroplast DNA dataset versus nrITS dataset were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70 % (Mason-Gamer and Kellogg *1996*). The trees gave no evidence of incongruence. Hence, the datasets were combined.

Bayesian searches were conducted using four simultaneous Markov chains over 25 million generations (heterogeneous model) and sampling every 2,500th generation. Tracer version 1.5 (Rambaut and Drummond *2003*) was used to examine the parameters and determine the number of trees needed to reach stationarity (burn-in). Bayesian posterior probability (BPP) confidence values were generated from trees saved after this initial burn-in. Values were regarded as significant when BPP ≥0.95 (Larget and Simon *1999*).

Results

Lejeuneeae dataset

Of the 2,015 investigated characters, 1,321 were constant and 441 parsimony informative (see Table 2 for character state distributions within the single markers). The MP analysis yielded four equally parsimonious trees with a length of 1,940 steps, a consistency index (CI) of 0.53 and a retention index (RI) of 0.47 (Fig. 1). *Bromeliophila natans* and *Prionolejeunea limpida* Herzog formed a clade with a BPV = 100 %; this clade was placed sister to *Cyclolejeunea luteola* (Spruce) Grolle with a BPV = 100 %. The *Bromeliophila–Cyclolejeunea–Prionolejeunea* clade was found as sister to a clade made up by *Rectolejeunea berteroana* (Gottsche ex Steph.) A. Evans and *Lepidolejeunea integristipula* (J.B. Jack & Steph.) R.M. Schust. in a moderately supported sister relationship (BPV = 67 %). *Lejeunea cavifolia* (Ehrh.) Lindb., *Harpalejeunea molleri* (Steph.) Grolle and *Microlejeunea africana* Steph. were placed in an independent, polytomous clade with a BPV = 100 %.

Bromeliophila–Cyclolejeunea–Prionolejeunea **dataset**

Five hundred and thirty of the 2,291 investigated characters were parsimony informative, 211 unique to a single specimen and 1,550 constant (Table 2). The strict consensus tree of 156 equally parsimonious trees with a length of 1,715 steps, a consistency index (CI) of 0.6 and a retention index (RI) of 0.84 is shown in Fig. 2. *Bromeliophila* was sister to *Prionolejeunea* with a BPV = 100 %. The *Bromeliophila* clade (BPV = 99 %) consisted of two accessions of *B. natans* from the states of São Paulo and Santa Catharina and a paratype of *B. helenae* from Dominica. The two *B. natans* accessions formed a sister relationship with a BPV of 100 %. The *Prionolejeunea* clade achieved a BPV = 99 %. One accession of *P. grata* (Gottsche) Schiffn. from Réunion was placed sister to a moderately supported Neotropical *Prionolejeunea* clade (BPV = 63 %). Multiple accessions of *P. schlimiana* (Gottsche) Steph. and *P. scaberula* (Spruce) Steph. formed monophyla. Three accessions of *P. decora* (Taylor) Steph. were placed in a polytomy with an accession of *P. mucronata*(Sande Lac.) Steph. (BPV = 67 %).

The *Bromeliophila–Prionolejeunea* clade was placed sister to the well-supported *Cyclolejeunea* clade (BPV = 100 %). Multiple accessions of all investigated *Cyclolejeunea* species formed monophyla with BPVs ranging from 85 to 100 %.*Cyclolejeunea* (subg. *Nephrolejeunea* Grolle) *luteola* was sister to the rest of the genus with a BPV = 55 %, followed by a clade with several accessions of *C*. (subg. *Hyalolejeunea* Grolle) *accedens* (Gottsche) A. Evans. *Cyclolejeunea peruviana* (Lehm. & Lindenb.) A. Evans formed a well supported sister relationship (BPV = 99 %) with a clade including accessions of *C. chitonia* (Taylor) A. Evans, *C. foliorum* (Nees) Grolle and *C. convexistipa* (Lehm. & Lindenb.) A. Evans (*C*. subg. *Cyclolejeunea*). A sister relationship of *C. chitonia* and *C. foliorum* has a BPV = 98 %.

The topology derived from the Bayesian analysis (Fig. 3) resembles the MP strict consensus tree (Fig. 2). In contrast to the MP topology, *Prionolejeunea aemula* (Gottsche) A. Evans from Guadeloupe is placed sister to the rest of this genus but with a BPP value <0.95. Three accessions of *P. decora* form a monophyletic lineage with a BPP = 1.00. *Cyclolejeunea luteola* was sister to the rest of this genus with a BPP = 1.00. The crown groups of most *Cyclolejeunea* species showed intra-species relationships with BPP > 0.95.

Discussion

Systematic position of *Bromeliophila*

Small or even monospecific genera of Lejeuneeae often prove to be nested in larger entities when molecular data are taken into account, e.g. *Metzgeriopsis* K.I. Goebel in *Cololejeunea* (Spruce) Schiffn. (Gradstein et al. *2006*), *Myriocolea* Spruce in *Colura*(Dumort.) Dumort. (Heinrichs et al. *2012b*) and *Oryzolejeunea* (R.M. Schust.) R.M. Schust. as well as *Sphaerolejeunea* Herzog in *Lejeunea* (Heinrichs et al. *2012a*; Ye et al. *2013*). We expected a similar result for *Bromeliophila* since several authors proposed a close relationship of *Bromeliophila* and *Lejeunea* based on their extensive morphological overlap (Schuster *1994*; Gradstein *1997*, *2013*). In addition, some authors treated the generitype *Peltolejeunea natans* as an element of *Lejeunea* (Grolle*1985*). *Bromeliophila* and *Lejeunea* share a soft texture, greenish or yellowish-green color, lejeuneoid gynoecial innovations, 2-cells broad ventral merophytes, bifid underleaves and noncompressed, equally 5-keeled perianths. However, despite these similarities, *Bromeliophila* is not closely related to *Lejeunea* but an element of the *Prionolejeunea*–*Cyclolejeunea* clade (Fig. 1). The close relationship of *Prionolejeunea* and *Cyclolejeunea* is morphologically well founded since both genera share 2-keeled perianths, denticulate leaf margins and vegetative branches of the *Lejeunea*-type (Schuster *1992*). This sister relationship has furthermore been supported by DNA sequence data (Ilkiu-Borges *2005*). In stark contrast, *Bromeliophila* has entire leaf margins and longly stipitate 5-keeled perianths of which each is double-winged (Fig. 4; Schuster *1994*; Gradstein *1997*). Despite these differences in leaf margin and perianth structure, nuclear as well as chloroplast sequence data recovered a sister relationship of *Bromeliophila* and *Prionolejeunea*. This relationship is consistent with the shared occurrence of lejeuneoid gynoecial innovations and lack of ocelli (Schuster *1994*; Bernecker-Lücking *1998*). *Cyclolejeunea* on the other hand has pycnolejeuneoid gynoecial innovations (Grolle *1984*) and—with the exception of *C. accedens*—ocelli.

Classification of *Cyclolejeunea* and *Prionolejeunea*

The species lineages of *Prionolejeunea* originate from a largely polytomous backbone (Fig. 3), hence a classification into supraspecific entities is unwarranted. In contrast, the *Cyclolejeunea* crown group splits into several well-separated lineages that can be recognized by combinations of morphological character states (Grolle *1984*). *Cyclolejeunea* subg. *Nephrolejeunea* includes the only species with ovate to elongate gemmae with a median constriction, *C. luteola* (Bernecker-Lücking *1998*), whereas *C.* subg. *Hyalolejeunea* includes *C. accedens*, the only species lacking ocelli.*Cyclolejeunea* subg. *Cyclolejeunea* comprises the species with ocelli and rounded gemmae (*C. chitonia*, *C. convexistipa*, *C. foliorum*, *C. peruvianum*).

Ecology of the *Bromeliophila*—*Cyclolejeunea*–*Prionolejeunea* clade

Cyclolejeunea and *Prionolejeunea* are epiphyllous or corticolous epiphytes in moist lowland and lower montane rainforests. They do not show an obvious preference to particular groups of angiosperms. In contrast, *Bromeliophila* is so far known only as an epiphyll, growing exclusively in the water-filled leaf axils of Bromeliaceae (Gradstein et al. *2001*; Benavides and Callejas *2004*). Due to this peculiar habitat, *Bromeliophila* stands are likely better supplied with nutrients than *Cyclolejeunea* and *Prionolejeunea* populations; however, the humid and rather nutrient-rich habitat cannot explain the deviant perianth structure of *Bromeliophila*. It is still somewhat unclear how *Bromeliophila* builds up local populations; however, the lack of propagules indicates that range formation is a result of spore dispersal. Given the unique habitat, *Bromeliophila* spores may be not only distributed through wind currents but also through animals, such as amphibians and insects, that are dependent on the watertight compartments formed by leaf bases of bromeliads. It has been shown that frogs and snakes can act as dispersal agents for limnetic invertebrates endemic to the bromelioid tanks (Lopez et al. *1999*, *2005*).

Bromeliophila and *Cyclolejeunea* are Neotropical genera whereas *Prionolejeunea* occurs in tropical America and Africa (Ilkiu-Borges *2005*). The limited genetic divergence among species of the *Prionolejeunea* crown group (Fig. 3) indicates that the African-American disjunctions are the result of dispersal events rather than vicariance. Similar patterns have been reported for other genera of liverworts (Heinrichs et al. *2005*; Hartmann et al. *2006*; Feldberg et al. *2007*) and also other land plants such as ferns (Janssen et al. *2007*; Rouhan et al. *2012*) and bromelioids (Givnish et al. *2004*).

Circumscription of species

Multiple accessions of morphologically–typologically circumscribed species form monophyletic lineages (Figs. 2, 3), indicating congruence of morphologically–typologically inferred and phylogenetically inferred species. In a study on *Cyclolejeunea*, Bernecker-Lücking (*1998*) recovered considerable infraspecific variation of characters of the sterile gametophyte. This variation/plasticity may reflect at least partly adaptation to environmental conditions. Several *Cyclolejeunea* species show a genetic differentiation among accessions, providing some evidence for the accumulation of genetic diversity, which may coincide with the mentioned morphological variation. An extension of the sampling is necessary to discover morphological disparity or geographical patterns within the currently accepted *Cyclolejeunea* species. Similar results have been reported for several genera of leafy liverworts such as *Diplasiolejeunea* (Spruce) Schiffn. (Dong et al. *2012*), *Frullania* Raddi (Ramaiya et al. *2010*), *Marchesinia* Gray (Heinrichs et al. *2009*), and *Porella* L. (Heinrichs et al. *2011*). Morphologically cryptic or semicryptic biologically relevant entities have been recovered in the majority of species complexes of liverworts that were studied exhaustively.

The infraspecific variation of *Bromeliophila* and *Prionolejeunea* species is comparatively low. Unfortunately, we were not able to sequence multiple accessions of *Bromeliophila helenae*. This species is considered to be morphologically variable and has a disjunct range with stands in the Lesser Antilles, Colombia and the Guyana Highland of Venezuela (Benavides and Callejas *2004*). Information on the internal molecular variation would allow for testing of the current species concept and a reconstruction of the formation of its range.

Perspectives

Our study provides evidence for a single origin of the obligate Bromeliaceae tank dwellers of Lejeuneaceae and clarifies their systematic position. The sister relationship of *Prionolejeunea* and *Bromeliophila* is proposed here for the first time, demonstrating the importance of molecular data for resolving relationships within Lejeuneaceae. Several genera with unclear taxonomic affinities need to be included in future studies, e.g. *Kymatolejeunea* Grolle and *Metalejeunea* Grolle. These studies will allow for a classification of Lejeuneaceae in monophyletic entities and a reconstruction of their morphological evolution and range formation.

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Table 1

Taxa used in the present study, including information about the origin of the studied material, voucher information, as well as GenBank accession numbers

Taxon	**Voucher**	***rbc*L**	***trn*L-F**	**nrITS**
***Anoplolejeunea conferta* (C.F.W. Meissn.) A. Evans**	Ecuador, *Wilson et al . 04-08* (GOET)	DQ983653	DQ987438	DQ987335
***Aureolejeunea quinquecarinata* R.M. Schust.**	Ecuador, *Schäfer-Verwimp & Preußing 23299/A* (GOET)	DQ983658	DQ987450	DQ987350
***Bromeliophila helenae*Gradst.**	Dominica, *Schäfer-Verwimp & Verwimp17887* (GOET, Paratype)	**KF039847**	–	**KF039810**
***Bromeliophila natans*(Steph.) R.M. Schust.**	Brazil, Santa Catarina,*Gehrig s.n.* (GOET)	**KF039849**	**KF039879**	**KF039812**
Bromeliophila natans	Brazil, São Paulo, *Peralta et al . 8112* (M)	**KF039848**	–	**KF039811**
***Ceratolejeunea cornuta*(Lindenb.) Steph.**	Bolivia, *Drehwald 4739*(GOET)	AY548088	DQ238570	DQ987257
***Cheilolejeunea rigidula*(Mont.) R.M. Schust.**	Suriname, *Muñoz 98-63*(GOET)	DQ983668	DQ987453	DQ987353
***Cololejeunea peculiaris* (Herzog) Benedix**	Malaysia, *Schäfer-Verwimp & Verwimp 18861/A* (GOET)	AY548095	DQ238572	DQ987280
***Colura tenuicornis* (A. Evans) Steph.**	Dominican Republic, *Schäfer-Verwimp & Verwimp 27039* (JE)	JX470967	JX470978	JX470990
Cyclolejeunea accedens	Costa Rica, San	**KF039826**	**KF039858**	**KF039788**

(Gottsche) A. Evans	José, *Schäfer-Verwimp & Holz*279/A (M)			
Cyclolejeunea accedens	Ecuador (I), Zamora-Chinchipe, *Schäfer-Verwimp & Nebel 31964/B*(M)	**KF039824**	–	**KF039786**
Cyclolejeunea accedens	Ecuador (II), Zamora-Chinchipe, *Schäfer-Verwimp & Nebel 31949/B*(M)	**KF039825**	–	**KF039787**
Cyclolejeunea chitonia(Taylor) A. Evans	French Guiana, *Holz FG-00-124* (GOET)	**KF039844**	**KF039875**	**KF039806**
Cyclolejeunea chitonia	Guadeloupe (I), *Schäfer-Verwimp & Verwimp 22130/A* (M)	**KF039845**	**KF039876**	**KF039807**
Cyclolejeunea chitonia	Guadeloupe (II), *Schäfer-Verwimp & Verwimp 22218/A* (M)	–	**KF039877**	**KF039808**
Cyclolejeunea chitonia	Panama, *Schäfer-Verwimp & Verwimp 30724* (M)	**KF039846**	**KF039878**	**KF039809**
Cyclolejeunea convexistipa (Lehm. & Lindenb.) A. Evans	Brazil, São Paulo, *Schäfer-Verwimp & Verwimp 11621* (M)	**KF039839**	**KF039871**	**KF039801**
Cyclolejeunea convexistipa	Costa Rica, *Schäfer-Verwimp & Holz 300/D* (M)	**KF039838**	**KF039870**	**KF039800**
Cyclolejeunea convexistipa	Ecuador (I), Orellana, *Schäfer-Verwimp & Nebel 32820* (M)	**KF039842**	**KF039873**	**KF039804**
Cyclolejeunea convexistipa	Ecuador (II), Zamora-Chinchipe, *Schäfer-Verwimp &*	**KF039835**	**KF039867**	**KF039797**

	Preussing 23427 (M)			
Cyclolejeunea convexistipa	Ecuador (III), Zamora-Chinchipe, *Schäfer-Verwimp & Preussing 23408/F* (M)	**KF039836**	**KF039868**	**KF039798**
Cyclolejeunea convexistipa	Ecuador (IV), Tunguragua, *Gradstein et al . 10039*(GOET)	**KF039843**	**KF039874**	**KF039805**
Cyclolejeunea convexistipa	French Guiana (I), *Hartmann & Riet 04/109*(GOET)	KF039840	–	**KF039802**
Cyclolejeunea convexistipa	French Guiana (II), *Holz FG00-0157* (GOET)	–	DQ207904	DQ207882
Cyclolejeunea convexistipa	Guyana, *Kennedy & Pulles 473*9 (GOET)	**KF039837**	**KF039869**	KF039799
Cyclolejeunea convexistipa	Panama, *Schäfer-Verwimp & Verwimp 30786* (M)	**KF039841**	**KF039872**	**KF039803**
Cyclolejeunea foliorum**(Nees) Grolle**	Ecuador (I), Orellana, *Schäfer-Verwimp & Nebel 32784* (M)	**KF039833**	**KF039865**	**KF039795**
Cyclolejeunea foliorum	Ecuador (II), Zamora-Chinchipe, *Schäfer-Verwimp & Nebel 32104/A*(M)	**KF039834**	**KF039866**	**KF039796**
Cyclolejeunea luteola**(Spruce) Grolle**	Colombia, *Balcaza & Ajarce 919* (GOET)	–	DQ207906	DQ207884
Cyclolejeunea luteola	Dominica, *Schäfer-Verwimp & Verwimp 17866* (M)	**KF039823**	**KF039857**	**KF039785**
Cyclolejeunea luteola	Ecuador, Zamora-Chinchipe, *Schäfer-Verwimp & Preussing 23356* (M)	**KF039821**	**KF039855**	**KF039783**

Cyclolejeunea luteola	Jamaica, *Gradstein 6353*(GOET)	**KF039822**	**KF039856**	**KF039784**
Cyclolejeunea luteola	Panama, *Schäfer-Verwimp & Verwimp 30955* (M)	**KF039820**	**KF039854**	**KF039782**
***Cyclolejeunea peruviana* (Lehm. & Lindenb.) A. Evans**	Brazil, São Paulo, *Schäfer-Verwimp & Verwimp 11621/A* (M)	**KF039831**	**KF039863**	**KF039793**
Cyclolejeunea peruviana	Colombia, *Gradstein 8546*(GOET)	DQ983672	DQ987383	DQ207885
Cyclolejeunea peruviana	Costa Rica, *Schäfer-Verwimp & Holz 280/A* (M)	**KF039827**	**KF039859**	**KF039789**
Cyclolejeunea peruviana	Dominica, *Schäfer-Verwimp & Verwimp 17737/A* (M)	**KF039828**	**KF039860**	**KF039790**
Cyclolejeunea peruviana	Ecuador (I), Pichincha, *Schäfer-Verwimp et al . 24126/A* (M)	KF039832	**KF039864**	**KF039794**
Cyclolejeunea peruviana	Ecuador (II), Zamora-Chinchipe, *Schäfer-Verwimp & Preussing 23352* (M)	**KF039829**	KF039861	**KF039791**
Cyclolejeunea peruviana	Ecuador (III), Zamora-Chinchipe, *Schäfer-Verwimp & Nebel 31956*(M)	**KF039830**	**KF039862**	**KF039792**
***Diplasiolejeunea pellucida* (C.F.W. Meissn. ex Spreng.) Schiffn.**	Ecuador, *Schäfer-Verwimp et al . 24134*(GOET)	JQ729500	JQ729607	JQ729383
***Drepanolejeunea anoplantha* (Spruce) Steph.**	Dominican Republic, *Schäfer-Verwimp & Verwimp 27059* (JE)	KC313120	KC313159	KC313080

***Echinolejeunea papillata* (Mitt.) R.M. Schust.**	New Zealand, *Schäfer-Verwimp & Verwimp 13967* (JE)	KC313135	KC313175	KC313097
***Harpalejeunea molleri*(Hook. f & Tayl.) Grolle**	Azores, *Schäfer-Verwimp & Verwimp 29334* (JE)	KC313148	KC313188	KC313110
***Lejeunea cavifolia*(Ehrh.) Lindb.**	Germany, *Heinrichs 3695*(GOET)	AY548102	DQ238581	DQ987259
***Lepidolejeunea delessertii* (Nees & Mont.) Grolle**	Réunion, *Schäfer-Verwimp & Verwimp 20355/B*(M)	**KF039819**	**KF039853**	**KF039781**
***Lepidolejeunea eluta*(Nees) R.M. Schust.**	Bolivia, *Churchill & Vasquez 21800* (GOET)	AY548066	DQ238579	DQ987266
***Lepidolejeunea integristipula* (J.B. Jack & Steph.) R.M. Schust.**	Fiji Isls., *Pócs 3307/AC*(GOET)	DQ983697	DQ987417	DQ987313
***Luteolejeunea herzogii*(Buchloh) Piippo**	Costa Rica, *Schäfer-Verwimp & Holz 0294/B*(GOET)	DQ983706	DQ987467	DQ987368
***Marchesinia robusta*(Mitt.) Schiffn.**	Ecuador, *Wilson et al . 04-05* (GOET)	DQ983710	DQ987436	DQ987332
***Mastigolejeunea auriculata* (Wilson & Hook.) Schiffn.**	Bolivia, *Churchill 21275*(GOET)	AY548070	DQ987385	DQ987268
***Microlejeunea africana*Steph.**	São Tomé & Principe, *Shevock 34576A* (GOET)	KC313150	KC313190	KC313112
***Myriocoleopsis gymnocolea* (Steph.) M.E. Reiner & Gradst.**	Ecuador, *Gradstein et al . 10020* (GOET)	DQ238568	DQ 238583	DQ987277

***Omphalanthus filiformis* Nees**	Ecuador, *Schäfer-Verwimp & Preußing 23543* (GOET)	DQ983716	DQ983793	DQ987283
***Prionolejeunea aemula*(Gottsche) A. Evans**	Guadeloupe, *Schäfer-Verwimp & Verwimp 22588* (M)	–	DQ207915	DQ207898
***Prionolejeunea decora*(Taylor) Steph.**	Dominica, *Schäfer-Verwimp & Verwimp 18028* (M)	**KF039851**	**KF039881**	**KF039816**
Prionolejeunea decora	Guadeloupe (I), *Schäfer-Verwimp & Verwimp 22308* (M)	–	DQ207919	DQ207890
Prionolejeunea decora	Guadeloupe (II), *Schäfer-Verwimp & Verwimp 22263* (M)	–	**KF039880**	**KF039815**
***Prionolejeunea galliotii*Steph.**	Panama, *Reiner-Drehwald 960026* (GOET)	–	DQ907923	DQ207886
***Prionolejeunea grata*(Gottsche) Schiffn.**	Réunion, *Pócs 08056/W*(M)	–	–	**KF039818**
***Prionolejeunea grollei*Ilkiu-Borges & Schäf.-Verw.**	Guadeloupe, *Schäfer-Verwimp & Verwimp 22355* (M, Paratype)	–	DQ907225	DQ207887
***Prionolejeunea guadalupensis*(Lindenb.) Steph.**	Dominica, *Schäfer-Verwimp & Verwimp 17922/A* (M)	–	DQ207926	DQ207892
***Prionolejeunea limpida*Herzog**	Brazil, *Schäfer-Verwimp & Verwimp 13291* (M)	**KF039850**	DQ207928	DQ207895
***Prionolejeunea magnistipula* Herzog**	Colombia, *Gradstein 8964*(GOET)	–	DQ207929	DQ207896
***Prionolejeunea mucronata* (Sande Lac.) Steph.**	Costa Rica, *Schäfer-Verwimp & Holz 287* (M)	**KF039852**	–	**KF039817**
Prionolejeunea scaberula	French Guiana, *Holz FG-0012*	–	DQ207934	DQ207889

(Spruce) Zwickel	(GOET)			
Prionolejeunea scaberula	Guadeloupe, *Schäfer-Verwimp & Verwimp 22184/B* (M)	–	DQ207933	DQ207888
***Prionolejeunea schlimiana* (Gottsche) Steph.**	Costa Rica, *Dauphin 2115*(GOET)	–	DQ207935	DQ207893
Prionolejeunea schlimiana	Ecuador (I), *Andersson & Kautz 3202* (GOET)	–	DQ207936	DQ207894
Prionolejeunea schlimiana	Ecuador (II), *Schäfer-Verwimp & Nebel 33210*(M)	–	–	**KF039814**
***Prionolejeunea trachyodes* (Spruce) Steph.**	Panama, *Schäfer-Verwimp & Verwimp 30778* (M)	–	–	**KF039813**
***Rectolejeunea berteroana* (Gottsche) A. Evans**	Guadeloupe, *Schäfer-Verwimp & Verwimp 22245/A* (GOET)	DQ983724	DQ987444	DQ987342
***Siphonolejeunea elegantissima* (Steph.) Grolle**	Australia, *Pócs & Brown*0026/AA (EGR)	DQ983726	DQ987452	DQ987352

Accession numbers of new sequences are in bold face

Table 2

Distribution of constant and phylogenetically informative sites for aligned positions of the three genomic regions (1. Lejeuneeae dataset, 2. *Bromeliophila-Cyclolejeunea-Prionolejeunea* dataset)

	***rbc*L**	***trn*L-*trn*F**	**ITS1-5.8S-ITS2**	**Total**
Number of sites in matrix	914/936	433/435	668/920	2,015/2,291
Constant	720/793	268/327	333/430	1,321/1,550
Unique to a single specimen	90/48	73/49	90/114	253/211
Parsimony informative	104/95	92/59	245/376	441/530

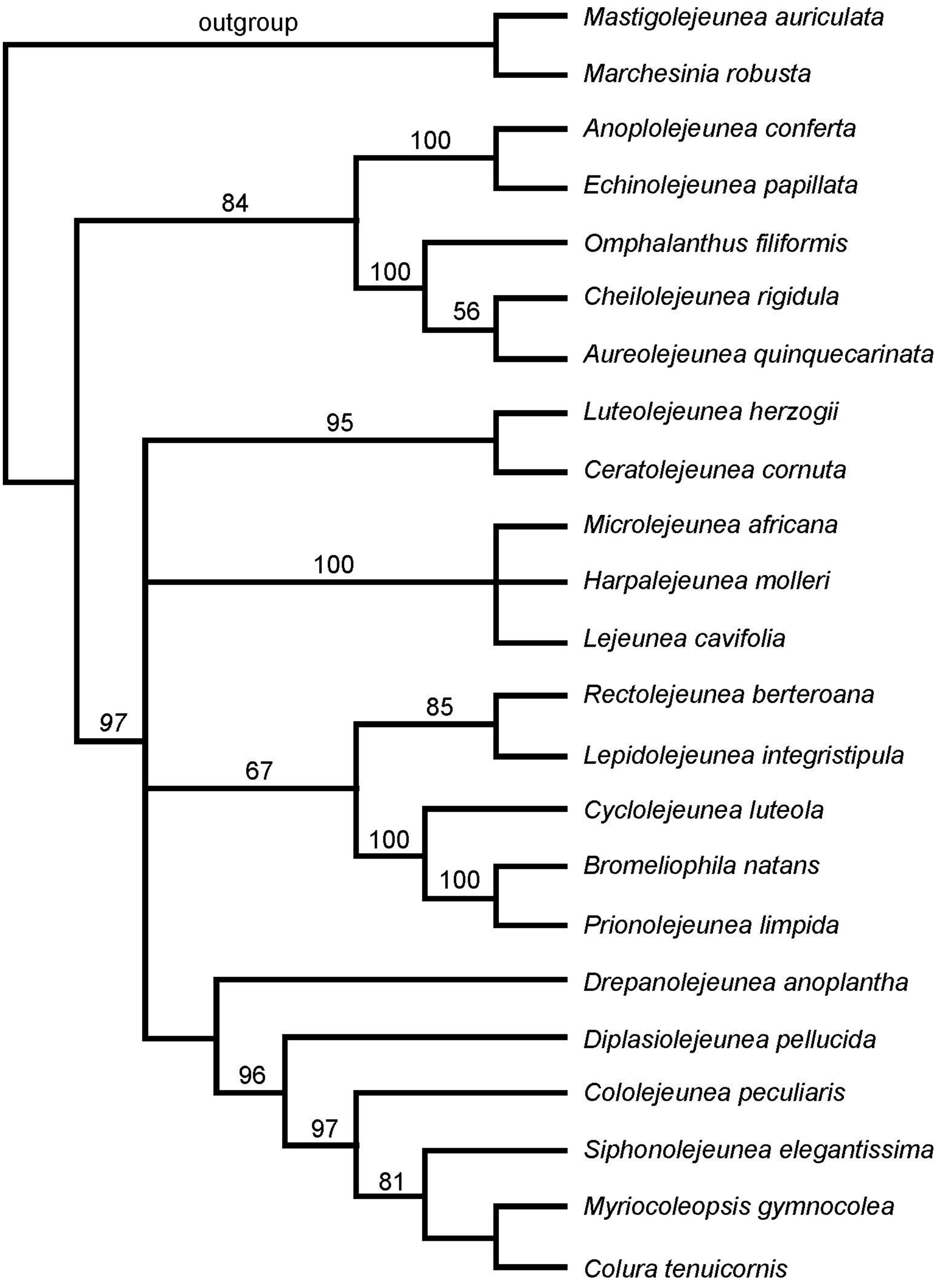

Fig. 1 Strict consensus of four equally parsimonious trees. *Bromeliophila* is placed sister to *Prionolejeunea* rather than *Lejeunea*

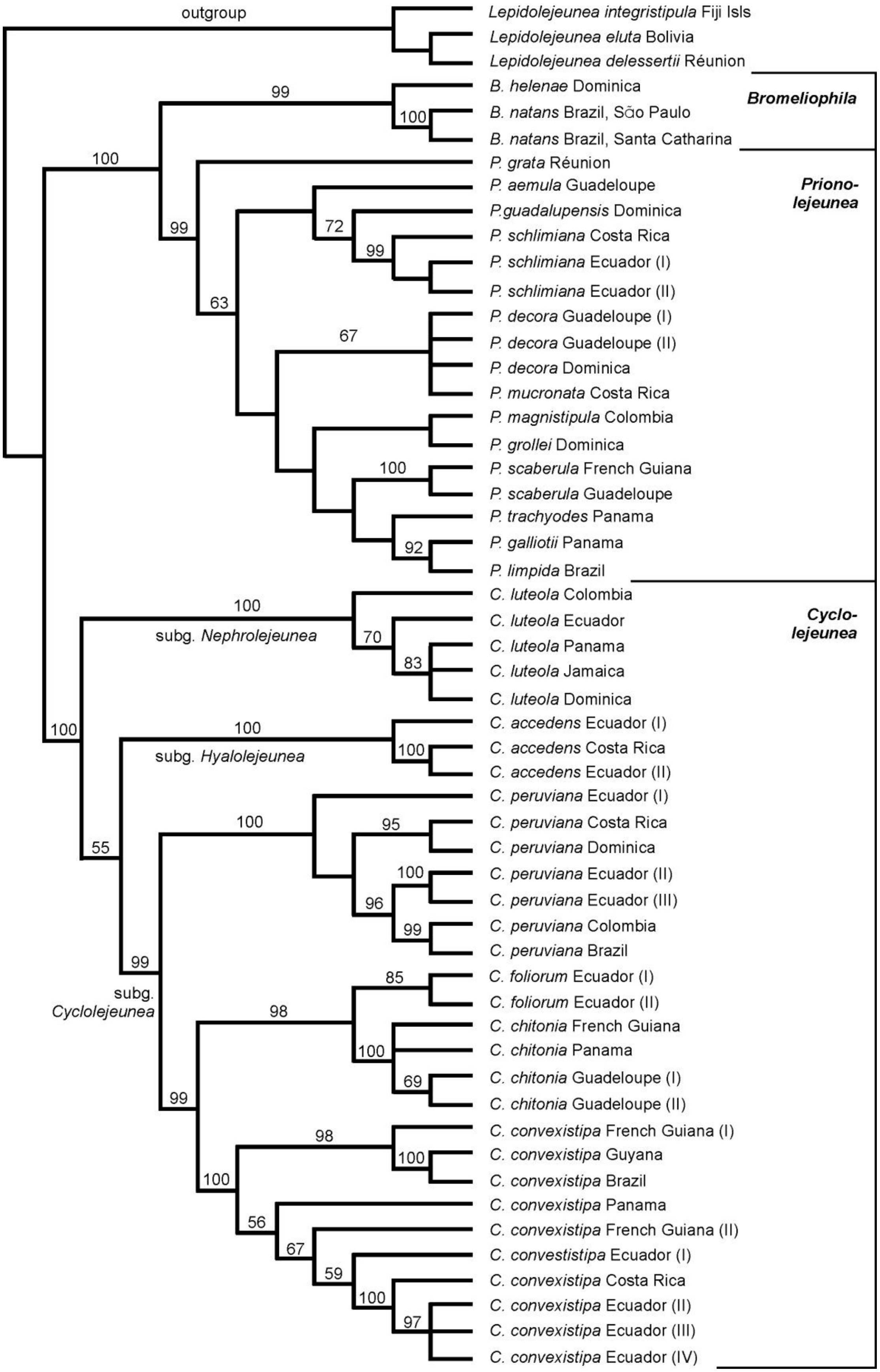

Fig. 2 Rooted strict consensus of 156 most parsimonious trees recovered during heuristic searches of the combined *rbc*L–*trn*L-F–nrITS dataset. Bootstrap percentage values >50 are indicated at branches

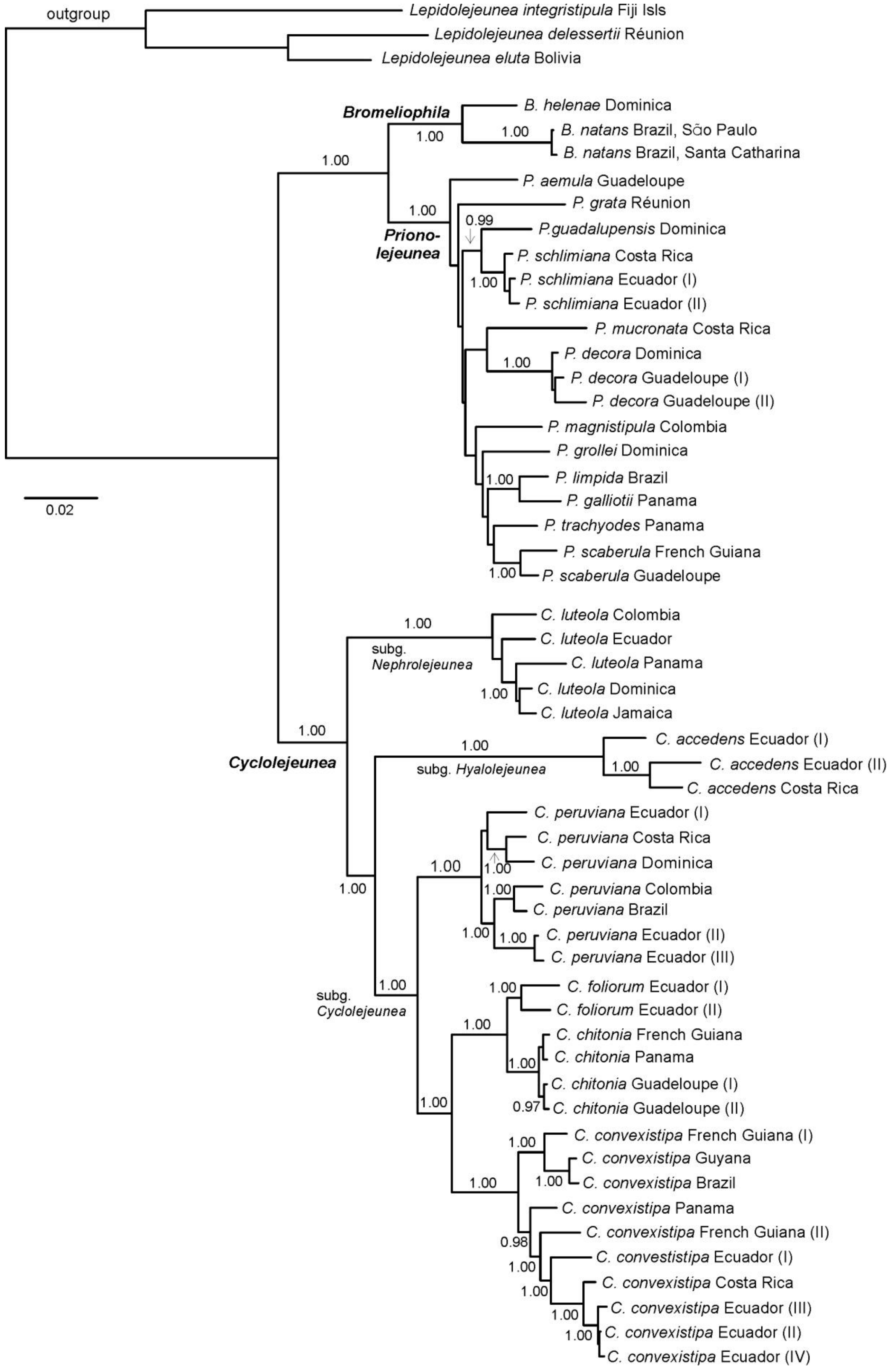

Fig. 3 Majority rule consensus tree of trees recovered in stationary phase of Bayesian search. Support (≥0.95) from Bayesian searches is indicated at branches

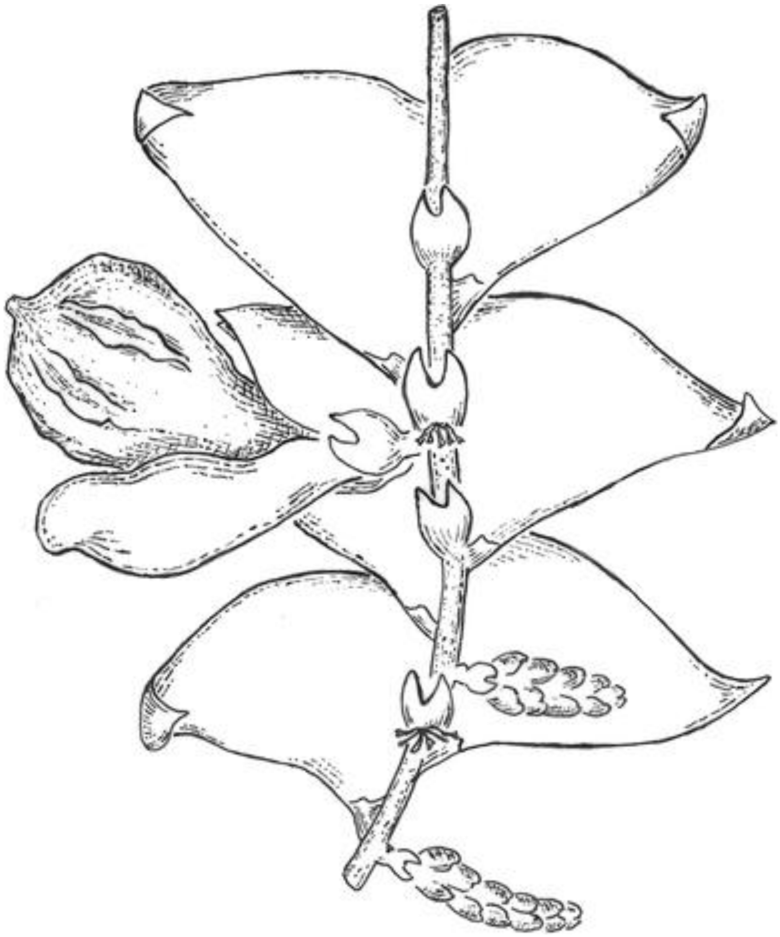

Fig. 4 *Bromeliophila natans*, part of shoot with perianth and two androecia, ventral view [drawn from Peralta et al. 8112 (M)]

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