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PHYLOGENY, BIOGEOGRAPHY, CLASSIFICATION, AND  
AMBER FOSSILS OF THE LIVERWORT FAMILIES  
LEJEUNEACEAE AND RADULACEAE

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München, 07. Dezember 2017



*für meine Familie*



# **PREFACE**

## **Statutory declaration**

### **Erklärung**

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Jochen Heinrichs betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

### **Eidesstattliche Erklärung**

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Julia Bechteler, 07. Dezember 2017

(Unterschrift)

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## Declaration of contribution

In this thesis, I present the results from my doctoral research, carried out in Munich from August 2014 to November 2017 under the guidance of Prof. Dr. Jochen Heinrichs. My thesis resulted in four manuscripts, presented in Chapters 2 to 5, of which all have been published. I generated all data and conducted all analyses myself except for Chapter 1 for which J. Heinrichs and A. Schäfer-Verwimp contributed the character matrix and G.E. Lee the drawing in Fig. 2. For Chapter 5, I conducted the analyses based on datasets provided by M.A.M. Renner, and photographs by A.R. Schmidt and J. Heinrichs. Writing and discussion involved collaboration with J. Heinrichs, with input from the co-authors.

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## List of publications

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- BECHTELER, J., LEE, G.E., SCHÄFER-VERWIMP, A., RENNER, M.A.M.,  
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# CONTENTS

<b>Preface</b> .....	v
Statutory Declaration .....	v
Erklärung .....	v
Eidesstattliche Erklärung .....	v
Declaration of contribution .....	vii
List of publications .....	vii
Funding .....	viii
<b>Contents</b> .....	ix
<b>Summary</b> .....	1
<b>Chapter 1: General Introduction</b> .....	3
1.1 Epiphyllous liverworts .....	7
1.2 The study systems: Lejeuneaceae and Radulaceae .....	9
1.3 Evolution of Lejeuneaceae and Radulaceae .....	11
1.4 Fossil record of Lejeuneaceae and Radulaceae .....	12
1.5 Specific research questions .....	14
<b>Chapter 2: Towards a monophyletic classification of Lejeuneaceae IV: Reinstatement of <i>Allorgella</i>, transfer of <i>Microlejeunea aphanella</i> to <i>Vitalianthus</i> and refinements of the subtribal classification</b> .....	17
<b>Chapter 3: Towards a monophyletic classification of Lejeuneaceae V: The systematic position of <i>Pictolejeunea</i></b> .....	35
<b>Chapter 4: Geographical structure, narrow species ranges and Cenozoic diversification in a pantropical clade of epiphyllous leafy liverworts</b> .....	49
<b>Chapter 5: Burmese amber fossil of <i>Radula</i> (Porellales, Jungermanniopsida) provides insights into the Cretaceous evolution of epiphytic lineages of leafy liverworts</b> .....	67
<b>Chapter 6: General Discussion</b> .....	93
6.1 Phylogeny and classification of Lejeuneaceae (Lejeuneaceae) .....	95
6.2 Evolution of epiphyllous liverworts: The case of <i>Leptolejeunea</i> .....	96
6.3 A Cretaceous amber fossil belonging to the genus <i>Radula</i> (Radulaceae) ....	99
6.4 General conclusion and perspective .....	101
<b>References</b> .....	103
<b>Acknowledgements</b> .....	119
<b>Curriculum vitae</b> .....	121



## SUMMARY

This dissertation deals with the molecular phylogeny and classification of the largest family of liverworts, the Lejeuneaceae, the historical biogeography of its predominantly epiphyllous genus *Leptolejeunea*, and the oldest known amber inclusion of the leafy liverwort family Radulaceae. Lejeuneaceae are leafy liverworts and include perhaps 1,700 species in 71 genera; they are closely related to the Radulaceae, which include only the genus *Radula* with c. 250 species. Both families are most species-rich in tropical and subtropical forests where most their species grow on other plants. In my research, I wanted to answer two main questions: During which geologic period did the epiphyllous genus *Leptolejeunea* diversify and reach its pantropical range and what can be inferred from the morphological traits of the oldest fossil of *Radula*, such as leaf shape, bract pair number, gemmae development, and the type of the lobule insertion. Additionally, I wanted to increase the generic sampling of the phylogeny of Lejeuneaceae, for what I generated numerous new plastid and nuclear DNA sequences from dried herbarium material of the family Lejeuneaceae, yielding a phylogeny with 30 of 44 genera of the tribe Lejeuneae that resolved the placement of taxonomically unclear genera (i.e., *Haplolejeunea*, *Metalejeunea*, *Pictolejeunea*, *Vitalianthus*). The type species of *Otolejeunea* does not group with a second species, and I therefore reinstated the genus *Allorgella*, which in my concept includes at least six species that occur in Madagascar, Southern China, Southeast Asia, and Australasia. DNA sequences and the morphological examination of the isolectotype of *Microlejeunea aphanella*, collected in Brazil in 1884, indicated, that this species is closely related to the type species of the genus *Vitalianthus* (one of its two species sampled); I therefore transferred it to that genus.

To answer my first question, I compiled a dataset with 216 new plastid and nuclear DNA sequences for 17 species of *Leptolejeunea*, generated a molecular clock-dated chronogram, and then used it for formal biogeographical analyses. For coding the species' geographic occurrence, I designated three large regions, but included some 14 accessions for each of two supposedly pantropical species (*L. elliptica* and *L. maculata*) that are implausible to represent single gene pools. The stem age of *Leptolejeunea* was estimated as 68 [48-94] Ma, and the diversification of *Leptolejeunea* likely benefitted from megathermal angiosperm forests of the Paleocene and Eocene, that provided the high atmospheric humidity required by epiphyllous liverworts. Dispersal from Asia to Africa

appears to have occurred around 2.5 to 2.1 Ma, probably by migratory birds or on floating islands, as inferred for many other plants and animals.

To get a better understanding of the morphological evolution of leafy liverworts, I focused on their amber inclusions. Chapter 5 of this thesis describes the oldest known fossil of the genus *Radula*, *R. cretacea* Bechteler, M.A.M. Renner, Schäf.-Verw., & Heinrichs, from Burmese amber of a minimum age of 98 Ma. The observed complicate bilobed leaf shape, absence of underleaves, numerous archegonia per gynoeceium, and *Radula*-type branching are characteristic for *Radula*. Ancestral state reconstructions, using further characters (as mentioned above) allowed me to securely assign the fossil to the subgenus *Odontoradula*, which has 18 species and occurs mainly in Asia and Oceania.

# Chapter 1

## GENERAL INTRODUCTION



The evolutionary history of organisms is of fundamental relevance to understanding biodiversity and can provide arguments for conservation, for example, by providing age estimates, revealing narrow geographic ranges (endemicity), or by demonstrating the uniqueness of adaptive traits. Fossils give valuable insights into the organismal diversity of the past, proof past geographic ranges, and can be used to reconstruct former environmental conditions. In the past 30 years, fossils have also gained the new role of calibrating molecular phylogenies to translate genetic distances into absolute times, leading to so-called time trees or chronograms. Such calibrated phylogenies, in which branch lengths are forced to be proportional to time allow one to infer relationships between taxa as well as the divergence times of the surviving lineages. Biogeographical analyses that build on such time trees, can help to understand today's distribution patterns of the species in the phylogeny (Ronquist & Sanmartín 2011; Sanmartín 2012). A large problem in using plant fossils as calibrations is that many are not sufficiently well preserved, taxonomically solidly placed, and stratigraphically known. This has been especially true of bryophytes (Krassilov & Schuster 1984; Miller 1984; Taylor *et al.* 2009; Heinrichs *et al.* 2018).

To study the evolutionary history of delicate and tiny organisms, amber inclusions are of particular value. Amber is fossilized tree resin that is produced by angiosperms as well as gymnosperms (Langenheim 1990). Organisms encased in amber are often well-preserved, even down to the level of cell organelles, and thus, amber inclusions are greatly enhancing morphological investigation (Grolle & Braune 1988; Poinar *et al.* 1996), including of arthropods (Poinar 1993; Peñalver *et al.* 2006; Schmidt *et al.* 2012), lichens (Kaasalainen *et al.* 2017b), plants (Taylor *et al.* 2009), and entire paleo-ecosystems (Schmidt *et al.* 2010; Sadowski *et al.* 2017). Some groups, despite their small size and their occurrence on tree bark, are still sparsely represented in the amber fossil record. One such group is bryophytes, which in some ecosystems contribute a higher living, above-ground phytomass than vascular plants (Longton 1988: Franz Josef Land, 98 g m<sup>-2</sup> [31% of 316 g m<sup>-2</sup> of complete live, above ground phytomass]; angiosperms 15 g m<sup>-2</sup> [4%]). Bryophytes have a high water holding capacity of up to 1,500% of their dry weight, and therefore play an important role in water retention (Proctor 2009). Additionally, they are involved in carbon and nutrient cycling (Turetsky 2003), and can host bacteria capable of atmospheric nitrogen fixation (DeLuca *et al.* 2002; Fűrnrkranz *et al.* 2008). Due their ability to take up water and solutes over their entire surface, they are sensitive to chemicals, making them bio-indicators for rain and air pollution (Pearson *et al.* 2000). Bryophytes comprise the mosses (Bryophyta), the liverworts (Marchantiophyta), and the hornworts

(Anthocerotophyta). Mosses have some 12,800 species (Crosby *et al.* 1999), liverworts about 7,500 (von Konrat *et al.* 2010), and hornworts about 215 (Söderström *et al.* 2016). Bryophytes may not be a natural group (Renzaglia *et al.* 2000; Qiu *et al.* 2006; Cox *et al.* 2014; Liu *et al.* 2014; Wickett *et al.* 2014) and their origin is therefore difficult to infer. The oldest known microfossils with bryophyte affinities, and at the same time one of the first records of land plants, are cryptospores (spore tetrads) from Ordovician rocks (c. 475 Ma) from Oman (Wellmann *et al.* 2003). The bryophyte macrofossil record extends back to the Middle Devonian of eastern New York, with *Metzgeriothallus sharonae* regarded to belong to the liverworts (Hernick *et al.* 2008). Bryophytes are the only land plants in which the haploid gametophyte is the dominant generation.

My doctoral research was aimed at increasing our understanding of the biogeography and morphological evolution of leafy liverworts. Many molecular studies in the last years have dealt with these liverworts' phylogenetic relationships, some proposing biogeographic hypotheses (Feldberg *et al.* 2007; Dong *et al.* 2012; Heinrichs *et al.* 2015a), but only a few used chronograms with absolute ages for more detailed insights (Hartmann *et al.* 2006; Devos & Vanderpoorten 2009; Scheben *et al.* 2016; Carter *et al.* 2017; Patiño *et al.* 2017). We therefore know little about the biogeography of epiphyllous and epiphytic liverworts, which are widespread throughout tropical and subtropical regions. These liverworts are adapted for living on trunks and leaves by having a flat habit, imbricate leaves, inflated lobules, and rhizoid discs. Based on these morphological traits, the life style of amber-preserved gametophytes as epiphytic (or not) can be inferred, with further insights about likely life styles coming from the shape of the leaf lobe and lobule, their relative sizes, the absence/presence and the shape of the underleaf, leaf position, and types of ventral and lateral branches. Sometimes, substrate fragments are preserved in amber, along with an epiphytic liverwort, which can give valuable information.

The morphological traits of a well-determined leafy liverwort amber fossil can also be compared to those of extant species, helping to understand morphological evolution. Cretaceous amber fossils are of special interest in this regard, since they provide insights about morphological change during the Cretaceous Terrestrial Revolution (Benton 2010; Vermeij 2011; Magallón *et al.* 2015), which is assumed to have triggered the diversification of epiphytic and epiphyllous leafy liverworts (Feldberg *et al.* 2014), ferns (Schneider *et al.* 2004), and insects (Wilf *et al.* 2000; Moreau *et al.* 2006). Studies on Cretaceous amber fossils of ferns (Regalado *et al.* 2017), mushrooms (Hibbett *et al.* 1997), and bees (Poinar & Danforth 2006) provide examples of such insights. When I started my Ph.D. research in



2014, only four liverworts representing two families were known from Cretaceous amber (Heinrichs *et al.* 2015b).

My study systems are the leafy liverwort families Lejeuneaceae and Radulaceae, which together have some 1,950 species in some 72 genera (Söderström *et al.* 2016) that are mainly distributed in tropical and subtropical ecosystems around the world. Lejeuneaceae make up more than 70% (39 of 53 species) of the epiphytic liverwort diversity in tropical lowland rain forests in Guyana (Cornelissen & Ter Steege 1989), and more than 80% (138 of 168 species) of epiphyllous liverworts in China (Zhu & So 2001). In the following paragraphs, I provide an overview on the epiphyllous lifestyle of liverworts and my two study systems.

### **1.1 Epiphyllous liverworts**

Liverworts can inhabit a variety of substrates, ranging from soil and rocks, to bark, branches, twigs, and leaves of trees and shrubs (Vanderpoorten & Goffinet 2009). They can also grow on artificial surfaces, such as plastic ribbon tape and iron railings (Monge-Nájera & Blanco 1995; Pócs 1996) and on animals (Gradstein & Equihua 1995; Lücking *et al.* 2010). The probably first description of an epiphyllous liverwort was made by Swartz 1788, who described *Jungermannia flava* (= *Lejeunea flava*, Lejeuneaceae) from Jamaica. Photosynthetic organisms that grow on surfaces of living vascular plant leaves are called epiphylls (Bentley 1987), and the habitat, provided by leaves of higher plants to epiphylls, is called the phyllosphere (Ruinen 1961). Epiphyllous liverworts belong to different families and are no natural group. The epiphyllous life form is mostly found in tropical and subtropical forests with a permanently high humidity and in areas with rainfall exceeding 3,000 mm/year, as in parts of North Madagascar and New Guinea, almost any liverwort and even mosses are able to occur in the phyllosphere (Pócs 1996). In epiphyllous communities of the phyllosphere, which are like a small ecosystem consisting of several organisms (bryophytes, algae, lichen, fungi, rotifers, bacteria), liverworts, and especially the Lejeuneaceae, followed by the Radulaceae, in terms of their abundance and diversity are the most important component (Richards 1984; Pócs 1996; Lücking 1997). Although, liverwort species counts per host leaf are generally ranging from two to nine (Zhu & So 2001), some leaf surfaces may host up to 24 liverwort species (Lücking 1997: Costa Rica).

Epiphyllous liverworts are not only abundant in tropical and subtropical rainforests (Pócs 1996), but also play important ecological roles in these ecosystems. In

general, bryophytes have a good water storage capacity (Köhler *et al.* 2007), which creates perfect conditions for bacteria capable of atmospheric nitrogen fixation (cyanobacteria, gamma-proteobacteria). Such bacteria are often associated with epiphyllous liverworts and then can contribute nitrogen to the host plants (Bentley & Carpenter 1984; Bentley 1987; Fürnkranz *et al.* 2008: up to 6  $\mu\text{mol N}_2$  per  $\text{m}^2$  per day in lowland rainforests of Costa Rica). Other studies have shown that epiphyllous liverworts are an “intermittent sink of nutrients” from rainfall in the canopy (Wanek & Pörtl 2005, p. 587). In addition, epiphyllous liverworts may act as protection against herbivores. Thus, a liverwort cover has been found to decrease the frequency of leaf cutter ants damaging host leaves (Mueller & Wolf-Mueller 1991). Possible reasons may be terpenoids in the liverwort’s oil bodies or that an epiphyll cover increases the workload for the ants cutting up the leaves.

It is still debated if a cover of epiphyllous liverworts negatively affects the photosynthesis of the host leaves (Roskoski 1981; Coley *et al.* 1993). One study (of epiphyllous lichens) found that host leaves can compensate the shading resulting from the epiphylls by photo-acclimation, what might also apply to a cover of epiphyllous liverworts (Anthony *et al.* 2002). *Radula flaccida*, an epiphyllous liverwort, can penetrate the cuticle and epidermis of its host leaf with its rhizoids (Berrie & Eze 1975), and the possibility of epiphyllous liverworts being hemi-parasites needs further testing (Richards 1984).

Local habitat conditions, and especially the atmospheric humidity, are among the most important factors influencing colonization of epiphyllous liverworts (Winkler 1967; Olarinmoye 1974; Coley *et al.* 1993; Sonnleitner *et al.* 2009). Epiphyllous liverworts germinate and grow on any suitable leaf, but angiosperm leaves and fern fronds are more suitable than gymnosperm needles (Winkler 1967; Zhu & So 2001). The absolute epiphyllous cover is always highest in large leaves (Monge-Nájera 1989), and smooth leaf surfaces seem to be more suitable for epiphyllous liverworts than rough leaf surfaces and leaves with waxy cuticles (Winkler 1967; Richards 1984). Leaf shape and drip tips did not influence the epiphyllous cover naturally established on artificial plastic leaves (Monge-Nájera & Blanco 1995: focus on bryophytes; Lücking & Bernecker-Lücking 2005: focus on lichens), as well as on living leaves in Costa Rica (Ivey & DeSilva 2001: several species, e.g. *Faramea parviflora*, *Ocotea meiziana*; experimentally manipulated by cutting off the drip-tips). The survival rate of young epiphyllous liverworts in habitats with optimal condition from colonization to the end of the first year is around 40% (Winkler 1967), and their habitat is normally short-lived, with life spans of individual leaves in tropical broad-leaved trees and shrubs generally ranging from 12 to 14 months (Winkler 1967; Bentley

1979), but hosts with shorter-lived leaves were found to exhibit a higher epiphyll cover than those with longer-lived leaves (Coley *et al.* 1993). Epiphyllous liverworts cope with such ephemeral conditions by having short generation times; they can become reproductively active within less than six months after colonizing leaf surfaces (Zartman & Nascimento 2006).

## 1.2 The study systems: Lejeuneaceae and Radulaceae

Lejeuneaceae are the largest family of liverworts (Gradstein 2013; Söderström *et al.* 2016), and a recent liverwort classification by Söderström *et al.* (2016) listed 1,887 species in 71 genera (Shi *et al.* 2015; Ye *et al.* 2015; Söderström *et al.* 2016; Wang *et al.* 2016; Sukkharak & Gradstein 2017; Chapter 2). Estimates of biological species of this family range from 1,000 (Sukkharak & Gradstein 2017) to 1,700 (He & Zhu 2011). The family Radulaceae consists only of the genus *Radula*, with 245 listed species (Söderström *et al.* 2016). In both groups, species delimitation is difficult due to a high degree of morphological homoplasy (Gradstein *et al.* 2003b; Yu *et al.* 2013; Renner 2015; Shi *et al.* 2015) and the occurrence of cryptic species (Heinrichs *et al.* 2009; Dong *et al.* 2012; Renner *et al.* 2013a, b; Sukkharak & Gradstein 2017). For *Radula*, the most up-to-date subgeneric classification based on a molecular phylogeny listed seven subgenera that mostly lack morphological synapomorphies (Devos *et al.* 2011). The Lejeuneaceae are divided in two subfamilies, the Ptychanthoideae and the Lejeuneoideae, the latter comprising three tribes (Brachiolejeuneae, Symbiezidiae, Lejeuneae) (Gradstein 2013), with over 80% of Lejeuneaceae species in the Lejeuneae (1,651 of 1,887 species; Söderström *et al.* 2016). In contrast to the well-resolved phylogenetic backbone of the Radulaceae (Devos *et al.* 2011), the deeper parts of the Lejeuneaceae phylogeny are poorly resolved, and hence it is unclear, if the Ptychanthoideae are a monophyletic group, as could be shown for the Lejeuneoideae (Wilson *et al.* 2007a). Many genera in the tribe Lejeuneae had never been studied in a molecular phylogenetic framework when I started my research. Additionally, information on within-genus relationships was completely lacking for many genera. My particular focus, the pantropical genus *Leptolejeunea* (Table 1) was estimated as having 25 to 48 species (Gradstein *et al.* 2001; Söderström *et al.* 2016). *Leptolejeunea elliptica* and *L. maculata* were supposedly pantropical (Table 1). The majority of *Leptolejeunea* species are dioicous (29 of the estimated 48 species), whereas ten species are monoicous, *L.*

*elliptica* is reported to be either dioicous or monoicous, and sexual forms of seven are unknown (Table 1).

Table 1. Information on the life form, sexual system and distribution of the 48 *Leptolejeunea* species, with taxon confidence levels according to Söderström *et al.* 2016 and Shu *et al.* 2016. \* = serious doubts, \*\* = knowledge problems, \*\*\* = accepted, E = epiphyllous, NE = epiphytic, A = Neotropics, B = Afromadagascar, C = Asia/Australasia.

Species	E	NE	Sexuality	Distribution	Reference
<i>L. amphiophthalmalma</i> ***	x		sterile	C	Herzog 1942 (as <i>L. picta</i> ); Yang 2014
<i>L. apiculata</i> **	x		dioicous	C	Zhu & So 2001; Yang 2014
<i>L. arunachalensis</i> **					unknown
<i>L. astroidea</i> ***	x		monoicous	B	Stephani 1913
<i>L. australis</i> **		x	sterile	C	Stephani 1913
<i>L. balansae</i> **	x		dioicous	C	Herzog 1942; Zhu & So 2001
<i>L. borneensis</i> *	x		dioicous	C	Herzog 1942
<i>L. brasiliensis</i> **	x		monoicous	A	Bischler 1969; Gradstein & Costa 2003a
<i>L. convexistipa</i> *	x		dioicous	A	Bischler 1969
<i>L. curvatifolia</i> *	x		sterile	C	Stephani 1923
<i>L. denticulata</i> **	x		dioicous	C	Stephani 1913
<i>L. dentistipula</i> **	x		sterile	C	Stephani 1913; Herzog 1942
<i>L. diversilobulata</i> **	x		monoicous	A	Bischler 1969; Gradstein & Costa 2003a
<i>L. dolabriformis</i> **	x		dioicous	C	Pearson 1922
<i>L. elliptica</i> ***	x		dioicous or monoicous	A, B, C	Schuster 1967; Bischler 1969; Zhu & So 2001
<i>L. emarginata</i> *	x		dioicous	C	Yang 2014
<i>L. epiphylla</i> ***	x		dioicous	B, C	Stephani 1913; Herzog 1942; Zhu & So 2001
<i>L. exocellata</i> ***	x		monoicous	A	Bischler 1969; Gradstein & Ilkiu-Borges 2009
<i>L. foliicola</i> **	x		monoicous	C	Stephani 1913; Herzog 1942
<i>L. integristipula</i> **	x		monoicous	C	Stephani 1923
<i>L. jamaicensis</i> *	x		dioicous	A	Schuster 1967
<i>L. lancifolia</i> **	x		dioicous	C	Stephani 1913; Herzog 1942
<i>L. latifolia</i> **		x	dioicous	C	Herzog 1950
<i>L. latilobula</i>	x		dioicous	C	Shu <i>et al.</i> 2016
<i>L. lepinii</i> **	x		dioicous	C	Stephani 1913
<i>L. ligulata</i> **	x		sterile	C	Herzog 1942
<i>L. maculata</i> ***	x		dioicous	A, B, C	Zhu & So 2001; Gradstein & Costa 2003a
<i>L. massartiana</i> *	x		dioicous	C	Herzog 1942
<i>L. micronesica</i> **	x		monoicous	C	Inoue & Miller 1965
<i>L. minima</i> **	x		sterile	C	Herzog 1950
<i>L. mirikana</i> **	x		dioicous	C	Dey & Singh 2010

Species	E	NE	Sexuality	Distribution	Reference
<i>L. moniliata</i> ***	x		monoicous	A	Bischler 1969; Gradstein & Ilkiu-Borges 2009
<i>L. obfuscata</i> ***	x		dioicous	A	Bischler 1969; Gradstein & Costa 2003a
<i>L. punctata</i> *	x		monoicous	C	Herzog 1942
<i>L. radicata</i> ***	x		sterile	A	Stephani 1913 (as <i>Ceratolejeunea radicata</i> )
<i>L. renneri</i> *	x		dioicous	C	Herzog 1942
<i>L. revoluta</i> *	x		dioicous	C	Chen 1955
<i>L. rosulans</i> **	x		dioicous	C	Stephani 1913
<i>L. serratifolia</i> **	x		dioicous	A	Bischler 1969; Gradstein & Costa 2003a
<i>L. spinistipula</i> **		x	monoicous	C	He 1997
<i>L. subdentata</i> **	x		dioicous	C	Herzog 1942; Zhu & So 2001
<i>L. subrotundifolia</i> ***		x	dioicous	C	Herzog 1942
<i>L. tridentata</i> ***	x		dioicous	A	Bischler 1969; Gradstein & Costa 2003a
<i>L. trigonostipa</i> *	x		dioicous	A	Stephani 1913
<i>L. tripuncta</i> **	x		dioicous	C	Herzog 1942 (as <i>L. serrulata</i> ); Shu <i>et al.</i> 2016
<i>L. truncatifolia</i> **	x		dioicous	C	Zhu & So 2001; Yang 2014
<i>L. udarii</i> *	x		dioicous	C	Dey & Singh 2010
<i>L. vitrea</i> ***	x		dioicous	C	Stephani 1913; Herzog 1942

### 1.3 Evolution of Lejeuneaceae and Radulaceae

For a long time, bryophytes and thus also liverworts were thought of as “unchanging, unmoving sphinxes of the past” (Crum 1972, p. 279). This resulted from broad species concepts, which in turn resulted in broad geographical ranges (Shaw 2001). It was also believed that many bryophyte species were of ancient origin, with some even dating back to the Jurassic (Stotler & Crandall-Stotler 1974). With the advent of DNA-based molecular methods in the late 20<sup>th</sup> century, morphologically determined species were often found to harbor considerable molecular variation, and the number of cryptic species rose (Shaw 2001; Heinrichs *et al.* 2009; Yu *et al.* 2013). Many of these former intercontinentally distributed species were split into separate lineages, many of them with restricted geographical ranges (Shaw 2001; Stech & Wagner 2005; Chapter 4). Additionally, molecular clock-based age estimates suggested young ages for most extant bryophyte species (Hartmann *et al.* 2006; Feldberg *et al.* 2014; Laenen *et al.* 2014; Patiño *et al.* 2017). This led to long-distance dispersal (LDD) becoming the new paradigm, replacing continental vicariance as the preferred explanation for disjunct ranges (Devos & Vanderpoorten 2009; Scheben *et al.* 2016).

Stem age estimates indicate a Late Triassic to Late Jurassic origin of Lejeuneaceae at 152.0 Ma (Laenen *et al.* 2014: 95% HPD interval not stated), 191 [158-226] Ma (Cooper

*et al.* 2012), and 216.3 Ma (Feldberg *et al.* 2014: 95% HPD interval not stated). This is well in agreement with the compression fossil, *Sinolejeunea yimaensis* of the Middle Jurassic Yima Formation of China, which likely belongs to the Lejeuneaceae (Yang & Wu 2010), but due to its poor preservation was not used in any of the three cited studies. Heinrichs *et al.* (2007), however, found that the Lejeuneaceae originated only during the Early Cretaceous, with its stem age at 131.8 [123.8-139.8] Ma.

*Radula* has been estimated as having originated sometime between the Mid-Permian and the Early Eocene in studies focused on liverwort evolution (Heinrichs *et al.* 2007; Cooper *et al.* 2012; Feldberg *et al.* 2014; Laenen *et al.* 2014; see Chapter 5 Suppl. Table S3 for details). Patiño *et al.* (2017) specifically dealt with the evolutionary history of *Radula* and inferred a Middle Jurassic origin of the stem of the genus, at approximately 175 Ma (taken from their Fig. 1) and a crown group origin of the genus at 144.6 [109.6-197.5] Ma. The crown group diversification of Lejeuneaceae was retrieved to have occurred during the Cretaceous at 78.7 [76.4-81.0] Ma (Heinrichs *et al.* 2007), 91.1-103.4 Ma (Wilson *et al.* 2007b), 113.6 Ma (Laenen *et al.* 2014: 95% HPD interval not stated), and 146.1 Ma (Feldberg *et al.* 2014: 95% HPD interval not stated). This coincides with the diversification of the angiosperms during the Cretaceous and Tertiary (Magallón *et al.* 2015) and the time of the Cretaceous Terrestrial Revolution (Benton 2010; Vermeij 2011). Before this time, gymnosperms dominated the forests (Peralta-Medina & Falcon-Lang 2012). Due to their high leaf vein density, angiosperms have a higher transpiration capacity and can thus provide higher humidity compared to gymnosperms (Boyce *et al.* 2010; Boyce & Leslie 2012). This might have led to a less warm, more humid and more non-seasonal climate, that could have triggered the diversification of several organisms (Boyce & Lee 2010), including Lejeuneaceae and *Radula* (Wilson *et al.* 2007b; Feldberg *et al.* 2014).

#### **1.4 Fossil record of Lejeuneaceae and Radulaceae**

As mentioned, the oldest known fossil thought to represent Lejeuneaceae is *Sinolejeunea yimaensis*, a compression fossil of the Middle Jurassic Yima Formation of China (Yang & Wu 2010). The remaining fossils of Lejeuneaceae and Radulaceae are inclusions in amber of different ages and deposits, together representing about 35 species (Heinrichs *et al.* 2018). Here I want to give a short summary of the amber deposits yielding inclusions of Lejeuneaceae and Radulaceae (Fig. 1), with information on their assumed ages and possible

resin-producing trees. Information about species numbers in this paragraph comes from the most recent review of liverwort amber inclusions by Heinrichs *et al.* (2018).

The youngest and by far the most important deposit regarding species numbers is the 15-20 million years old, Early-Middle Miocene Dominican amber (Iturralde-Vinent & MacPhee 1996), which so far has yielded 20 Lejeuneaceae and two *Radula* species. This amber was probably produced by the Fabaceae genus *Hymenaea* (Langenheim 1995; Poinar & Brown 2002) and can be found on the Greater Antilles, particularly the Dominican Republic (Iturralde-Vinent 2001). Another source with Miocene age is the Mexican amber from which four Lejeuneaceae species are known. It may have been produced by the same legume trees as the Dominican amber (Langenheim 1995; Solórzano Kraemer 2007).

The Paleogene Baltic and Bitterfeld ambers from northern Europe are another important source of liverwort fossils. Two *Radula* species and one species of Lejeuneaceae have been described from Baltic amber, and one *Radula* species and two Lejeuneaceae species from Baltic and Bitterfeld ambers. The absolute age of Baltic and Bitterfeld amber is still unclear (Kaasalainen *et al.* 2017b). Baltic amber may be of Late Eocene age (Sadowski *et al.* 2017) and Bitterfeld amber may be, too (Grolle & Meister 2004; Frahm 2010; Wolfe *et al.* 2016) or else may date to the late Oligocene (Standke 2008). The minimum age of Bitterfeld amber is accepted to be 24 Ma (Kaasalainen *et al.* 2017b). The resin of conifers is assumed to have produced both, Baltic and Bitterfeld amber (Wolfe *et al.* 2016; Sadowski *et al.* 2016, 2017).

The deposits of Rovno amber in the Ukraine have so far yielded one species of Lejeuneaceae. Rovno amber is assumed to have a Middle-Late Eocene age (Perkovsky *et al.* 2007, 2010). Another deposit for Eocene amber from which one Lejeuneaceae inclusion is known is the 52 million years old Ypresian Cambay Indian amber, most likely produced by Dipterocarpaceae trees (Rust *et al.* 2010). During my Ph.D. research, the first Cretaceous fossil of *Radula* from the Burmese amber of Myanmar with a minimum age of 98 Ma (Shi *et al.* 2012) became available for study. Most likely Burmese amber consists of gymnosperm resin, probably from *Metasequoia* (Grimaldi *et al.* 2002). The well-preserved morphology and the Cretaceous origin of this new fossil make it important for studying the evolution of *Radula*, and leafy liverworts in general. A more detailed treatment of this fossil is given in Chapter 5 and in the Discussion of this thesis.

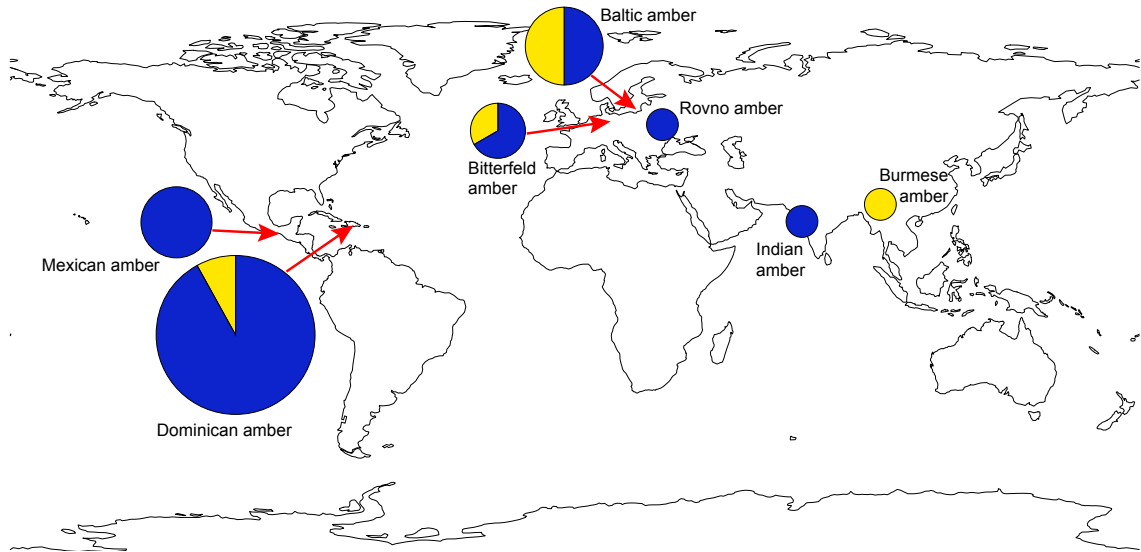


Figure 1. Amber deposits yielding inclusions of Lejeuneaceae (blue) and Radulaceae (yellow). Pie chart size represents the proportion of species found in each locality.

### 1.5 Specific research questions

The goal of my research was to gain insights into the evolution of epiphytic and epiphyllous leafy liverworts, and I chose to work with Lejeuneaceae and Radulaceae because of the abundant and important material, and unique expertise, available in the laboratory of my Ph.D. advisor Jochen Heinrichs. When I started to work on Lejeuneaceae, many genera had never been studied molecularly and the phylogenetic backbone of the family phylogeny was poorly resolved, hampering our understanding of the evolution of these plants. During my research, I contributed to the completion of the Lejeuneaceae phylogeny by analyzing generic relationships and clarifying generic boundaries in the tribe Lejeuneae. The results of this work are presented in Chapter 2 and 3.

To better understand the factors that shaped the evolution of an epiphyllous liverwort genus, I selected *Leptolejeunea* of the Lejeuneaceae, which was thought to include several pantropical species, raising interesting questions about gene flow in such species and dispersal (Grolle 1976; Schuster 1980; Pócs & Lye 1999; Zhu & So 2001; Chapter 1.2, Table 1). With molecular data, I could test for possible hidden molecular diversity in morphologically determined species. Another aim was to uncover the biogeographical history of *Leptolejeunea* to gain a better understanding of the age of its current distribution and continental subgroups. To find answers, I compiled a dataset



consisting of published sequence data and newly generated data from herbarium specimens, and then applied molecular-clock dating and complementary biogeographical analyses (Chapter 4).

Lastly, I studied a Cretaceous liverwort fossil from Burmese amber in extensive detail and assigned it to the leafy liverwort genus *Radula* of the family Radulaceae (Chapter 5). My research on this fossil was aimed at gaining insights into liverwort morphology during the time of the Cretaceous Terrestrial Revolution (Benton 2010; Vermeij 2011). I employed ancestral character reconstructions and divergence time estimations to evaluate its potential assignments in the phylogeny of *Radula*, thereby testing and increasing the future utility of this fossil as calibration for molecular age estimations.



## Chapter 2

TOWARDS A MONOPHYLETIC CLASSIFICATION OF  
LEJEUNEACEAE IV: REINSTATEMENT OF *ALLORGELLA*,  
TRANSFER OF *MICROLEJEUNEA APHANELLA* TO  
*VITALIANTHUS* AND REFINEMENTS OF THE SUBTRIBAL  
CLASSIFICATION.

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## Towards a monophyletic classification of Lejeuneaceae IV: reinstatement of *Allorgella*, transfer of *Microlejeunea aphanella* to *Vitalianthus* and refinements of the subtribal classification

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**Abstract** The classification of Lejeuneaceae, the most speciose tribe of the largely epiphytic leafy liverwort family Lejeuneaceae, is deemed notoriously difficult and hampered by extensive morphological homoplasy. We employed sequences of two chloroplast regions (*trnL-trnF*, *rbcl*) and the nuclear ribosomal ITS region of several critical taxa to explore their phylogenetic position. Based on our phylogenetic analyses and accompanying morphological investigations, we transfer *Microlejeunea aphanella* to *Vitalianthus*, reinstate the genus *Allorgella*, transfer *Vitalianthus* from Drepanolejeuneinae to Lepidolejeuneinae, identify

*Metalejeunea* as another element of Lepidolejeuneinae, and place *Allorgella* and *Haplolejeunea* in Echinolejeuneinae. We map selected morphological characters on the reconstructed phylogenetic hypothesis to demonstrate the importance of subgynoecial innovations and ocelli for the circumscription of genera and subtribes of Lejeuneaceae.

**Keywords** *Haplolejeunea* · Lejeuneaceae · Liverwort · *Metalejeunea* · Molecular phylogeny · *Oiolejeunea*

### Introduction

Porellales are a mainly epiphytic lineage of leafy liverworts that is characterized by complicate bilobed or trilobed, incubously inserted leaves, fasciculate rhizoids, endosporous protonemata as well as a lack of ventral branching and mycorrhiza-like mutualisms (Heinrichs et al. 2005). Their most species-rich family are the Lejeuneaceae that include more than 1000 species (Frey and Stech 2009; He and Zhu 2011; Heinrichs et al. 2015) in two subfamilies, Ptychanthoideae with undivided underleaves, ventral merophytes 4–20 cells wide and 6–20 functional elaters per capsule valve; and Lejeuneoideae with ventral merophytes 1–4(–16) cells wide, bifid, undivided or absent underleaves and 5–6 functional elaters per valve (Gradstein 2013). Lejeuneoideae contain the vast majority of Lejeuneaceae species (Wilson et al. 2007a, b). Representatives of this lineage are abundant in humid tropical forests where they grow not only on trunks, branches and twigs but also as epiphylls on living leaves (Lücking 1995; Pócs 1996; Gradstein 1997; Cornelissen and Ter Steege 1989; Sonnleitner et al. 2009).

Lejeuneaceae and other Porellales lineages exhibit an increase in lineage accumulation during the late Mesozoic and Cenozoic and thus likely show a pronounced response

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to the ecological opportunities provided by humid, angiosperm-dominated megathermal forests (Feldberg et al. 2014). Schuster (2001) assumed that a rapid adaptation to new niches in these forests led to enormous morphological homoplasy in Lejeuneoideae. Morphology-based phylogenetic data matrices are indeed of limited information content and more than half of the investigated characters proved to be homoplastic (Gradstein et al. 2003). The high homoplasy content and the lack of stable morphological diagnostic characters hamper the delimitation of Lejeuneaceae genera and the reconstruction of their relationships (Sukkharak et al. 2011).

Gradstein (2013) summarized current knowledge on Lejeuneaceae phylogeny and provided a revised classification of the family that he divided into 68 genera of which he assigned 49 to the subfamily Lejeuneoideae. He split Lejeuneoideae into three tribes, Brachiolejeuneae, Symbiezidiaceae and Lejeuneae. Lejeuneae are characterized by thin stems with ventral merophytes usually less than four cells wide, predominance of bifid underleaves, a seta with only twelve outer rows of cells and a butterfly- to broadly quadrate-shaped group of differentiated capsule wall cells (Weis 2001; Wilson et al. 2007a; Gradstein 2013). They include 40 of the genera of Lejeuneoideae, among them the species-rich genera *Cololejeunea* (Spruce) Steph. (Yu et al. 2013) and *Lejeunea* Lib. (Lee 2013). Classification of genera of Lejeuneae is largely based on gametophytical characters. Gradstein (2013) accepted eight subtribes of Lejeuneae but abstained from assigning seven genera because they were not investigated in published phylogenetic studies. Since then, four further subtribes were added based on extensions of the molecular datasets of Lejeuneae (Heinrichs et al. 2014a, b; Schäfer-Verwimp et al. 2014) and several genus and subtribe concepts were changed (Yu et al. 2014; Ye et al. in press). In the present study, we include sequences of several taxa in a molecular dataset of Lejeuneae which have not yet been integrated in phylogenetic analyses using DNA sequence variation. As a result of our investigation, we assign these taxa to subtribes of Lejeuneae, demonstrate the polyphyly of *Otolejeunea* Grolle and Tixier, and transfer *Microlejeunea aphanella* (Spruce) Steph. to *Vitalianthus* R.M.Schust. and Giacotti.

## Materials and methods

### Taxa studied, DNA extraction, PCR amplification and sequencing

Gametophytical plant tissue was isolated from dried herbarium specimens of *Haplolejeunea cucullata* (Steph.) Grolle, *Haplolejeunea sticta* Grolle, *Metalejeunea*

*crassitexta* (J.B.Jack & Steph.) Pócs, *Metalejeunea cucullata* (Reinw., Blume & Nees) Grolle, *Microlejeunea aphanella*, *Otolejeunea moniliata* Grolle, *Otolejeunea semperiana* (Steph.) Grolle, *Rectolejeunea flagelliformis* A.Evans, *Vitalianthus bischlerianus* (K.C.Pôrto & Grolle) R.M.Schust. & Giacotti and *Xylolejeunea grolleana* (Pócs) Xiao L.He & Grolle (Table 1). Total genomic DNA was isolated using the Invisorb Spin Plant Mini Kit (Stratag Molecular GmbH, Berlin, Germany). Fifty-one sequences were newly generated for this study and 239 sequences were downloaded from Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>).

The *rbcL* gene was amplified using the PCR protocol in Gradstein et al. (2006) with the modification that the PCR was carried out with 0.2 µL MyTaq DNA Polymerase (Bioline Reagents Ltd., UK), 10 µL reaction buffer, 1 µL of upstream primer (*rbcL*-1PI-F; 10 µM), 1 µL of downstream primer (M1390-R; 10 µM) and 1 µL of template DNA. This mix was filled up with double-distilled water to a total volume of 50 µL. When products were not obtained in the initial PCR with the primers *rbcL*-1PI-F and M1390-R, a nested PCR was conducted by running the same program with 1 µL of the previous PCR and the primer pair *rbcL*-210-F and *rbcL*-1200-R. Amplification of the *trnL-trnF* region was carried out with 0.4 µL MyTaq DNA Polymerase, 11 µL reaction buffer, 1 µL of upstream primer (10 µM), 1 µL of downstream primer (10 µM), and 1 µL of template DNA. This mix was filled up to a total volume of 50 µL with double-distilled water. The primer pair *trnL*LejF and *trnL/trnF*-R was employed and the PCR program as described in Gradstein et al. (2006). When no product was obtained in the first PCR, a second PCR was conducted using the same protocol but with 1 µL of the previous PCR mix replacing the template DNA. For the nrITS1-5.8S-ITS2 region, the PCR primers and program of Hartmann et al. (2006) were used, with the same amount of reagents as described above for the *trnL-trnF* region. The nrITS1 and ITS2 regions were amplified separately. If this method failed, the nrITS1-5.8S-ITS2 region was amplified and a semi-nested PCR was carried out following the same protocol, yet amplifying the nrITS1 and ITS2 regions separately. The primer pair for the first PCR was Bryo18SF and Bryo26SR. For the semi-nested PCR, the primers Bryo18SF, Bryo5.8SR, Bryo5.8SF and Bryo26SR were used (Hartmann et al. 2006).

Bidirectional sequences were generated by an ABI 3730 48 capillary sequencing machine using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing primers were the same as used for the PCR. Newly generated sequences were assembled and edited with PhyDE v.0.9971 (<http://www.phyde.de/index.html>).

**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	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Acrolejeunea fertilis</i> (Reinw. et al.) Schiffn.	Bali, Schäfer-Verwimp and Verwimp 17009 (GOET)	AY684929	DQ987391	DQ987281
<i>Allorgella semperiana</i> (Steph.) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs	Australia, Streimann 57955 (EGR)	<b>KT626926</b>	<b>KT626942</b>	<b>KT626909</b>
<i>Allorgella semperiana</i>	Indonesia, Schäfer-Verwimp and Verwimp 24836/C (M)	<b>KT626927</b>	–	<b>KT626910</b>
<i>Anopolejeunea conferta</i> (C.F.W.Meissn. ex Spreng.) A.Evans	Ecuador (I), Schäfer-Verwimp and Nebel 33081/B (M)	KJ408336	KJ408359	KJ408310
<i>Anopolejeunea conferta</i>	Ecuador (II), Wilson et al. 04–08 (GOET)	DQ983653	DQ987438	DQ987335
<i>Anopolejeunea conferta</i>	Venezuela, Pócs et al. 9712/BA (M)	KJ408337	KJ408360	KJ408311
<i>Anopolejeunea conferta</i>	Costa Rica, Schäfer-Verwimp and Holz 154 (M)	KJ408335	KJ408358	KJ408309
<i>Archilejeunea fuscescens</i> (Hampe ex Lehm.) Fulford	Costa Rica, Bernecker 97–53 (GOET)	DQ983655	DQ987384	DQ987267
<i>Aureolejeunea quinquecarinata</i> R.M.Schust.	Ecuador, Schäfer-Verwimp and Preußing 23299/A (GOET)	DQ983658	DQ987450	DQ987350
<i>Bromeliophila natans</i> (Steph.) R.M.Schust.	Brazil, Gehrig s.n. (GOET)	KF039849	KF039879	KF039812
<i>Bryopteris diffusa</i> (Sw.) Nees	Bolivia, Acebey and Villavicencio 855 (GOET)	AY548085	AM237147	AM237095
<i>Ceratolejeunea cubensis</i> (Mont.) Schiffn.	Guadeloupe, Schäfer-Verwimp and Verwimp 22279/B (M)	KF606946	KF606941	KF606936
<i>Ceratolejeunea laetefusca</i> (Austin) R.M.Schust.	Brazil, Schäfer-Verwimp 33995 (M)	KF606947	KF606942	KF606937
<i>Cheilolejeunea asperima</i> (Steph.) Grolle	Costa Rica, van Melick 214551 (M)	KJ408339	KJ408362	KJ408313
<i>Cheilolejeunea choachina</i> (Gottsche) Gradst.	Ecuador, Schäfer-Verwimp and Nebel 33152/A (M)	KJ408341	KJ408364	KJ408315
<i>Cheilolejeunea clausa</i> (Nees & Mont.) Steph.	Brazil, Teixeira 39 (M)	KJ716751	KJ716776	KJ716763
<i>Cheilolejeunea clypeata</i> (Schwein.) W.Ye & R.L.Zhu	USA, Davis s.n. (GOET)	DQ983699	DQ987426	DQ987322
<i>Cheilolejeunea discoidea</i> (Lehm. & Lindenb.) R.M.Schust. & Kachroo	Panama, Schäfer-Verwimp and Verwimp 34530 (M)	KJ408343	KJ408366	KJ408317
<i>Cheilolejeunea insecta</i> Grolle & Gradst.	Brazil, Schäfer-Verwimp and Verwimp 14725 (M)	KJ716749	KJ716774	KJ716761
<i>Cheilolejeunea holostipa</i> (Spruce) Grolle & R.L.Zhu	Panama, Schäfer-Verwimp and Verwimp 34526 (M)	KJ408351	KJ408375	KJ408326
<i>Cheilolejeunea lindenberghii</i> (Gottsche) Mizut.	Malaysia, Pócs and Pócs 1006/AG (M)	KJ408344	KJ408367	KJ408318
<i>Cheilolejeunea mimosa</i> (Hook.f. & Taylor) R.M.Schust.	New Zealand, Schäfer-Verwimp and Verwimp 13745 (M)	KJ408345	KJ408368	KJ408319
<i>Cheilolejeunea norisiae</i> G.Dauphin & Gradst.	Panama, Schäfer-Verwimp and Verwimp 30821 (M)	KJ408346	KJ408369	KJ408320
<i>Cheilolejeunea oncophylla</i> (Ångstr.) Grolle & M.E.Reiner	Brazil, Peralta 11914 (M)	KJ408347	KJ408370	KJ408321
<i>Cheilolejeunea revoluta</i> (Herzog) Gradst. & Grolle	Brazil, Peralta 15116 (M)	KJ408349	KJ408372	KJ408323
<i>Cheilolejeunea rigidula</i> (Mont.) R.M.Schust.	Suriname, Muñoz 98–62 (GOET)	DQ983668	DQ987453	DQ987353
<i>Cheilolejeunea serpentina</i> (Mitt.) Mizut.	São Tomé and Príncipe, Shevock 39658 (M)	KJ716753	KJ716778	KJ716765
<i>Cheilolejeunea uncioloba</i> (Lindenb.) Malombe	Brazil, Yano 32383 (M)	KJ716755	KJ716780	KJ716767
<i>Cololejeunea cardiocarpa</i> (Mont.) A.Evans	Brazil, Borhidi and Pereira BB25 (GOET)	JQ991163	JQ991278	JQ991048
<i>Cololejeunea inflectens</i> (Mitt.) Benedix	Malaysia, Schäfer-Verwimp and Verwimp 18861/A (GOET, as <i>C. peculiaris</i> )	AY548095	DQ238572	DQ987280

Table 1 continued

Taxon	Voucher	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Colura cylindrica</i> Herzog	Guadeloupe, Schäfer-Verwimp and Verwimp 22154/B (JE)	JX470969	JX470980	JX470992
<i>Colura irrorata</i> (Spruce) Heinrichs, Y. Yu, Schäf.-Verw. & Pócs	Ecuador, Gradstein et al. 10033 (GOET)	AY548073	DQ238584	DQ987279
<i>Cyclolejeunea chitonia</i> (Taylor) A.Evans	French Guiana, Holz FG-00-124 (GOET)	KF039844	KF039875	KF039806
<i>Cyclolejeunea luteola</i> (Spruce) Grolle	Dominica, Schäfer-Verwimp and Verwimp 17866 (M)	KF039823	KF039857	KF039785
<i>Cystolejeunea lineata</i> (Lehm. & Lindenb.) A.Evans	Guadeloupe, Schäfer-Verwimp and Verwimp 22183 (GOET)	AY548092	DQ987401	DQ987295
<i>Diplasiolejeunea cavifolia</i> Steph.	Malaysia, Schäfer-Verwimp and Verwimp 19036/A (GOET)	JQ729531	JQ729641	JQ729418
<i>Diplasiolejeunea paucertii</i> Steph.	Panama, De Gracia et al. 338 (GOET)	JQ729541	JQ729652	JQ729429
<i>Drepanolejeunea anoplantha</i> (Spruce) Steph.	Dominican Republic, Schäfer-Verwimp and Verwimp 27059 (JE)	KC313120	KC313159	KC313080
<i>Drepanolejeunea granatensis</i> (J.B.Jack & Steph.) Bischl.	Ecuador, Schäfer-Verwimp et al. 24383/B (JE)	KC313121	KC313160	KC313081
<i>Drepanolejeunea navicularis</i> Steph.	Ecuador, Schäfer-Verwimp and Nebel 31772/A (JE)	KC313124	KC313163	KC313085
<i>Echinolejeunea papillata</i> (Mitt.) R.M.Schust. ex Hamlin	New Zealand, Schäfer-Verwimp and Verwimp 14195/A (JE)	KC313135	KC313175	KC313097
<i>Frullanoides corticalis</i> (Lehm. & Lindenb.) van Slageren	French Guiana, Hartmann 04–080 (GOET)	DQ983681	AM237196	AM237143
<i>Fulfordianthus evansii</i> (Fulford) Gradst.	Costa Rica, Dauphin s.n. (GOET)	DQ983683	AM237197	AM237144
<i>Haplolejeunea cucullata</i> (Steph.) Grolle	Brazil (I), Peralta et al. 16390 (SP)	<b>KT626925</b>	<b>KT626939</b>	<b>KT626906</b>
<i>Haplolejeunea cucullata</i>	Brazil (II), Schäfer-Verwimp and Verwimp 14560 (M)	–	<b>KT626940</b>	<b>KT626907</b>
<i>Haplolejeunea stricta</i> Grolle	Reunion Island, Kis and Gyarmati 9604/G (EGR)	–	<b>KT626941</b>	<b>KT626908</b>
<i>Harpalejeunea grandistipula</i> R.M.Schust.	Ecuador, Schäfer-Verwimp et al. 24163/B (JE)	KC313145	KC313185	KC313107
<i>Harpalejeunea marginalis</i> (Hook.f. & Taylor) Steph.	Chile, Schäfer-Verwimp and Verwimp 8082 (JE)	KC313147	KC313187	KC313109
<i>Harpalejeunea molleri</i> (Steph.) Grolle	Portugal, Schäfer-Verwimp and Verwimp 29334 (GOET)	KC313148	KC313188	KC313110
<i>Leiolejeunea grandiflora</i> A.Evans	Jamaica (I), Schäfer-Verwimp 35394 (M)	KJ716757	KJ716782	KJ716769
<i>Leiolejeunea grandiflora</i>	Jamaica (II), Schäfer-Verwimp 35422 (M)	KJ716756	KJ716781	KJ716768
<i>Lejeunea cavifolia</i> (Ehrh.) Lindb.	Germany, Heinrichs 3695 (GOET)	AY548102	DQ238581	DQ987259
<i>Lejeunea flava</i> (Sw.) Nees	Brazil, Gradstein s.n. (GOET)	DQ983692	DQ987413	DQ987309
<i>Lejeunea mimula</i> Hürl.	Indonesia, Schäfer-Verwimp 20930 (GOET)	AY548104	DQ238580	DQ987261
<i>Lejeunea pterigonia</i> (Lehm. & Lindenb.) Mont.	Ecuador, Nöske 164 (GOET)	KF5 56549	KF556328	KF556081
<i>Lepidolejeunea cuspidata</i> (Gottsche) Heinrichs & Schäf.-Verw.	Guadeloupe, Schäfer-Verwimp and Verwimp 22193 (M)	KP635323	KP635348	KP635295
<i>Lepidolejeunea delessertii</i> (Nees & Mont.) Grolle	Réunion, Schäfer-Verwimp and Verwimp 20355/B (M)	KF039819	KF039853	KF039781
<i>Lepidolejeunea integrispula</i> (J.B.Jack & Steph.) R.M.Schust.	Fiji, Pócs 03307/AC (GOET)	DQ983697	DQ987417	DQ987313
<i>Lepidolejeunea involuta</i> (Gottsche) Grolle	Dominica, Schäfer-Verwimp and Verwimp 17855 (JE)	KP635314	KP635339	KP635285
<i>Leptolejeunea convexistipa</i> Bischl.	Panama, Schäfer-Verwimp and Verwimp 30861(JE)	KF954161	KF954151	KF954154
<i>Leptolejeunea vitrea</i> (Nees) Schiffn.	Malaysia, Dürhammer D148 (JE)	KF954164	KF954152	KF954157
<i>Lopholejeunea eulopha</i> (Taylor) Schiffn.	Australia, Pócs and Streimann 9987/H1 (GOET)	AY548067	DQ987381	DQ987262
<i>Luteolejeunea herzogii</i> (Buchloh) Piippo	Costa Rica, Schäfer-Verwimp and Holz 0294/B (GOET)	DQ983706	DQ987467	DQ987368



Table 1 continued

Taxon	Voucher	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Marchesinia robusta</i> (Mitt.) Schiffn.	Ecuador, Wilson et al. 04–05 (GOET)	DQ983710	DQ987436	DQ987332
<i>Mastigolejeunea auriculata</i> (Wilson & W.J.Hooker) Schiffn.	Bolivia, Churchill 21275 (GOET)	AY548070	DQ987385	DQ987268
<i>Metalejeunea crassitexta</i> (J.B.Jack & Steph.) Pócs	Fiji (I), Pócs and Pócs 03303/R (EGR)	<b>KT626918</b>	<b>KT626934</b>	<b>KT626899</b>
<i>Metalejeunea crassitexta</i>	Fiji (II), Pócs and Pócs 03303/AS (EGR)	<b>KT626917</b>	<b>KT626933</b>	<b>KT626898</b>
<i>Metalejeunea cucullata</i> (Reinw., Blume & Nees) Grolle	Bali (I), Schäfer-Verwimp and Verwimp 16631/B (JE)	<b>KT626919</b>	<b>KT626935</b>	–
<i>Metalejeunea cucullata</i>	Bali (II), Schäfer-Verwimp and Verwimp 16727/B (JE)	<b>KT626915</b>	<b>KT626931</b>	<b>KT626896</b>
<i>Metalejeunea cucullata</i>	Fiji (I), Pócs and Pócs 03307/N (EGR)	<b>KT626912</b>	–	<b>KT626893</b>
<i>Metalejeunea cucullata</i>	Fiji (II), Pócs and Pócs 03305/Q (EGR)	<b>KT626913</b>	<b>KT626929</b>	<b>KT626894</b>
<i>Metalejeunea cucullata</i>	Fiji (III), Pócs 1163/AR (EGR)	<b>KT626916</b>	<b>KT626932</b>	<b>KT626897</b>
<i>Metalejeunea cucullata</i>	Thailand, Schäfer-Verwimp and Verwimp 16267 (JE)	<b>KT626914</b>	<b>KT626930</b>	<b>KT626895</b>
<i>Microlejeunea africana</i> Steph.	São Tomé and Príncipe, Shevock 34576 A (GOET)	KC313150	KC313190	KC313112
<i>Microlejeunea capillaris</i> (Gottsche) Steph.	Costa Rica, Schäfer-Verwimp and Holz SV/H-0489/B (JE)	KC313152	KC313192	KC313114
<i>Microlejeunea squarrosa</i> (Steph.) Heinrichs, Schäf.-Verw., Pócs & S.Dong	Brazil, Schäfer-Verwimp 13376 (GOET)	DQ983720	DQ987446	DQ987344
<i>Microlejeunea ulicina</i> (Taylor) Steph.	Canary Islands, Schäfer-Verwimp and Verwimp 24666 (GOET)	KC313155	KC313195	KC313116
<i>Myriocoleopsis gymnocolea</i> (Steph.) M.E.Reiner & Gradst.	Ecuador, Gradstein et al. 10020 (GOET)	DQ238568	DQ238583	DQ987277
<i>Neurolejeunea breutelii</i> (Gottsche) A.Evans	Brazil, Schäfer-Verwimp 14740 (GOET)	DQ983714	DQ987405	EF011779
<i>Omphalanthus filiformis</i> Nees	Ecuador, Schäfer-Verwimp and Preußing 23543 (GOET)	DQ983716	DQ987393	DQ987283
<i>Otigoniolejeunea portoricensis</i> (Hampe & Gottsche) Y.M.Weï, R.L.Zhu & Gradst.	Guadeloupe, Schäfer-Verwimp and Verwimp 22615, as <i>Physantholejeunea</i> (M)	DQ983719	DQ987448	DQ987346
<i>Otolejeunea moniliata</i> Grolle	Madagascar (I), Pócs 9448/L (EGR)	<b>KT626923</b>	<b>KT626937</b>	<b>KT626904</b>
<i>Otolejeunea moniliata</i>	Madagascar (II), Pócs et al. 90113/EA (EGR)	<b>KT626922</b>	<b>KT626936</b>	<b>KT626903</b>
<i>Otolejeunea moniliata</i>	Madagascar (III), Pócs et al. 9472/AD (M)	KJ408352	KJ408376	KJ408327
<i>Prionolejeunea limpida</i> Herzog	Brazil, Schäfer-Verwimp and Verwimp 13291 (M)	KF039850	DQ207928	DQ207895
<i>Ptychanthus striatus</i> (Lehm. & Lindenb.) Nees	Java, Gradstein 10215 (GOET)	DQ983721	DQ987406	DQ987300
<i>Pycnolejeunea densistipula</i> (Lehm. & Lindenb.) Steph.	Ecuador, Schäfer-Verwimp and Preussing 23368 (M)	KJ408353	KJ408377	KJ408328
<i>Pycnolejeunea macroloba</i> (Nees & Mont.) Schiffn.	Brazil, Yano 32740 (M)	KJ408354	KJ408378	KJ408329
<i>Rectolejeunea versifolia</i> (Schiffn.) L.Söderstr. & A.Hagborg	Guadeloupe, Schäfer-Verwimp and Verwimp 22245/A, as <i>R. berteroaana</i> (GOET)	DQ983724	DQ987444	DQ987342
<i>Rectolejeunea flagelliformis</i> A.Evans	Panama, Schäfer-Verwimp and Verwimp 34286 (JE)	<b>KT626924</b>	<b>KT626938</b>	<b>KT626905</b>
<i>Rectolejeunea truncatilobula</i> C.J.B.Bastos	Brazil, Peralta 8083 (M)	–	KJ408380	KJ408331
<i>Schiffneriolejeunea nymannii</i> (Steph.) Gradst. & Terken	Malaysia, Gradstein et al. 10321 (GOET)	DQ983725	DQ987424	DQ987320
<i>Siphonolejeunea elegantissima</i> (Steph.) Grolle	Australia, Pócs and Brown 0026/AA (E)	DQ983726	DQ987452	DQ987352
<i>Spruceanthus thozetianus</i> (Gottsche & F.v.Müll.) B.M.Thiers	Australia, Pócs 01107/M (GOET)	AM384877	DQ987460	DQ987362

**Table 1** continued

Taxon	Voucher	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Thysananthus spathulistipus</i> (Reinw. et al.) Lindenb.	Bali, Schäfer-Verwimp and Verwimp 20790 (GOET)	DQ983739	DQ987392	DQ987282
<i>Vitalianthus aphanellus</i> (Spruce) Bechteler, G.E.Lee, Schäfer-Verw. & Heinrichs	Brazil, Yano and Zartman 32771 (SP)	<b>KT626920</b>	–	<b>KT626900</b>
<i>Vitalianthus bischlerianus</i> (K.C.Pôrto & Grolle) R.M.Schust. & Giancotti	Brazil (I), Schäfer-Verwimp and Verwimp 9505 (M)	<b>KT626921</b>	–	<b>KT626901</b>
<i>Vitalianthus bischlerianus</i>	Brazil (II), Schäfer-Verwimp and Verwimp 12913 (M)	–	–	<b>KT626902</b>
<i>Xylolejeunea crenata</i> (Mont.) Xiao L.He & Grolle	Ecuador, Schäfer-Verwimp and Nebel 32827/A (M)	KJ408356	KJ408382	KJ408333
<i>Xylolejeunea grolleana</i> (Pócs) Xiao L.He & Grolle	Madagascar, Pócs and Szabó 9878/EM (EGR)	<b>KT626911</b>	<b>KT626928</b>	<b>KT626892</b>

New sequences in bold face

### Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall 1999). Ambiguous positions were excluded and lacking parts of sequences marked as missing (Online Resource 1). Thirteen representatives of Brachiolejeuneae and Ptychanthoideae were chosen as outgroup according to the topologies presented in Wilson et al. (2007a). The ingroup consisted of 88 accessions of Lejeuneae.

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, 100 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 as a strict consensus tree. Non-parametric bootstrapping values (Felsenstein 1985) were generated as heuristic searches with 500 replicates. Rearrangements were restricted to ten millions per replicate. Bootstrap percentage values (BPV)  $\geq 70\%$  were regarded as good support (Hillis and Bull 1993). The individual marker sets and the combined chloroplast DNA dataset vs nrITS dataset were first analyzed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes. Conflicting nodes with BPV of each  $>70$  were taken as evidence for incongruent phylogenetic signals (Mason-Gamer and Kellogg 1996). The trees gave no evidence of incongruence. Hence the datasets were combined.

Maximum likelihood (ML) analyses of the combined datasets were conducted with RAxML version 8 (Stamatakis 2006, 2014). jModelTest version 2 (Darriba et al.

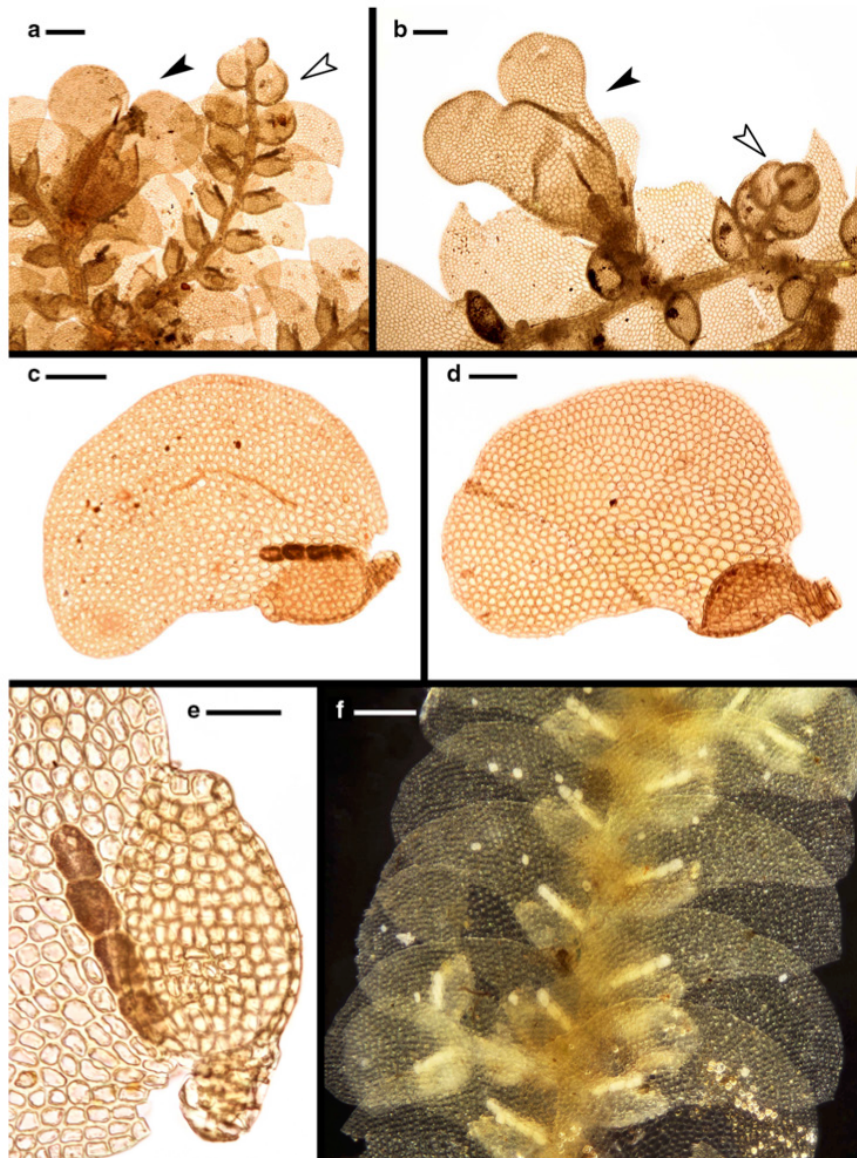
2012) was used to determine the appropriate DNA substitution model, rate of invariable sites and gamma rate heterogeneity according to the Akaike information criterion (AIC; Akaike 1973). The analyses resulted in a GTR+G+I model for the chloroplast as well as the nuclear data partition and an ML analysis was conducted with a chloroplast and a nuclear partition. Trees were generated by selecting 10 independent runs and the multiparametric bootstrap option autoMRE resulting in 250 bootstrap replicates. ML bootstrap values (BV) of each node were visualized using FigTree 1.4 (<http://beast.bio.ed.ac.uk/figtree>).

### Morphological investigation

Specimens were studied using a Leica M50 dissection microscope and Carl Zeiss AxioScope A1 compound microscope, the latter equipped with a Canon 60D digital camera. Incident and transmitted light were used simultaneously or separately. The illustrations accompanying our study (Fig. 1) represent digitally stacked photomicrographic composites of moistened plants obtained with the software package HeliconFocus 6.0. Drawings (Fig. 2) were produced using a Leica DM1000 microscope equipped with a drawing tube.

### Distribution of morphological character states

We scored several morphological characters (Table 2) that were considered to be relevant for the taxonomy of Lejeuneae. The evolution of these characters was reconstructed by plotting them on the strict consensus tree obtained from the most parsimonious trees recovered. Investigated characters include gynoecial innovations that may be lejeuneoid (i.e., with a lateral leaf as basalmost

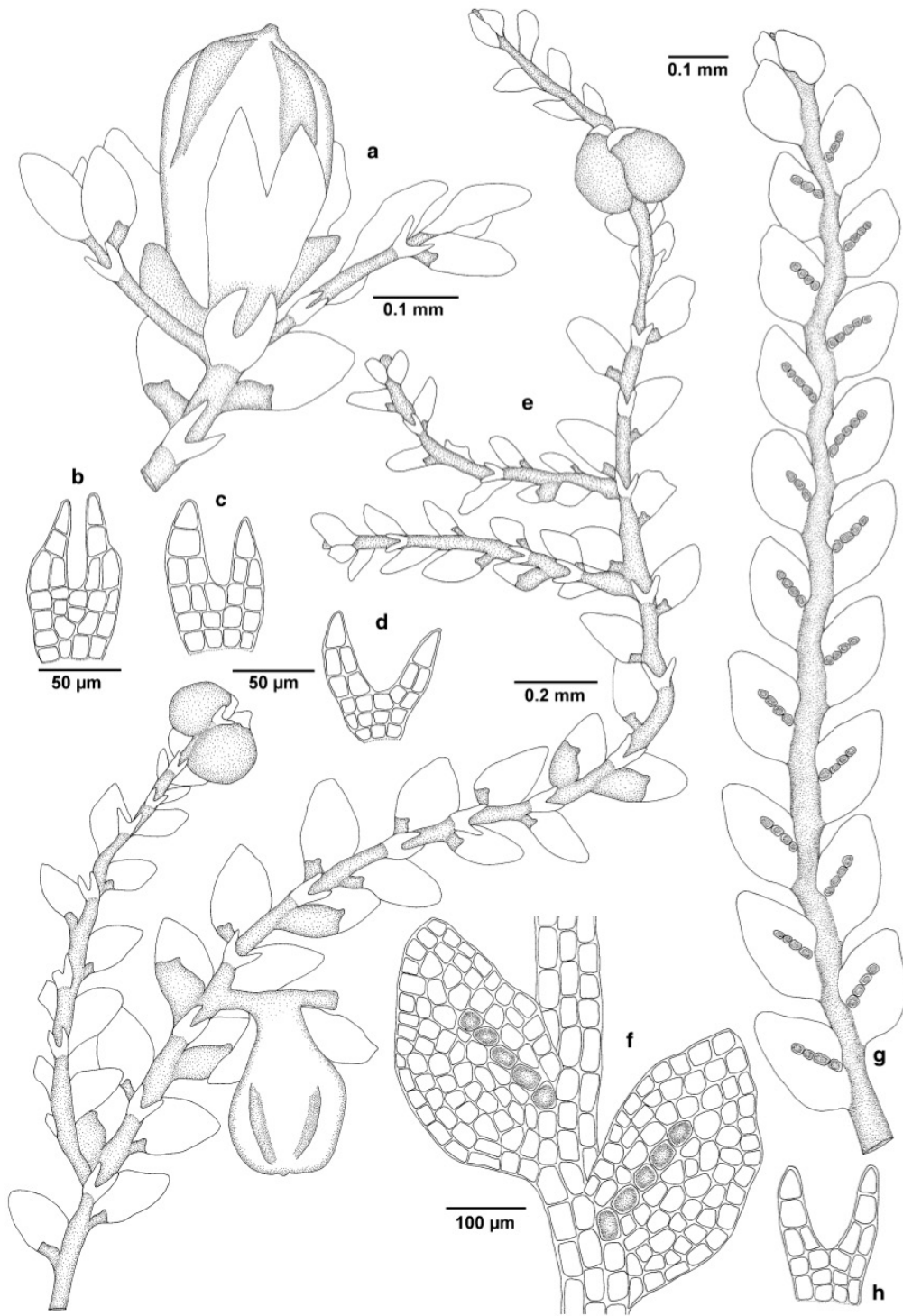


**Fig. 1** Photomicrographs of *Otolejeunea moniliata* (a, c, e, f) and *Allorgella semperiana* (b, d). a, b Autoicous shoots in ventral view. Black arrowheads point to perianths, and white arrowheads to androecia. c, d Leaves in ventral view. The leaf in c with a moniliate row of four ocelli. e Lowermost portion of leaf in ventral view showing lobule and lobe base with a moniliate row of ocelli. The free

margin of the lobule covers at least parts of the lowermost three ocelli. f Part of shoot in dorsal view showing each one basal row of ocelli per leaf lobe [a, c, e from Pócs et al. 901131/EA(EGR); b, d from Schäfer-Verwimp and Verwimp 24836/C (M), f from Pócs 9448/L (EGR)]

leafy appendage) or pycnolejeuneoid (i.e., with an underleaf as basalmost appendage) (Gradstein et al. 2003). Both types cannot be discriminated in genera lacking underleaves (*Cololejeunea*, *Myriocoleopsis* Schiffn.) or having one underleaf per leaf rather than per leaf pair [*Diplasiolejeunea* (Spruce) Schiffn., *Colura* (Dumort.)

Dumort.]. We also coded sex distribution, absence or presence of ocelli [modified cells containing a single, large oil body (He and Piippo 1999)], shape of underleaves and the orientation of the outer leaf lobule tooth. Character states were determined based on data from the literature and investigation of herbarium material.



**Fig. 2** *Vitalianthus aphanellus*. **a** Part of shoot in ventral view with perianth and two pycnolejeuneoid subgynoecial innovations. **b–d**, **h** Underleaves. **e** autoicous gametophyte in ventral view with one perianth and two androecia. **f** part of shoot in dorsal view with two leaves, each with a five-cell long moniliate row of ocelli (*dotted cells*). **g** top of shoot in dorsal view, ocelli indicated [**a–e** drawn from isolectotype (M), **f–h** drawn from DNA voucher]

**Table 2** Morphological character states of the investigated taxa as mapped onto the MP topology (Fig. 3)

<b>1. subgynoecial innovation</b>	<ul style="list-style-type: none"> <li><span style="color: red;">■</span> pycnolejeuneoid</li> <li><span style="color: grey;">■</span> lejeuneoid</li> <li><span style="color: yellow;">■</span> present, type unknown</li> <li><span style="color: blue;">■</span> absent</li> </ul>
<b>2. ocelli</b>	<ul style="list-style-type: none"> <li><span style="color: red;">■</span> present</li> <li><span style="color: grey;">■</span> absent</li> </ul>
<b>3. sex distribution</b>	<ul style="list-style-type: none"> <li><span style="color: red;">■</span> autoicous</li> <li><span style="color: grey;">■</span> dioicous</li> </ul>
<b>4. outer lobule tooth</b>	<ul style="list-style-type: none"> <li><span style="color: red;">■</span> pointing outwards</li> <li><span style="color: grey;">■</span> pointing forward</li> <li><span style="color: yellow;">■</span> obsolete or lacking</li> </ul>
<b>5. underleaves</b>	<ul style="list-style-type: none"> <li><span style="color: red;">■</span> one underleaf per leaf pair</li> <li><span style="color: grey;">■</span> one underleaf per leaf</li> <li><span style="color: yellow;">■</span> absent</li> </ul>

## Results

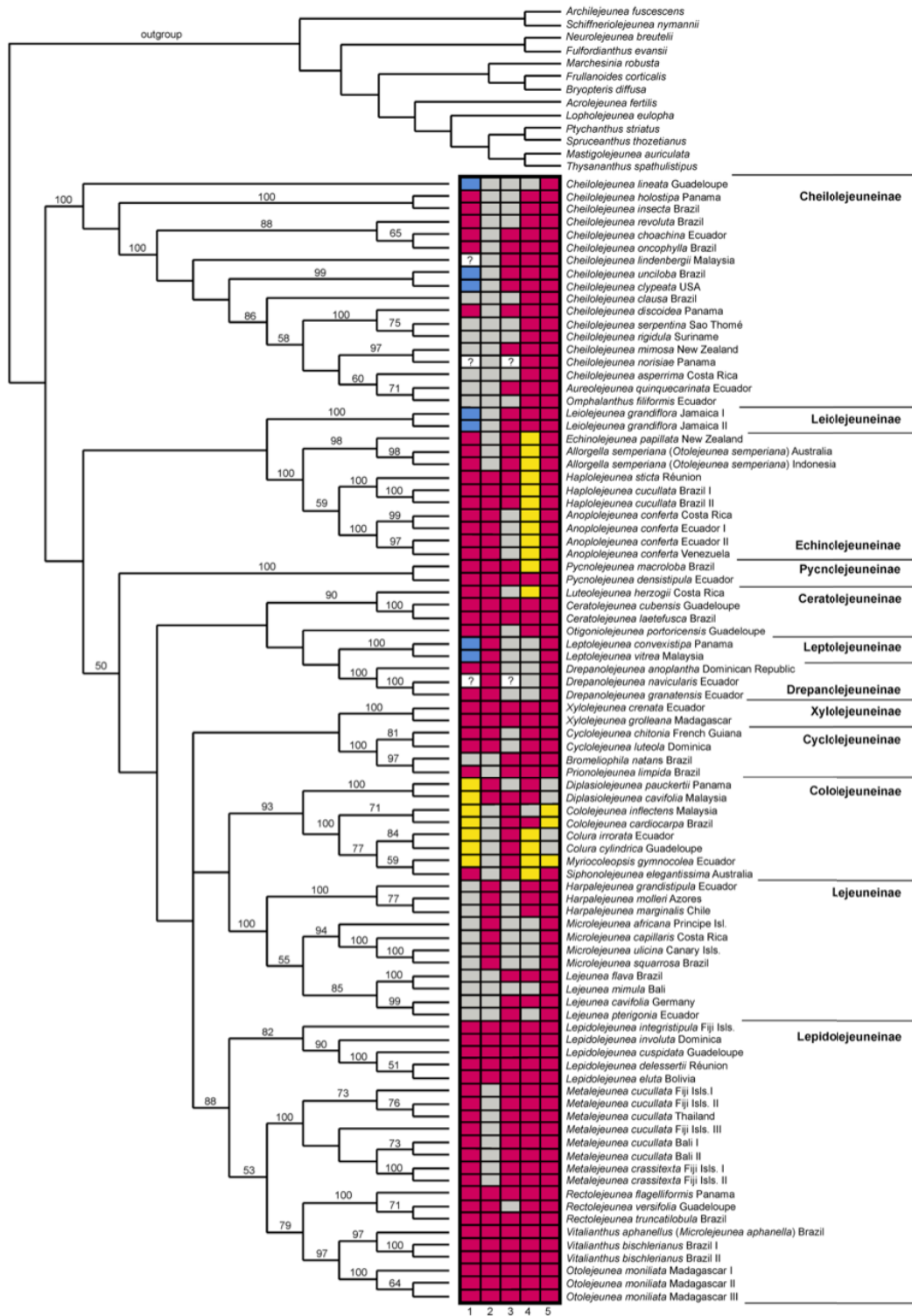
Of a total of 2059 character sites, 730 were parsimony informative, 219 variable, but parsimony uninformative and 1110 constant. The MP analysis resulted in two equally parsimonious trees with a length of 5142 steps, a consistency index (CI) of 0.31 and a retention index (RI) of 0.63. The strict consensus tree is depicted in Fig. 3. Clades assigned to subtribes achieve BPVs of 88–100 yet their sister relationships do not get BPVs above 50. *Otigoniolejeunea* (Spruce) Schiffn. forms a sister relationship with *Leptolejeunea* (Spruce) Steph. albeit without support. Cheilolejeuneinae are placed in an unsupported sister relationship with the remainder of Lejeuneaceae. *Haplolejeunea* Grolle nests in Echinolejeuneinae; *Metalejeunea* Grolle and *Vitalianthus* in Lepidolejeuneinae. *Microlejeunea aphanella* is placed sister to *Vitalianthus bischlerianus* whereas the other investigated *Microlejeunea* (Spruce) Steph. species form a subclade of Lejeuneinae. *Microlejeunea aphanella* is autoicous, has pycnolejeuneoid innovations and leaves with moniliate rows of ocelli. The other investigated *Microlejeunea* elements are dioicous, have lejeuneoid innovations and lack moniliate ocelli (Fig. 3). Based on the molecular phylogeny and shared morphological character states, *Microlejeunea aphanella* is

transferred to *Vitalianthus* (see below). *Otolejeunea* (subg. *Otolejeunea*) *moniliata* and *Vitalianthus* form a subclade of Lepidolejeuneinae whereas *Otolejeunea* [subg. *Allorgella* (Tixier) Grolle] *semperiana* nests in Echinolejeuneinae. We thus reinstate the genus *Allorgella* Tixier (see below). The ML analysis (Fig. 4) recovered the same subtribal clades than found in the MP analysis, but *Otigoniolejeunea* is placed in Ceratolejeuneinae (BPV = 55). The other subtribal clades have BPVs of 98–100. Echinolejeuneinae are placed sister to Cheilolejeuneinae plus Leiolejeuneinae with a BPV of 77. A clade with a BPV of 86 includes all other subtribes of Lejeuneaceae, with Pycnolejeuneinae separated from the rest of this clade with a BPV of 85.

## Discussion

### *Otolejeunea* and *Microlejeunea*

The epiphyllous genera *Otolejeunea* and *Allorgella* were simultaneously described in a publication of Tixier (1980). They share autoicy (Fig. 1a, b), subgynoecial innovations of the pycnolejeuneoid type, and the presence of obcordate perianths with two lateral keels that are apically expanded into auricles (Fig. 1a, b) yet differ by the presence of a linear, “moniliate” row of ocelli in the leaves of *Otolejeunea* (Fig. 1c, e, f) and a total absence of ocelli in *Allorgella* (Grolle 1985; Grolle and Reiner-Drehwald 2000; Pócs 2004) (Fig. 1d). Grolle (1985) lowered *Allorgella* to a subgenus of *Otolejeunea*; however, the expanded genus is polyphyletic (Figs. 3, 4). We thus reject the concept of Grolle (1983, 1985) and reinstate *Allorgella* which differs from *Otolejeunea* not only by the absence of ocelli but also by its tendency to have reduced leaf lobules, in contrast to the well-developed lobules of *Otolejeunea moniliata* (Grolle 1985). Furthermore, the former genus is characterized by irregular, “*Allorgella*-type” marginal leaf denticulations (Tixier 1980; Zhu and So 1997) that are lacking in *Otolejeunea moniliata*. The presence of plants with obcordate, auriculate perianths in two different main lineages of Lejeuneaceae is somewhat unexpected; however, this type of perianth occurs also in *Cyclolejeunea* A.Evans and *Prionolejeunea* (Spruce) Schiffn. of Cyclolejeuneinae. We abstain from a transfer of *Otolejeunea* (subg. *Phoxolejeunea* Grolle) *streimannii* Grolle to *Allorgella* since this species has perianth keels with triangular rather than auriculate protrusions and may represent an independent lineage (Grolle 1985). *Otolejeunea philippinensis* was placed in subg. *Phoxolejeunea* by Zhu and So (1998) but transferred to subg. *Allorgella* by Grolle and Reiner-Drehwald (2000). In the near future, an extension of the molecular sampling of *Allorgella* would be desirable, given



◀ **Fig. 3** Rooted strict consensus of two most parsimonious trees recovered during heuristic searches of the nrITS-trnL-trnF-rbcL dataset. Bootstrap percentage values >50 are indicated at branches. Morphological character states (Table 2) are mapped onto the tree

access to further *Allorgella* specimens suitable for DNA extraction.

Our results corroborate the importance of ocelli for the classification of Lejeuneaceae (Schuster 1992; He and Piippo 1999; Dong et al. 2013; Heinrichs et al. 2015). The example *Microlejeunea aphanella* demonstrates that not only the presence/absence of ocelli is of taxonomical importance but also their position and arrangement. He and Piippo (1999) recognized different ocelli arrangements in the leaves of Lejeuneaceae and distinguished four types of ocelli arrangements, basal, suprabasal, linear and scattered. Basal ocelli are typical for *Microlejeunea* (Gradstein et al. 2001) whereas *Microlejeunea aphanella* has a linear “moniliate” row of ocelli (Fig. 2f, g). These ocelli were not only visible in our DNA voucher but also still present in some leaves of the more than 150 years old isoelectotype specimen. This material has pycnolejeuneoid innovations (Fig. 2a) that are completely absent in Lejeuneinae but regularly present in Lepidolejeuneinae (Fig. 3). Furthermore, *M. aphanella* is autoicous (Fig. 2e), whereas other species of *Microlejeunea* are dioicous. *Microlejeunea* leaf lobules are usually very large (Bischler et al. 1962, 1963; Miller et al. 1967; Lee et al. 2015), in contrast to the rather small lobules seen in *M. aphanella*. The morphological differences between *M. aphanella* and other *Microlejeunea* elements are reflected in their different phylogenetic position (Figs. 3, 4). *Microlejeunea* is an element of Lejeuneinae whereas *Microlejeunea aphanella* is placed in the Lepidolejeuneinae, in a well-supported sister relationship with *Vitalianthus bischlerianus* (Pôrto and Grolle 1987; Schuster 1994). *Microlejeunea aphanella* and *V. bischlerianus* are not only closely related in molecular phylogenies, but also morphologically similar. Both species share the pycnolejeuneoid innovation type, 4–5 keeled perianths, autoicy, a moniliate arrangement of ocelli, narrowly bifid underleaves, oblique to rather wide spreading leaf lobes, and reduced to rather large leaf lobules with an outwards directed outer tooth. Based on molecular and morphological evidence, we transfer *M. aphanella* to *Vitalianthus*. *Vitalianthus aphanellus* differs from *V. bischlerianus* by its rather remotely inserted leaves (Fig. 2e, g), a curved rather than  $\pm$  straight ventral leaf margin and unwinged rather than winged male bracts (Pôrto and Grolle 1987; Schuster and Giancotti 1993; own observations). The two *Vitalianthus* species form a clade which is placed sister to *Otolejeunea moniliata* (Figs. 3, 4). *Vitalianthus* and *Otolejeunea* share the presence of moniliate ocelli, autoicy and pycnolejeuneoid innovations; however, *Otolejeunea*

has two-keeled perianths with auricles whereas the perianths of *Vitalianthus* are 4–5 keeled and lack auricles. We thus prefer to retain both genera, despite a trend towards wider genus concepts (Humphreys and Linder 2009).

### Refinements of subtribes

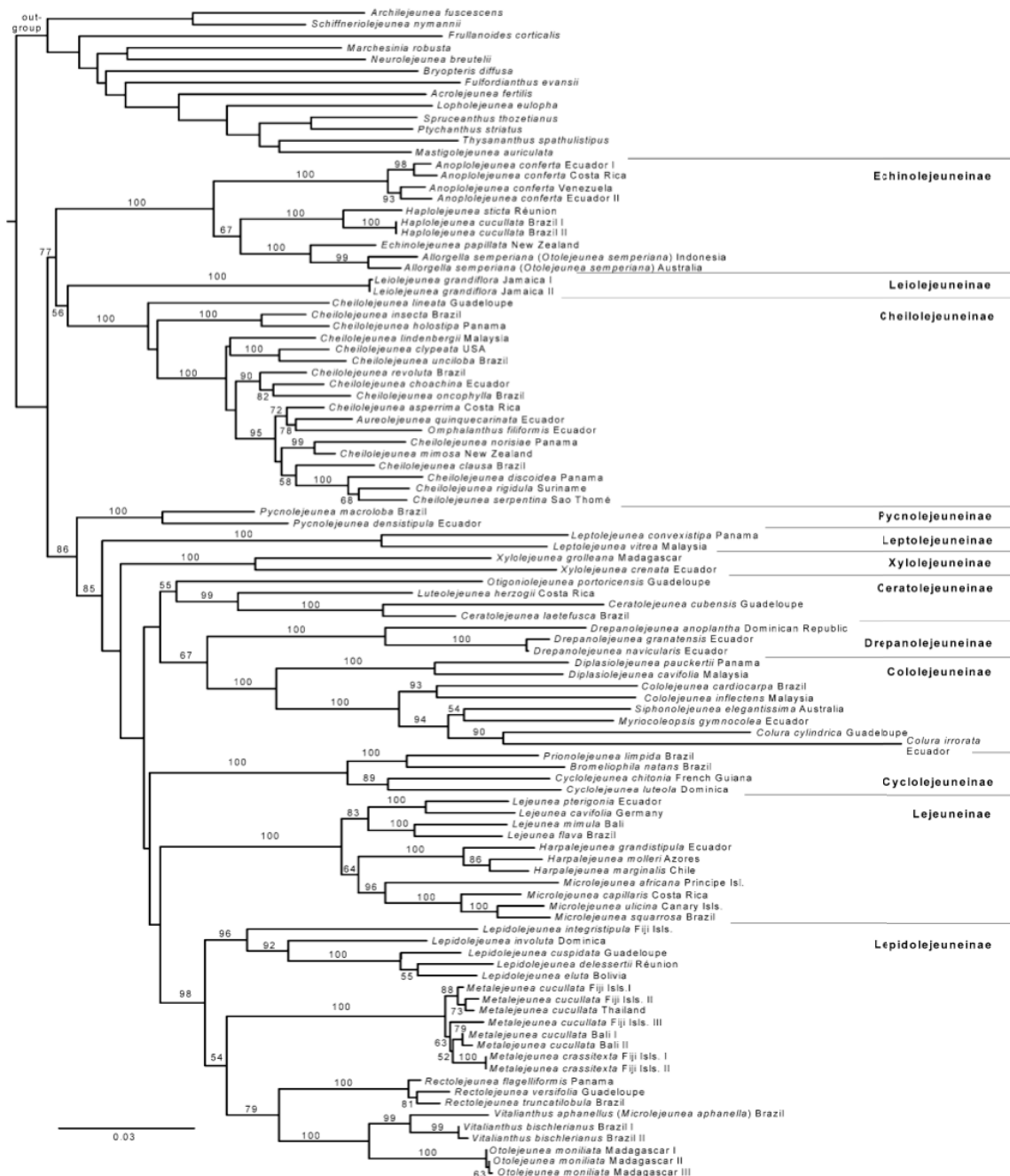
Gradstein (2013) placed *Vitalianthus* in Drepanolejeuneinae yet mentioned that its upright rather than widely diverging underleaf lobes are atypical for species of this relationship. *Vitalianthus bischlerianus* was originally described as a species of *Drepanolejeunea* (Spruce) Steph. (Pôrto and Grolle 1987); however, its moniliate rows of ocelli are hardly seen in this genus (Schuster 1994). Molecular data place *Vitalianthus* in Lepidolejeuneinae, together with *Rectolejeunea* A.Evans, *Otolejeunea* (Heinrichs et al. 2014a) and *Metalejeunea*. The latter genus has not yet been classified in a subtribe of Lejeuneaceae. It resembles *Microlejeunea* in general appearance but differs by its autoicy, pycnolejeuneoid innovations, and a complete lack of ocelli (Grolle 1995). Six accessions of the generitype *M. cucullata* (Reinw., Blume & Nees) Grolle from the Fiji Islands, Thailand and Bali form a robust monophyletic lineage, together with a clade consisting of two accessions of the poorly known Fijian endemic *Metalejeunea crassitexta* (J.B.Jack & Steph.) Pócs (Pócs et al. 2011). This clade is nested in *M. cucullata* yet its position does not get high bootstrap support (Figs. 1, 2). Furthermore, Fijian accessions of *M. cucullata* form a separate subclade rather than clustering with *M. crassitexta*. We thus keep both taxa as separate species; however, *Metalejeunea* deserves more work with an extension of the sampling towards the Neotropics.

Lepidolejeuneinae consist of a rather heterogeneous assemblage of species with or without ocelli. They share the presence of pycnolejeuneoid subgynoecial innovations, autoicy, a lobule tooth that points outwards, and bifid underleaves (Fig. 3).

*Haplolejeunea* is another genus that has not yet been included in molecular phylogenetic studies. Based on morphology (Gradstein et al. 2003) it was placed in Cololejeuneinae; however, in our study it nests in the pantropical Echinolejeuneinae. Echinolejeuneinae represent a rather heterogeneous assemblage of genera that share bifid underleaves and pycnolejeuneoid subgynoecial innovations. Outwards pointing outer lobule teeth are absent (Fig. 3) as well as hyaline papillae positioned distal to the lobule apex.

### Genus concepts

Despite the high amount of morphological homoplasy in Lejeuneaceae (Fig. 3), previous morphology-based genus



**Fig. 4** A most likely phylogram resulting from maximum likelihood analysis of the combined comprehensive dataset. Bootstrap percentage values >50 are indicated at branches

concepts are well supported by molecular evidence. The African *Haplolejeunea sticta* is found in a well-supported sister relationship with the Neotropical *H. cucullata* (Steph.) Grolle, as are the Madagascan *Xylolejeunea*

*grolleana* and the Neotropical *X. crenata*. The transfer of *Microlejeunea aphanella* to *Vitalianthus* is in good accordance with morphology and reflects the previous misinterpretation of morphological character states of this taxon



rather than an incongruence of morphology and molecules. Molecular data, on the other hand, enforced a compromise between two different genus concepts, the narrow *Otolejeunea* concept of Tixier (1980) and the wide *Otolejeunea* concept of Grolle (1983).

### Perspectives

Despite a nearly complete generic sampling, the backbone of Lejeuneaceae remains poorly resolved (Figs. 3, 4). This finding lends some support to Schuster's (2001) hypothesis of a rapid radiation of lejeuneoid liverworts into angiosperm-dominated forests. Although the largely polytomous topologies hamper the reconstruction of ancestral character states, it is evident that Lejeuneaceae exhibit a high content of morphological homoplasy, as was already suggested by Schuster (2001) and Gradstein et al. (2003). This phenomenon is not an exception in Porellales and has also been demonstrated for the Porellales genus *Radula* Dumort. (Renner 2015). Morphological homoplasy necessitates the inclusion of molecular data in reconstructions of evolutionary processes (Dong et al. 2012; Heinrichs et al. 2013). An extension of the present taxon sampling and inclusion of *Acantholejeunea* (R.M.Schust) R.M.Schust., *Hattoriolejeunea* Mizut., *Kymatolejeunea* Grolle, *Schusterolejeunea* Grolle as well as *Tuyamaella* S.Hatt. is urgently needed to arrive at a natural classification of Lejeuneaceae and a deeper understanding of the morphological evolution of lejeuneoid liverworts.

### Taxonomic treatment

*Vitalianthus aphanellus* (Spruce) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Lejeunea aphanella* Spruce, Trans. & Proc. Bot. Soc. Edinburgh 15:290. 1884  $\equiv$  *Microlejeunea aphanella* (Spruce) Steph., Sp. Hep. 5:816. 1915. —TYPE: (Hepaticae Spruceanae) Brazil, Rio Negro, *Spruce s.n.* (lectotype: G; isolectotype, M!).

*Allorgella* Tixier, Nova Hedwigia 32:612. 1980  $\equiv$  *Otolejeunea* subg. *Allorgella* (Tixier) Grolle, Haussknechtia 2:53. 1985. —TYPE: *Allorgella hoana* Tixier.

*Allorgella semperiana* (Steph.) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Prionolejeunea semperiana* Steph., Sp. Hep. 5:227. 1913  $\equiv$  *Otolejeunea semperiana* (Steph.) Grolle, Haussknechtia 2:53. 1985. —TYPE: Philippines, Luzon, *Semper 768* (lectotype: G 14277).

*Allorgella zantenii* (Grolle) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Otolejeunea zantenii*

Grolle, Haussknechtia 2:54. 1985. —TYPE: Papua New Guinea, *van Zanten 683801/K4* (holotype: JE).

*Allorgella rabenorii* (Tixier) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Otolejeunea rabenorii* Tixier, Nova Hedwigia 46: 376. 1988. —TYPE: Madagascar, *Tixier 12591* (holotype: PC).

*Allorgella australiensis* (B.M.Thiers) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Otolejeunea australiensis* B.M.Thiers, Brittonia 44: 162. 1992. —TYPE: Australia, Queensland, *Thiers* and *Halling 2264* (holotype: AD).

*Allorgella philippinensis* (R.L.Zhu & M.L.So) Bechteler, G.E.Lee, Schäf.-Verw. & Heinrichs, **comb. nov.**  $\equiv$  *Otolejeunea philippinensis* R.L.Zhu & M.L.So, Syst. Bot. 23:231. 1998. —TYPE: Philippines, *Micholitz 20* (holotype: G 00069720).

*Allorgella subana* (Pócs) Pócs, **comb. nov.**  $\equiv$  *Otolejeunea subana* Pócs, Acta Acad. Paed. Agriensis, Sect. Biol. 25:50. 2004. —TYPE: Madagascar, Mantady Forest Reserve, *Pócs* and *Szabó 9485/R* (holotype: EGR!).

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### References

- Akaike H (1973) Information theory as an extension of the maximum likelihood principle. In: Petrov BN, Csaki F (eds) Second international symposium on information theory. Akadémiai Kiadó, Budapest, pp 267–281
- Bischler H, Miller HA, Bonner CEB (1962) Studies in Lejeuneaceae. IV. The typification of the genus *Microlejeunea*. Nova Hedwigia 4:173–187
- Bischler H, Bonner CEB, Miller HA (1963) Studies in Lejeuneaceae VI. The genus *Microlejeunea* Steph. in Central and South America. Nova Hedwigia 5:359–411
- Cornelissen JHC, Ter Steege H (1989) Distribution and ecology of epiphytic bryophytes and lichens in dry evergreen forest of Guyana. J Trop Ecol 5:131–150. doi:10.1017/S0266467400003400
- Darriba D, Taboada GL, Doallo R, Posada D (2012) JModelTest 2: more models, new heuristics and parallel computing. Nature Meth 9:772. doi:10.1038/nmeth.2109
- Dong S, Schäfer-Verwimp A, Meinecke P, Feldberg K, Bombosch A, Pócs T, Schmidt AR, Reitner J, Schneider H, Heinrichs J (2012) Tramps, narrow endemics and morphologically cryptic species in the epiphyllous liverwort *Diplasiolejeunea*. Molec Phylogen Evol 65:582–594. doi:10.1016/j.ympev.2012.07.009
- Dong S, Schäfer-Verwimp A, Pócs T, Feldberg K, Czumaj A, Schmidt AR, Schneider H, Heinrichs J (2013) Size doesn't matter—recircumscription of *Microlejeunea* based on molecular

- and morphological evidence. *Phytotaxa* 85:41–55. doi:10.11646/phytotaxa.85.2.2
- Feldberg K, Schneider H, Stadler T, Schäfer-Verwimp A, Schmidt AR, Heinrichs J (2014) Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Sci Rep* 4:5974. doi:10.1038/srep05974
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. doi:10.2307/2408678
- Frey W, Stech M (2009) Marchantiophyta, Bryophyta, Anthocerotophyta. In: Frey W (ed), *Syllabus of plant families—a. Engler's Syllabus der Pflanzenfamilien*, 13th ed., part 3, Bryophytes and seedless vascular plants, Schweizerbart, Stuttgart, pp 13–263
- Gradstein SR (1997) The taxonomic diversity of epiphyllous bryophytes. *Abstr Bot* 21:15–19
- Gradstein SR (2013) A classification of Lejeuneaceae (Marchantiophyta) based on molecular and morphological evidence. *Phytotaxa* 100:6–20. doi:10.11646/phytotaxa.100.1.2
- Gradstein SR, Churchill SP, Salazar-Allen N (2001) Guide to the bryophytes of tropical America. *Mem New York Bot Gard* 86:1–577
- Gradstein SR, Reiner-Drehwald ME, Schneider H (2003) A phylogenetic analysis of the genera of Lejeuneaceae (Hepaticae). *Bot J Linn Soc* 143:391–410. doi:10.1111/j.1095-8339.2003.00235.x
- Gradstein SR, Wilson R, Ilkiu-Borges AL, Heinrichs J (2006) Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Bot J Linn Soc* 151:293–308. doi:10.1111/j.1095-8339.2006.00531.x
- Grolle R (1983) Nomina generica Hepaticarum; references, types and synonymies. *Acta Bot Fenn* 121:1–62
- Grolle R (1985) Zur Kenntnis der Lebermoosgattung *Otolejeunea*. *Haussknechtia* 2:45–56
- Grolle R (1995) The Hepaticae and Anthocerotae of the East African Islands. An annotated catalogue. *Bryophyt Biblioth* 48:1–178
- Grolle R, Reiner-Drehwald ME (2000) *Otolejeunea* (Hepaticae, Lejeuneaceae) in the neotropics. *Cryptog Bryol* 21:101–107. doi:10.1016/S1290-0796(00)00109-7
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Oxford University Press. Nucleic Acids Symposium Series No* 41:95–98
- Hartmann FA, Wilson R, Gradstein SR, Schneider H, Heinrichs J (2006) Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *Int J Pl Sci* 167:1205–1214. doi:10.1086/508023
- He XL, Piippo S (1999) On the taxonomic significance of ocelli characters in the hepatic family Lejeuneaceae. *Bryobrothera* 5:93–97
- He Q, Zhu RL (2011) Spore output in selected species of Lejeuneaceae. *Cryptog Bryol* 32:107–112. doi:10.7872/cryb.v32.iss1.2011.107
- Heinrichs J, Gradstein SR, Wilson R, Scheider H (2005) Towards a natural classification of liverworts (Marchantiophyta) based on the chloroplast gene *rbcL*. *Cryptog Bryol* 26:131–150
- Heinrichs J, Dong S, Schäfer-Verwimp A, Pócs T, Feldberg K, Czumaj A, Schmidt AR, Reitner J, Renner MAM, Hentschel J, Stech M, Schneider H (2013) Molecular phylogeny of the leafy liverwort *Lejeunea* (Porellales): evidence for a Neotropical origin, uneven distribution of sexual systems and insufficient taxonomy. *PLoS ONE* 8:e82547. doi:10.1371/journal.pone.0082547
- Heinrichs J, Dong S, Schäfer-Verwimp A, Peralta DF, Feldberg K, Schmidt AR, Schneider H (2014a) Towards a monophyletic classification of Lejeuneaceae II: subtribes Pycnolejeuneinae and Xylolejeuneinae subtr. nov., transfer of *Otolejeunea* to Lepidolejeuneinae, and generic refinements. *Phytotaxa* 163:61–76. doi:10.11646/phytotaxa.163.2.1
- Heinrichs J, Schäfer-Verwimp A, Czumaj A, Dong S, Scheben A, Feldberg K, Schneider H (2014b) Towards a monophyletic classification of Lejeuneaceae I: subtribe Leptolejeuneinae subtr. nov. *Phytotaxa* 156:165–174. doi:10.11646/phytotaxa.156.3.7
- Heinrichs J, Feldberg K, Bechteler J, Scheben A, Czumaj A, Pócs T, Schneider H, Schäfer-Verwimp A (2015) Integrative taxonomy of *Lepidolejeunea* (Porellales, Jungermanniopsida): ocelli allow the recognition of two neglected species. *Taxon* 64:216–228. doi:10.12705/642.5
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing the confidence in phylogenetic analysis. *Syst Biol* 42:182–192. doi:10.1093/sysbio/42.2.182
- Humphreys AM, Linder HP (2009) Concept versus data in delimitation of plant genera. *Taxon* 58:1054–1074
- Lee GE (2013) A systematic revision of the genus *Lejeunea* Lib. (Marchantiophyta: Lejeuneaceae) in Malaysia. *Cryptog Bryol* 34:381–484. doi:10.7872/cryb.v34.iss4.2013.381
- Lee GE, Bechteler J, Schäfer-Verwimp A, Heinrichs J (2015) *Microlejeunea miocenica* sp. nov. (Porellales, Jungermanniopsida) in Dominican amber, the first fossil of a subcosmopolitan genus of leafy liverworts. *Rev Palaeobot Palynol* 222:16–21. doi:10.1016/j.revpalbo.2015.07.002
- Lücking A (1995) Diversität und Mikrohabitatpräferenzen epiphyller Moose in einem tropischen Regenwald in Costa Rica unter besonderer Berücksichtigung der Familie Lejeuneaceae. PhD Thesis, University of Ulm, Ulm
- Mason-Gamer RJ, Kellogg EA (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst Biol* 45:524–545. doi:10.1093/sysbio/45.4.524
- Miller HA, Bonner CEB, Bischler H (1967) *Microlejeunea* in Asia and Australia. *Nova Hedwigia* 14:61–67
- Pócs T (1996) Epiphyllous liverwort diversity at worldwide level and its threat and conservation. *Anales Inst Biol Univ Nac Autón México Bot* 67:109–127
- Pócs T (2004) New or little known epiphyllous liverworts, XI. *Otolejeunea subana* sp. nov. from Madagascar. *Acta Acad Paedagogicae Agriensis Sect Biol* 25:49–57
- Pócs T, Sass-Gyarmati A, Naikatiní A, Tuiwawa M, Braggins J, Pócs S, von Konrat M (2011) New liverwort (Marchantiophyta) records for the Fiji Islands. *Telopea* 13:455–494
- Pôrto KC, Grolle R (1987) *Drepanolejeunea bischleriana* sp. nov. du Brésil. *Cryptog Bryol Lichenol* 8:301–304
- Renner MAM (2015) Lobule shape evolution in *Radula* (Jungermanniopsida): one rate fits all? *Bot J Linn Soc* 178:222–242. doi:10.1111/boj.12279
- Schäfer-Verwimp A, Feldberg K, Dong S, Van Melick H, Peralta DF, Schmidt AR, Schneider H, Heinrichs J (2014) Towards a monophyletic classification of Lejeuneaceae III: the systematic position of *Leiolejeunea*. *Phytotaxa* 170:187–193. doi:10.11646/phytotaxa.170.3.4
- Schuster RM (1992) The oil-bodies of the Hepaticae. II. Lejeuneaceae (Part 2). *J Hattori Bot Lab* 72:163–359
- Schuster RM (1994) Studies on Lejeuneaceae, I. Preliminary studies on new genera of Lejeuneaceae. *J Hattori Bot Lab* 75:211–235
- Schuster RM (2001) Studies on Lejeuneaceae, IV. On the circumscription and subdivision of the subfamily Lejeuneoideae. *J Hattori Bot Lab* 91:137–172
- Schuster RM, Giancotti C (1993) On *Vitalianthus* Schust. & Giancotti, a new genus of Lejeuneaceae. *Nova Hedwigia* 57:445–456
- Sonnleitner M, Dullinger S, Wanek W, Zechmeister H (2009) Microclimatic patterns correlate with the distribution of epiphyllous bryophytes in a tropical lowland rain forest in Costa

- Rica. J Trop Ecol 25:321–330. doi:10.1017/S0266467409006002
- Stamatakis A (2006) RAxML-VI-HPC: maximum-likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690. doi:10.1093/bioinformatics/btl446
- Stamatakis A (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. doi:10.1093/bioinformatics/btu033
- Sukkharak P, Gradstein SR, Stech M (2011) Phylogeny, taxon circumscriptions, and character evolution in the core Ptychanthoideae (Lejeuneaceae, Marchantiophyta). *Taxon* 60:1607–1622
- Swofford DL (2000) PAUP\*, phylogenetic analyses using parsimony (\* and other methods), version 4.01b10. Sinauer Associates, Sunderland, Massachusetts
- Tixier P (1980) Deux nouveaux genres de Lejeunéacées *Otolejeunea* Grolle & P. Tx. et *Allorgella* P. Tx. *Nova Hedwigia* 32:607–622
- Weis G (2001) Morphologische und anatomische Untersuchungen der Sporophyten bei den Jubulaceae Klinggr. und Lejeuneaceae Casares-Gil (Hepaticae) und deren systematische Bedeutung. *Bryophyt Biblioth* 57:1–302
- Wilson R, Gradstein SR, Schneider H, Heinrichs J (2007a) Unravelling the phylogeny of Lejeuneaceae (Jungermanniopsida): evidence for four main lineages. *Molec Phylogen Evol* 43:270–282. doi:10.1016/j.ympev.2006.10.017
- Wilson R, Heinrichs J, Hentschel J, Gradstein SR, Schneider H (2007b) Steady diversification of derived liverworts under Tertiary climatic fluctuations. *Biol Lett* 3:566–569. doi:10.1098/rsbl.2007.0287
- Ye W, Gradstein SR, Shaw AJ, Shaw B, Ho BC, Schäfer-Verwimp A, Pócs T, Heinrichs J, Zhu RL (in press) Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers. *Cryptog Bryol* 36(4)
- Yu Y, Pócs T, Schäfer-Verwimp A, Heinrichs J, Zhu RL, Schneider H (2013) Evidence for rampant homoplasy in the phylogeny of the epiphyllous liverwort genus *Cololejeunea* (Lejeuneaceae). *Syst Bot* 38:553–563. doi:10.1600/036364413X670304
- Yu Y, Pócs T, Zhu RL (2014) Notes on Early Land Plants Today. 62. A synopsis of *Myriocoleopsis* (Lejeuneaceae, Marchantiophyta) with special reference to transfer of *Cololejeunea minutissima* to *Myriocoleopsis*. *Phytotaxa* 183:293–297. doi:10.11646/phytotaxa.183.4.11
- Zhu RL, So ML (1997) A new record of the genus *Otolejeunea* (Hepaticae, Lejeuneaceae) in subtropical China. *Ann Bot Fenn* 34:285–289
- Zhu RL, So ML (1998) A new species of *Otolejeunea* (Hepaticae, Lejeuneaceae) from the Philippines. *Syst Bot* 23:231–234. doi:10.2307/2419590



## Chapter 3

### TOWARDS A MONOPHYLETIC CLASSIFICATION OF LEJEUNEACEAE V: THE SYSTEMATIC POSITION OF *PICTOLEJEUNEA*.

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## Towards a monophyletic classification of Lejeuneaceae V: the systematic position of *Pictolejeunea*

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### Abstract

With more than 1,000 species in some 70 genera, Lejeuneaceae represent the largest family of liverworts. Although much progress has been made in recent years, the supraspecific classification of Lejeuneaceae has not yet been satisfactorily solved. Maximum likelihood and Bayesian analyses of a three marker dataset (nrITS, cp DNA *rbcL* and *trnL-trnF*) derived from 85 accessions of Lejeuneaceae and 13 outgroup species revealed *Pictolejeunea picta* in a sister relationship to the remainder of Lejeuneaceae, the most speciose tribe of Lejeuneaceae. Based on the outcome of the phylogenetic analyses, we establish subtribe Pictolejeuneinae *subtr. nov.*

**Key words:** classification, Lejeuneaceae, Pictolejeuneinae, Porellales, liverwort, molecular phylogeny

### Introduction

With more than 1,000 species (He & Zhu 2011), Lejeuneaceae are the largest family of the liverworts (Söderström *et al.* 2016). They are morphologically well circumscribed by lateral branches of the *Lejeunea*- or *Frullania*-type, fascicled rhizoids from underleaf base, 2-3-lobed leaves with the ventral lobule broadly attached to the dorsal lobe along a keel, gynoecia with a single archegonium and a single series of bracts and bracteoles, beaked perianths, sporophytes enclosed by a stalked calyptra, 2-stratose capsule walls, elaters adherent to the capsule valves, and spores with rosette markings in the exine (Crandall-Stotler *et al.* 2009). Centres of diversity are the humid tropics where Lejeuneaceae constitute a considerable portion of the epiphytic, especially epiphyllic diversity (Lücking 1995; Pócs 1996; Gradstein 1997; Gradstein *et al.* 2001). Lejeuneaceae are notorious for their complicated supraspecific classification and some 90 genera were accepted at the beginning of the millennium (Gradstein *et al.* 2003). Since then, molecular phylogenetic studies resolved several genera nested in others, e.g. *Chondriolejeunea* (Benedix 1953: 75) Kis & Pócs (2001: 293) and *Metzgeriopsis* Goebel (1888: 54) in *Cololejeunea* (Spruce 1884: 291) Schiffner (1893: 121) (Gradstein *et al.* 2006; Yu *et al.* 2013), *Myriocolea* Spruce (1884: 305) in *Colura* (Dumortier 1831: 32) Dumortier (1835: 12) (Heinrichs *et al.* 2011), and *Oryzolejeunea* (Schuster 1970: 338) Schuster (1992: 249), *Sphaerolejeunea* Herzog (1938: 88) and *Taxilejeunea* (Spruce 1884: 212) Schiffner (1893: 125) in *Lejeunea* Libert (1820: 372) (Wilson *et al.* 2007; Heinrichs *et al.* 2012, 2013; Ye *et al.* 2013). A revised classification of Lejeuneaceae thus accepted only 68 genera in two subfamilies, three tribes and eight subtribes, however several genera had not, at that stage, been included in molecular phylogenies and were thus not assignable to subtribes (Gradstein 2013). Subsequently, several changes to the classification of Gradstein (2013) were introduced including the reinstatement of *Allorgella* Tixier (1980: 612) (Bechteler *et al.* 2016), description of *Gradsteinianthus* R.L.Zhu & Jian Wang in Wang *et al.* (2016: 29) and the synonymy of *Aureolejeunea* Schuster (1978: 428), *Cyrtolejeunea* Evans (1903: 553), *Cystolejeunea* Evans (1906: 16), *Evansiolejeunea* Vanden Berghen (1948: 86), *Omphalanthus* Lindenberg & Nees in Gottsche *et al.* (1845: 303) and *Leucolejeunea* Evans (1907: 225) with *Cheilolejeunea* (Spruce 1884: 251) Stephani (1890: 284) (Schäfer-Verwimp

*et al.* 2014; Ye *et al.* 2015). Several genera were moved from one subtribe to another, e.g. *Vitalianthus* Schuster & Giancotti (1993: 447) from Drepanolejeuneinae to Lepidolejeuneinae (Bechteler *et al.* 2016), *Otolejeunea* Grolle & Tixier in Tixier (1980: 609) from Cyclolejeuneinae to Lepidolejeuneinae (Heinrichs *et al.* 2014b) and *Bromeliophila* Schuster (1994: 226) from Lejeuneinae to Cyclolejeuneinae (Heinrichs *et al.* 2014a). In addition, the classification of Lejeuneaceae was further completed by introduction of the subtribes Leptolejeuneinae (Heinrichs *et al.* 2014c), Leiolejeuneinae (Schäfer-Verwimp *et al.* 2014), Pycnolejeuneinae and Xylolejeuneinae (Heinrichs *et al.* 2014b).

*Pictolejeunea* Grolle (1977: 248) is a small Neotropical-Asian genus with six currently accepted species (Söderström *et al.* 2016) whose exact systematic position is unknown (Gradstein 2013). Ilkiu-Borges (2005) found the genus in a polytomy with several other lejeuneoid genera using a cp DNA *trnL-trnF* alignment, and rightly considered this marker alone insufficient to resolve the phylogeny of Lejeuneaceae. Here, we include nrITS, *rbcL* and *trnL-trnF* sequence data of *Pictolejeunea* in an alignment of Lejeuneaceae and investigate its phylogenetic position. We demonstrate its sister relationship with the remainder of Lejeuneaceae, and introduce the new subtribe Pictolejeuneinae.

## Materials & Methods

### Taxon sampling, DNA extraction, PCR amplification, sequencing and alignment

Dried specimens of the generitype *Pictolejeunea picta* (Gottsche ex Stephani 1913a: 223) Grolle (1977: 252) from the herbarium SP were used to isolate gametophytical tissue (Table 1). DNA extraction, PCR amplification and sequencing were carried out as described in Bechteler *et al.* (2016). Three markers were amplified: the chloroplast *rbcL* gene and *trnL-trnF* region and the nuclear ITS region (ITS1-5.8S-ITS2). In a first step the newly obtained sequences were compared with GenBank sequences using the nucleotide BLAST search ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE\\_TYPE=BlastSearch](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch); Altschul *et al.* 1990), and then added to the Lejeuneaceae alignment of Wilson *et al.* (2007) to test the hypothesis that *Pictolejeunea picta* belongs to the Lejeuneaceae (Gradstein 2013). Since this hypothesis was confirmed (results not shown here) the newly obtained sequences were aligned with sequences of 83 Lejeuneaceae specimens downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). The outgroup consisted of 13 Ptychanthoideae and Brachiolejeuneae according to the phylogenetic hypotheses presented in Wilson *et al.* (2007). Sequences were aligned manually in PhyDE v.0.9971 (<http://www.phyde.de/index.html>) and ambiguous positions were excluded for phylogenetic analyses.

**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	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Acrolejeunea fertilis</i> (Reinw. <i>et al.</i> ) Schiffn.	Bali, Schäfer-Verwimp & Verwimp 17009 (GOET)	AY684929	DQ987391	DQ987281
<i>Allorgella semperiana</i> (Steph.) Bechteler <i>et al.</i>	Australia, Streimann 57955 (EGR)	KT626926	KT626942	KT626909
<i>Allorgella semperiana</i>	Indonesia, Schäfer-Verwimp & Verwimp 24836/C (M)	KT626927	-----	KT626910
<i>Anoplolejeunea conferta</i> (C.F.W.Meissn. ex Spreng.) A.Evans	Ecuador, Schäfer-Verwimp & Nebel 33081/B (M)	KJ408336	KJ408359	KJ408310
<i>Anoplolejeunea conferta</i>	Venezuela, Pócs <i>et al.</i> 9712/BA (M)	KJ408337	KJ408360	KJ408311
<i>Anoplolejeunea conferta</i>	Costa Rica, Schäfer-Verwimp & Holz 154 (M)	KJ408335	KJ408358	KJ408309
<i>Archilejeunea fuscescens</i> (Hampe ex Lehm.) Fulford	Costa Rica, Bernecker 97-53 (GOET)	DQ983655	DQ987384	DQ987267
<i>Bromeliophila natans</i> (Steph.) R.M.Schust.	Brazil, Santa Catarina, Gehrig s.n. (GOET)	KF039849	KF039879	KF039812
<i>Bryopteris diffusa</i> (Sw.) Nees	Bolivia, Acebey & Villavicencio 855 (GOET)	AY548085	AM237147	AM237095
<i>Ceratolejeunea cubensis</i> (Mont.) Schiffn.	Guadeloupe, Schäfer-Verwimp & Verwimp 22279/B (M)	KF606946	KF606941	KF606936
<i>Ceratolejeunea laetefusca</i> (Austin) R.M. Schust.	Brazil, Schäfer-Verwimp 33995 (M)	KF606947	KF606942	KF606937

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TABLE 1. (Continued)

Taxon	Voucher	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Cheilolejeunea asperrima</i> (Steph.) Grolle	Costa Rica, van Melick 214551 (M)	KJ408339	KJ408362	KJ408313
<i>Cheilolejeunea choachina</i> (Gottsche) Gradst.	Ecuador, Schäfer-Verwimp & Nebel 33152/A (M)	KJ408341	KJ408364	KJ408315
<i>Cheilolejeunea clausa</i> (Nees & Mont.) Steph.	Brazil, Teixeira 39 (M)	KJ716751	KJ716776	KJ716763
<i>Cheilolejeunea clypeata</i> (Schwein.) W.Ye & R.L.Zhu	USA, Davis s.n. (GOET)	DQ983699	DQ987426	DQ987322
<i>Cheilolejeunea discoidea</i> (Lehm. & Lindenb.) R.M.Schust. & Kachroo	Panama, Schäfer-Verwimp & Verwimp 34530 (M)	KJ408343	KJ408366	KJ408317
<i>Cheilolejeunea filiformis</i> (Sw.) W.Ye, R.L.Zhu & Gradst.	Ecuador, Schäfer-Verwimp & Preußing 23543 (GOET)	DQ983716	DQ987393	DQ987283
<i>Cheilolejeunea gaoi</i> R.L.Zhu, M.L.So & Grolle	China (I), Ye & Wei 20090717-1 (HSNU)	-----	KT190781	KT190897
<i>Cheilolejeunea gaoi</i>	China (II), Ye & Wei 20090716-17 (HSNU)	-----	KT190780	KT190896
<i>Cheilolejeunea holostipa</i> (Spruce) Grolle & R.L.Zhu	Panama, Schäfer-Verwimp & Verwimp 34526 (M)	KJ408351	KJ408375	KJ408326
<i>Cheilolejeunea insecta</i> Grolle & Gradst.	Brazil, Schäfer-Verwimp & Verwimp 14725 (M)	KJ716749	KJ716774	KJ716761
<i>Cheilolejeunea lineata</i> (Lehm. & Lindenb.) Steph.	Guadeloupe, Schäfer-Verwimp & Verwimp 22183 (GOET)	AY548092	DQ987401	DQ987295
<i>Cheilolejeunea lindenbergii</i> (Gottsche) Mizut.	Malaysia, Pócs & Pócs 1006/AG (M)	KJ408344	KJ408367	KJ408318
<i>Cheilolejeunea mimosa</i> (Hook. f. & Taylor) R.M.Schust.	New Zealand, Schäfer-Verwimp & Verwimp 13745 (M)	KJ408345	KJ408368	KJ408319
<i>Cheilolejeunea norisiae</i> G.Dauphin & Gradst.	Panama, Schäfer-Verwimp & Verwimp 30821 (M)	KJ408346	KJ408369	KJ408320
<i>Cheilolejeunea oncophylla</i> (Ångstr.) Grolle & M.E.Reiner	Brazil, Peralta 11914 (M)	KJ408347	KJ408370	KJ408321
<i>Cheilolejeunea quinquecarinata</i> (R.M.Schust.) W.Ye, R.L.Zhu & Gradst.	Ecuador, Schäfer-Verwimp & Preußing 23299/A (GOET)	DQ983658	DQ987450	DQ987350
<i>Cheilolejeunea revoluta</i> (Herzog) Gradst. & Grolle	Brazil, Peralta 15116 (M)	KJ408349	KJ408372	KJ408323
<i>Cheilolejeunea rigidula</i> (Mont.) R.M.Schust.	Suriname, Muñoz 98-62 (GOET)	DQ983668	DQ987453	DQ987353
<i>Cheilolejeunea rigidula</i>	São Tomé and Príncipe, Shevock 39658 (M)	KJ716753	KJ716778	KJ716765
<i>Cheilolejeunea unciloba</i> (Lindenb.) Malombe	Brazil, Yano 32383 (M)	KJ716755	KJ716780	KJ716767
<i>Cololejeunea cardiocarpa</i> (Mont.) A.Evans	Brazil, Borhidi & Pereira BB25 (GOET)	JQ991163	JQ991278	JQ991048
<i>Cololejeunea inflectens</i> (Mitt.) Benedix	Malaysia, Schäfer-Verwimp & Verwimp 18861/A (GOET, as <i>C. peculiaris</i> )	AY548095	DQ238572	DQ987280
<i>Colura cylindrica</i> Herzog	Guadeloupe, Schäfer-Verwimp & Verwimp 22154/B (JE)	JX470969	JX470980	JX470992
<i>Colura irrorata</i> (Spruce) Heinrichs, Y.Yu, Schäfer-Verw. & Pócs	Ecuador, Gradstein <i>et al.</i> 10033 (GOET)	AY548073	DQ238584	DQ987279
<i>Cyclolejeunea chitonina</i> (Taylor) A.Evans	French Guiana, Holz FG-00-124 (GOET)	KF039844	KF039875	KF039806
<i>Cyclolejeunea luteola</i> (Spruce) Grolle	Dominica, Schäfer-Verwimp & Verwimp 17866 (M)	KF039823	KF039857	KF039785
<i>Diplasiolejeunea cavifolia</i> Steph.	Malaysia, Schäfer-Verwimp & Verwimp 19036/A (GOET)	JQ729531	JQ729641	JQ729418
<i>Diplasiolejeunea pauckertii</i> Steph.	Panama, De Gracia <i>et al.</i> 338 (GOET)	JQ729541	JQ729652	JQ729429

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**TABLE 1.** (Continued)

<b>Taxon</b>	<b>Voucher</b>	<b><i>rbcL</i></b>	<b><i>trnL-F</i></b>	<b>nrITS</b>
<i>Drepanolejeunea anoplantha</i> (Spruce) Steph.	Dominican Republic, Schäfer-Verwimp & Verwimp 27059 (JE)	KC313120	KC313159	KC313080
<i>Drepanolejeunea granatensis</i> (J.B.Jack & Steph.) Bischl.	Ecuador, Schäfer-Verwimp <i>et al.</i> 24383/B (JE)	KC313121	KC313160	KC313081
<i>Drepanolejeunea navicularis</i> Steph.	Ecuador, Schäfer-Verwimp & Nebel 31772/A (JE)	KC313124	KC313163	KC313085
<i>Echinolejeunea papillata</i> (Mitt.) R.M.Schust. Ex Hamlin	New Zealand, Schäfer-Verwimp & Verwimp 14195/A (JE)	KC313135	KC313175	KC313097
<i>Frullanoides corticalis</i> (Lehm. & Lindenb.) van Slageren	French Guiana, Hartmann 04-080 (GOET)	DQ983681	AM237196	AM237143
<i>Fulfordianthus evansii</i> (Fulford) Gradst.	Costa Rica, Dauphin s.n. (GOET)	DQ983683	AM237197	AM237144
<i>Haplolejeunea cucullata</i> (Steph.) Grolle	Brazil (I), Peralta <i>et al.</i> 16390 (SP)	KT626925	KT626939	KT626906
<i>Haplolejeunea cucullata</i>	Brazil (II), Schäfer-Verwimp & Verwimp 14560 (M)	-----	KT626940	KT626907
<i>Haplolejeunea sticta</i> Grolle	Reunion Island, Kis & Gyarmati 9604/G (EGR)	-----	KT626941	KT626908
<i>Harpalejeunea grandistipula</i> R.M.Schust.	Ecuador, Schäfer-Verwimp <i>et al.</i> 24163/B (JE)	KC313145	KC313185	KC313107
<i>Harpalejeunea marginalis</i> (Hook. f & Taylor) Steph.	Chile, Schäfer-Verwimp & Verwimp 8082 (JE)	KC313147	KC313187	KC313109
<i>Harpalejeunea molleri</i> (Steph.) Grolle	Portugal, Schäfer-Verwimp & Verwimp 29334 (GOET)	KC313148	KC313188	KC313110
<i>Leiolejeunea grandiflora</i> A.Evans	Jamaica (I), Schäfer-Verwimp 35394 (M)	KJ716757	KJ716782	KJ716769
<i>Leiolejeunea grandiflora</i>	Jamaica (II), Schäfer-Verwimp 35422 (M)	KJ716756	KJ716781	KJ716768
<i>Lejeunea cavifolia</i> (Ehrh.) Lindb.	Germany, Heinrichs 3695 (GOET)	AY548102	DQ238581	DQ987259
<i>Lejeunea flava</i> (Sw.) Nees	Brazil, Gradstein s.n. (GOET)	DQ983692	DQ987413	DQ987309
<i>Lejeunea mimula</i> Hürl.	Indonesia, Schäfer-Verwimp 20930 (GOET)	AY548104	DQ238580	DQ987261
<i>Lejeunea pterigonia</i> (Lehm. & Lindenb.) Mont.	Ecuador, Nöske 164 (GOET)	KF5 56549	KF556328	KF556081
<i>Lepidolejeunea cuspidata</i> (Gottsche) Heinrichs & Schäf.-Verw.	Guadeloupe, Schäfer-Verwimp & Verwimp 22193 (M)	KP635323	KP635348	KP635295
<i>Lepidolejeunea delessertii</i> (Nees & Mont.) Grolle	Réunion, Schäfer-Verwimp & Verwimp 20355/B (M)	KF039819	KF039853	KF039781
<i>Lepidolejeunea integristipula</i> (Jack & Steph.) R.M.Schust.	Fiji, Pócs 03307/AC (GOET)	DQ983697	DQ987417	DQ987313
<i>Lepidolejeunea involuta</i> (Gottsche) Grolle	Dominica, Schäfer-Verwimp & Verwimp 17855 (JE)	KP635314	KP635339	KP635285
<i>Leptolejeunea convexistipa</i> Bischl.	Panama, Schäfer-Verwimp & Verwimp 30861 (JE)	KF954161	KF954151	KF954154
<i>Leptolejeunea vitrea</i> (Nees) Schiffn.	Malaysia, Dürhammer D148 (JE)	KF954164	KF954152	KF954157
<i>Lopholejeunea eulopha</i> (Taylor) Schiffn.	Australia, Pócs & Streimann 9987/H1 (GOET)	AY548067	DQ987381	DQ987262
<i>Luteolejeunea herzogii</i> (Buchloh) Piippo	Costa Rica, Schäfer-Verwimp & Holz 0294/B (GOET)	DQ983706	DQ987467	DQ987368
<i>Marchesinia robusta</i> (Mitt.) Schiffn.	Ecuador, Wilson <i>et al.</i> 04-05 (GOET)	DQ983710	DQ987436	DQ987332
<i>Mastigolejeunea auriculata</i> (Wilson & W.J. Hooker) Schiffn.	Bolivia, Churchill 21275 (GOET)	AY548070	DQ987385	DQ987268
<i>Metalejeunea crassitexta</i> (J.B.Jack & Steph.) Pócs	Fiji (I), Pócs & Pócs 03303/R (EGR)	KT626918	KT626934	KT626899
<i>Metalejeunea cucullata</i> (Reinw., Blume & Nees) Grolle	Fiji (II), Pócs & Pócs 03305/Q (EGR)	KT626913	KT626929	KT626894

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**TABLE 1.** (Continued)

Taxon	Voucher	<i>rbcL</i>	<i>trnL-F</i>	nrITS
<i>Microlejeunea africana</i> Steph.	São Tomé and Príncipe, Shevock 34576 A (GOET)	KC313150	KC313190	KC313112
<i>Microlejeunea capillaris</i> (Gottsche) Steph.	Costa Rica, Schäfer-Verwimp & Holz SV/H-0489/B (JE)	KC313152	KC313192	KC313114
<i>Microlejeunea squarrosa</i> (Steph.), Heinrichs, Schäf.-Verw., Pócs & S.Dong	Brazil, Schäfer-Verwimp 13376 (GOET)	DQ983720	DQ987446	DQ987344
<i>Microlejeunea ulicina</i> (Taylor) Steph.	Canary Islands, Schäfer-Verwimp & Verwimp 24666 (GOET)	KC313155	KC313195	KC313116
<i>Myriocoleopsis gymnocolea</i> (Steph.) M.E.Reiner & Gradst.	Ecuador, Gradstein <i>et al.</i> 10020 (GOET)	DQ238568	DQ238583	DQ987277
<i>Neurolejeunea breutelii</i> (Gottsche) A.Evans	Brazil, Schäfer-Verwimp 14740 (GOET)	DQ983714	DQ987405	EF011779
<i>Otolejeunea moniliata</i> Grolle	Madagascar (I), 9448/L (EGR)	KT626923	KT626937	KT626904
<i>Otolejeunea moniliata</i>	Madagascar (II), Pócs <i>et al.</i> 90113/EA (EGR)	KT626922	KT626936	KT626903
<i>Physantholejeunea portoricensis</i> (Hampe & Gottsche) R.M.Schust.	Guadeloupe, Schäfer-Verwimp & Verwimp 22615 (M)	DQ983719	DQ987448	DQ987346
<i>Pictolejeunea picta</i> (Gott. ex Steph.) Grolle	French Guiana, Holz FG00-227 (GOET)	-----	DQ207914	-----
<i>Pictolejeunea picta</i>	Brazil I, Lopes 202 (SP)	-----	-----	KX765484
<i>Pictolejeunea picta</i>	Brazil II, Lopes 192 (SP)	KX765481	KX765483	KX765483
<i>Prionolejeunea limpida</i> Herzog	Brazil, Schäfer-Verwimp & Verwimp 13291 (M)	KF039850	DQ207928	DQ207895
<i>Ptychanthus striatus</i> (Lehm. & Lindenb.) Nees	Java, Gradstein 10215 (GOET)	DQ983721	DQ987406	DQ987300
<i>Pycnolejeunea densistipula</i> (Lehm. & Lindenb.) Steph.	Ecuador, Schäfer-Verwimp & Preussing 23368 (M)	KJ408353	KJ408377	KJ408328
<i>Pycnolejeunea macroloba</i> (Nees & Mont.) Schiffn.	Brazil, Yano 32740 (M)	KJ408354	KJ408378	KJ408329
<i>Rectolejeunea versifolia</i> (Schiffn.) L.Söderstr. & A.Hagborg	Guadeloupe, Schäfer-Verwimp & Verwimp 22245/A (GOET)	DQ983724	DQ987444	DQ987342
<i>Rectolejeunea flagelliformis</i> A.Evans	Panama Schäfer-Verwimp & Verwimp 34286 (JE)	KT626924	KT626938	KT626905
<i>Rectolejeunea truncatilobula</i> C.J.B. Bastos	Brazil, Peralta 8083 (M)	-----	KJ408380	KJ408331
<i>Schiffneriolejeunea nymannii</i> (Steph.) Gradst. & Terken	Malaysia, Gradstein <i>et al.</i> 10321 (GOET)	DQ983725	DQ987424	DQ987320
<i>Siphonolejeunea elegantissima</i> (Steph.) Grolle	Australia, Pócs & Brown 0026/AA (E)	DQ983726	DQ987452	DQ987352
<i>Spruceanthus thozetianus</i> (Gottsche & F. v. Müll.) B.M.Thiers	Australia, Pócs 01107/M (GOET)	AM384877	DQ987460	DQ987362
<i>Thysananthus spathulistipus</i> (Reinw. <i>et al.</i> ) Lindenb.	Bali, Schäfer-Verwimp & Verwimp 20790 (GOET)	DQ983739	DQ987392	DQ987282
<i>Vitalianthus aphanellus</i> (Spruce) Bechteler <i>et al.</i>	Brazil, Yano & Zartman 32771 (SP)	KT626920	-----	KT626900
<i>Vitalianthus bischlerianus</i> (K.C.Pôrto & Grolle) R.M.Schust. & Giancotti	Brazil (I), Schäfer-Verwimp & Verwimp 9505 (M)	KT626921	-----	KT626901
<i>Vitalianthus bischlerianus</i>	Brazil (II), Schäfer-Verwimp & Verwimp 12913 (M)	-----	-----	KT626902
<i>Xylolejeunea crenata</i> (Mont.) Xiao L.He & Grolle	Ecuador, Schäfer-Verwimp & Nebel 32827/A (M)	KJ408356	KJ408382	KJ408333
<i>Xylolejeunea grolleana</i> (Pócs) Xiao L.He & Grolle	Madagascar, Pócs & Szabó 9878/EM (EGR)	KT626911	KT626928	KT626892

### Phylogenetic analyses

jModelTest2 (Darriba *et al.* 2012) was used to determine the best fit models of evolution according to the Akaike Information Criterion (Akaike 1973). This resulted in a TVM+I+G model for the *trnL-trnF* region and in a GTR+I+G model for *rbcL*, ITS, as well as the dataset comprising all three markers.

Maximum likelihood (ML) analyses were conducted using RAxML 8.2.4 (Stamatakis 2014). Since the three markers showed no incongruence when analysed separately, the single datasets were combined. Clades with bootstrap values (BP) of 70-94 % were regarded as moderately supported and those with BP  $\geq$  95 % as strongly supported (Erixon *et al.* 2003). For the final run the dataset was partitioned by markers and a GTR+G model was employed for each of them, following the recommendation given by jModelTest and Stamatakis (2016). Ten thorough ML searches in combination with the multi-parametric bootstrapping using the autoMRE function (Pattengale *et al.* 2010) were executed.

MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003) was used for Bayesian Inference (BI). Again the dataset was partitioned by markers and the GTR+I+G model as suggested by jModelTest was used for each partition. Two Metropolis-coupled Markov Chain Monte Carlo (MCMC) analyses, including three heated chains and one cold chain, were run for 10 million generations, sampled every 1000 generations. TRACER 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) was used to check for convergence and stationarity and an average standard deviation of split frequency below 0.01 indicated a sufficiently long run. The initial 25 % of sampled trees were discarded as burn-in. The remainder was summarized with TreeAnnotator 1.8.2 (Drummond *et al.* 2012). The resulting maximum clade credibility (MCC) tree was visualized using FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bayesian Posterior Probability (PP) values were regarded as significant when PP  $\geq$  0.95 (Larget & Simon 1999).

### Morphology

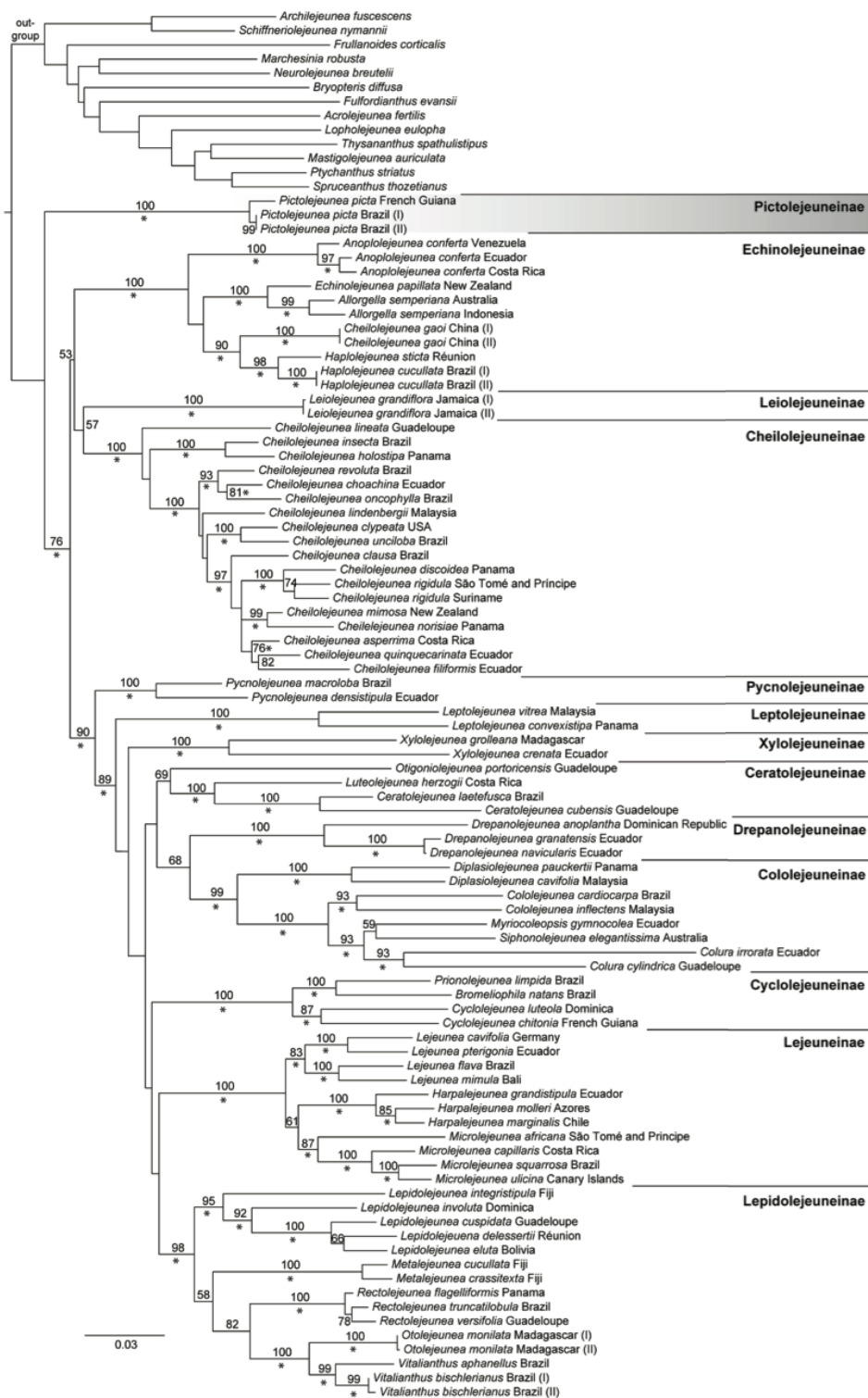
The DNA vouchers of *Pictolejeunea picta* were studied under a Carl Zeiss AxioScope A1 compound microscope equipped with a Canon 60D digital camera. The images printed in Fig. 2 are digitally stacked photomicrographic composites of up to 12 individual focal planes obtained using the software package HeliconFocus 6.7.1.

## Results

The ML and BI analyses recovered highly concordant phylogenetic hypotheses (Fig. 1). A clade with three accessions of *Pictolejeunea picta* (BP=100, PP=1.00) was placed sister to the remainder of Lejeuneae (BP=76, PP=1.00). This remainder split into two main lineages. A lineage with BP=53 and PP=0.86 included members of Cheilolejeuneinae, Echinolejeuneinae and Leiolejeuneinae. *Cheilolejeunea gaoi* Zhu *et al.* (2000: 499) was resolved in Echinolejeuneinae rather than Cheilolejeuneinae and placed sister to *Haplolejeunea* Grolle (1975: 205) (BP=90; PP=1.00). The other lineage (BP=90, PP=1.00) included representatives of Ceratolejeuneinae, Cololejeuneinae, Cyclolejeuneinae, Drepanolejeuneinae, Lejeuneinae, Lepidolejeuneinae, Leptolejeuneinae, Pycnolejeuneinae and Xylolejeuneinae. Pycnolejeuneinae were separated from the remainder of this lineage with a BP=89 and PP=1.00. All subtribes of Lejeuneae except Ceratolejeuneinae (BP=69, PP=0.82) achieved BP percentage values of 98-100 and PP of 1.00.

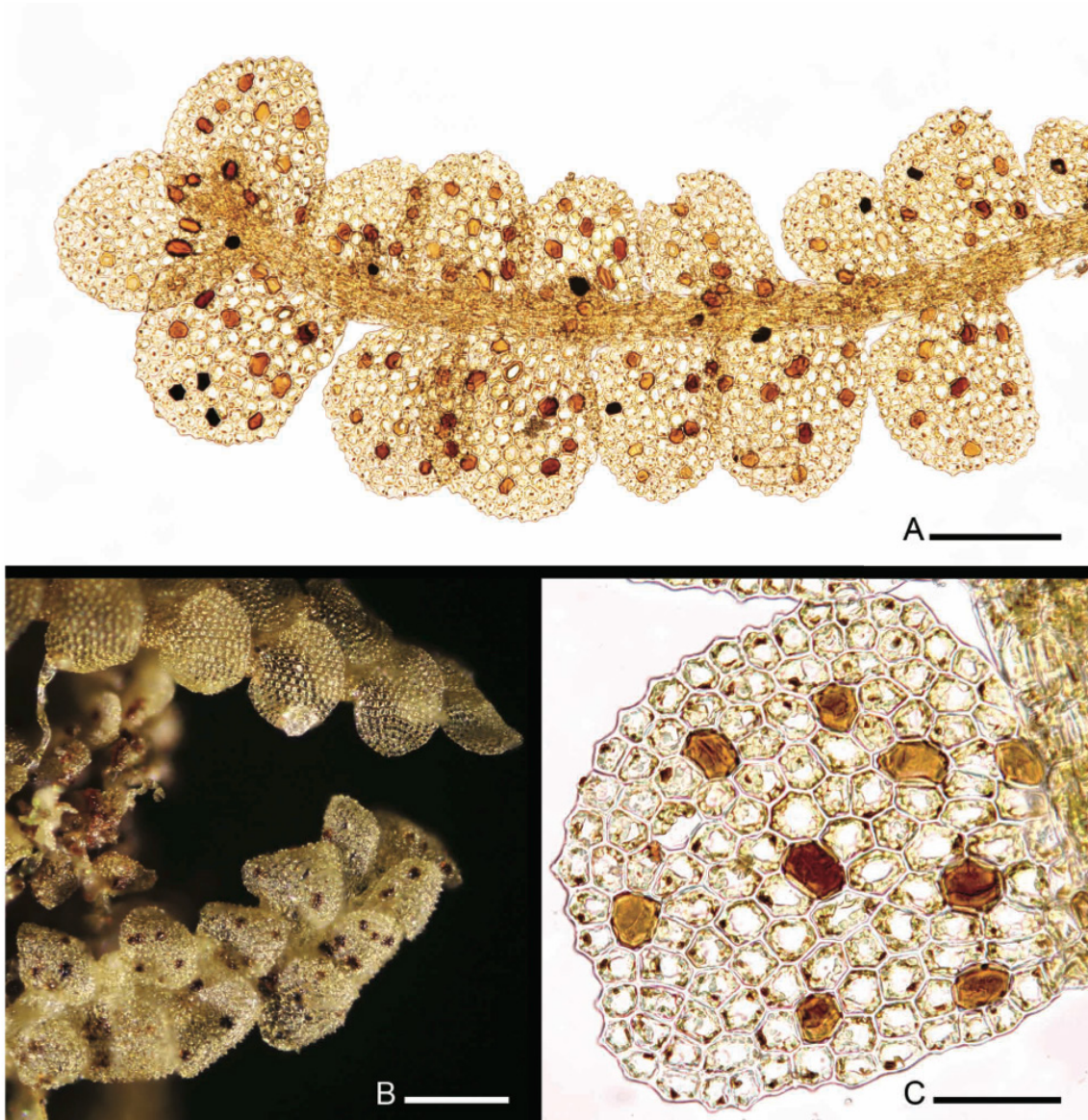
## Discussion

Our sampling includes 30 genera of Lejeuneae and thus represents the most comprehensive generic sampling of this tribe available so far. As *Pictolejeunea* was resolved sister to the remainder of Lejeuneae it should be placed in its own subtribe (see taxonomic treatment). Members of *Pictolejeunea* were originally placed within *Prionolejeunea* Spruce (1884: 152) Schiffner (1893: 127) or *Cheilolejeunea* yet these genera differ by their lack of brownish or reddish ocelli and their marginal lobule papillae. The papillae is on the free lobule margin, not positioned inside of the lobule as in *Pictolejeunea* (Grolle 1977). Ental papillae are known from several representatives of Ptychanthoideae, however, they also occur in the lejeuneoid genera *Diplasiolejeunea* (Spruce 1884: 301) Schiffner (1893: 121) and *Tuyamaella* Hattori (1951: 60) (Grolle 1977). Brownish or reddish ocelli are also not an exclusive character of *Pictolejeunea* but also occur in *Leptolejeunea moniliata* Stephani (1913b: 371) (Gradstein & Costa 2003) and *Leptolejeunea amphiophthalmia* Zwickel (1933: 117) [Yang & Lin 2008, as *Leptolejeunea picta* Herzog (1942: 430)].



**FIGURE 1** Most likely phylogram resulting from maximum likelihood analysis of the nrITS-*trnL-trnF-rbcL* dataset. Bootstrap percentage values  $\geq 50$  are indicated at branches. Stars indicate Bayesian Posterior Probability values  $\geq 0.95$ .

*Pictolejeunea* somewhat resembles *Cheilolejeunea gaoi* “in delicate plants without secondary pigmentation, absence of gynoeical innovation, obsolete first tooth and indistinct second tooth of leaf lobule, and thin-walled cells without trigones and intermediate thickenings” (Zhu *et al.* 2000). However, according to the latter authors, *Cheilolejeunea gaoi* differs from *Pictolejeunea* in its distant, marginal hyaline papilla and the absence of ocelli in any part of the plant. According to our phylogenetic hypothesis, it is not closely related to *Pictolejeunea*. Ye *et al.* (2015) already demonstrated that *Cheilolejeunea gaoi* does not belong to *Cheilolejeunea* s.l.; however, our extended sampling indicates a close relationship with *Haplolejeunea* (Echinolejeuneinae), a genus that differs from *C. gaoi* in the presence of ocelli and pycnolejeuneoid innovations, and a lack of teeth on the free margin of the lobule (Gradstein *et al.* 2001). Considering these morphological differences, it may be appropriate to either erect a new genus to accommodate this species or alternatively to reassign this species to genus *Haplolejeunea*. However, this hypothesis should be confirmed with extended taxon sampling.



**FIGURE 2** *Pictolejeunea picta* **A.** Top of shoot in ventral view. Note the reddish brown ocelli in leaves and underleaves. **B.** Portion of dried herbarium specimen. Note the ocelli and papillose leaf surface of *Pictolejeunea*. The upper plant belongs to *Lejeunea* sp. **C.** Leaf with ten scattered ocelli (A, C from DNA voucher *Lopes 202*; B from DNA voucher *Lopes 192*; scale bars A, 1.5 mm; B, 200  $\mu$ m; C, 50  $\mu$ m).

### Perspectives

The classification of Lejeuneaceae is hampered by extensive morphological homoplasy (Gradstein *et al.* 2003, Dong *et al.* 2012, Heinrichs *et al.* 2013, Bechteler *et al.* 2016) which is also evident from the numerous taxonomical improvements since the last comprehensive supraspecific classification of the family by Gradstein (2013). Our generic sampling includes only the Neotropical type species *Pictolejeunea picta* since no material of the other five *Pictolejeunea* species was available for molecular investigation. An extension of the sampling is necessary to confirm the current genus concept of *Pictolejeunea* and its disjunct range. Of special interest are the Bornean *Pictolejeunea mizutanii* Grolle (1977: 255), the Cuban *Pictolejeunea levis* Grolle & Reiner-Drehwald (2005: 81), the only species of the genus with (lejeuneoid) innovations, and the Venezuelan *P. reginae* Ilkiu-Borges (2002: 318) which has entire underleaves and was placed in its own subgenus *Neopictolejeunea* Ilkiu-Borges (2002: 320).

### Taxonomic treatment

**Lejeuneaceae** subtribe **Pictolejeuneinae** Bechteler, G.E.Lee, Schäf.-Verw., D.F.Peralta, M.A.M.Renner & Heinrichs, subtr. nov.

Typus: *Pictolejeunea* Grolle (Fig. 2)

Plants incubous with lateral branches, stem hyalodermis and ventral merophyte two cells wide; complicate-bilobed leaves with wide spreading lobes and lobules connected to lobes with a long keel; ental lobule papilla present, differentiated lobule teeth lacking, scattered pale brown to reddish ocelli in leaves, underleaves, involucre and perianths. Gynoecia usually without innovations; perianths somewhat flattened with two broad lateral keels expanded into short auricles.

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### References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.  
[http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2)
- Akaike, H. (1973) Information theory as an extension of the maximum likelihood principle. In: Petrov, B.N. & Csáki, F. (Eds.) *Second International Symposium on Information Theory*, Akadémiai Kiadó, Budapest, pp. 267–281.
- Bechteler, J., Lee, G.E., Schäfer-Verwimp, A., Pócs, T., Peralta, D.F., Renner, M.A.M., Schneider, H. & Heinrichs, J. (2016) Towards a monophyletic classification of Lejeuneaceae IV: reinstatement of *Allorgella*, transfer of *Microlejeunea aphanella* to *Vitalianthus* and refinements of the subtribal classification. *Plant Systematics and Evolution* 302: 187–201.  
<http://dx.doi.org/10.1007/s00606-015-1252-8>
- Benedix, E.H. (1953) Indomalayische Cololejeuneen. Eine Revision tropischer Lebermoose. *Feddes Repertorium Beiheft* 134: 1–86.
- Crandall-Stotler, B., Stotler, R.E. & Long, D.G. (2009) Phylogeny and classification of the Marchantiophyta. *Edinburgh Journal of Botany* 66: 155–198.  
<http://dx.doi.org/10.1017/S0960428609005393>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.  
<http://dx.doi.org/10.1038/nmeth.2109>
- Dong, S., Schäfer-Verwimp, A., Meineke, P., Feldberg, K., Bombosch, A., Pócs, T., Schmidt, A.R., Reitner, J., Schneider, H. & Heinrichs, J. (2012) Tramps, narrow endemics and morphologically cryptic species in the epiphyllous liverwort genus *Diplasiolejeunea*. *Molecular Phylogenetics and Evolution* 65: 582–594.

- <http://dx.doi.org/10.1016/j.ympbev.2012.07.009>
- Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.  
<http://dx.doi.org/10.1093/molbev/mss075>
- Dumortier, B.C.J. (1831) *Sylloge Jungermannidearum Europae indigenarum: earum genera et species systematice complectens*. Casterman, Tournay, 100 pp.  
<http://dx.doi.org/10.5962/bhl.title.22343>
- Dumortier, B.C.J. (1835) *Recueil d'Observations sur les Jungermanniacées*. Blanquart, Tournay, 27 pp.  
<http://dx.doi.org/10.5962/bhl.title.731>
- Erixon, P., Svennblad, B., Britton, T. & Oxelman, B. (2003) Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* 52: 665–673.  
<http://dx.doi.org/10.1080/10635150390235485>
- Evans, A.W. (1903) Hepaticae of Puerto Rico III. *Harpalejeunea*, *Cyrtolejeunea*, *Euosmolejeunea* and *Trachylejeunea*. *Bulletin of the Torrey Botanical Club* 30: 544–563.  
<http://dx.doi.org/10.2307/2478516>
- Evans, A.W. (1906) Hepaticae of Puerto Rico VI. *Cheilolejeunea*, *Rectolejeunea*, *Cystolejeunea*, and *Pycnolejeunea*. *Bulletin of the Torrey Botanical Club* 33: 1–25.  
<http://dx.doi.org/10.2307/2478618>
- Evans, A.W. (1907) *Leucolejeunea*, a new genus of Hepaticae. *Torreyia* 7: 225–229.
- Goebel, K.I.E. (1888) Morphologische und biologische Studien I: Über epiphytische Farne und Muscineen. *Annales du Jardin Botanique de Buitenzorg* 7: 1–73.
- Gottsche, C.M., Lindenbergh, J.B.W. & Nees von Esenbeck, C.G. (1845) *Synopsis Hepaticarum*, Part 2. Meissner, Hamburg, pp. 145–304.
- Gradstein, S.R. (1997) The taxonomic diversity of epiphyllous bryophytes. *Abstracta Botanica* 21: 15–19.
- Gradstein, S.R. (2013) A classification of Lejeuneaceae (Marchantiophyta) based on molecular and morphological evidence. *Phytotaxa* 100 (1): 6–20.  
<http://dx.doi.org/10.11646/phytotaxa.100.1.2>
- Gradstein, S.R. & Costa, D.P. (2003) The Hepaticae and Anthocerotae of Brazil. *Memoirs of the New York Botanical Garden* 87: 1–318.
- Gradstein, S.R., Churchill, S.P. & Salazar-Allen, N. (2001) Guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden* 86: 1–577.
- Gradstein, S.R., Reiner-Drehwald, M.E. & Schneider, H. (2003) A phylogenetic analysis of the genera of Lejeuneaceae (Hepaticae). *Botanical Journal of the Linnean Society* 143: 391–410.  
<http://dx.doi.org/10.1111/j.1095-8339.2003.00235.x>
- Gradstein, S.R., Wilson, R., Ilkiu-Borges, A.L. & Heinrichs, J. (2006) Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Botanical Journal of the Linnean Society* 151: 293–308.  
<http://dx.doi.org/10.1111/j.1095-8339.2006.00531.x>
- Grolle, R. (1975) *Haplolejeunea* aus Madagaskar - eine weitere neue Gattung der Tuyamaelloideae. *Journal of the Hattori Botanical Laboratory* 39: 203–210.
- Grolle, R. (1977) *Pictolejeunea* - eine neue Gattung der Lejeuneoideae aus der Neotropis und Borneo. *Feddes Repertorium* 88: 247–256.  
<http://dx.doi.org/10.1002/fedr.19770880402>
- Grolle, R. & Reiner-Drehwald, M.E. (2005) *Pictolejeunea levis*, a new species of Lejeuneaceae from Cuba. *Journal of Bryology* 27: 281–283.  
<http://dx.doi.org/10.1179/174328205X70038>
- Hattori, S. (1951) Contributio ad floram hepaticarum Yakusimensis, V. *Journal of the Hattori Botanical Laboratory* 5: 43–68.
- He, Q. & Zhu, R.L. (2011) Spore output in selected species of Lejeuneaceae. *Cryptogamie, Bryologie* 32: 107–112.  
<http://dx.doi.org/10.7872/cryb.v32.iss1.2011.107>
- Heinrichs, J., Dong, S., Yu, Y., Schäfer-Verwimp, A., Pócs, T., Feldberg, K., Hentschel, J., Schmidt, A.R. & Schneider, H. (2011) A 150 year old mystery solved: Transfer of the rheophytic liverwort *Myriocolea irrorata* to *Colura*. *Phytotaxa* 66 (1): 55–64.  
<http://dx.doi.org/10.11646/phytotaxa.66.1.9>
- Heinrichs, J., Dong, S., Feldberg, K., Schäfer-Verwimp, A. & Schmidt, A.R. (2012) *Sphaerolejeunea* is a synonym of *Lejeunea*. *Phytotaxa* 69 (1): 7–15.  
<http://dx.doi.org/10.11646/phytotaxa.69.1.3>
- Heinrichs, J., Dong, S., Schäfer-Verwimp, A., Pócs, T., Feldberg, K., Czumaj, A., Schmidt, A.R., Reitner, J., Renner, M.A.M., Hentschel, J., Stech, M. & Schneider, H. (2013) Molecular phylogeny of the leafy liverwort *Lejeunea* (Porellales): Evidence for a Neotropical



- origin, uneven distribution of sexual systems and insufficient taxonomy. *PLoS ONE* 8: e82547.  
<http://dx.doi.org/10.1371/journal.pone.0082547>
- Heinrichs, J., Czumaj, A., Dong, S., Scheben, A., Schäfer-Verwimp, A., Peralta, D.F., Feldberg, K., Schmidt, A.R. & Schneider, H. (2014a) The Bromeliaceae tank dweller *Bromeliophila* (Lejeuneaceae, Porellales) is a member of the *Cyclolejeunea-Prionolejeunea* clade. *Plant Systematics and Evolution* 300: 63–73.  
<http://dx.doi.org/10.1007/s00606-013-0860-4>
- Heinrichs, J., Dong, S., Schäfer-Verwimp, A., Peralta, D.F., Feldberg, K., Schmidt, A.R. & Schneider, H. (2014b) Towards a monophyletic classification of Lejeuneaceae I: subtribes Pycnolejeuneinae and Xylolejeuneinae subtr. nov., transfer of *Otolejeunea* to Lepidolejeuneinae, and generic refinements. *Phytotaxa* 163 (2): 61–76.  
<http://dx.doi.org/10.11646/phytotaxa.163.2.1>
- Heinrichs, J., Schäfer-Verwimp, A., Czumaj, A., Dong, S., Scheben, A., Feldberg, K. & Schneider, H. (2014c) Towards a monophyletic classification of Lejeuneaceae I: subtribe Leptolejeuneinae subtr. nov. *Phytotaxa* 156 (3): 165–174.  
<http://dx.doi.org/10.11646/phytotaxa.156.3.7>
- Herzog, T. (1938) *Sphaerolejeunea*, eine neue Gattung der Lejeuneaceae Schizostipae. *Annales Bryologici* 11: 86–89.
- Herzog, T. (1942) Revision der Lebermoosgattung *Leptolejeunea* Spr. in der Indomalaya. *Flora Neue Folge* 35: 377–434.
- Ilkiu-Borges, A.L. (2002) *Pictolejeunea reginae*, a new species of Lejeuneaceae (Hepaticae) from Venezuela. *Brittonia* 54: 318–321.  
[http://dx.doi.org/10.1663/0007-196X\(2003\)54\[318:PRANSO\]2.0.CO;2](http://dx.doi.org/10.1663/0007-196X(2003)54[318:PRANSO]2.0.CO;2)
- Ilkiu-Borges, A.L. (2005) A taxonomic monograph of the genus *Prionolejeunea* (Lejeuneaceae, Jungermanniopsida). Cuvillier, Göttingen.
- Kis, G. & Pócs, T. (2001) What is *Chondriolejeunea*? *Cryptogamie Bryologie* 22: 237–246.  
[http://dx.doi.org/10.1016/S1290-0796\(01\)01076-8](http://dx.doi.org/10.1016/S1290-0796(01)01076-8)
- Larget, B. & Simon, D.L. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750–759.  
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026160>
- Libert, M. (1820) Sur un genre nouveau d'Hépatiques, *Lejeunia*. *Annales Générales des Sciences Physiques* 6: 372–374.
- Lücking, A. (1995) *Diversität und Mikrohabitatpräferenzen epiphyller Moose in einem tropischen Regenwald in Costa Rica unter besonderer Berücksichtigung der Familie Lejeuneaceae*. Dissertation University of Ulm.
- Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R., Moret, B.M. & Stamatakis, A. (2010) How many bootstrap replicates are necessary? *Journal of Computational Biology* 17: 337–354.  
<http://dx.doi.org/10.1089/cmb.2009.0179>
- Pócs, T. (1996) Epiphyllous liverwort diversity at worldwide level and its threat and conservation. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Serie Botánica* 67: 109–127.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.  
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Schäfer-Verwimp, A., Feldberg, K., Dong, S., Van Melick, H., Peralta, D.F., Schmidt, A.R., Schneider, H. & Heinrichs, J. (2014) Towards a monophyletic classification of Lejeuneaceae III: the systematic position of *Leiolejeunea*. *Phytotaxa* 170 (3): 187–193.  
<http://dx.doi.org/10.11646/phytotaxa.170.3.4>
- Schiffner, V. (1893) Hepaticae. In: Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien* 1. Engelmann, Leipzig, pp. 97–141.
- Schuster, R.M. (1970) Studies on Hepaticae, XLIX–LIII. New Lejeuneaceae from Dominica and Jamaica. *Bulletin of the Torrey Botanical Club* 97: 336–352.  
<http://dx.doi.org/10.2307/2483854>
- Schuster, R.M. (1978) Studies on Venezuelan Hepaticae, II. *Phytologia* 39: 425–432.  
<http://dx.doi.org/10.5962/bhl.part.7622>
- Schuster, R.M. (1992) The oil-bodies of the Hepaticae. II. Lejeuneaceae (part 2). *Journal of the Hattori Botanical Laboratory* 72: 163–359.
- Schuster, R.M. (1994) Studies on Lejeuneaceae, I. Preliminary studies on new genera of Lejeuneaceae. *Journal of the Hattori Botanical Laboratory* 75: 211–235.
- Schuster, R.M. & Giancotti, C. (1993) On *Vitalianthus* Schust. & Giancotti, a new genus of Lejeuneaceae. *Nova Hedwigia* 57: 445–456.
- Söderström, L., Hagborg, A., Von Konrat, M., Bartholomew-Began, S., Bell, D., Briscoe, L., Brown, E., Cargill, D.C., Cooper, E.D., Costa, D.P., Crandall-Stotler, B.J., Dauphin, G., Engel, J.J., Feldberg, K., Glenny, D., Gradstein, S.R., He, X., Heinrichs, J., Hentschel, J., Ilkiu-Borges, A.L., Katagiri, T., Konstantinova, N.A., Larrain, J., Long, D.G., Nebel, M., Pócs, T., Puche, F., Reiner-Drehwald, M.E., Renner, M.A.M., Sass-Gyarmati, A., Schäfer-Verwimp, A., Segarra-Moragues, J.G., Stotler, R.E., Sukkharak, P., Thiers, B.M., Uribe, J. Váña, J., Villarreal, J.C., Wigginton, M., Zhang, L. & Zhu, R.L. (2016) World checklist of hornworts and liverworts.

- PhytoKeys* 59: 1–828.  
<http://dx.doi.org/10.3897/phytokeys.59.6261>
- Spruce, R.M. (1884) Hepaticae Amazonicae et Andinae. Tribus I: Jubuleae. *Transactions and Proceedings of the Botanical Society of Edinburgh* 15: 1–308.
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.  
<http://dx.doi.org/10.1093/bioinformatics/btu033>
- Stamatakis, A. (2016) The RAxML v8.2.X Manual. Heidelberg Institute for Theoretical Studies. Available from: <http://sco.h-its.org/exelixis/web/software/raxml/#documentation> (accessed 20 July 2016)
- Stephani, F. (1890) Hepaticae africanae novae in insulis Bourbon, Maurice et Madagascar lectae. *Botanical Gazette* 15: 281–292.  
<http://dx.doi.org/10.1086/326585>
- Stephani, F. (1913a) *Prionolejeunea*. Species Hepaticarum V, Georg & Cie, Geneva, pp. 196–228.
- Stephani, F. (1913b) *Leptolejeunea*. Species Hepaticarum V, Georg & Cie, Geneva, pp. 360–390.
- Tixier, P. (1980) Deux nouveaux genres de Lejeunéacées, *Otolejeunea* Grolle & P. Tx. et *Allorgella* P. Tx. *Nova Hedwigia* 32: 607–622.
- Vanden Berghen, C. (1948) Un nouveau genre d'Hépatiques *Evansiolejeunea* nov. gen. *Revue Bryologique et Lichénologique* 17: 86–90.
- Wang, J., Zhu, R.-L. & Gradstein, S.R. (2016) Taxonomic revision of Lejeuneaceae subfamily Ptychanthoideae (Marchantiophyta) in China. *Bryophytorum Bibliotheca* 65: 1–142.
- Wilson, R., Gradstein, S.R., Schneider, H. & Heinrichs, J. (2007) Unravelling the phylogeny of Lejeuneaceae (Jungermanniopsida): evidence for four main lineages. *Molecular Phylogenetics and Evolution* 43: 270–282.  
<http://dx.doi.org/10.1016/j.ympev.2006.10.017>
- Yang, J.-D. & Lin, S.H. (2008) *Leptolejeunea picta* Herz., a liverwort new to Taiwan. *Taiwania* 53: 308–310.
- Ye, W., Wei, Y.M., Schäfer-Verwimp, A. & Zhu, R.L. (2013) Phylogenetic position of *Oryzolejeunea* (Lejeuneaceae, Marchantiophyta): Evidence from molecular markers and morphology. *Journal of Systematics and Evolution* 51: 468–475.  
<http://dx.doi.org/10.1111/j.1759-6831.2012.00238.x>
- Ye, W., Gradstein, S.R., Shaw, A.J., Shaw, B., Ho, B.C., Schäfer-Verwimp, A., Pócs, T., Heinrichs, J. & Zhu, R.L. (2015) Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers. *Cryptogamie Bryologie* 36: 313–333.  
<http://dx.doi.org/10.7872/cryb/v36.iss4.2015.313>
- Yu, Y., Pócs, T., Schäfer-Verwimp, A., Heinrichs, J., Zhu, R.L. & Schneider, H. (2013) Evidence for rampant homoplasy in the phylogeny of the epiphyllous liverwort genus *Cololejeunea* (Lejeuneaceae). *Systematic Botany* 38: 1–11.  
<http://dx.doi.org/10.1600/036364413X670304>
- Zhu, R.L., So, M.L. & Grolle, R. (2000) *Cheilolejeunea gaoi* (Hepaticae, Lejeuneaceae), a new species from Guangxi, China. *The Bryologist* 103: 499–522.  
[http://dx.doi.org/10.1639/0007-2745\(2000\)103\[0499:CGHLAN\]2.0.CO;2](http://dx.doi.org/10.1639/0007-2745(2000)103[0499:CGHLAN]2.0.CO;2)
- Zwicker, W. (1933) Zwei neue Gattungen, einige neue Arten und Umstellungen bei den Lejeuneaceen. *Annales Bryologici* 6: 105–121.

## Chapter 4

### GEOGRAPHICAL STRUCTURE, NARROW SPECIES RANGES AND CENOZOIC DIVERSIFICATION IN A PANTROPICAL CLADE OF EPIPHYLLOUS LEAFY LIVERWORTS.

Bechteler, J., A. Schäfer-Verwimp, G.E. Lee, K. Feldberg, O.A.  
Pérez-Escobar, T. Pócs, D.F. Peralta, M.A.M. Renner, and J.  
Heinrichs

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## ORIGINAL RESEARCH

# Geographical structure, narrow species ranges, and Cenozoic diversification in a pantropical clade of epiphyllous leafy liverworts

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## Abstract

The evolutionary history and classification of epiphyllous cryptogams are still poorly known. *Leptolejeunea* is a largely epiphyllous pantropical liverwort genus with about 25 species characterized by deeply bilobed underleaves, elliptic to narrowly obovate leaf lobes, the presence of ocelli, and vegetative reproduction by cladia. Sequences of three chloroplast regions (*rbcl*, *trnL-F*, *psbA*) and the nuclear ribosomal ITS region were obtained for 66 accessions of *Leptolejeunea* and six outgroup species to explore the phylogeny, divergence times, and ancestral areas of this genus. The phylogeny was estimated using maximum-likelihood and Bayesian inference approaches, and divergence times were estimated with a Bayesian relaxed clock method. *Leptolejeunea* likely originated in Asia or the Neotropics within a time interval from the Early Eocene to the Late Cretaceous (67.9 Ma, 95% highest posterior density [HPD]: 47.9–93.7). Diversification of the crown group initiated in the Eocene or early Oligocene (38.4 Ma, 95% HPD: 27.2–52.6). Most species clades were established in the Miocene. *Leptolejeunea epiphylla* and *L. schiffneri* originated in Asia and colonized African islands during the Plio-Pleistocene. Accessions of supposedly pantropical species are placed in different main clades. Several monophyletic morphospecies exhibit considerable sequence variation related to a geographical pattern. The clear geographic structure of the *Leptolejeunea* crown group points to evolutionary processes including rare long-distance dispersal and subsequent speciation. *Leptolejeunea* may have benefitted from the large-scale distribution of humid tropical angiosperm forests in the Eocene.

## KEYWORDS

ancestral area estimation, bryophyte, cryptic speciation, divergence time estimation, epiphyte, *Leptolejeunea*, phylogeny.

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## 1 | INTRODUCTION

Range estimation is a challenging theme in morphologically little differentiated groups of organisms and suitable to improve understanding of species diversity and evolution. Many bryophyte genera belong to these critical groups and are in need of thorough reinvestigation including integrative molecular–morphological approaches; however, to date, only a limited number of studies is available (Dong et al., 2012; Forrest, Salazar-Allen, Gudiño, Korpelainen, & Long, 2011; Hedenäs et al., 2014; Heinrichs et al., 2015; Renner et al., 2013; Vanderpoorten, Patiño, Dirkse, Blockeel, & Hedenäs, 2015; Vigalondo et al., 2016). These studies identified numerous morphologically not or weakly differentiated bryophyte species of which many have rather narrow ranges.

Prior to the advent of DNA-based investigations, many bryophyte species were considered to have broad, often intercontinental ranges equivalent to the ranges of angiosperm genera (Shaw, 2001; Vanderpoorten, Gradstein, Carine, & Devos, 2010). The intercontinental distributions of these species were interpreted as a vicariant pattern within species of ancient origin (Schuster, 1983) some of which were thought to date back to the Jurassic (Stotler & Crandall-Stotler, 1974). However, inferences of Mesozoic ages of bryophyte species have been contradicted by DNA-based divergence time estimates that have identified crown-group diversification events within the Cenozoic in many lineages (Cooper, Henwood, & Brown, 2012; Feldberg et al., 2014; Laenen et al., 2014; Wilson, Heinrichs, Hentschel, Gradstein, & Schneider, 2007). Divergence time estimates suggest long-distance dispersal (LDD) is more likely than vicariance as the process resulting in extant intercontinental ranges (Devos & Vanderpoorten, 2009; Dong et al., 2012; Hartmann, Wilson, Gradstein, Schneider, & Heinrichs, 2006; Scheben, Bechteler, Lee, Pócs, Schäfer-Verwimp, & Heinrichs, 2016; Sun, He, & Glenn, 2014). Divergence time estimates also suggested an important role of angiosperm-dominated forests in shaping the diversity of epiphyllous cryptogams (Feldberg et al., 2014).

*Leptolejeunea* (Spruce) Steph. is a pantropical genus of nearly exclusively epiphyllous leafy liverworts that grow in lowland and lower montane rainforests, occasionally also in high montane rainforests up to ca. 3,000 m (Bischler, 1969). The genus includes both local endemics (Shu, Zhu, & Pócs, 2016) and intercontinentally distributed species such as *L. elliptica*, *L. epiphylla*, and *L. maculata* (Grolle, 1976; Pócs & Lye, 1999; Schuster, 1980; Zhu & So, 2001). *Leptolejeunea* is characterized by its minute size, deeply bilobed underleaves with two widely divergent and subulate lobes, elliptic to narrowly obovate leaf lobes often with dentate margins, the presence of one to several ocelli in leaf lobes, and vegetative reproduction by cladia (Figure 1). Several species show a tendency for dry leaves to become elevated and produce monoterpenes that emit a strong fragrance (Gradstein, Churchill, & Salazar-Allen, 2001) meaning the genus can be readily identified even in the field; yet identification of species is notoriously difficult. Söderström et al. (2016) accepted 48 species but indicated knowledge problems or serious doubts about the taxonomic value of many. An earlier study estimated global diversity at 25 species (Gradstein et al.,

2001). So far, only a few accessions have been included in molecular phylogenetic studies (Ahonen, Muonen, & Piippo, 2003; Heinrichs et al., 2014; Wilson, Gradstein, Schneider, & Heinrichs, 2007). Results from these studies rejected a previously hypothesized close relationship between *Leptolejeunea* and *Drepanolejeunea* based on shared underleaf shape and the presence of ocelli in leaves of both genera (Gradstein, 2013), and resolved *Leptolejeunea* in a relatively isolated position within Lejeuneaceae subf. Lejeuneoideae (Heinrichs et al., 2014). Lejeuneaceae subtribe Leptolejeuneinae was established as a result to accommodate *Leptolejeunea* (Heinrichs et al., 2014). However, molecular phylogenetic investigations conducted to date have not improved current morphology-based species concepts nor resolved biogeographic patterns.

Currently, in contradiction to more traditional views of morphological species, widespread *Leptolejeunea* species are believed to be the result of recent LDD out of Asia, a hypothesis promoted by Schuster (1983: 618): “taxa such as *Leptolejeunea elliptica*... have shown dispersal, clearly in geologically recent times, well out from Asia into the Pacific, to South America, Central and southern North America.” Here, we extend the sampling of Heinrichs et al. (2014) and test previous hypotheses on origins and extant distribution of *Leptolejeunea* species. We provide evidence for a Cenozoic origin of the *Leptolejeunea* crown group and reject pantropical species ranges.

## 2 | MATERIALS AND METHODS

### 2.1 | Taxon sampling, DNA extraction, PCR amplification, sequencing, and alignment

Tissue for DNA extraction was isolated from *Leptolejeunea* specimens from the herbaria EGR, GOET, SP, and Schäfer-Verwimp (SV). Specimens were revised based on literature and considering results from phylogenetic analyses. Total genomic DNA was isolated using the Invisorb Spin Plant Mini Kit (Stratag Molecular GmbH, Berlin, Germany). Four markers were amplified: the nuclear ribosomal internal transcribed spacer region (ITS1–5.8S–ITS2), the chloroplast *rbcL* gene, the *trnL-trnF* region, and the *psbA* gene together with the *psbA-trnH* intergenic spacer. PCR amplification of the first three markers follows Bechteler, Lee, Schäfer-Verwimp, Pócs, et al. (2016). The *psbA/psbA-trnH* region was amplified using the PCR program and primers (*trnK2F*, 510F, 576R, *trnHR*) described in Forrest and Crandall-Stotler (2004). This protocol was modified as follows: 0.4 µL of MyTaq Polymerase (Bioline Reagents Ltd., UK), 11 µL of reaction buffer, 1 µL of upstream primer, 1 µL of downstream primer, and 1 µL of template DNA. The mix was filled up with double-distilled water to a total volume of 50 µL. Representatives of *Pycnolejeunea* and *Xylolejeunea* were chosen as outgroups following phylogenetic hypotheses of Wilson, Gradstein, et al. (2007), Bechteler, Lee, Schäfer-Verwimp, Pócs, et al. (2016) and Bechteler, Lee, Schäfer-Verwimp, Renner, et al. (2016). Corresponding sequences were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>), in addition to published sequences of *Leptolejeunea*. The resulting dataset comprised 66 specimens



**FIGURE 1** Images of two species of *Leptolejeunea*. (a) Habitats of dried herbarium specimen of *Leptolejeunea convexistipa* showing epiphyllous growth on a fern leaf. (b) Leaf of *Leptolejeunea epiphylla* with four ocelli in a broken row indicated by red stars. (c) Part of shoot of *Leptolejeunea convexistipa* focusing on a leaf with one basal ocellus (red star). Note the characteristic underleaf of the genus *Leptolejeunea* at the bottom left corner (black arrowhead)

of *Leptolejeunea* and three specimens each of *Pycnolejeunea* and *Xylolejeunea* (Table 1). All sequences were aligned manually with BioEDIT 7.1.3.0 (Hall, 1999), and ambiguous sites were excluded.

## 2.2 | Phylogenetic analyses

Maximum-likelihood (ML) analyses were conducted using RAxML 8.2.4 (Stamatakis, 2014). The best fit models of evolution selected by JMODELTEST 2 (Darriba, Taboada, Doallo, & Posada, 2012) under the Akaike information criterion (AIC; Akaike, 1973) were as follows: TIM3+I+G for *rbcL*, TPM1uf+G for *trnL-trnF*, TIM3+I+G for *psbA/psbA-trnH*, and TIM3+I+G for *nrITS1-5.8S-ITS2*. These were not available in RAxML so the best fitting overparameterized model, GTR+G, was used for all markers (Posada, 2008). First, all markers were analyzed separately on the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010) using the "thorough ML" option, and an additional analysis was carried out for a combined chloroplast DNA dataset. Clades with bootstrap

values (BP) of 70%–94% were regarded as moderately supported and those with BP  $\geq 95\%$  as strongly supported (Erixon, Svennblad, Britton, & Oxelman, 2003). No strongly supported topological contradictions between single markers or the nuclear and plastid datasets were detected. Accordingly, all matrices were concatenated, resulting in an alignment of 3,694 nucleotide positions. Ten thorough ML searches in combination with multiparametric bootstrapping using the autoMRE function (Pattengale, Alipour, Bininda-Emonds, Moret, & Stamatakis, 2010) were conducted.

Bayesian inference was undertaken with MRBAYES 3.2.6 (Ronquist & Huelsenbeck, 2003) using a partition for each marker and a GTR substitution model with rate of invariable sites and gamma rate heterogeneity as recommended by JMODELTEST 2. Two metropolis-coupled Markov chain Monte Carlo (MCMC) analyses, including three heated chains and one cold chain, were run for 10 million generations, sampled every 1,000 generations. TRACER 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) was used to check for convergence and stationarity,

**TABLE 1** Taxa used in this study, including information about the geographical origin, voucher details, as well as GenBank accession numbers. Accession numbers in bold were obtained from GenBank

Taxon	Origin	Collector, voucher number, and herbarium	GenBank accession numbers			
			<i>rbcL</i>	<i>trnLF</i>	<i>psbA</i>	nrITS
<i>Leptolejeunea amphiothyma</i> Zwickel	Malaysia	Pócs et al. 13168/AA (EGR)	KX808754	KX808806	KY006551	KX808704
<i>Leptolejeunea astroidea</i> (Mitt.) Steph.	Príncipe Island	Shevock 40015A (EGR)	KX808792	KX808851	KY006539	KX808742
<i>L. astroidea</i>	Uganda	Pócs et al. 97108/O (EGR)	KX808791	KX808850	–	KX808741
<i>Leptolejeunea balansae</i> Steph.	Malaysia	Pócs et al. 13184/F (EGR)	KX808777	KX808832	KY006538	KX808725
<i>Leptolejeunea brasiliensis</i> Bischl.	Brazil (I)	Peralta & Carmo 14222 (SP)	KX808758	KX808810	KY006502	KX808708
<i>L. brasiliensis</i>	Brazil (II)	Yano 28424 (SP)	KX808756	KX808808	KY006500	KX808706
<i>L. brasiliensis</i>	Brazil (III)	Peralta & Guiglota 13863 (SP)	KX808757	KX808809	KY006501	KX808707
<i>Leptolejeunea convexistipa</i> Bischl.	Dominican Republic	Schäfer-Verwimp & Verwimp 27206/B (SV)	KX808800	–	KY006540	–
<i>L. convexistipa</i>	Ecuador (I)	Schäfer-Verwimp 24419/C (SV)	KX808799	–	–	KX808748
<i>L. convexistipa</i> published as <i>elliptica</i> (Lehm. & Lindenb.) Schiffn.	Ecuador (II)	Wilson et al. 04-18 (GOET)	<b>DQ983698</b>	–	<b>EF011862</b>	<b>DQ987375</b>
<i>L. convexistipa</i>	Ecuador (III)	Schäfer-Verwimp et al. 24407/E (SV)	KX808798	KX808856	KY006533	KX808747
<i>L. convexistipa</i>	Panama (I)	Schäfer-Verwimp & Verwimp 30861 (JE)	<b>KF954161</b>	<b>KF954151</b>	–	<b>KF954154</b>
<i>L. convexistipa</i>	Panama (II)	Schäfer-Verwimp & Verwimp 30937/A (SV)	KX808801	KX808857	KY006534	KX808749
<i>Leptolejeunea dapitana</i> Steph.	Malaysia (I)	Pócs et al. 13160/Q (EGR)	KX808772	KX808824	KY006513	KX808719
<i>L. dapitana</i>	Malaysia (II)	Pócs et al. 13160/L (EGR)	KX808771	KX808823	KY006512	KX808718
<i>L. dapitana</i>	Vietnam	Luong TP211-004b (EGR)	KX808770	KX808822	KY006511	KX808717
<i>Leptolejeunea elliptica</i> (Lehm. & Lindenb.) Schiffn.	Dominican Republic	Pócs & Pócs 03157/AB (GOET)	KX808795	KX808854	KY006532	KX808744
<i>L. elliptica</i>	Ecuador (I)	Schäfer-Verwimp & Nebel 32794 (SV)	KX808794	KX808853	KY006531	KX808743
<i>L. elliptica</i>	Ecuador (II)	Schäfer-Verwimp & Nebel 32834/A (SV)	KX808797	–	KY006552	KX808746
<i>L. elliptica</i>	Guadeloupe	Schäfer-Verwimp & Verwimp 22518 (SV)	KX808793	KX808852	KY006541	–
<i>L. elliptica</i>	Jamaica	Schäfer-Verwimp 34834/E (SV)	KX808796	KX808855	KY006549	KX808745
<i>Leptolejeunea epiphylla</i> (Mitt.) Steph.	Cambodia	Pócs s.n. (SV)	KX808765	KX808817	KY006546	KX808703
<i>L. epiphylla</i>	Malaysia (I)	Pócs et al. 13172/F (EGR)	KX808764	KX808816	KY006550	KX808713
<i>L. epiphylla</i>	Malaysia (II)	Schäfer-Verwimp & Verwimp 19081 (JE)	<b>KF954163</b>	–	–	<b>KF954156</b>
<i>L. epiphylla</i>	Mayotte	Pócs et al. 9288/AA (EGR)	–	KX808818	KY006508	KX808714
<i>L. epiphylla</i>	Príncipe Island (I)	Shevock 40133 (SV)	KX808767	KX808819	KY006509	KX808715
<i>L. epiphylla</i>	Príncipe Island (II)	Shevock 42132 (EGR)	KX808768	KX808820	KY006545	KX808702

(Continues)



TABLE 1 (Continued)

Taxon	Origin	Collector, voucher number, and herbarium	GenBank accession numbers			
			<i>rbcL</i>	<i>trnLF</i>	<i>psbA</i>	<i>nrITS</i>
<i>L. epiphylla</i>	Indonesia, Sumatra	Schäfer-Verwimp & Verwimp 24962/A (SV)	KX808769	KX808821	KY006510	KX808716
<i>L. epiphylla</i>	Thailand	Schäfer-Verwimp 16245 (SV)	KX808766	–	–	KX808701
<i>Leptolejeunea exocellata</i> (Spruce) A.Evans	Argentina	Schäfer-Verwimp & Verwimp 9330 (GOET)	KX808760	KX808812	KY006504	KX808700
<i>L. exocellata</i>	Dominican Republic (I)	Schäfer-Verwimp & Verwimp 27018/A (SV)	KX808763	KX808815	KY006507	KX808712
<i>L. exocellata</i>	Dominican Republic (II)	Schäfer-Verwimp & Verwimp 27197/A (SV)	KX808761	KX808813	KY006505	KX808710
<i>L. exocellata</i>	Dominican Republic (III)	Schäfer-Verwimp & Verwimp 27215/C (SV)	KX808762	KX808814	KY006506	KX808711
<i>L. exocellata</i>	Ecuador	Schäfer-Verwimp et al. 24407/C (SV)	KX808759	KX808811	KY006503	KX808709
<i>Leptolejeunea foliicola</i> Steph.	Indonesia, Bali	Schäfer-Verwimp & Verwimp 16689/E (SV)	–	KX808843	–	KX808734
<i>L. foliicola</i>	Malaysia (I)	Schäfer-Verwimp & Verwimp 18903/C (SV)	KX808785	KX808842	KY006525	KX808733
<i>L. foliicola</i>	Malaysia (II)	Schäfer-Verwimp & Verwimp 18976 (SV)	KX808786	KX808844	KY006526	KX808735
<i>Leptolejeunea maculata</i> (Mitt.) Schiffn.	Malaysia (I)	Pócs et al. 13171/G (EGR)	KX808782	KX808839	KY006523	KX808731
<i>L. maculata</i>	Malaysia (II)	Pócs et al. 13168/AE (EGR)	KX808783	KX808840	KY006542	–
<i>L. maculata</i>	Malaysia (III)	Pócs et al. 13167/AM (EGR)	–	KX808837	KY006521	KX808729
<i>L. maculata</i>	Malaysia (IV)	Schäfer-Verwimp & Verwimp 18599/A (SV)	KX808781	KX808838	KY006522	KX808730
<i>Leptolejeunea moniliata</i> Steph.	Guadeloupe	Schäfer-Verwimp & Verwimp 22117/A (SV)	KX808755	KX808807	KY006499	KX808705
<i>Leptolejeunea radicata</i> (Nees ex Mont.) Grolle	Dominica	Schäfer-Verwimp & Verwimp 17723/C (JE)	KF954165	–	–	KF954158
<i>L. radicata</i>	Guadeloupe (I)	Schäfer-Verwimp & Verwimp 22305/A (SV)	KX808804	KX808860	KY006537	KX808753
<i>L. radicata</i>	Guadeloupe (II)	Schäfer-Verwimp & Verwimp 22417/E (SV)	KX808803	KX808859	KY006536	KX808751
<i>L. radicata</i>	Guadeloupe (III)	Schäfer-Verwimp & Verwimp 22414/D (SV)	KX808805	–	–	KX808752
<i>L. radicata</i>	Panama	Schäfer-Verwimp & Verwimp 30795 (SV)	KX808802	KX808858	KY006535	KX808750
<i>Leptolejeunea schiffneri</i> Steph.	Malaysia	Schäfer-Verwimp & Verwimp 18619/A (SV)	KX808773	KX808826	KY006548	–
<i>L. schiffneri</i>	Mayotte (I)	Pócs et al. 05106/BK (SV)	KX808776	KX808829	KY006516	KX808722
<i>L. schiffneri</i>	Mayotte (II)	Pócs et al. 05105/E (EGR)	–	KX808830	KY006544	–
<i>L. schiffneri</i>	Indonesia, Sumatra (I)	Schäfer-Verwimp & Verwimp 25233/B (SV)	KX808775	KX808828	KY006515	KX808723
<i>L. schiffneri</i>	Indonesia, Sumatra (II)	Schäfer-Verwimp & Verwimp 25233/B1 (SV)	KX808774	KX808827	KY006547	KX808721
<i>L. schiffneri</i>	Indonesia, Sumatra (III)	Schäfer-Verwimp & Verwimp 25228 (SV)	–	KX808825	KY006514	KX808720

(Continues)

TABLE 1 (Continued)

Taxon	Origin	Collector, voucher number, and herbarium	GenBank accession numbers			
			<i>rbcl</i>	<i>trnLF</i>	<i>psbA</i>	<i>nrlITS</i>
<i>Leptolejeunea</i> spec.	Thailand (I)	Chantanaorrapint 1352 (EGR)	–	KX808831	KY006517	KX808724
<i>Leptolejeunea</i> spec.	Thailand (II)	Schäfer-Verwimp & Verwimp 16177 (SV)	KX808779	KX808835	KY006520	KX808728
<i>Leptolejeunea subacuta</i> Steph. ex A.Evans published as <i>elliptica</i> (Lehm. & Lindenb.) Schiffn.	China	Koponen et al. 50179 (H)	AY125939	AY144480	–	–
<i>L. subacuta</i>	Laos	Peregovits NoLaos/8 (EGR)	KX808789	KX808847	KY006498	KX808737
<i>L. subacuta</i>	Japan, Ryukyu Islands	Yamaguchi 15722 (GOET)	KX808787	KX808845	KY006527	KX808736
<i>L. subacuta</i>	Thailand (I)	Schäfer-Verwimp & Verwimp 23785/C (SV)	–	KX808848	KY006529	KX808739
<i>L. subacuta</i>	Thailand (II)	Schäfer-Verwimp & Verwimp 23791/B (SV)	KX808790	KX808849	KY006530	KX808740
<i>L. subacuta</i>	Thailand (III)	Schäfer-Verwimp & Verwimp 23834/A (SV)	KX808788	KX808846	KY006528	KX808738
<i>Leptolejeunea</i> cf. <i>subrotundifolia</i> Herzog	Madagascar	Pócs & Szabo 9875/AZ (EGR)	KX808780	KX808836	KY006543	–
<i>Leptolejeunea</i> cf. <i>subrotundifolia</i>	Thailand	Pócs & Somadee 1228/C (EGR)	KX808784	KX808841	KY006524	KX808732
<i>Leptolejeunea vitrea</i> (Nees) Schiffn.	Malaysia (I)	Dürhammer D148 (JE)	KF954164	KF954152	–	KF954157
<i>L. vitrea</i>	Malaysia (II)	Pócs et al. 13175/O (EGR)	–	KX808833	KY006518	KX808726
<i>L. vitrea</i>	Philippines	Schumm & Schwarz 6425 (SV)	KX808778	KX808834	KY006519	KX808727
<i>Pycnolejeunea densistipula</i> (Lehm. & Lindenb.) Steph.	Ecuador	Schäfer-Verwimp & Preussing 23368 (GOET)	AY548075	DQ987400	EF011774	DQ987294
<i>Pycnolejeunea macroloba</i> (Nees & Mont.) Schiffn.	Brazil	Yano 32740 (M)	KJ408354	KJ408378	–	KJ408329
<i>Pycnolejeunea sphaeroides</i> (Sande Lac.) J.B.Jack & Steph.	Malaysia	Schäfer-Verwimp & Verwimp 18615/B (M)	KJ408355	KJ408379	–	KJ408330
<i>Xylolejeunea crenata</i> (Nees & Mont.) X.L.He & Grolle	Brazil	Schäfer-Verwimp 11225 (GOET)	DQ983740	DQ987443	EF011822	DQ987341
<i>X. crenata</i>	Ecuador	Schäfer-Verwimp & Nebel 32827/A (M)	KJ408356	KJ408382	–	KJ408333
<i>Xylolejeunea grolleana</i> (Pócs) X.L.He & Grolle	Madagascar	Pócs & Szabó 9878/EM (EGR)	KT626911	KT626928	–	KT626892

TABLE 2 Marginal-likelihood estimations using stepping-stone sampling in BEAST and ln Bayes factor calculation resulting in an uncorrelated log-normal (UCLN) relaxed clock model and a birth–death tree prior accounting for incomplete sampling (BDincompl.) for the *Leptolejeunea* dataset

	Model 1	BDincompl., UCLN	BD, UCLN	Yule, UCLN	BDincompl., strict clock
Model 2	Log marginal likelihood	–17,905.64	–17,911.82	–17,940.59	–17,945.11
BDincompl., UCLN	–17,905.64	0.00	–6.17	–34.95	–39.46
BD, UCLN	–17,911.82	6.17	0.00	–28.78	–33.29
Yule, UCLN	–17,940.59	34.95	28.78	0.00	–4.51
BDincompl., strict clock	–17,945.11	39.46	33.29	4.51	0.00

and an average standard deviation (SD) of split frequency below 0.01 indicated a sufficiently long run. The initial 25% of sampled trees were discarded as burn-in. The remainder were summarized with TREEANNOTATOR 1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012), and the resulting maximum clade credibility (MCC) tree was visualized using FIGTREE 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). BPP values  $\geq 0.95$  were regarded as good support (Larget & Simon, 1999).

### 2.3 | Divergence time estimates and biogeography

Dating analyses were performed using BEAST 1.8.2 (Drummond et al., 2012) using the same partitioning scheme and substitution models as the MrBAYES analyses. An ultrametric starting tree without time scale was generated by setting the ingroup monophyletic, using linked trees over all partitions, 60 million generations and sampling every 6,000 generations. An uncorrelated log-normal (UCLN) relaxed clock and a birth-death prior accounting for incomplete sampling (Stadler, 2009) were used. The result was inspected in TRACER, and ESS values  $>200$  indicated good mixing of the MCMC and a sufficient number of generations. A MCC tree was generated with TREEANNOTATOR 1.8.2 after discarding the first 10% of trees as burn-in and visualized in FIGTREE. This tree was used as a starting tree for subsequent divergence time estimates. Again, the ingroup was constrained as monophyletic, trees were linked over all partitions, and this analysis ran for 100 million generations sampling every 10,000 generations. As no *Leptolejeunea* fossils are known, a plastid genome substitution rate of  $5 \times 10^{-4}$  subst./sites/my (Palmer, 1991; Villarreal & Renner, 2012) was used for the three chloroplast markers with a SD of  $1 \times 10^{-4}$  and a normal prior distribution. For the nrITS region, a substitution rate of  $1.35 \times 10^{-3}$  subst./sites/my was adopted from Les, Crawford, Kimball, Moody, & Landolt (2003). A normal prior distribution in combination with the truncate option and upper and lower bounds of  $0.4\text{--}8.3 \times 10^{-3}$  subst./sites/my was implemented to allow the rate to vary over the large spectrum of reported nrITS rates (Kay, Whittall, & Hodges, 2006; Villarreal & Renner, 2014). The stepping-stone sampling in BEAST (Baele, Li, Drummond, Suchard, & Lemey, 2013; Baele et al., 2012; Xie, Lewis, Fan, Kuo, & Chen, 2011) and the Bayes factor (Kass & Raftery, 1995) were used to compare between pure-birth (Yule), birth-death, and birth-death incomplete sampling tree priors, as well as an UCLN relaxed clock and a strict clock. This resulted in choosing a birth-death incomplete sampling prior in combination with a UCLN relaxed clock model. Log marginal-likelihood values and Bayes factor values are shown in Table 2. Results of the BEAST run were examined in TRACER, summarized in TREEANNOTATOR by median branch lengths, and visualized in FIGTREE.

Afromadagascar, Asia-Australasia, and tropical-subtropical America were chosen as putative areas of endemism, and each specimen was assigned to one of these regions according to the label information. Ancestral areas of distribution were reconstructed using maximum parsimony criteria as implemented in MESQUITE 3.1 (Maddison & Maddison, 2016) based on the MCC topology from the divergence time analysis. In addition, the R-package BIOGEOBEARS (Matzke, 2013a, 2013b, 2014) was employed to infer the ancestral

history of *Leptolejeunea*. This likelihood-based method implements the LAGRANGE DEC model (Ree & Smith, 2008), DIVA (dispersal-variance analysis; Ronquist, 1997), and BayArea (Landis, Matzke, Moore, & Huelsenbeck, 2013), each of which can be extended with an additional free parameter  $j$  accounting for founder-event speciation. To obtain the recommended operational taxonomic units consisting of monophyletic populations and not individual specimens, specimens of one species with the same putative area of endemism were merged together into a single terminal using the R-script provided on the BIOGEOBEARS webpage ([http://phylo.wikidot.com/example-bio-geobears-scripts#pruning\\_a\\_tree](http://phylo.wikidot.com/example-bio-geobears-scripts#pruning_a_tree)). All six models were compared using likelihood values, the AIC, and the AIC corrected for small sample size (AIC<sub>c</sub>) (Matzke, 2014). The maximum number of areas was set to three to account for the assumed pantropical ranges of *Leptolejeunea* species (Grolle, 1976; Pócs, 2012; Pócs & Lye, 1999; Schuster, 1983).

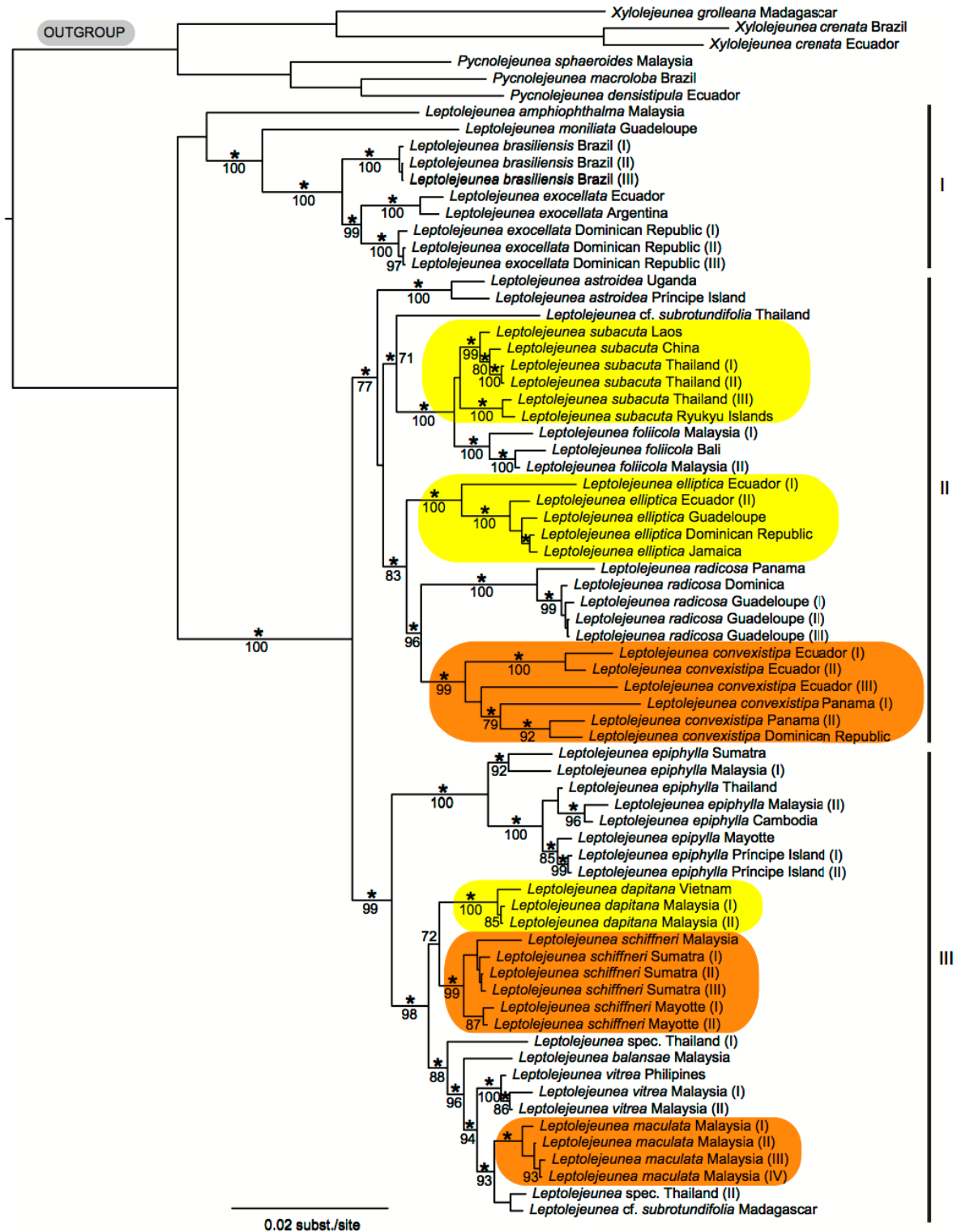
### 2.4 | Morphological investigation

Specimens were studied under a Carl Zeiss AxioScope A1 compound microscope equipped with a Canon 60D digital camera using transmitted or incident light. The *Leptolejeunea convexistipa* voucher Schäfer-Verwimp 35198/A (M) and the *L. epiphylla* voucher Schäfer-Verwimp 16245 (M) were digitized (Figure 1). All presented images are digitally stacked photomicrographic composites of up to 20 individual focal planes obtained using the software package HeliconFocus 6.7.1.

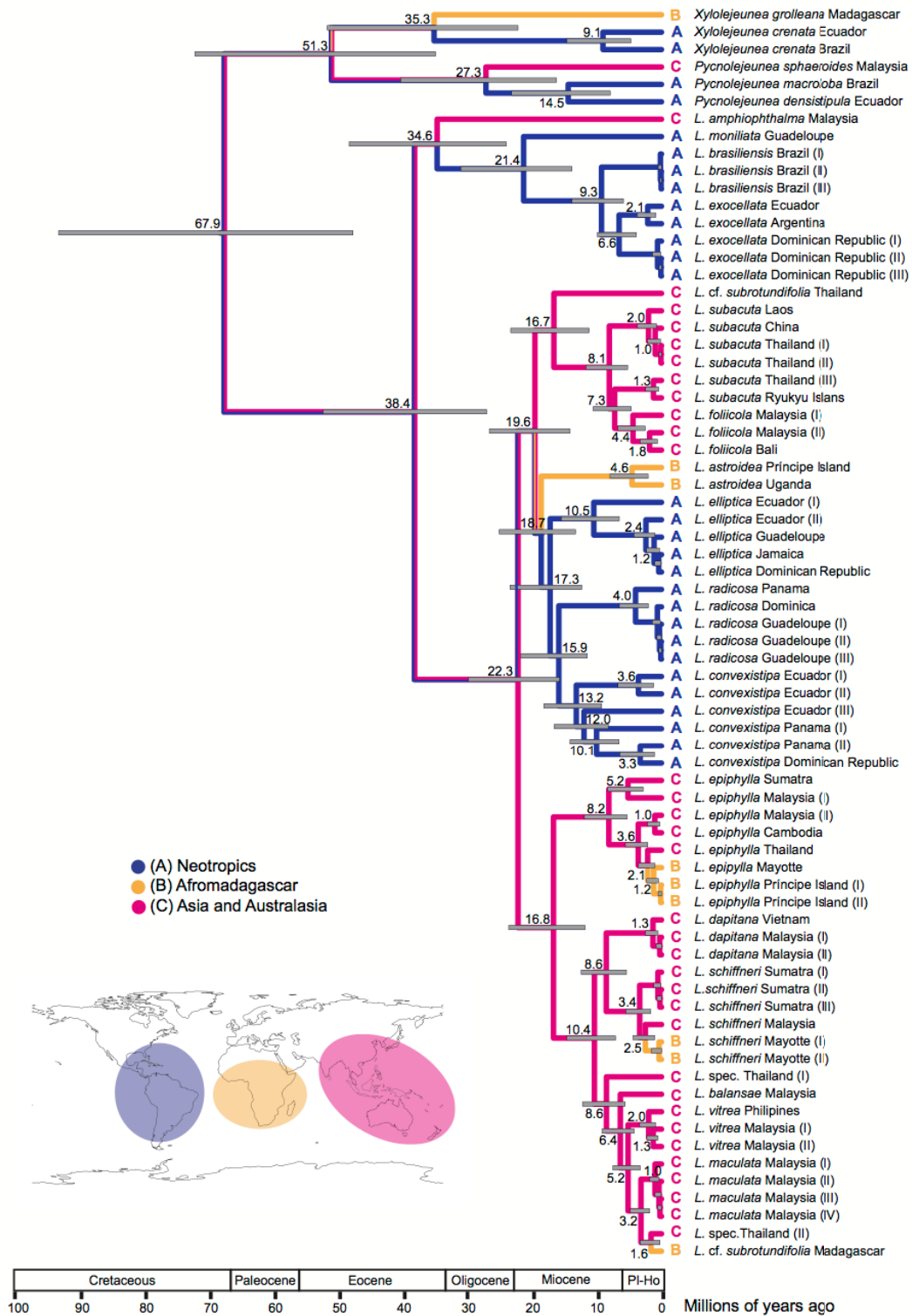
## 3 | RESULTS

### 3.1 | Phylogeny

*Leptolejeunea* splits into three main clades (labeled I, II, III) with clade I placed sister to the remainder of the genus (Figure 2). Clade I includes a Malaysian accession of *L. amphiphthalma* in an unsupported sister relationship to a robust Neotropical clade consisting of *L. moniliata*, *L. brasiliensis*, and *L. exocellata*. Three accessions of *L. brasiliensis* were placed sister to a clade with five accessions of *L. exocellata*. A clade with three accessions of *L. exocellata* from the Dominican Republic was placed sister to a clade with *L. exocellata* accessions from Argentina and Ecuador. Clade II achieved a BPP of 1.00 and a BP of 77 and included a lineage with accessions of *L. astroidea* from Uganda and Príncipe Island, a lineage with Asian accessions of *L. subacuta*, *L. cf. subrotundifolia* and *L. foliicola*, and a Neotropical lineage with accessions of *L. convexistipa*, *L. elliptica*, and *L. radicata*. An accession of *L. radicata* from Panama was placed sister to a clade with accessions from Dominica and Guadeloupe. Clade III comprised Paleotropical accessions (BPP 1.00, BP 99). *Leptolejeunea epiphylla* split into a clade with two accessions from Sumatra and Malaysia, and a clade with accessions from Cambodia, Malaysia, and Thailand in a sister relationship with accessions from Mayotte and Príncipe Island. The *L. epiphylla* clade was sister to a clade with accessions assigned to *L. dapitana*, *L. maculata*, *L. schiffneri*, *L. vitrea*, *L. balansae*, *L. cf. subrotundifolia*, and *L. spec. indet.* The *L. schiffneri* clade included an Asian lineage and a lineage with accessions from Mayotte. Representatives of other clade



**FIGURE 2** Majority rule consensus tree of trees recovered in stationary phase of Bayesian search. A star indicates a Bayesian Posterior probability  $>.97$ . Maximum-likelihood bootstrap percentage values  $>70$  are also shown at branches. Orange highlighted accessions were earlier considered to belong to *Leptolejeunea maculata*, and yellow highlighted accessions were earlier considered to belong to *L. elliptica*



**FIGURE 3** BEAST chronogram with 95% highest posterior density (HPD) intervals and branches colored according to the most parsimonious reconstruction of distributions of *Leptolejeunea*. Putative areas of endemism are indicated for every accession rather than morphospecies. Node ages  $\geq 1$  Ma are reported

**TABLE 3** Results of the BioGeoBEARS analyses favoring a DIVALIKE+J model, as shown in bold, according to model selection by log-likelihood values (lnL), Akaike information criterion (AIC), and AIC corrected for small sample size (AIC<sub>c</sub>)

	lnL	n	d	e	j	AIC	AIC <sub>c</sub>
DEC	-42.11	2	0.008	0.003	0	88.22	88.72
DEC+J	-24.95	3	10 <sup>-12</sup>	10 <sup>-12</sup>	0.15	55.89	56.94
DIVALIKE	-38.08	2	0.009	10 <sup>-12</sup>	0	80.16	80.66
<b>DIVALIKE+J</b>	<b>-24.79</b>	<b>3</b>	<b>10<sup>-12</sup></b>	<b>10<sup>-12</sup></b>	<b>0.14</b>	<b>55.59</b>	<b>56.63</b>
BAYAREALIKE	-54.58	2	0.009	0.03	0	113.2	113.7
BAYAREALIKE+J	-25.77	3	10 <sup>-7</sup>	10 <sup>-7</sup>	0.14	57.54	58.58

n, number of parameters; d, rate of dispersal; e, rate of extinction; j, relative probability of founder-event speciation.

**TABLE 4** Estimated ancestral area probabilities for selected nodes obtained from the BioGeoBEARS analysis of *Leptolejeunea* rounded in percent. Node numbers are displayed in Figure 4. Areas are coded as follows: A, Neotropics; B, Afromadagascar; C, Australasia; AB, AC, BC, ABC are combinations of these areas

Node	Estimated ancestral area (DIVALIKE+J)
1	A 17, B 3, C 18, AB 7, AC 31, BC 8, ABC 16
2	A 16, B 3, C 76, AC 3, BC 3
3	A 30, C 70
4	A 6, B 8, C 85, BC 1
5	A 12, B 13, C 75
6	A 50, B 50
7	B 8, C 92
8	B 20, C 80
9	B 16, C 84
10	B 16, C 84

III species originated exclusively from Asia. Most species represented by multiple accessions achieved BPPs of 1.00 and BPs >98; *L. maculata* achieved a BPP of 0.99 and a BP of 62; the monophyly of *L. subcuta* was unsupported.

### 3.2 | Divergence time estimates and biogeography

The divergence time analyses (Figure 3) provided evidence for a split between the outgroup and *Leptolejeunea* in a time interval from the Early Eocene to the Late Cretaceous (67.9 Ma, 95% HPD: 47.9–93.7) and an Oligocene to Eocene (38.4 Ma, 95% HPD: 27.2–52.6) age of the *Leptolejeunea* crown group. Most of the species clades were established in the Miocene. The BioGeoBEARS analyses favored a DIVALIKE+J model for the estimation of ancestral areas (Table 3), and results obtained with this model are shown in Figure 4 in combination with the modified BEAST chronogram. Estimated ancestral area probabilities for selected nodes are given in Table 4. The origin of *Leptolejeunea* is ambiguous, with the highest probability of an origin in Asia or the Neotropics. Similar results were achieved using maximum parsimony criteria (Figure 3). Neotropical–Paleotropical disjunctions occurred during the Miocene to Eocene. *Leptolejeunea epiphylla* and *L. schiffneri* originated in Asia and colonized African islands during the Plio-Pleistocene.

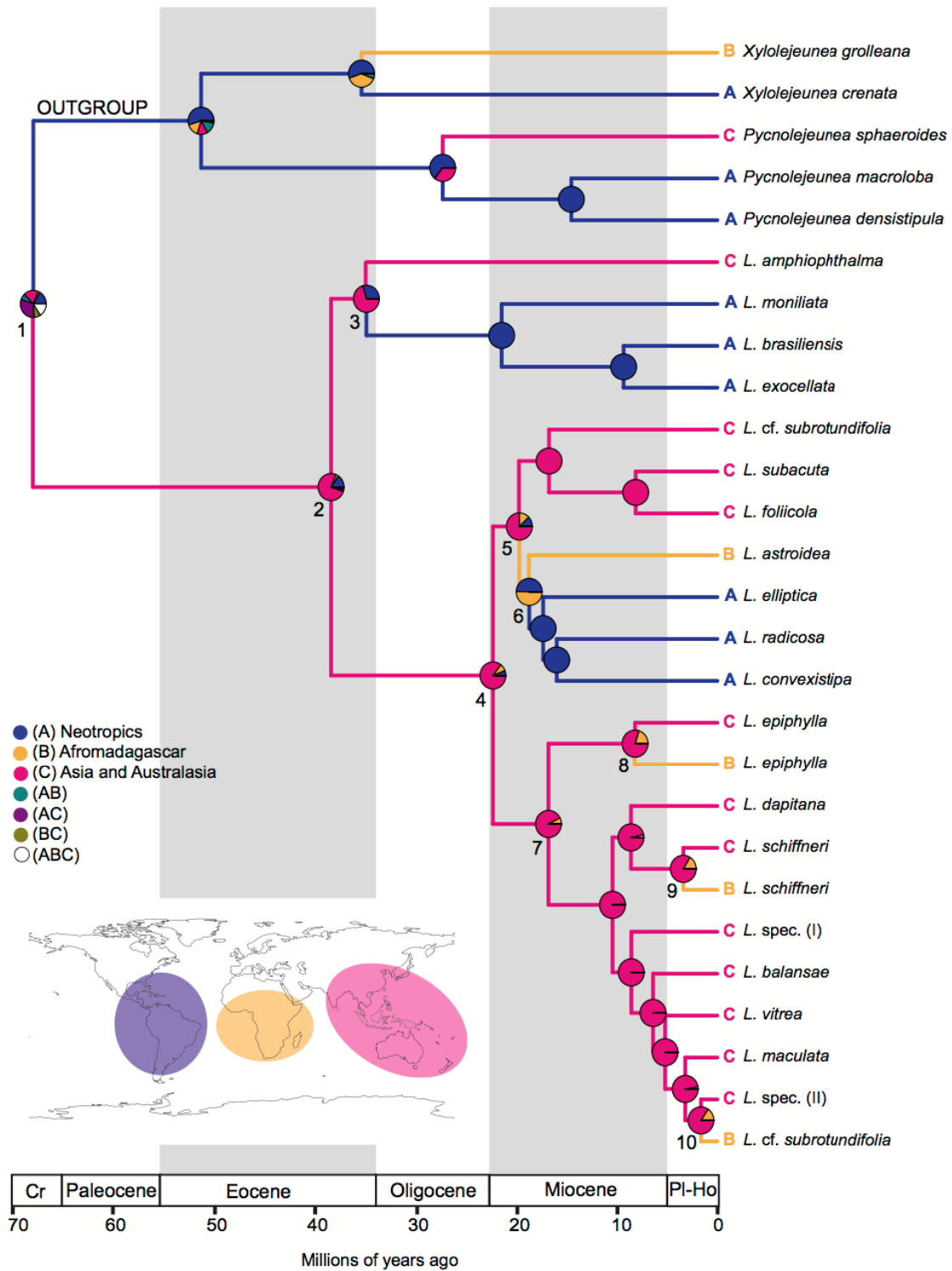
## 4 | DISCUSSION

### 4.1 | Bryophyte species in the molecular age

Although intercontinentally disjunct bryophyte species often form monophyla (Heinrichs et al., 2010; Vigalondo et al., 2016), accessions from different continents are often resolved in sister clades (Heinrichs et al., 2011). This pattern of geographically structured phylogenetic relationships suggests gene flow and interbreeding between populations on different continents has ceased, and this may be confirmed by detailed study (Medina, Lara, Goffinet, Garilleti, & Mazimpaka, 2013). Other studies point to the polyphyly of supposedly intercontinentally distributed species (Huttunen & Ignatov, 2010; Renner, 2014) and indicate that monophyletic bryophyte species often have restricted ranges (Medina, Lara, Goffinet, Garilleti, & Mazimpaka, 2012; Medina et al., 2013; Renner et al., 2013). That patterns of phylogenetic and morphological diversification are often decoupled in bryophytes is now well recognized, and many instances of morphologically cryptic species complexes have been documented (Baczkiewicz & Buczkowska, 2016; Kyrkjeeide, Hassel, Flatberg, Shaw, Yousefi, et al., 2016; Odrzykoski & Szweykowski, 1991; Ramaiya et al., 2010; Shaw, Boles, & Shaw, 2008). However, the prevalence of morphologically cryptic divergence, and the number of species resulting from such events, remains unknown. Species circumscription based on morphology may overlook two important features: firstly, the existence of higher phylogenetic diversity than suggested by patterns of morphological variation and secondly, higher geographic structuring than suggested by the distribution of morphological variation (Medina et al., 2013; Ramaiya et al., 2010; Renner, Brown, & Wardle, 2011; Renner et al., 2013).

### 4.2 | *Leptolejeunea* species ranges and taxonomy

Our study contradicts hypothesized pantropical ranges for two *Leptolejeunea* species (Figure 2, note highlighted specimens) and supports the hypothesis of Shaw (2001) that morphological uniformity of bryophytes often belies a complex genetic structure. According to our sampling, *L. elliptica* is restricted to the Neotropics rather than representing a pantropical species (Pócs, 2012; Schuster, 1980). Paleotropical accessions that were earlier assigned to *L. elliptica* are placed in separate lineages and have been revised to *L. dapitana* and



**FIGURE 4** Result of the BioGEOBEARS analysis of *Leptolejeunea* in combination with the modified BEAST chronogram. Circles at nodes represent probabilities for ancestral areas resulting from DIVALIKE analysis accounting for founder-event speciation. See Table 4 for percent values. Branches are colored according to the most probable area for splits as indicated by BioGEOBEARS

*L. subacuta* (Figure 2). The supposedly pantropical *L. maculata* (Grolle, 1976; Pócs & Lye, 1999) forms three independent lineages (Figure 2). Asian *L. maculata* s.str. is placed in main clade III, together with a Palearctic lineage here identified as *L. schiffneri*. Neotropical accessions of *L. maculata* belong to main clade II and have been identified as *L. convexistipa*. Such findings have frequently been explained as instances of cryptic or near cryptic speciation (Shaw, 2001); however, molecular topologies may allow revision of morphological evidence and the identification of morphological character states supporting the different lineages (Forrest et al., 2011; Heinrichs et al., 2015; Renner et al., 2013). Revision of *Leptolejeunea* specimens is challenging as the taxonomy of this genus relies heavily on the number and distribution of ocelli in the leaves, that is, specialized cells containing only a single large rather than several small oil bodies (He & Piippo, 1999). These often disappear from herbarium specimens. Exceptionally large or small leaf cells in herbarium specimens may be indicative of ocelli; however, ocelli sharing the size of the surrounding leaf cells may not be recognizable in dried materials. A thorough revision of *Leptolejeunea* thus needs to be based on the investigation of living plants from all parts of the range and sequencing of a comprehensive number of specimens including types or topotypes. New sources of species circumscribing characters also need to be sought. Such work is beyond the scope of this study; however, our data facilitate discrimination between alternative interpretations of species circumscription and to reconstruct the distribution of the main clades. Our data also support the finding of Renner (2015) that morphologically similar leafy liverworts may be placed in different main lineages, despite considerable morphological overlap. Accessions originally assigned to the same species were resolved in different main clades, and the supposedly closely related species *L. brasiliensis* and *L. elliptica* (Schuster, 1980) were resolved in main clade I or II (Figure 2). Phylogenies of Lejeuneaceae genera often show a geographical pattern related to the distribution of lineages rather than a morphological pattern. Examples include the genera *Lejeunea* (Heinrichs et al., 2013) and *Diplasiolejeunea* (Dong et al., 2012) which exhibit separation into predominantly Neotropical and predominantly Palearctic lineages. A similar situation manifests in *Leptolejeunea*.

### 4.3 | Divergence time estimates, biogeography, and infraspecific variation

Our divergence time estimates suggest Cenozoic diversification of *Leptolejeunea* and contradict Gondwanan vicariance (Raven & Axelrod, 1974) as an explanation for the observed disjunctions. Establishment of the *Leptolejeunea* crown group in the Eocene accordances well with the appearance of humid megathermal angiosperm forests (Morley, 2011) which provided the preferred epiphyllous habitat of extant *Leptolejeunea* representatives. Cretaceous gymnosperm forests differed in structure and evaporated less water than tropical angiosperm forests (Boyce & Lee, 2010). Thus, they may not have hosted as diverse epiphyll communities or supported Lejeuneaceae representatives adapted to other niches than modern species (Feldberg et al., 2014). Similar evolutionary processes have been reconstructed for

the genera *Lejeunea*, *Harpalejeunea*, and *Microlejeunea* based on molecular and fossil evidence (Heinrichs et al., 2016).

Our reconstruction failed to unambiguously identify the area of origin of *Leptolejeunea*; however, we need to consider the wide distribution of humid angiosperm forests in the Eocene including the northern "boreotropical" region (Morley, 2011). Lack of fossils and extant species precludes inference of a northern range for *Leptolejeunea*; however, the Eocene range of *Leptolejeunea* likely differed from the current distribution. Cooling during the Neogene (Zachos, Pagani, Sloan, Thomas, & Billups, 2001) may have resulted in range contraction and extinction in the north, and possibly the extinction of some early lineages. Caution interpreting biogeographical reconstructions utilizing standard substitution rates is always required; however, our chronogram suggests either lower speciation or higher extinction rates during the early Oligocene cooling phase (Liu et al., 2009), and the establishment of extant *Leptolejeunea* species predominantly in the Miocene. This pattern could relate to a Miocene reorganization of tropical forests. Miocene origins for extant diversity have also been observed in mosses (Lewis, Rozzi, & Goffinet, 2014; Shaw et al., 2010) and leptosporangiate ferns (Schneider et al., 2010; Wei et al., 2015). The age of the oldest Neotropical–Palearctic disjunctions could relate to boreotropical migration (Davis, Bell, Matthews, & Donoghue, 2002; Le Péchon et al., 2016) although a thorough reconstruction is precluded by the lack of fossils. Miocene disjunctions are better explained by LDD, as are the island occurrences of several species. Liverworts have dispersed to the African continent and associated islands from both the Neotropics and Asia (Feldberg et al., 2007; Heinrichs et al., 2005). Both biogeographical analyses (Figures 3 and 4) provide evidence for an Asian origin of the African accessions of *L. epiphylla*, *L. schiffneri*, and *L. cf. subrotundifolia*, whereas the origin of the African *L. astroidea* remains unclear. African taxa nesting in Asian clades have also been described for ferns (Hennequin, Hovenkamp, Christenhusz, & Schneider, 2010; Janssen, Kreier, & Schneider, 2007) and angiosperms (Kulju, Sierra, Draisma, Samuel, & van Welzen, 2007; Li, Dressler, Zhang, & Renner, 2009; Richardson, Chatrou, Mols, Erkens, & Pirie, 2004). Monsoon trade winds were proposed as dispersal agent from Asia to Africa (Li et al., 2009) and could also be responsible for the observed pattern in *Leptolejeunea*. Alternatively, animal-mediated dispersal may contribute to current disjunctions. At small spatial scales, millipedes have been demonstrated to move gemmae of species of the moss genus *Calymperes* (Zona, 2013). Larger animals that move over correspondingly larger spatial scales may also transport propagules and plant fragments (Lewis et al., 2014). In New Zealand, the isolated occurrences of the tropical *Calymperes tenerum* are congruent with known visitation sites of the predominantly tropical black-winged petrel (P. J. de Lange, personal communication). Seabirds are known to visit potential or actual breeding sites, even though visiting individuals may not nest there. To visit these sites, which are often forested, birds literally crash through the canopy to the ground, thus coming into close, vigorous contact with leaf and twig surfaces, providing ample opportunity for plant fragments to become deeply embedded within the bird's feather matrix. Seabirds roam widely during their nonbreeding season and routinely traverse



oceans and have been known to traverse land masses bridging oceanic waterways.

The island occurrences provide evidence for the ability of *Leptolejeunea* species to disperse over long distances either by vegetative propagules (Laenen et al., 2016) or by spores (Van Zanten & Gradstein, 1988). However, successful LDD seems rare in *Leptolejeunea*, as indicated by the plurispecies clades being restricted to either the Neotropics or the Paleotropics, but also by the genetic variation within single morphospecies. Although our data support a narrower species concept and reinstatement of several putative synonyms, some species clades still have a considerable molecular variation, with initial splits in the late Miocene (Figure 3). Examples include a split between mainland South American *L. exocellata* and accessions from the West Indian Islands, splits within Asian *L. epiphylla*, and splits within Neotropical *L. convexistipa*. Considerable molecular variation related to a geographical rather than a morphological pattern has been observed for a larger number of liverworts (Fuselier et al., 2009; Heinrichs et al., 2015; Ramaiya et al., 2010) although it is still somewhat unclear whether this variation is in general indicative of genetically independent entities. Follow-up studies should thus involve denser sampling and additional markers including microsatellites. Intercontinental gene flow has already been demonstrated for bryophytes, especially for holarctic species of the moss genus *Sphagnum* (Kyrkjeeide, Hassel, Flatberg, Shaw, Brochmann, et al., 2016; Shaw et al., 2014); however, the epiphyllous habitat of *Leptolejeunea* species in the understory of tropical forests may lower the LDD success rate compared to *Sphagnum* species which occur in open wetland systems.

#### 4.4 | Perspectives

Every disjunction has its first day; hence, we cannot generally reject intercontinental or even pantropical species ranges (Lewis et al., 2014). On the other hand, a growing body of evidence indicates that LDD occurs only infrequently in bryophytes and that it is thus often associated with speciation. The accumulation of genetic disparity in bryophytes is often not associated with the accumulation of a similar amount of morphological disparity (Bacziewicz & Buczkowska, 2016; Ramaiya et al., 2010), although there are exceptions (Heinrichs, Gradstein, Groth, & Lindner, 2003). Lack of molecular support for morphology-based supraspecific taxa such as sections and subgenera (Devos, Renner, Gradstein, Shaw, & Vanderpoorten, 2011) further complicates the understanding of bryophyte evolution and appropriate choice of ingroup representatives. A reliable reconstruction of the evolutionary history and biogeography of bryophytes thus needs to be based on comprehensive molecular phylogenies with complete population-level sampling. Only such phylogenies will facilitate species identification and refined estimation of bryophyte global diversity and origins.

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#### CONFLICT OF INTEREST

None declared.

#### REFERENCES

- Ahonen, I., Muonen, J., & Piippo, S. (2003). Inferring the phylogeny of Lejeuneaceae: A first appraisal of molecular data. *The Bryologist*, 106, 297–308.
- Akaike, H. (1973). Information theory as an extension of the maximum likelihood principle. In B. N. Petrov, & F. Csáki (Eds.), *Second International Symposium on Information Theory* (pp. 267–281). Budapest: Akadémia Kiado.
- Bacziewicz, A., & Buczkowska, J. (2016). Differentiation and genetic variability of three cryptic species within the *Aneura pinguis* complex (Jungermanniidae, Marchantiophyta). *Cryptogamie, Bryologie*, 37, 3–18.
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. V. (2012). Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, 29, 2157–2167.
- Baele, G., Li, W. L. S., Drummond, A. J., Suchard, M. A., & Lemey, P. (2013). Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution*, 30, 239–243.
- Bechteler, J., Lee, G. E., Schäfer-Verwimp, A., Pócs, T., Peralta, D. F., Renner, M. A. M., ... Heinrichs, J. (2016). Towards a monophyletic classification of Lejeuneaceae IV: Reinstatement of *Allorgella*, transfer of *Microlejeunea aphanella* to *Vitalianthus* and refinements of the subtribal classification. *Plant Systematics and Evolution*, 302, 187–201.
- Bechteler, J., Lee, G. E., Schäfer-Verwimp, A., Renner, M. A. M., Peralta, D. F., & Heinrichs, J. (2016). Towards a monophyletic classification of Lejeuneaceae V: The systematic position of *Pictolejeunea*. *Phytotaxa*, 280, 259–270.
- Bischler, H. (1969). Le genre *Leptolejeunea* (Spruce) Steph. en Amérique. *Nova Hedwigia*, 17, 265–350.
- Boyce, C. K., & Lee, J.-E. (2010). An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity. *Proceedings of the Royal Society of London B: Biological Sciences*, 277, 3437–3443.
- Cooper, E. D., Henwood, M. J., & Brown, E. A. (2012). Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution. *Biological Journal of the Linnean Society*, 107, 425–441.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
- Davis, C. C., Bell, C. D., Matthews, S., & Donoghue, M. J. (2002). Laurasian migration explains Gondwanan disjunctions: Evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 6833–6837.
- Devos, N., Renner, M. A. M., Gradstein, S. R., Shaw, J., & Vanderpoorten, A. (2011). Molecular data challenge traditional subgeneric divisions in the leafy liverwort *Radula*. *Taxon*, 60, 1623–1632.
- Devos, N., & Vanderpoorten, A. (2009). Range disjunctions, speciation, and morphological transformation rates in the liverwort genus *Leptoscyphus*. *Evolution*, 63, 779–792.
- Dong, S., Schäfer-Verwimp, A., Meinecke, P., Feldberg, K., Bombosch, A., Pócs, T., ... Heinrichs, J. (2012). Tramps, narrow endemics and morphologically

- cryptic species in the epiphyllous liverwort *Diplasiolejeunea*. *Molecular Phylogenetics and Evolution*, 65, 582–594.
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973.
- Erixon, P., Svennblad, B., Britton, T., & Oxelman, B. (2003). Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology*, 52, 665–673.
- Feldberg, K., Hentschel, J., Wilson, R., Rycroft, D. S., Glenny, D., & Heinrichs, J. (2007). Phylogenetic biogeography of the leafy liverwort *Herbertus* (Jungermanniales, Herbertaceae) based on nuclear and chloroplast DNA sequence data: Correlation between genetic variation and geographical distribution. *Journal of Biogeography*, 34, 688–698.
- Feldberg, K., Schneider, H., Stadler, T., Schäfer-Verwimp, A., Schmidt, A. R., & Heinrichs, J. (2014). Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Scientific Reports*, 4, 5974.
- Forrest, L. L., & Crandall-Stotler, B. J. (2004). A phylogeny of the simple thalloid liverworts (Jungermanniopsida, subclass Metzgeriidae) as inferred from five chloroplast genes. *Monographs in Systematic Botany from the Missouri Botanical Garden*, 98, 119–140.
- Forrest, L. L., Salazar-Allen, N., Gudiño, J. A., Korpelainen, H., & Long, D. G. (2011). Molecular and morphological evidence for distinct species in *Dumortiera* (Dumortieraceae). *The Bryologist*, 114, 102–115.
- Fuselier, L., Davison, P. G., Clements, M., Shaw, B., Devos, N., Heinrichs, J., ... Shaw, A. J. (2009). Phylogeographic analyses reveal distinct lineages of *Metzgeria furcata* and *M. conjugata* (Metzgeriaceae) in Europe and North America. *Biological Journal of the Linnean Society*, 98, 745–756.
- Gradstein, S. R. (2013). A classification of Lejeuneaceae based on molecular and morphological evidence. *Phytotaxa*, 100, 6–20.
- Gradstein, S. R., Churchill, S. P., & Salazar-Allen, N. (2001). Guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden*, 86, 1–577.
- Grolle, R. (1976). *Drepanolejeunea* subgen. *Kolpoleonjeunea*—eine neue Untergattung aus der Paläotropis. *Journal of the Hattori Botanical Laboratory*, 40, 191–216.
- Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98, Oxford University Press.
- Hartmann, F. A., Wilson, R., Gradstein, S. R., Schneider, H., & Heinrichs, J. (2006). Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *International Journal of Plant Sciences*, 167, 1205–1214.
- He, X. L., & Piippo, S. (1999). On the taxonomic significance and classification of ocelli characters in the hepatic family Lejeuneaceae. *Bryobrothera*, 5, 93–97.
- Hedenäs, L., Désamoré, A., Laenen, B., Papp, B., Quandt, D., Gonzáles-Mancebo, J. M., ... Stech, M. (2014). Three species for the price of one within the moss *Homalothecium sericeum* s.l. *Taxon*, 63, 249–257.
- Heinrichs, J., Dong, S., Schäfer-Verwimp, A., Pócs, T., Feldberg, K., Czumaj, A., ... Schneider, H. (2013). Molecular phylogeny of the leafy liverwort *Lejeunea* (Porellales): Evidence for a Neotropical origin, uneven distribution of sexual systems and insufficient taxonomy. *PLoS One*, 8, e82547.
- Heinrichs, J., Feldberg, K., Bechteler, J., Scheben, A., Czumay, A., Pócs, T., ... Schäfer-Verwimp, A. (2015). Integrative taxonomy of *Lepidolejeunea* (Porellales, Jungermanniopsida): Ocelli allow the recognition of two neglected species. *Taxon*, 64, 216–228.
- Heinrichs, J., Gradstein, S. R., Groth, H., & Lindner, M. (2003). *Plagiochila cucullifolia* var. *anomala* var. nov. from Ecuador, with notes on discordant molecular and morphological variation in *Plagiochila*. *Plant Systematics and Evolution*, 242, 205–216.
- Heinrichs, J., Hentschel, J., Bombosch, A., Fiebig, A., Reise, J., Edelmann, M., ... Shaw, A. J. (2010). One species or at least eight? Delimitation and distribution of *Frullania tamarisci* (L.) Dumort. (Jungermanniopsida, Porellales) inferred from nuclear and chloroplast DNA markers. *Molecular Phylogenetics and Evolution*, 56, 1105–1114.
- Heinrichs, J., Kreier, H.-P., Feldberg, K., Schmidt, A. R., Zhu, R. L., Shaw, B., ... Wissemann, V. (2011). Formalizing morphologically cryptic biological entities: New insights from DNA taxonomy, hybridization, and biogeography in the leafy liverwort *Porella platyphylla* (Jungermanniopsida, Porellales). *American Journal of Botany*, 98, 1252–1262.
- Heinrichs, J., Lindner, M., Gradstein, S. R., Groth, H., Buchbender, V., Solga, A., & Fischer, E. (2005). Origin and subdivision of *Plagiochila* (Jungermanniidae: Plagiochilaceae) in tropical Africa based on evidence from nuclear and chloroplast DNA sequences and morphology. *Taxon*, 54, 317–333.
- Heinrichs, J., Schäfer-Verwimp, A., Czumay, A., Dong, S., Scheben, A., Feldberg, K., & Schneider, H. (2014). Towards a monophyletic classification of Lejeuneaceae I: Subtribe Leptolejeuneinae subtr. nov. *Phytotaxa*, 156, 165–174.
- Heinrichs, J., Scheben, A., Bechteler, J., Lee, G. E., Schäfer-Verwimp, A., Hedenäs, L., ... Schmidt, A. R. (2016). Crown group Lejeuneaceae and *Pleurocarpus* mosses in Early Eocene (Ypresian) Indian amber. *PLoS One*, 11, e0156301.
- Hennequin, S., Hovenkamp, P., Christenhusz, M., & Schneider, H. (2010). Phylogenetics and biogeography of *Nephrolepis*—A tale of old settlers and young tramps. *Botanical Journal of the Linnean Society*, 164, 113–127.
- Huttunen, S., & Ignatov, M. S. (2010). Evolution and taxonomy of aquatic species in the genus *Rhynchostegium* (Brachytheciaceae, Bryophyta). *Taxon*, 59, 791–808.
- Janssen, T., Kreier, H.-P., & Schneider, H. (2007). Origin and diversification of African ferns with special emphasis on Polypodiaceae. *Brittonia*, 59, 159–181.
- Kass, R. E., & Raftery, A. E. (1995). Bayes factor. *Journal of the American Statistical Association*, 90, 773–795.
- Kay, K. M., Whittall, J. B., & Hodges, S. A. (2006). A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: An approximate molecular clock with life history effects. *BMC Evolutionary Biology*, 6, 36.
- Kulju, K. K. M., Sierra, S. E. C., Draisma, S. G. A., Samuel, R., & van Welzen, P. C. (2007). Molecular phylogeny of *Macaranga*, *Mallotus*, and related genera (Euphorbiaceae s.s.): Insights from plastid and nuclear DNA sequence data. *American Journal of Botany*, 94, 1726–1743.
- Kyrkjæide, M. O., Hassel, K., Flatberg, K. I., Shaw, A. J., Brochmann, C., & Stenøien, H. K. (2016). Long-distance dispersal and barriers shape genetic structure of peatmosses (*Sphagnum*) across the Northern Hemisphere. *Journal of Biogeography*, 43, 1215–1226.
- Kyrkjæide, M. O., Hassel, K., Flatberg, K. I., Shaw, A. J., Yousefi, N., & Stenøien, H. K. (2016). Spatial genetic structure of the abundant and widespread peatmoss *Sphagnum magellanicum* Brid. *PLoS One*, 11, e0148447.
- Laenen, B., Machac, A., Gradstein, S. R., Shaw, B., Patiño, J., Désamoré, A., ... Vanderpoorten, A. (2016). Geographical range in liverworts: Does sex really matter? *Journal of Biogeography*, 43, 627–635.
- Laenen, B., Shaw, B., Schneider, H., Goffinet, B., Paradis, E., Désamoré, A., ... Shaw, A. J. (2014). Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications*, 5, 6134.
- Landis, M., Matzke, N. J., Moore, B. R., & Huelsenbeck, J. P. (2013). Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, 62, 789–804.
- Large, B., & Simon, D. L. (1999). Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution*, 16, 750–759.
- Le Péchon, T., Zhang, L., He, H., Zhou, X.-M., Bytebier, B., Gao, X.-F., & Zhang, L.-B. (2016). A well-sampled phylogenetic analysis of the polystichoid ferns (Dryopteridaceae) suggests a complex biogeographical history involving both boreotropical migrations and recent transoceanic dispersals. *Molecular Phylogenetics and Evolution*, 98, 324–336.

- Les, D. H., Crawford, D. J., Kimball, R. T., Moody, M. L., & Landolt, E. (2003). Biogeography of discontinuously distributed hydrophytes, a molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences*, 164, 917–932.
- Lewis, L. R., Rozzi, R., & Goffinet, B. (2014). Direct long-distance dispersal shapes a New World amphitropical disjunction in the dispersal-limited dung moss *Tetraplodon* (Bryopsida: Splachnaceae). *Journal of Biogeography*, 41, 2385–2395.
- Li, Y., Dressler, S., Zhang, D., & Renner, S. (2009). More Miocene dispersal between Africa and Asia—The case of *Bridelia* (Phyllanthaceae). *Systematic Botany*, 34, 521–529.
- Liu, Z., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., ... Pearson, A. (2009). Global cooling during the Eocene-Oligocene climate transition. *Science*, 323, 1187–1190.
- Maddison, D. R., & Maddison, W. P. (2016). *Mesquite: A modular system for evolutionary analysis*. Version 3.10. Retrieved from <http://mesquiteproject.org>.
- Matzke, N. J. (2013a). *BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts*. Vienna, Austria: CRAN: The Comprehensive R Archive Network. Retrieved from <http://cran.r-project.org/package=BioGeoBEARS>.
- Matzke, N. J. (2013b). Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography*, 5, 242–248.
- Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, 63, 951–970.
- Medina, R., Lara, F., Goffinet, B., Garilleti, R., & Mazimpaka, V. (2012). Integrative taxonomy successfully resolves the pseudo-cryptic complex of the disjunct epiphytic moss *Orthotrichum consimile* s.l. (Orthotrichaceae). *Taxon*, 61, 1180–1198.
- Medina, R., Lara, F., Goffinet, B., Garilleti, R., & Mazimpaka, V. (2013). Unnoticed diversity within the disjunct moss *Orthotrichum tenellum* s.l. validated by morphological and molecular approaches. *Taxon*, 62, 1133–1152.
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees*. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, pp. 1–8.
- Morley, R. J. (2011). Cretaceous and tertiary climate change and the past distribution of megathermal rainforests. In M. B. Bush, J. R. Flenley, & W. D. Gosling (Eds.), *Tropical Rainforest Responses to Climatic Change* (pp. 1–34). Berlin: Springer.
- Odrzykoski, I. J., & Szwedkowski, J. (1991). Genetic differentiation without concordant morphological divergence in the thallose liverwort *Conocephalum conicum*. *Plant Systematics and Evolution*, 178, 135–151.
- Palmer, J. D. (1991). Plastid chromosome, structure and evolution. In L. Bogorad, & I. K. Vasil (Eds.), *The Molecular Biology of Plastids* (pp. 5–53). San Diego, CA: Academic Press.
- Pattengale, N. D., Alipour, M., Bininda-Emonds, O. R., Moret, B. M., & Stamatakis, A. (2010). How many bootstrap replicates are necessary? *Journal of Computational Biology*, 17, 337–354.
- Pócs, T. (2012). New or little known epiphyllous liverworts, XVI. A small collection from Laos. *Acta Biologica Plantarum Agriensis*, 2, 5–10.
- Pócs, T., & Lye, K. A. (1999). New records and additions to the hepatic flora of Uganda. 2. *Tropical Bryology*, 17, 23–33.
- Posada, D. (2008). jModeltest: Phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 415–430.
- Ramaiya, M., Johnson, M. G., Shaw, B., Heinrichs, J., Hentschel, J., von Konrat, M., ... Shaw, A. J. (2010). Morphologically cryptic biological species within the liverwort *Frullania asagrayana*. *American Journal of Botany*, 97, 1707–1718.
- Raven, P. H., & Axelrod, D. I. (1974). Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden*, 61, 539–673.
- Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57, 4–14.
- Renner, M. A. M. (2014). *Radula* subg. *Radula* in Australasia and the Pacific (Jungermanniopsida). *Telopea*, 17, 107–167.
- Renner, M. A. M. (2015). Lobule shape evolution in *Radula* (Jungermanniopsida): One rate fits all? *Botanical Journal of the Linnean Society*, 178, 222–242.
- Renner, M. A. M., Brown, E. A., & Wardle, G. M. (2011). The *Lejeunea tumida* species group is positively polyphyletic (Lejeuneaceae: Jungermanniopsida). *Australian Systematic Botany*, 23, 443–462.
- Renner, M. A. M., Devos, N., Patiño, J., Brown, E. A., Orme, A., Elgey, M., ... von Konrat, M. J. (2013). Integrative taxonomy resolves the cryptic and pseudo-cryptic *Radula buccinifera* complex (Porellales, Jungermanniopsida), including two reinstated and five new species. *PhytoKeys*, 27, 1–113.
- Richardson, J. E., Chatrou, L. W., Mols, J. B., Erkens, R. H. J., & Pirie, M. D. (2004). Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 359, 1495–1508.
- Ronquist, F. (1997). Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology*, 46, 195–203.
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Scheben, A., Bechteler, J., Lee, G. E., Pócs, T., Schäfer-Verwimp, A., & Heinrichs, J. (2016). Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *Journal of Biogeography*, 43, 1739–1749.
- Schneider, H., Kreier, H.-P., Janssen, T., Otto, E., Muth, H., & Heinrichs, J. (2010). Key innovations versus key opportunities: Identifying causes of rapid radiations in derived ferns. In M. Glaubrecht (Ed.), *Evolution in Action* (pp. 61–75). Berlin: Springer.
- Schuster, R. M. (1980). *The Hepaticae and Anthocerotae of North America east of the Hundredth Meridian*, Vol. 4. New York, NY: Columbia University Press.
- Schuster, R. M. (1983). Phytogeography of the Bryophyta. In R. M. Schuster (Ed.), *New Manual of Bryology* (pp. 463–626). Nichinan: The Hattori Botanical Laboratory.
- Shaw, A. J. (2001). Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography*, 28, 253–261.
- Shaw, A. J., Boles, S., & Shaw, B. (2008). A phylogenetic delimitation of the *Sphagnum subsecundum* complex. *American Journal of Botany*, 95, 731–744.
- Shaw, A. J., Devos, N., Cox, C. J., Boles, S. B., Shaw, B., Buchanan, A. M., ... Seppelt, R. (2010). Peatmoss (*Sphagnum*) diversification associated with Miocene Northern Hemisphere climatic cooling? *Molecular Phylogenetics and Evolution*, 55, 1139–1145.
- Shaw, A. J., Golinski, G. K., Clark, E. G., Shaw, B., Stenøien, H. K., & Flatberg, K. I. (2014). Intercontinental genetic structure in the amphipacific peatmoss *Sphagnum miyabearum* (Bryophyta: Sphagnaceae). *Biological Journal of the Linnean Society*, 11, 17–37.
- Shu, L., Zhu, R.-L., & Pócs, T. (2016). A new species of *Leptolejeunea* (Lejeuneaceae, Marchantiophyta) from Fiji with special reference to *Leptolejeunea tripuncta*. *Cryptogamie, Bryologie*, 37, 157–165.
- Söderström, L., Hagborg, A., von Konrat, M., Bartoomew-Began, S., Bell, D., Briscoe, L., ... Zhu, R. L. (2016). World checklist of hornworts and liverworts. *PhytoKeys*, 59, 1–828.
- Stadler, T. (2009). On incomplete sampling under birth-death models and connections to the sampling-based coalescent. *Journal of Theoretical Biology*, 261, 58–66.
- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
- Stotler, R. E., & Crandall-Stotler, B. (1974). A monograph of the genus *Bryopteris* (Swartz) Nees von Esenbeck. *Bryophytorum Bibliotheca*, 3, 1–159, + 219 figures on 32 plates.

- Sun, Y., He, X., & Glenny, D. (2014). Transantarctic disjunctions in Schistochilaceae (Marchantiophyta) explained by early extinction events, post-Gondwanan radiations and palaeoclimatic changes. *Molecular Phylogenetics and Evolution*, 76, 189–201.
- Van Zanten, B. O., & Gradstein, S. R. (1988). Experimental dispersal geography of neotropical liverworts. *Nova Hedwigia Beihefte*, 90, 41–94.
- Vanderpoorten, A., Gradstein, S. R., Carine, M. A., & Devos, N. (2010). The ghosts of Gondwana and Laurasia in modern liverwort distributions. *Biological Reviews*, 85, 471–487.
- Vanderpoorten, A., Patiño, J., Dirkse, G., Blockeel, T., & Hedenäs, L. (2015). Early divergence of an Azorean endemic species in the moss genus *Rhynchostegiella* (Brachytheciaceae). *Phytotaxa*, 210, 60–69.
- Vigalondo, B., Lara, F., Draper, I., Valcárcel, V., Garillete, R., & Mazimpaka, V. (2016). Is it really you, *Orthotrichum acuminatum*? Ascertaining a new case of intercontinental disjunction in mosses. *Botanical Journal of the Linnean Society*, 180, 30–49.
- Villarreal, J. C., & Renner, S. S. (2012). Hornwort pyrenoids, a carbon-concentrating mechanism, evolved and were lost at least five times during the last 100 million years. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 18873–18878.
- Villarreal, J. C., & Renner, S. S. (2014). A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a taxonomically cleaned-up genus *Nothoceros*. *Molecular Phylogenetics and Evolution*, 78, 25–35.
- Wei, R., Xiang, Q., Schneider, H., Sundue, M. A., Kessler, M., Kamau, P. W., ... Zhang, X. (2015). Eurasian origin, boreotropical migration and trans-oceanic dispersal in the pantropical fern genus *Diplazium* (Athryiaceae). *Journal of Biogeography*, 42, 1809–1819.
- Wilson, R., Gradstein, S. R., Schneider, H., & Heinrichs, J. (2007). Unravelling the phylogeny of Lejeuneaceae (Jungermanniopsida): Evidence for four main lineages. *Molecular Phylogenetics and Evolution*, 43, 270–282.
- Wilson, R., Heinrichs, J., Hentschel, J., Gradstein, S. R., & Schneider, H. (2007). Steady diversification of derived liverworts under Tertiary climatic fluctuations. *Biology Letters*, 3, 566–569.
- Xie, W., Lewis, P. O., Fan, Y., Kuo, L., & Chen, M.-H. (2011). Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, 60, 150–160.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 ma to present. *Science*, 292, 686–693.
- Zhu, R. L., & So, M. L. (2001). Epiphyllous liverworts of China. *Nova Hedwigia Beihefte*, 121, 1–418.
- Zona, S. (2013). Millipedes transport gemmae of *Calymperes palisotii* (Bryophyta: Calymperaceae). *Nova Hedwigia*, 97, 477–483.

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## Chapter 5

A BURMESE AMBER FOSSIL OF *RADULA* (PORELLALES, JUNGERMANNIOPSIDA) PROVIDES INSIGHTS INTO THE CRETACEOUS EVOLUTION OF EPIPHYTIC LINEAGES OF LEAFY LIVERWORTS.

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## A Burmese amber fossil of *Radula* (Porellales, Jungermanniopsida) provides insights into the Cretaceous evolution of epiphytic lineages of leafy liverworts

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**Abstract.** DNA-based divergence time estimates suggested major changes in the composition of epiphyte lineages of liverworts during the Cretaceous; however, evidence from the fossil record is scarce. We present the first Cretaceous fossil of the predominantly epiphytic leafy liverwort genus *Radula* in ca. 100 Myr old Burmese amber. The fossil's exquisite preservation allows first insights into the morphology of early crown group representatives of *Radula* occurring in gymnosperm-dominated forests. Ancestral character state reconstruction aligns the fossil with the crown group of *Radula* subg. *Odontoradula*; however, corresponding divergence time estimates using the software BEAST lead to unrealistically old age estimates. Alternatively, assignment of the fossil to the stem of subg. *Odontoradula* results in a stem age estimate of *Radula* of 227.8 Ma (95 % highest posterior density (HPD): 165.7–306.7) and a crown group estimate of 176.3 Ma (135.1–227.4), in agreement with analyses employing standard substitution rates (stem age 235.6 Ma (142.9–368.5), crown group age 183.8 Ma (109.9–289.1)). The fossil likely belongs to the stem lineage of *Radula* subg. *Odontoradula*. The fossil's modern morphology suggests that switches from gymnosperm to angiosperm phorophytes occurred without changes in plant body plans in epiphytic liverworts. The fossil provides evidence for striking morphological homoplasy in time. Even conservative node

assignments of the fossil support older rather than younger age estimates of the *Radula* crown group, involving origins for most extant subgenera by the end of the Cretaceous and diversification of their crown groups in the Cenozoic.

### 1 Introduction

DNA-based divergence time estimates suggest major changes in the composition of epiphyte lineages of liverworts, mosses, and ferns during the Cretaceous radiation of main angiosperm lineages (Schuettpelz and Pryer, 2006; Newton et al., 2007; Hennequin et al., 2008; Cooper et al., 2012; Feldberg et al., 2014). These lineages may have benefited from the more humid climate of angiosperm-dominated forests compared to gymnosperm forests (Boyce et al., 2010; Boyce et al., 2010; Boyce and Leslie, 2012; Zwieniecki and Boyce, 2014); however, evidence from the fossil record is scarce (Taylor et al., 2009). Only very few well-preserved Cretaceous fossils of leafy liverworts have been observed, of which some have been placed in the extant genera *Frullania* Raddi (Heinrichs et al., 2012) and *Gackstroemia* Trevis. (Heinrichs et al., 2014), whereas others have been assigned to fossil genera with somewhat unclear relationships (*Kaolakia* Heinrichs, M. E. Reiner, Feldberg, von Konrat &

A. R. Schmidt, Heinrichs et al., 2011; *Diettertia* J. T. Br. & Robison, Schuster and Janssens, 1989). Cretaceous fossils of thallose liverworts are more numerous but generally poorly preserved (Fletcher et al., 2008; Li et al., 2014, 2016; Tomescu, 2016) and can hardly be aligned with extant genera or families (Laenen et al., 2014; Villarreal et al., 2016). Considering the importance of the Cretaceous for the evolution of epiphytic lineages of liverworts (Feldberg et al., 2014) and our scarce knowledge on the morphology of these plants, an extension of the Cretaceous fossil record of liverworts is very desirable.

With some 250 species (Yamada, 1986; Söderström et al., 2016), *Radula* Dumort. is one of the largest genera of the Porellales, a predominantly epiphytic clade of leafy liverworts (Heinrichs et al., 2005). *Radula* is well known for its rather monotonous, reduced morphology, for example in the absence of underleaves and the rather uniformly shaped, predominantly entire-margined leaves. It has been included in several integrative taxonomic studies that identified numerous inconsistencies in previous morphology-based classifications and molecular topologies (Devos et al., 2011b; Renner et al., 2013c, 2014; Renner, 2014). These studies led to new hypotheses on species ranges and species circumscriptions (Patiño et al., 2013, 2017; Renner et al., 2013a) and a new supraspecific classification (Devos et al., 2011b). Most importantly, these studies demonstrated the presence of morphologically very similar plants in different main lineages, and morphological convergence caused by lineages repeatedly traversing shared regions of morphospace appeared commonplace (Renner, 2015). As a consequence, many *Radula* species cannot be assigned with confidence to the recently established subgenera using morphological evidence alone (Devos et al., 2011b).

Until now, five fossil species of *Radula* were known, all from the Cenozoic. These include the Eocene–Oligocene Bitterfeld or Baltic amber fossils *R. baltica* Heinrichs, Schäf.-Verw. & M. A. M. Renner; *R. oblongifolia* Casp.; and *R. sphaerocarpoides* Grolle (Heinrichs et al., 2016b) and the Miocene Dominican amber fossils *R. steerei* Grolle (Grolle, 1987) and *R. intacta* M. A. M. Renner, Schäf.-Verw. & Heinrichs (Kaasalainen et al., 2017). The subgeneric affiliation of these fossils is unclear and, accordingly, they were not assigned to any crown group node of *Radula* in divergence time estimates based on DNA sequence data of extant species (Patiño et al., 2017). These studies relied on standard substitution rates of chloroplast DNA of seed-free land plants (Palmer, 1991; Villarreal and Renner, 2014) and suggested a Jurassic origin of *Radula* and divergence of the extant subgenera in the Cretaceous (Patiño et al., 2017).

Here, we present a well-preserved fossil of *Radula* in ca. 100 Myr old Burmese amber from Myanmar, which extends by some 65 Myr the temporal range encompassed by *Radula* fossils. We reconstruct the character states of the fossil on a comprehensive phylogeny of *Radula* and discuss the possible relationships of the fossil to extant subgenera. We present

a series of divergence time estimates to consider possible crown and stem group assignments and examine the fossils' importance for understanding the evolutionary history of *Radula*.

## 2 Material and methods

### 2.1 Amber fossil

Burmese amber derives from the amber localities near the village of Tanai in Kachin State, Myanmar (Grimaldi et al., 2002; Kania et al., 2015). Biostratigraphic studies (Cruikshank and Ko, 2003) and U–Pb dating of zircons (Shi et al., 2012) revealed a late Albian to earliest Cenomanian age of Burmese amber, with a minimum age of 98 Ma. The amber inclusion was examined under a Carl Zeiss Stereo Discovery V8 dissection microscope and a Carl Zeiss Axio Scope A1 compound microscope using incident and transmitted light simultaneously. Images were taken with Canon EOS 5D digital cameras attached to the microscopes. For enhanced illustration of three-dimensional structures, all figures are photomicrographic composites that were digitally stacked from up to 43 focal planes, using the software package Helicon Focus 6.7 (Fig. 1).

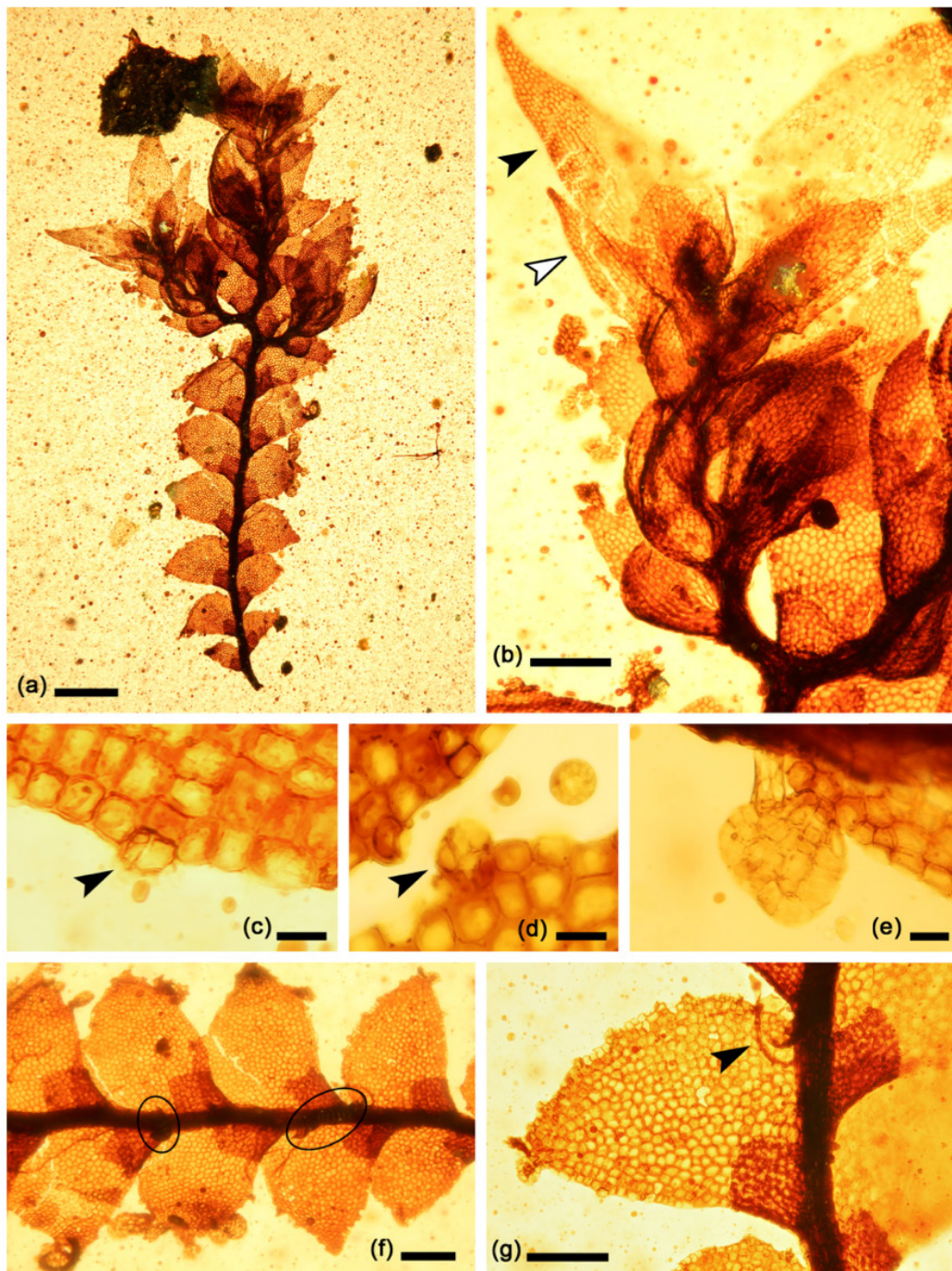
### 2.2 Phylogenetic analyses

A molecular dataset for *Radula* species was compiled based on GenBank sequences used in previous studies by Devos et al. (2011a) and Patiño et al. (2017). Plastid *trnL-F*, *trnG*, *atpB*, *psbT*, *rps4*, and *psbA* sequences of 99 *Radula* accessions were downloaded from GenBank, and sequences of *R. pugioniformis* M. A. M. Renner were newly generated for this study following the protocol given in Devos et al. (2011a, b). *Lepidolaena clavigera* Hook., Dumort. ex Trevis.; *Porella navicularis* (Lehm. & Lindenb.) Pfeiff.; *Lejeunea tuberculosa* Steph.; and *Frullania* sp. served as out-group taxa. Herbarium voucher numbers and their GenBank accession numbers are given in the Supplement. Sequences were manually aligned in Geneious version 6 (Kearse et al., 2012). The Akaike information criterion (AIC; Akaike, 1973) in jModelTest 2 (Darriba et al., 2012) was employed to select the best-fit models of evolution for each of the six plastid markers. This resulted in a TIM1+I+G model for *trnL-F*; a TVM+G model for *trnG*; a TVM+I+G model for *atpB*, *psbT*, and *rps4*; and a GTR+I+G model for *psbA* and the concatenated six-marker dataset.

### 2.3 Phylogeny reconstruction

An ultrametric starting tree was generated in BEAST 1.8.2 for further analyses (Drummond et al., 2012) by using an uncorrelated log-normal (UCLN) relaxed clock model and a birth–death prior accounting for incomplete sampling (Stadler, 2009), running the analysis for 40 million gen-





**Figure 1.** *Radula cretacea* sp. nov. from Cretaceous Burmese amber (PB22484). (a) Overview of the fossil. (b) Upper portion of shoot with female bract pairs. The white arrowhead points to the outer bract; the black arrowhead points to the inner female bract. (c–e) Gemmae in different developmental stages. (f) Portion of the shoot. Two fern sporangia (encircled) are attached to the stem of the *Radula* fossil. (g) Leaf. Note the acute leaf apex and gemmae development at its margin. The arrowhead points to a degraded fern sporangium. Scale bars: (a) 500  $\mu\text{m}$ , (b, f) 200  $\mu\text{m}$ , (g) 150  $\mu\text{m}$ , and (c, d, e) 20  $\mu\text{m}$ . (a–e, g) Fossil in ventral view and (f) in dorsal view.

erations and sampling every 4000 generations. A single GTR+I+G model as suggested by jModelTest2 was employed for the concatenated dataset in all analyses. The quality of the run was assessed in TRACER (Rambaut et al., 2014) in which effective sample size (ESS) values >200 indicated good mixing and a sufficient number of generations. The resulting maximum clade credibility (MCC) tree was generated in TreeAnnotator 1.8.2 (Drummond et al., 2012) using median node heights, excluding the first 10 % of trees as burn-in. The tree was visualized using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>).

#### 2.4 Ancestral character state reconstruction

Four characters possessed by the fossil are potentially informative regarding the fossil's phylogenetic relationships. These characters are the acute to acuminate leaf lobe apex, the two pairs of female bracts, the longitudinal lobule insertion, and the production of gemmae from the leaf-lobe margin. Other potentially informative character systems such as perianth structure, sporophyte anatomy, and spores are not preserved in the fossil. Ancestral character states were estimated on the MCC topology after reducing the outgroup taxa to *Porella navicularis*. A maximum likelihood approach implemented in the ape package v3.5 (Paradis et al., 2004) in R v3.3.0 (R Core Team, 2016) was employed to infer the evolution of the following discrete morphological characters for *Radula*: presence and absence of gemmae, number of female bract pairs, shape of the lobule insertion (transverse, longitudinal), and shape of the leaf apex (round, acute). The coding matrices can be found in the Supplement. Two models, parameterizing the transition rates among the states were compared using the log likelihood values, namely an equal rates model and an all-rates-different model.

#### 2.5 Divergence time estimates

Divergence time estimates were obtained using BEAST 1.8.2. The MCC tree was employed as the starting tree for all subsequent analyses, in which the ingroup was constrained monophyletic, with run lengths of 100 million generations, sampling every 10 000 generations. Three different calibration approaches were conducted. In the first, a plastid standard substitution rate of  $5 \times 10^{-4}$  subst./sites/Myr (Palmer, 1991; Villarreal and Renner, 2014) with a standard deviation (SD) of  $1 \times 10^{-4}$ , and a normal prior distribution was used. In the second, the fossil was given a crown group assignment within *R.* subgenus *Odontoradula* K. Yamada according to morphological similarity of the fossil with extant taxa of this group and character state reconstructions (Fig. 2). In detail, the clade consisting of *Radula ocellata* K. Yamada, *R. pulchella* Mitt., *R. cuspidata* Steph., *R. acuta* Mitt., *R. novae-hollandiae* Hampe, *R. kojana* Steph., and *R. apiculata* Sande Lac. ex Steph. was set to monophyletic and the include stem option was activated. A normal prior distribution with a mean

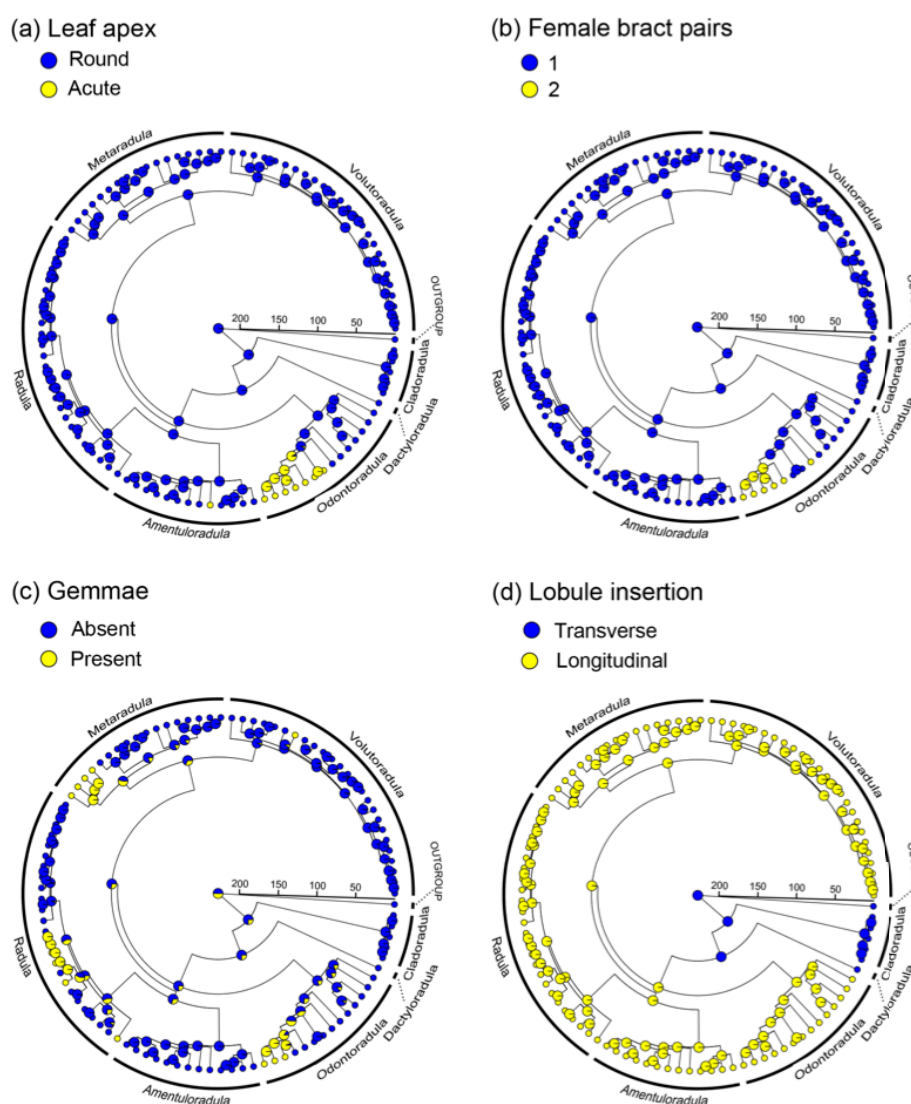
of 98.0 Ma and a SD of 1.0 was used, corresponding to the age of the Burmese amber fossil. In the third approach, the fossil was placed on the stem of *R.* subgenus *Odontoradula* and the same prior information for the fossil age was used. This approach considered the distribution of the fossil's character states suggesting *R.* subgenus *Odontoradula*. Published divergence time estimates of *Radula* were gathered from the literature (Heinrichs et al., 2007; Fiz-Palacios et al., 2011; Cooper et al., 2012; Feldberg et al., 2014; Laenen et al., 2014; see Supplement) and compared with the results of the present analyses.

Stepping-stone sampling in BEAST (Xie et al., 2011; Baele et al., 2012, 2013) and the resulting log-marginal likelihood values and ln-Bayes factor (Kass and Raftery, 1995) values helped to select between pure birth (Yule), birth-death, and birth-death incomplete sampling tree priors, as well as between the UCLN relaxed clock and a strict clock model. The model comparison was conducted using the first calibration approach, and the resulting combination of a birth-death tree prior and an UCLN relaxed clock model was assigned to the other calibration approaches. Log-marginal likelihood values and ln-Bayes factor values are shown in Table 1. All results were examined in TRACER, summarized in TreeAnnotator, and visualized in FigTree as reported above.

### 3 Results

#### 3.1 Ancestral character state reconstruction

The all-rates-different model was selected as the best-fit model for all four ancestral character state reconstructions (Table 2). Results are shown in Fig. 2 in which a yellow color coding refers to the character state of the *Radula* fossil. A round leaf apex was inferred as the ancestral state for *Radula*, whereas a transition to an acute leaf apex was reconstructed for the common ancestor of *Radula pugioniformis* M. A. M. Renner, *R. ocellata*, *R. pulchella* Mitt., *R. cuspidata*, *R. acuta*, *R. novae-hollandiae*, *R. kojana*, and *R. apiculata*. Acute leaf apices also occur in a single species of *R.* subg. *Amentuloradula* Devos, M. A. M. Renner, Gradst., A. J. Shaw & Vanderp. among those included in the phylogeny. The same pattern was observed for the number of female bract pairs, which changed from one to two female bract pairs within subgenus *Odontoradula*. The lack of gemmae production was inferred as the ancestral state for *Radula* and a transition to the development of gemmae occurred independently within the subgenera *Odontoradula*, *Volutoradula* Devos, M. A. M. Renner, Gradst., A. J. Shaw & Vanderp., *Radula*, and *Metaradula* R. M. Schust. Only one transition from a transverse to a longitudinal lobule insertion was inferred at the most recent common ancestor of the subgenera *Odontoradula*, *Amentuloradula*, *Radula*, *Metaradula*, and *Volutoradula*.



**Figure 2.** Results of ancestral character state reconstructions. (a) Shape of leaf apex. (b) Number of female bract pairs. (c) Gemmae development. (d) Type of lobule insertion. Yellow color coding refers to the morphological characters observed in the fossil.

**Table 1.** Marginal likelihood estimations using stepping-stone sampling in BEAST and ln-Bayes factor calculation resulting in an uncorrelated log-normal (UCLN) relaxed clock model. Since the birth–death (BD) tree prior and the birth–death tree prior accounting for incomplete sampling (BD incompl.) did not differ significantly, the less complex BD tree prior was used.

	Model 1	UCLN, Yule	UCLN, BD	UCLN, BD incompl.	Strict clock, BD
Model 2	Log-marginal likelihood	−23 875.92	−23 865.00	−23 864.05	−23 965.95
UCLN, Yule	−23 875.92	0.00	10.92	11.87	−90.03
UCLN, BD	−23 865.00	−10.92	0.00	0.95	−100.95
UCLN, BD incompl.	−23 864.05	−11.87	−0.95	0.00	−101.90
Strict clock, BD	−23 965.95	90.03	100.95	101.90	0.00

**Table 2.** Results of the ancestral character state reconstruction favoring an all-rates-different model for all analyzed morphological characters.

Morphological character	Equal-rates model (ER) log likelihood	All-rates-different model (ARD) log likelihood
Leaf apex	-11.149	-11.145
Female bract pairs	-10.853	-10.632
Gemmae	-34.241	-29.532
Lobule insertion	-4.919	-3.696

### 3.2 Molecular dating analyses

Calibration of the dataset with the plastid standard substitution rate resulted in an estimated divergence between *Radula* and the outgroup ranging from Devonian to early Cretaceous (235.6 Ma, 95 % HPD: 142.9–368.5), and crown group divergences between the early Permian and early Cretaceous (183.8 Ma, 95 % HPD: 109.9–289.1). Establishment of most extant species likely took place within the Neogene. Divergence time estimates resulting from an assignment of the fossil to the crown of subgenus *Odontoradula* resulted in much older age estimates. Under this crown-assignment dating strategy, the *Radula* stem was estimated to originate sometime from the Neoproterozoic (Cryogenian) to the early Carboniferous (508.1 Ma, 95 % HPD: 340.7–713.8) and its crown group from the late Neoproterozoic (Ediacaran) to the middle Permian (392.2 Ma, 95 % HPD: 266.5–551.5). The calibration approach placing the fossil on the stem of subgenus *Odontoradula* results in a stem age estimate of *Radula* dating back to the late Triassic (227.8 Ma, 95 % HPD: 165.7–306.7) and the origin of its crown group is estimated to the early Jurassic (176.3 Ma, 95 % HPD: 135.1–227.4). Since all analyses yielded the same topology, a phylogenetic chronogram with three scale axes referring to the three calibration approaches is presented as Fig. 3, while Table 3 shows the corresponding estimated divergence times and their 95 % HPD intervals for selected nodes.

### 3.3 Systematic palaeontology

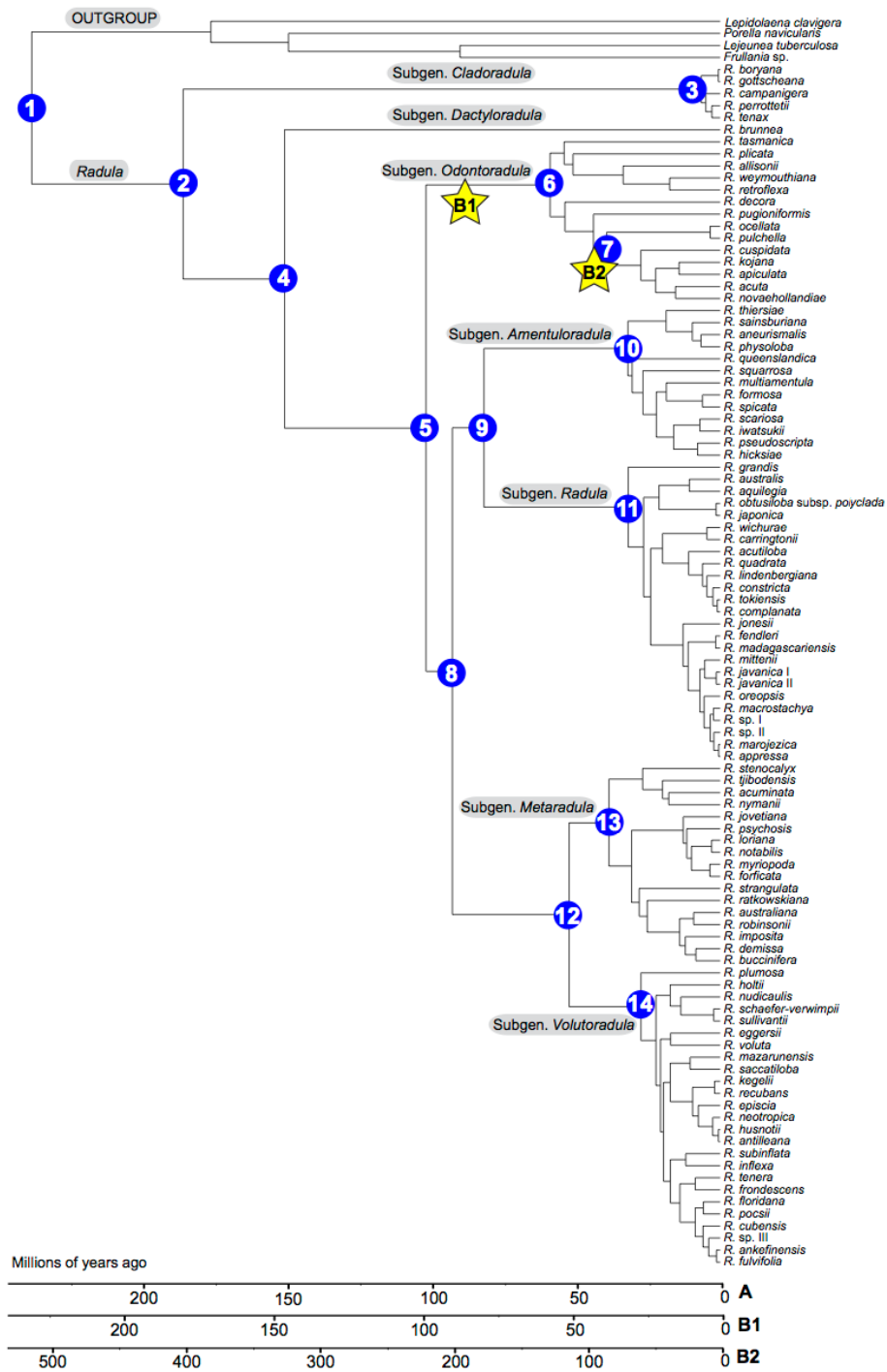
*Radula cretacea* Bechteler, M. A. M. Renner, Schäf.-Verw. & Heinrichs, sp. nov.

Holotype: Single liverwort fossil in Burmese amber piece PB22484 of the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences (Fig. 1; syninclusions: composed plant hairs and degraded sporangia of leptosporangiate ferns).

Diagnosis: Species of *Radula* distinctive in its possession of leaves whose apex is acute to acuminate, gemmae produced from leaf-lobe marginal cells only, and female

bracts in two pairs. From other species of *Radula* sharing these characters, the fossil differs in the production of subfloral innovations from the base of the upper pair of female bracts, in the cochleariform lobules on leaves transitional between female bracts and vegetative leaves, and the lanceolate lobes of the female bracts.

Description: Shoot 800–1160 µm wide; stem 65–85 µm wide and four or five cortical cell rows across. Branching *Radula* type. Leaves remote at shoot base, becoming contiguous then imbricate as stature increases along shoot, ovate, spreading, not obliquely patent, 270–600 µm long by 200–560 µm wide, postical margin straight to slightly curved along inner half, curved toward apex along outer half, apex acute to slightly acuminate, antical margin more or less straight or weakly curved near apex, curvature increasing toward stem, interior margin curved but not ampliate, hardly extending onto the dorsal stem surface, leaving the stem visible from above. Lobules around the area of leaf lobes; quadrate to trapeziform, 90–165 µm long by 110–210 µm wide, insertion longitudinal; keel arising from stem at 45° angle, running flush into the lobe outline, or meeting at a slight angle straight to slightly arched; exterior and antical margins straight to slightly curved, slightly irregular due to bulging marginal cells, apex obtuse to slightly attenuate, with shallow notch between two cells wherein papilla is situated (observed in one lobule); interior margin not ampliate, not extending onto ventral stem surface. Ventral leaf-free strip present, perhaps two cortical cell rows only; presence or absence of dorsal leaf-free strip not ascertained. Cells on leaf margin quadrate to rectangular, 12.5–27.5 µm long by 12.5–17.5 µm wide, long axis either perpendicular or parallel with leaf margin; medial cells isodiametric to slightly elongate, irregularly sized and arranged, 20–30 µm long by 15–25 µm wide, basal cells slightly larger, 30–35 µm long by 22.5–30 µm wide; cell medial walls unthickened, small concave trigones, possibly consisting entirely of primary wall material, present at cell angles, free exterior wall of marginal cells unthickened. Asexual reproduction by gemmae produced from cells of leaf margin, gemmae unistratose, subdiscoid to obcordate to thalloid as size increases, 50–185 µm or more in length and 45–125 µm in width. (?)Dioicous. Gynoecia terminal on leading axes and short lateral branches. Female bracts in two pairs, both larger than preceding leaves, transitional leaves bearing enlarged obovate and cochleariform lobules present between bracts and leaves; upper female bract lobe lanceolate, 990–1150 µm long by 300–410 µm wide, apex acuminate, medial cells 25–40 µm long by 20–25 µm wide, walls unthickened; upper bract lobule broad-elliptic to obovate or obtrullate, 480–570 µm long by 280–340 µm wide; lower female bract lobe lanceolate 620–820 µm long by 190–210 µm wide, apex acuminate, lobule obovate, 340–390 µm long by 160–250 µm wide, bracts imbricate, long axis orientated at around 30° to stem. A single *Radula*-type subfloral innovation present



**Figure 3.** Phylogenetic chronogram for *Radula* with timescales resulting from different calibration approaches using BEAST. Scale bar A results from the divergence time estimation using a plastid standard substitution rate. Scale bar B1 results from an assignment of the fossil to the stem of subgenus *Odontoradula* whereas scale bar B2 results from a fossil assignment to the crown group of this subgenus. Stars indicate alternative fossil assignments.

**Table 3.** Divergence time estimates for nodes of interest (see Fig. 3) in millions of years (Ma) before present with corresponding 95 % highest posterior density (HPD) intervals in square brackets shown for three different dating approaches. Approach A, calibration with the chloroplast standard substitution rate; approach B1, assignment of the *Radula* fossil to the stem of subgenus *Odontoradula*; approach B2, crown group assignment of the *Radula* fossil to the stem of a clade within subgenus *Odontoradula*. See material and methods for details.

	Approach A	Approach B1	Approach B2
Node	Node age [95 % HPD]	Node age [95 % HPD]	Node age [95 % HPD]
1	235.6 [142.9–368.5]	227.8 [165.7–306.7]	508.1 [340.7–713.8]
2	183.8 [109.9–289.1]	176.3 [135.1–227.4]	392.2 [266.5–551.5]
3	6.5 [2.7–12.3]	6.3 [2.9–11.1]	14.0 [6.4–24.6]
4	149.1 [91.6–236.6]	142.7 [116.9–179.8]	319.3 [221.4–444.5]
5	100.7 [62.1–155.1]	98.1 [95.5–101.0]	216.6 [156.5–285.8]
6	58.2 [35.8–90.8]	57.4 [43.0–72.9]	126.1 [105.4–159.5]
7	38.9 [22.7–62.6]	38.2 [25.7–51.0]	88.9 [74.9–97.6]
8	91.7 [56.6–142.3]	90.1 [79.0–97.3]	197.2 [143.9–265.9]
9	80.9 [47.5–126.7]	79.7 [62.1–93.4]	174.1 [121.2–239.2]
10	31.6 [19.2–50.2]	31.0 [21.6–42.0]	68.5 [44.7–95.2]
11	31.5 [18.6–50.6]	30.7 [20.3–43.3]	67.9 [42.7–97.0]
12	51.7 [32.0–81.3]	51.1 [37.7–65.2]	111.8 [75.8–153.2]
13	38.1 [22.7–58.9]	37.4 [27.4–49.3]	82.4 [54.0–111.6]
14	27.1 [16.0–42.9]	26.7 [18.5–36.4]	58.1 [38.1–82.9]

at the base of one of the bracts in the uppermost pair, again fertile. Gynoecial disc bearing around five archegonia 100–110  $\mu\text{m}$  long. Perianths not seen. Male reproductive structures not seen.

#### 4 Discussion

The majority of extant and fossil *Radula* species have rounded rather than acute leaf lobes and one rather than two female bract pairs (Castle, 1936). On the basis of the acute leaf lobe apex, the female bracts in two pairs, and the production of gemmae, the fossil plant would be assigned to *Radula* subg. *Odontoradula* and placed into Yamada's (1979) Asian sect. *Acutifoliae* series *Acutifoliae*, were it extant. Ancestral state reconstructions support this placement (Fig. 2). However, extending this confidence to time calibrating the phylogeny by enforcing a minimum age of 98 million years on the node corresponding with series *Acutifoliae* results in divergence time estimates that are unrealistic (Fig. 3) as they even exceed most age estimates of the land plant crown group (Clarke et al., 2011; Fiz-Palacios et al., 2011; Magallón et al., 2013). Possibly, strongly deviating substitution rates within the main clades of *Radula* and related epiphyte lineages of liverworts account for the apparent young age of ser. *Acutifoliae*. We have no biological explanation for this scenario and were also not able to observe the deviant branch length of *R.* subg. *Odontoradula* species and related lineages in plastid DNA phylograms of *Radula* (e.g., Devos et al., 2011b). Accordingly, we consider a position of *R. cretacea* in the crown group of *R.* subg. *Odontoradula* as unlikely. Assignment of *R. cretacea* to the stem of *R.* subg. *Odontoradula* leads to

divergence time estimates that are in good accordance with those based on standard substitution rates of seed-free land plants (Fig. 3; Patiño et al., 2017). They are also in good agreement with most other age estimates of *Radula* generated in dating analyses of major liverwort or land plant lineages (Heinrichs et al., 2007; Fiz-Palacios et al., 2011; Cooper et al., 2012; Feldberg et al., 2014; Laenen et al., 2014). These estimates were based on DNA sequence variation with integrated information from the fossil record. Some of these estimates seem to differ considerably; however, the large confidence intervals of the respective node age estimates broadly overlap and demonstrate the uncertainty in current age estimates of epiphyte lineages of liverworts (Supplement). We used similar datasets to Patiño et al. (2017); however, Patiño et al. (2017) applied a Yule prior. Our stepping stone analyses supported a birth–death rather than a Yule model to best fit our dataset. The corresponding analyses resulted in slightly older age estimates than those of Patiño et al. (2017) and our additional analyses with the Yule prior (Table 4). However, the older estimates obtained with the birth–death prior get support from the *Radula* fossil which indicates the presence of subg. *Odontoradula* already in the earliest late Cretaceous. Condamine et al. (2015) demonstrated the crucial importance of model choice in divergence time analyses and presented an example from cycads in which different priors resulted in strongly deviant divergence time estimates based on the same sequence dataset. Our choice of a birth–death model was supported not only by the stepping stone analyses but also seems reasonable when considering the Mesozoic or Paleozoic origin and the long stem lineages of *Radula* and its main crown group clades. These lineages, and indeed the fossil itself, both provide some evidence for extinction events in

**Table 4.** Comparison of divergence time estimates resulting from BEAST analyses only differing in their tree prior. A plastid standard substitution rate was used to calibrate the dataset. In approach A, a birth–death tree prior was used, while in approach AY a pure-birth (Yule) tree prior was implemented. Bayes factor values favor a birth–death tree prior (see Table 1). Node numbers correspond to Fig. 3. Node ages and their 95 % highest posterior density (HPD) intervals are given in millions of years (Ma) before present.

	Approach A	Approach AY
Node	Node age [95 % HPD]	Node age [95 % HPD]
1	235.6 [142.9–368.5]	184.5 [113.3–284.9]
2	183.8 [109.9–289.1]	154.1 [96.3–241.6]
3	6.5 [2.7–12.3]	7.2 [2.9–14.1]
4	149.1 [91.6–236.6]	127.7 [78.6–198.6]
5	100.7 [62.1–155.1]	91.6 [56.2–139.3]
6	58.2 [35.8–90.8]	56.2 [35.4–87.6]
7	38.9 [22.7–62.6]	38.4 [22.4–60.3]
8	91.7 [56.6–142.3]	84.1 [51.6–128.3]
9	80.9 [47.5–126.7]	73.94 [44.7–115.9]
10	31.6 [19.2–50.2]	33.4 [19.9–52.6]
11	31.5 [18.6–50.6]	33.5 [19.4–53.9]
12	51.7 [32.0–81.3]	51.9 [31.9–81.0]
13	38.1 [22.7–58.9]	39.2 [23.5–60.9]
14	27.1 [16.0–42.9]	29.2 [17.1–45.7]

the early history of the genus that are not considered in a pure birth model.

Since a position of the fossil *Radula cretacea* in the crown group of subg. *Odontoradula* resulted in unrealistically old age estimates, we prefer to treat it as a stem group element of this subgenus. This hypothesis contradicts our ancestral character state reconstructions, which suggest that early *Odontoradula* taxa had rounded lobe apices, a single pair of female bracts, and no asexual reproduction by gemmae. However, the high amount of homoplasy within some character systems in extant *Radula* species (Renner, 2015) and implied rapid changes of character states within extant subgenera (Renner et al., 2013b) give reason to assume gains and losses of character states during earlier radiations on the *Radula* stem lineages. Such a scenario would explain that early stem group species of *R.* subg. *Odontoradula* share character states with derived crown group representatives in the *R. pugioniformis*–*R. apiculata* clade. It is possible that *Radula* lineages explore a certain morphospace and that a certain suite of character states can be repeatedly combined in new ways. Unfortunately, the poor Mesozoic fossil record of *Radula* disables a detailed reconstruction, yet *Radula cretacea* provides a note of caution for node calibrations using evidence from the fossil record (Parham et al., 2012). We thus propose balancing different lines of evidence, including information from standard substitution rates, results generated using secondary calibrations, and data based on the morphology of the fossil and related taxa (Lóriga et al., 2014;

Heinrichs et al., 2015; Schneider et al., 2015, 2016; Feldberg et al., 2017). The recently proposed fossilized birth–death approach was designed to overcome the problem of assigning fossils to certain nodes in divergence time analyses (Heath et al., 2014); however, this approach requires a dense fossil record and numerous morphological character states of both fossils and extant taxa to be coded (Arcila et al., 2015; Warnock et al., 2015). We were unable to successfully employ this approach because of the small number of *Radula* fossils, their incomplete preservation, and the monotonous morphology of both the majority of extant and fossil species (Grolle, 1987; Renner and Braggins, 2004; Renner, 2015; Heinrichs et al., 2016b; Kaasalainen et al., 2017).

Treating the *Radula* fossil as an early stem group element of *R.* subg. *Odontoradula* leads to results that are in good accordance with most other published divergence time estimates, especially with reconstructions based on published standard substitution rates of seed-free land plants. The corresponding phylogenetic chronograms provide evidence for a late Cretaceous origin of most subgenera of *Radula* and for an establishment of their crown groups in the Paleogene. This pattern possibly relates to changes in the terrestrial ecosystems during the Cretaceous Terrestrial Revolution (Meredith et al., 2011), especially the establishment of major angiosperm lineages in the Late Cretaceous (Wang et al., 2009; Couvreur et al., 2011; Coiffard et al., 2012), connected with a decline in gymnosperm diversity (Becker, 2000). The wide distribution of megathermal angiosperm forests in the early Cenozoic (Morley, 2011), their new canopy structure, and their more humid microclimate (Boyce et al., 2010) likely led to major changes in the main epiphyte lineages of liverworts and the establishment of their modern crown groups. This process was likely initiated in the Late Cretaceous, when angiosperms started to dominate many terrestrial ecosystems. The impact of the Cretaceous–Paleogene mass extinction on plant evolution is still incompletely understood (Vajda and Bercovici, 2014); however, the long stem lineages of the *Radula* subgenera may to some extent relate to these extinction processes. Similar topologies suggestive of the same pattern have been reconstructed in other Porellales genera such as *Leptolejeunea* (Spruce) Steph. (Bechteler et al., 2017), *Lejeunea* Lib. and *Microlejeunea* (Spruce) Steph. (Heinrichs et al., 2016a), and *Frullania* (Silva et al., 2016).

The way *Radula cretacea* has been preserved provides minimal insight into what microhabitat the plant occupied in life; however, its inclusion in amber is consistent with the hypothesis that a bark epiphyte or a trunk-base dweller is at hand. Burmese amber was produced by gymnosperm trees in a tropical environment (Grimaldi et al., 2002) and, although angiosperms occurred in this amber forest (Santiago-Blay et al., 2005; Chambers et al., 2010), it was likely dominated by gymnosperms. The modern morphology of the fossil leads to the question of if a switch from gymnosperm to angiosperm carrier trees required major morphological changes in plant bodies. The switch to angiosperm phorophytes likely

involved an adaptation to a more humid microclimate and to a different light regime and possibly also to a somewhat deviant nutrient availability (Schneider et al., 2004). It does, however, not necessarily require changes of the general plant body plan, especially if adaptations to epiphyte growth such as complicated bilobed leaves, solely lateral branching, and fascicled rhizoids (Heinrichs et al., 2005) were already present in the liverwort lineages growing on gymnosperm bark. It is thus not surprising that other Burmese amber fossils of liverworts also have the morphological characteristics of extant genera (Heinrichs et al., 2012, 2014).

## 5 Conclusions

The first Cretaceous fossil of the leafy liverwort genus *Radula* provides crucial insights into the early evolution of predominantly epiphytic lineages of leafy liverworts. Character state reconstructions and a series of divergence time estimates suggest that the fossil is an early stem lineage representative of *Radula* subg. *Odontoradula*. Its modern morphology illustrates that switches from gymnosperm to angiosperm phorophytes did not require changes in plant body plans of epiphytic liverworts and provides evidence for morphological homoplasy in time. Even conservative node assignments of the fossil support older rather than younger age estimates of the *Radula* crown group, involving an establishment of most extant subgenera by the end of the Cretaceous and diversification of their crown groups in the Cenozoic.

**Data availability.** All necessary data are available in the Supplement.

**The Supplement related to this article is available online at <https://doi.org/10.5194/fr-20-201-2017-supplement>.**

**Competing interests.** The authors declare that they have no conflict of interest.

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## References

- Akaike, H.: Information theory as an extension of the maximum likelihood principle, in: Second International Symposium on Information Theory, edited by: Petrov, B. N and Csáki, F., Akadémia Kiado, Budapest, Hungary, 267–281, 1973.
- Arcila, D., Pyron, R. A., Tyler, J. C., Orti, G., and Betancur, R. R.: An evaluation of fossil tip-dating versus node-age calibrations in tetraodontiform fishes (Teleostei: Percormorphaceae), *Mol. Phylogenet. Evol.*, 82, 131–145, 2015.
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., and Alekseyenko, A. V.: Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty, *Mol. Biol. Evol.*, 29, 2157–2167, 2012.
- Baele, G., Li, W. L. S., Drummond, A. J., Suchard, M. A., and Lemey, P.: Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics, *Mol. Biol. Evol.*, 30, 239–243, 2013.
- Bechteler, J., Schäfer-Verwimp, A., Lee, G. E., Feldberg, K., Pérez-Escobar, O. A., Pócs, T., Peralta, D. F., Renner, M. A. M., and Heinrichs, J.: Geographical structure, narrow species ranges, and Cenozoic diversification in a pantropical clade of epiphyllous leafy liverworts, *Ecol. Evol.*, 7, 638–653, 2017.
- Becker, P.: Competition in the regeneration niche between conifers and angiosperms: Bond's slow seedling hypothesis, *Funct. Ecol.*, 14, 401–412, 2000.
- Boyce, C. K. and Lee, J. E.: An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity, *Proc. R. Soc. B*, 277, 3437–3443, 2010.
- Boyce, C. K. and Leslie, A. B.: The paleontological context of angiosperm vegetative evolution, *Int. J. Plant Sci.*, 173, 561–568, 2012.
- Boyce, C. K., Lee, J. E., Field, T. S., Brodribb, T. J., and Zwieniecki, M. A.: Angiosperms helped put the rain in the rainforests: the impact of plant physiological evolution on tropical biodiversity, *Ann. Mo. Bot. Gard.*, 97, 527–540, 2010.
- Castle, H.: A revision of the genus *Radula*. Introduction and part I. Subgenus *Cladoradula*, *Ann. Bryol.*, 9, 13–56, 1936.
- Chambers, K. L., Poinar, G., and Buckley, R.: *Tropidogyne*, a new genus of Early Cretaceous Eudicots (Angiospermae) from Burmese amber, *Novon*, 20, 23–29, 2010.
- Clarke, J., Warnock, R. C. M., and Donoghue, P. C. J.: Establishing a time-scale for plant evolution, *New Phytol.*, 192, 266–301, 2011.
- Coiffard, C., Gomez, B., Daviero-Gomez, V., and Dilcher, D. L.: Rise to dominance of angiosperm pioneers in European Cretaceous environments, *P. Natl. Acad. Sci. USA*, 109, 20955–20959, 2012.
- Condamine, F. L., Nagalingum, N. S., Marshal, C. R., and Morlon, H.: Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating, *BMC Evol. Biol.*, 15, 65, 2015.
- Cooper, E. D., Henwood, M. J., and Brown, E. A.: Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution, *Biol. J. Linn. Soc.*, 107, 425–441, 2012.
- Couvreur, T. L. P., Forest, F., and Baker, W. J.: Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms, *BMC Biol.*, 9, 44, 2011.



- Cruickshank, R. D. and Ko, K.: Geology of an amber locality in the Hukawng valley, Northern Myanmar, *J. Asian Earth Sci.*, 21, 441–445, 2003.
- Darriba, D., Taboada, G. L., Doallo, R., and Posada D.: JModelTest 2: More models, new heuristics and parallel computing, *Nature Meth.*, 9, 772, 2012.
- Devos, N., Renner, M. A. M., Gradstein, S. R., Shaw, A. J., Laenen, B., and Vanderpoorten, A.: Evolution of sexual systems, dispersal strategies and habitat selection in the liverwort genus *Radula*, *New Phytol.*, 192, 225–236, 2011a.
- Devos, N., Renner, M. A. M., Gradstein, S. R., Shaw, J., and Vanderpoorten, A.: Molecular data challenge traditional subgeneric divisions in the leafy liverwort *Radula*, *Taxon*, 60, 1623–1632, 2011b.
- Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A.: Bayesian phylogenetics with BEAUti and the BEAST 1.7, *Mol. Biol. Evol.*, 29, 1969–1973, 2012.
- Feldberg, K., Schneider, H., Stadler, T., Schäfer-Verwimp, A., Schmidt, A. R., and Heinrichs, J.: Epiphytic leafy liverworts diversified in angiosperm-dominated forests, *Sci. Rep.*, 4, 5974, 2014.
- Feldberg, K., Vána, J., Schäfer-Verwimp, A., Krings, M., Gröhn, C., Schmidt, A. R., and Heinrichs, J.: Problems related to the taxonomic placement of incompletely preserved amber fossils: transfer of the Paleogene liverwort *Cylindrocolea dimorpha* (Cephaloziaceae) to the extant *Odontoschisma* sect. *Iwatsukia* (Cephaloziaceae), *Foss. Rec.*, 20, 147–157, <https://doi.org/10.5194/fr-20-147-2017>, 2017.
- Fiz-Palacios, O., Schneider, H., Heinrichs, J., and Savolainen, V.: Diversification of land plants. Insights from a family-level phylogenetic analysis, *BMC Evol. Biol.*, 11, 341, 2011.
- Fletcher, B. J., Brentnall, S. J., Anderson, C. W., Berner, R. A., and Beerling, D. J.: Atmospheric carbon dioxide linked with Mesozoic and early Cenozoic climate change, *Nature Geosci.*, 1, 43–48, 2008.
- Grimaldi, D. A., Engel, M. S., and Nascimbene, P. C.: Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance, *Amer. Mus. Novit.*, 3361, 1–71, 2002.
- Grolle, R.: *Radula steerei* sp. nov. – A further hepatic in Dominican Amber, *Mem. New York Bot. Gard.*, 45, 259–263, 1987.
- Heath, T. A., Huelsenbeck, J., and Stadler, T.: The fossilized birth-death process for coherent calibration of divergence time estimates, *P. Natl. Acad. Sci. USA*, 111, E2957–E2966, 2014.
- Heinrichs, J., Gradstein, S. R., and Wilson, H.: Towards a natural classification of liverworts (Marchantiophyta) based on the chloroplast gene *rbcL*, *Cryptogam. Bryol.*, 26, 131–150, 2005.
- Heinrichs, J., Hentschel, J., Wilson, R., Feldberg, K., and Schneider, H.: Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence, *Taxon*, 56, 31–44, 2007.
- Heinrichs, J., Reiner-Drehwald, M. E., Feldberg, K., Grimaldi, D. A., Nascimbene, P. C., von Konrat, M., and Schmidt, A. R.: *Kaolakia borealis* nov. gen. et sp. (Porellales, Jungermanniopsida), a leafy liverwort from the Cretaceous of Alaska, *Rev. Palaeobot. Palyno.*, 165, 235–240, 2011.
- Heinrichs, J., Reiner-Drehwald, M. E., Feldberg, K., von Konrat, M., Hentschel, J., Vána, J., Grimaldi, D. A., Nascimbene, P. C., and Schmidt, A. R.: The leafy liverwort *Frullania* (Jungermanniopsida) in the Cretaceous amber forest of Myanmar, *Rev. Palaeobot. Palyno.*, 169, 21–28, 2012.
- Heinrichs, J., Schäfer-Verwimp, A., Feldberg, K., and Schmidt, A. R.: The extant liverwort *Gackstroemia* (Lepidolaenaceae, Porellales) in Cretaceous amber from Myanmar, *Rev. Palaeobot. Palyno.*, 203, 48–52, 2014.
- Heinrichs, J., Scheben, A., Lee, G. E., Vána, J., Schäfer-Verwimp, A., Krings, M., and Schmidt, A.: Molecular and morphological evidence challenges the records of the extant liverwort *Ptilidium pulcherrimum* in Eocene Baltic amber, *PLoS ONE*, 10, e0140977, <https://doi.org/10.1371/journal.pone.0140977>, 2015.
- Heinrichs, J., Scheben, A., Bechteler, J., Lee, G. E., Schäfer-Verwimp, A., Hedenäs, L., Singh, H., Pócs, T., Nascimbene, P. C., Peralta, D. F., Renner, M., and Schmidt, A. R.: Crown group Lejeuneaceae and pleurocarpous mosses in early Eocene (Ypresian) Indian Amber, *PLoS ONE*, 11, e0156301, <https://doi.org/10.1371/journal.pone.0156301>, 2016a.
- Heinrichs, J., Schmidt, A. R., Schäfer-Verwimp, A., Bauerschmidt, L., Neumann, C., Gröhn, C., Krings, M., and Renner, M. A. M.: Revision of the leafy liverwort genus *Radula* in Baltic and Bitterfeld amber, *Rev. Palaeobot. Palyno.*, 235, 157–164, 2016b.
- Hennequin, S., Schuettelpelz, E., Pryer, K. M., Ebihara, A., and Dubuisson, J.-Y.: Divergence times and the evolution of epiphytism in filmy ferns (Hymenophyllaceae) revisited, *Int. J. Plant Sci.*, 169, 1278–1287, 2008.
- Kaasalainen, U., Heinrichs, J., Renner, M. A. M., Hedenäs, L., Schäfer-Verwimp, A., Lee, G. E., Ignatov, M. S., Rikkinen, J., and Schmidt, A. R.: A Caribbean epiphyte community preserved in Miocene Dominican amber, *Earth Environm. Sci. Trans. R. Soc. Edinb.*, in press, 2017.
- Kania, I., Wang, B., and Szewdo, J.: *Dicranoptycha* Osten Sacken, 1860 (Diptera, Limoniidae) from the earliest Upper Cretaceous Burmese amber, *Cretaceous Res.*, 52, 522–530, 2015.
- Kass, R. E. and Raftery, A. E.: Bayes factor, *J. Am. Stat. Assoc.*, 90, 773–795, 1995.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buyton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., and Drummond, A.: Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data, *Bioinformatics*, 28, 1647–1649, 2012.
- Laenen, B., Shaw, B., Schneider, H., Goffinet, B., Paradis, E., Désamoré, A., Heinrichs, J., Villarreal, J. C., Gradstein, S. R., McDaniel, S. F., Long, D. G., Forrest, L. L., Hollingsworth, M. L., Crandall-Stotler, B., Davis, E. C., Engel, J., Von Konrat, M., Cooper, E. D., Patiño, J., Vanderpoorten, A., and Shaw, A. J.: Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts, *Nature Com.*, 5, 6134, <https://doi.org/10.1038/ncomms6134>, 2014.
- Li, R., Sun, B., Wang, H., He, Y., Yang, G., Yan, D., and Lin, Z.: *Marchantites huolinshensis* sp. nov. (Marchantiales) – A new fossil liverwort with gemmae cups from the Lower Cretaceous of Inner Mongolia, China, *Cretaceous Res.*, 50, 16–26, 2014.
- Li, R., Wang, X., Jin, P., Ma, F., Yan, D., Lin, Z., and Sun, B.: Fossil liverworts from the Lower Cretaceous Huolinhe formation in Inner Mongolia, China, *Acta Geol. Sin.*, 90, 838–846, 2016.
- Lóriga, J., Schmidt, A. R., Moran, R. C., Feldberg, K., Schneider, H., and Heinrichs, J.: The first fossil of a bolbitoid fern

- belongs to the early-divergent lineages of *Elaphoglossum* (Dryopteridaceae), *Am. J. Bot.*, 101, 1466–1475, 2014.
- Magallón, S., Hilu, K., and Quandt, D.: Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates, *Am. J. Bot.*, 100, 556–573, 2013.
- Meredit, R. W., Janečka, J. E., Gatesy, J., Ryder, O. A., Fisher, C. A., Teeling, E. C., Goodbla, A., Eizirik, E., Simão, T. L. L., Stadler, T., Rabosky, D. L., Honeycutt, R. L., Flynn, J. J., Ingram, C. M., Steiner, C., Williams, T. L., Robinson, T. J., Burk-Herrick, A., Westerman, M., Ayoub, N. A., Springer, M. S., and Murphy, W. J.: Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification, *Science*, 334, 521–524, 2011.
- Morley, R. J.: Cretaceous and Tertiary climate change and the past distribution of megathermal rainforests, in: *Tropical Rainforest Responses to Climatic Change*, edited by: Bush, M. B., Flenley, J. R., and Gosling, W. D., Springer, Berlin, Heidelberg, Germany, 1–34, 2011.
- Newton, A. E., Wikström, N., Bell, N., Forrest, L. L., and Ignatov, M. S.: Dating the diversification of the pleurocarpous mosses, in: *Pleurocarpous mosses: Systematics and evolution*, Systematics Association Special Volume 71, edited by: Newton, A. E. and Tangney, R., Taylor & Francis/CRC Press, Boca Raton, 337–366, 2007.
- Palmer, J. D.: Plastid chromosome, structure and evolution, in: *The Molecular Biology of Plastids*, edited by: Bogorad, L. and Vasil, I. K., Academic Press, San Diego, California, USA, 5–53, 1991.
- Paradis, E., Claude, J., and Strimmer, K.: APE: Analyses of phylogenetics and evolution in R language, *Bioinformatics*, 20, 289–290, 2004.
- Parham, J. F., Donoghue, P. C., Bell, C. J., Calway, T. D., Head, J. J., Holroyd, P. A., Inoue, J. G., Irmis, R. B., Joyce, W. G., Ksepka, D. T., Patané, J. S. L., Smith, N. D., Tarver, J. E., van Tuinen, M., Yang, Z., Angielczyk, K. D., Greenwood, J. M., Hipsley, C. A., Jacobs, L., Makovicky, P. J., Müller, J., Smith, K. T., Theodor, J. M., Warnock, R. C. M., and Benton, M. J.: Best practices for justifying fossil calibrations, *Syst. Biol.*, 61, 346–359, 2012.
- Patiño, J., Devos, N., Vanderpoorten, A., Schäfer-Verwimp, A., and Renner, M. A. M.: The identity of *Radula carringtonii* Jack, *J. Bryol.*, 35, 314–316, 2013.
- Patiño, J., Wang, J., Renner, M. A. M., Gradstein, S. R., Laenen, B., Devos, N., Shaw, A. J., and Vanderpoorten, A.: Range size heritability and diversification patterns in the liverwort *Radula*, *Mol. Phylogenet. Evol.*, 106, 73–85, 2017.
- Rambaut, A., Suchard, M. A., Xie, D., and Drummond, A. J.: Tracer v1.6, available at: <http://beast.bio.ed.ac.uk/Tracer> (last access: 13 January 2017), 2014.
- R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, available at: <http://www.r-project.org> (last access: 13 January 2017), 2016.
- Renner, M. A. M.: *Radula* subg. *Radula* in Australasia and the Pacific (Jungermanniopsida), *Telopea*, 17, 107–167, 2014.
- Renner, M. A. M.: Lobule shape evolution in *Radula* (Jungermanniopsida): one rate fits all?, *Bot. J. Linn. Soc.*, 178, 222–242, 2015.
- Renner, M. A. M. and Braggins, J. E.: The sterile gametophyte as a source of informative characters in the genus *Radula* (Radulaceae: Hepaticae), *Nova Hedwigia*, 78, 243–268, 2004.
- Renner, M. A. M., Devos, N., Brown, E. A., and von Konrat, M.: New records, replacements, reinstatements and four new species in the *Radula parvixeta* and *R. ventricosa* species groups (Jungermanniopsida) in Australia: cases of mistaken identity, *Aust. Syst. Bot.*, 26, 298–345, 2013a.
- Renner, M. A. M., Devos, N., Brown, E. A., and von Konrat, M.: Three modes of heterochrony explain lobule diversity in *Radula* subgenus *Cladoradula* (Porellales, Jungermanniopsida), a small lineage of early land plants, *Bot. J. Linn. Soc.*, 173, 153–175, 2013b.
- Renner, M. A. M., Devos, N., Patiño, J., Brown, E. A., Orme, A., Elgey, M., Wilson, T. C., Gray, L. J., and von Konrat, M. J.: Integrative taxonomy resolves the cryptic and pseudo-cryptic *Radula buccinifera* complex (Porellales, Jungermanniopsida), including two reinstated and five new species, *Phytokeys*, 27, 1–113, 2013c.
- Renner, M. A. M., Devos, N., Brown, E. A., and von Konrat, M.: A revision of Australian species of *Radula* subg. *Odontoradula*, *Aust. Syst. Bot.*, 26, 408–447, 2014.
- Santiago-Blay, J. A., Anderson, S. R., and Buckley, R. T.: Possible implications of two new angiosperm flowers from Burmese amber (Lower Cretaceous) for well-established-and diversified insect-plant associations, *Entomol. News*, 116, 341–346, 2005.
- Schneider, H., Schuettelpelz, E., Pryer, K. M., Cranfill, R., Magallón, S., and Lupia, R.: Ferns diversified in the shadow of angiosperms, *Nature*, 428, 553–557, 2004.
- Schneider, H., Schmidt, A. R., Nascimbene, P. C., and Heinrichs, J.: A new Dominican amber fossil of the derived genus *Pleopeltis* confirms generic stasis in the epiphytic fern diversity of the West Indies, *Organisms Diversity Evol.*, 15, 277–283, 2015.
- Schneider, H., Schmidt, A. R., and Heinrichs, J.: Burmese amber fossils bridge the gap in the Cretaceous record of polypod ferns, *Perspect. Pl. Ecol. Evol. Syst.*, 18, 70–78, 2016.
- Schuettelpelz, E. and Pryer, K. M.: Reconciling extreme branch length differences: decoupling time and rate through the evolutionary history of filmy ferns, *Syst. Biol.*, 55, 485–502, 2006.
- Schuster, R. M. and Janssens, J. A.: On *Diettertia*, an isolated Mesozoic member of the Jungermanniales, *Rev. Palaeobot. Palyno.*, 57, 277–287, 1989.
- Shi, G. H., Grimaldi, D. A., Harlow, G. E., Wang, J., Wang, J., Yang, M., Lei, W., Li, Q., and Li, X.: Age constraint on Burmese amber based on U–Pb dating of zircons, *Cretaceous Res.*, 37, 155–163, 2012.
- Silva, S. E., Silva, D. N., Almeida, T., Garcia, C. A., Paulo, O. A., and Sim-Sim, M.: Age estimates of *Frullania* (Frullaniaceae, Porellales) main lineages: another example of rapid and recent diversification in liverwort evolution, *Syst. Biodivers.*, 15, 156–165, 2016.
- Söderström, L., Hagborg, A., Von Konrat, M., Bartolomew-Began, S., Bell, D., Briscoe, L., Brown, E., Cargill, D. C., Cooper, E. D., Costa, D. P., Crandall-Stotler, B. J., Cooper, E. D., Dauphin, G., Engel, J., Feldberg, K., Glenn, D., Gradstein, S. R., He, X., Heinrichs, J., Hentschel, J., Ilkiu-Borges, A. L., Katagiri, T., Konstantinova, N. A., Larráin, J., Long, D., Nebel, M., Pócs, T., Puche, F., Reiner-Drehwald, E., Renner, M. A. M., Sassi-Gyarmati, A., Schäfer-Verwimp, A., Segarra-Moragues, J. G.,

- Stotler, R. E., Sukkharak, P., Thiers, B., Uribe, J., Vana, J., Villarreal, J., Wigginton, M., Zhang, L., and Zhu, R.-L.: World checklist of hornworts and liverworts, *PhytoKeys*, 59, 1–828, 2016.
- Stadler, T.: On incomplete sampling under birth-death models and connections to the sampling-based coalescent, *J. Theor. Biol.*, 261, 58–66, 2009.
- Taylor, T. N., Taylor, E. L., and Krings, M.: *Paleobotany. The Biology and Evolution of Fossil Plants*, 2nd edition, Elsevier/Academic Press Inc, Burlington MA, London, San Diego, New York, 2009.
- Tomescu, A. M. F.: The Early Cretaceous Apple Bay flora of Vancouver Island: a hotspot of fossil bryophyte diversity, *Botany*, 94, 683–695, 2016.
- Vajda, V. and Bercovici, A.: The global vegetation pattern across the Cretaceous–Paleogene mass extinction interval: A template for other extinction events, *Global Planet. Change*, 122, 29–49, 2014.
- Villarreal, J. C. and Renner, S. S.: A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a taxonomically cleaned-up genus *Nothoceros*, *Mol. Phylogenet. Evol.*, 78, 25–35, 2014.
- Villarreal, J. C., Crandall-Stotler, B. J., Hart, M. J., Long, D. G., and Forrest, L. L.: Divergence times and the evolution of morphological complexity in an early land plant lineage (Marchantiopsida) with a slow molecular rate, *New Phytol.*, 209, 1734–1746, 2016.
- Wang, H., Moore, M. J., Soltis, P. S., Bell, C. D., Brockington, S. F., Alexandre, R., Davis, C. C., Latvis, M., Manchester, S. R., and Soltis, D. E.: Rosid radiation and the rapid rise of angiosperm-dominated forests, *P. Natl. Acad. Sci. USA*, 106, 3853–3858, 2009.
- Warnock, R. C. M., Parham, J. F., Joyce, W. G., Lyson, T. R., and Donoghue, P. C. J.: Calibration uncertainty in molecular dating analyses: there is no substitute for the prior evaluation of time priors, *Proc. Roy. Soc. B*, 282, 20141013, <https://doi.org/10.1098/rspb.2014.1013>, 2015.
- Xie, W., Lewis, P. O., Fan, Y., Kuo, L., and Chen, M.-H.: Improving marginal likelihood estimation for Bayesian phylogenetic model selection, *Syst. Biol.*, 60, 150–160, 2011.
- Yamada, K.: A revision of Asian taxa of *Radula*, Hepaticae, *J. Hattori Bot. Lab.*, 45, 201–322, 1979.
- Yamada, K.: An alphabetical list of the species and infrspecific taxa of the genus *Radula* (Radulaceae), *J. Hattori Bot. Lab.*, 60, 175–186, 1986.
- Zwieniecki, M. A. and Boyce, C. K.: Evolution of a unique anatomical precision in angiosperm leaf venation lifts constraints on vascular plant ecology, *Proc. Roy. Soc. B*, 281, 2013289, <https://doi.org/10.1098/rspb.2013.2829>, 2014.

**Table S1.** Taxa used in this study with their corresponding GenBank accession numbers.

Taxon	Isolate	<i>atpB-rbcL</i>	<i>psbT-psbH</i>	<i>psbA-trnH</i>	<i>rps4</i>	<i>trnG</i>	<i>trnL-F</i>
<i>Frullania sp.</i>	L1535	KF852046	JF513426	AY507480	AY507439	-	-
<i>Lejeunea tuberculosa</i>	E24	KF852094	KF852247	KF851964	KF851488	-	-
<i>Lepidolaena clavigera</i>	IBC39	KF851996	JF513410	JF513394	JF513472	-	-
<i>Porella navicularis</i>	IBC163	KF852000	KF852118	KF851826	-	-	-
<i>Radula acuminata</i>	ND_227	HM992384	HM992127	HM992463	HM992295	HM992034	HM992208
<i>Radula acuta</i>	NSW890194	KF495334	-	KX827524	KX827553	KF495272	KF495393
<i>Radula acutiloba</i>	NSW976716	KM220231	-	KX827544	KX827585	KM220093	KM220162
<i>Radula allisonii</i>	NSW896403	KF495342	-	-	KX827557	KF495280	KF495397
<i>Radula aneurismalis</i>	ND_126	HM992358	HM992100	-	HM992269	HM992007	-
<i>Radula ankefinensis</i>	ND_222	HM992382	HM992125	HM992461	HM992293	HM992032	HM992207
<i>Radula antilleana</i>	ND_088	HM992343	HM992085	HM992429	HM992254	HM991992	HM992175
<i>Radula apiculata</i>	ND_339	-	HM992143	HM992478	HM992311	HM992050	-
<i>Radula appressa</i>	ND_229	HM992386	HM992129	HM992465	HM992297	HM992036	HM992209
<i>Radula aquilegia</i>	ND_078	HM992341	HM992083	HM992427	HM992252	HM991990	HM992173
<i>Radula australiana</i>	ND_119	HM992356	HM992098	HM992442	HM992267	HM992005	HM992183
<i>Radula australis</i>	ND_299	HM992395	HM992138	HM992473	HM992306	HM992045	HM992217
<i>Radula boryana</i>	ND_178	HM992375	HM992117	-	HM992285	HM992024	HM992200
<i>Radula brunnea</i>	ND_001	HM992315	HM992054	HM992403	-	HM991961	HM992147
<i>Radula buccinifera</i>	ND_127	HM992359	HM992101	HM992444	HM992270	HM992008	HM992185
<i>Radula campanigera</i>	ND_042	HM992330	HM992070	-	HM992239	HM991977	HM992160
<i>Radula carringtonii</i>	ND_018	HM992323	HM992062	HM992409	HM992231	HM991969	HM992153
<i>Radula complanata</i>	ND_311	HM992396	HM992139	HM992474	HM992307	HM992046	HM992218
<i>Radula constricta</i>	ND_004	HM992317	HM992056	-	HM992225	HM991963	-
<i>Radula cubensis</i>	ND_068	HM992337	HM992078	HM992422	HM992247	HM991985	HM992168

<i>Radula cuspidata</i>	ND_111	HM992353	HM992095	HM992439	HM992264	HM992002	-
<i>Radula decora</i>	ND_026	HM992327	HM992066	HM992413	HM992235	HM991973	-
<i>Radula demissa</i>	ND_053	HM992332	HM992072	HM992417	HM992241	HM991979	HM992162
<i>Radula eggersii</i>	ND_058	HM992334	HM992075	HM992420	HM992244	HM991982	HM992165
<i>Radula episcia</i>	ND_148	HM992366	HM992108	HM992449	HM992277	HM992015	HM992191
<i>Radula fendleri</i>	ND_074	HM992339	HM992080	HM992424	HM992249	HM991987	HM992170
<i>Radula floridana</i>	ND_323	HM992379	HM992122	HM992458	HM992290	HM992029	HM992204
<i>Radula foefnicata</i>	NSW878032	KF440561	KX827458		KX827552	KF440626	KF440490
<i>Radula formosa</i>	NSW979086	KX827519	KX827487	KX827545	KX827586	KX827496	KX827508
<i>Radula frondescens</i>	ND_091	HM992345	HM992087	HM992431	HM992256	HM991994	HM992177
<i>Radula fulvifolia</i>	ND_215	HM992379	HM992122	HM992458	HM992290	HM992029	HM992204
<i>Radula gottscheana</i>	ND_060	HM992335	HM992076	-	HM992245	HM991983	HM992166
<i>Radula grandis</i>	ND_212	-	HM992121	HM992457	HM992289	HM992028	HM992203
<i>Radula hickstiae</i>	ND_120	HM992357	HM992099	HM992443	HM992268	HM992006	HM992184
<i>Radula holtii</i>	ND_281	HM992394	HM992137	HM992472	HM992305	HM992044	HM992216
<i>Radula husnotii</i>	ND_015	HM992322	HM992061	HM992408	HM992230	HM991968	HM992152
<i>Radula imposita</i>	NSW875821	KF432252	KX827456	KX827523	KX827550	KF432338	KF440419
<i>Radula inflexa</i>	ND_039	-	HM992069	-	HM992238	HM991976	HM992159
<i>Radula iwatsukii</i>	ND_076	-	HM992082	HM992426	HM992251	HM991989	HM992172
<i>Radula japonica</i>	ND_353	HM992402	HM992146	HM992481	HM992314	HM992053	HM992223
<i>Radula javanica I</i>	AK323599	KM220207	KX827454	KX827521	KX827547	KM220070	KM220137
<i>Radula javanica II</i>	ND_220	HM992381	HM992124	HM992460	HM992292	HM992031	HM992206
<i>Radula jonesii</i>	ND_267	HM992393	HM992136	-	HM992304	HM992043	-
<i>Radula jovetiana</i>	NSW896752	KF440596	KX827463	KX827529	KX827560	KF440660	KF440530
<i>Radula kegelii</i>	ND_012	HM992320	HM992059	HM992406	HM992228	HM991966	HM992150
<i>Radula kojana</i>	ND_137	HM992364	HM992106	HM992447	HM992275	HM992013	-
<i>Radula lindenbergiana</i>	ND_063	HM992336	HM992077	HM992421	HM992246	HM991984	HM992167

<i>Radula loriana</i>	NSW970882	KF440604	KX827474	KX827538	KX827573	KF440668	KF440538
<i>Radula macrostachya</i>	ND_007	HM992318	HM992057	HM992404	HM992226	HM991964	HM992148
<i>Radula madagascariensis</i>	ND_232	HM992387	HM992130	HM992466	HM992298	HM992037	HM992210
<i>Radula marojezica</i>	ND_233	HM992388	HM992131	HM992467	HM992299	HM992038	HM992211
<i>Radula mazarunensis</i>	ND_081	HM992342	HM992084	HM992428	HM992253	HM991991	HM992174
<i>Radula mitenii</i>	NSW897201	KF432391	KX827467	KX827533	KX827565	KF432391	KF440473
<i>Radula multiamentula</i>	ND_108	HM992352	HM992094	HM992438	HM992263	HM992001	HM992182
<i>Radula myriopoda</i>	NSW970376	KF440611	KX827471	KX827536	KX827570	KF440675	KF440545
<i>Radula neotropica</i>	ND_160	HM992371	HM992113	HM992452	HM992281	HM992020	HM992196
<i>Radula notabilis</i>	NSW909500	KF432293	KX827469	KX827535	KX827568	KF432378	KF440458
<i>Radula novae-hollandiae</i>	NSW896816	KF495348		KX827530	KX827561	KF495286	KF495403
<i>Radula nudicaulis</i>	ND_020	HM992325	HM992064	HM992411	HM992233	HM991971	HM992155
<i>Radula nymanii</i>	NSW898712	KF432310		KX827534	KX827566	KF432396	KF440478
<i>Radula obtusiloba</i> subsp. <i>polyclada</i>	ND_315	HM992378	HM992120	-	HM992288	HM992027	-
<i>Radula ocellata</i>	ND_116	HM992354	HM992096	HM992440	HM992265	HM992003	-
<i>Radula oreopsis</i>	NSW970377	KM220174	KX827472		KX827571	KM220042	KM220104
<i>Radula perrottetii</i>	ND_158	HM992369	HM992111	-	HM992279	HM992018	HM992194
<i>Radula physoloba</i>	ND_211	HM992378	HM992120	-	HM992288	HM992027	-
<i>Radula plicata</i>	ND_103	HM992351	HM992093	HM992437	HM992262	HM992000	-
<i>Radula plumosa</i>	ND_011	HM992319	HM992058	HM992405	HM992227	HM991965	HM992149
<i>Radula pocsii</i>	ND_150	HM992367	HM992109	HM992450	-	HM992016	HM992192
<i>Radula pseudoscripta</i>	NSW895495	KX827511	KX827459	KX827525	KX827554	KX827490	KX827499
<i>Radula psychosis</i>	NSW970878	KF440592	KX827473	KX827537	KX827572	KF440656	KF440526
<i>Radula pugioniformis</i>	NSW870344	MF346528	-	MF346529	-	MF346530	MF346531
<i>Radula pulchella</i>	ND_219	HM992380	HM992123	HM992459	HM992291	HM992030	HM992205
<i>Radula quadrata</i>	ND_225	HM992383	HM992126	HM992462	HM992294	HM992033	-

<i>Radula queenslandica</i>	ND_118	HM992355	HM992097	HM992441	HM992266	HM992004	-
<i>Radula ratkowskiana</i>	ND_102	HM992350	HM992092	HM992436	HM992261	HM991999	HM992181
<i>Radula recubans</i>	ND_092	HM992346	HM992088	HM992432	HM992257	HM991995	HM992178
<i>Radula retroflexa</i>	ND_228	HM992385	HM992128	HM992464	HM992296	HM992035	-
<i>Radula robinsonii</i>	NSW875795	KF440568	KX827455	KX827522	KX827548	KF440634	KF440501
<i>Radula saccatiloba</i>	ND_075	HM992340	HM992081	HM992425	HM992250	HM991988	HM992171
<i>Radula sainsburiana</i>	ND_098	HM992347	HM992089	HM992433	HM992258	HM991996	HM992179
<i>Radula scariosa</i>	NSW895626	KX827512	KX827460	KX827527	KX827556	KX827500	KX827500
<i>Radula schaefer-vervimpii</i>	ND_019	HM992324	HM992063	HM992410	HM992232	HM991970	HM992154
<i>Radula sp. I</i>	ND_142	HM992365	HM992107	HM992448	HM992276	HM992014	HM992190
<i>Radula sp. II</i>	ND_234	HM992389	HM992132	HM992468	HM992300	HM992039	HM992212
<i>Radula sp. III</i>	ND_045	HM992331	HM992071	HM992416	HM992240	HM991978	HM992161
<i>Radula squarrosa</i>	NSW896832	KX827514	KX827464	KX827531	KX827562	KX827502	KX827502
<i>Radula stenocalyx</i>	ND_235	HM992390	HM992133	HM992469	HM992301	HM992040	HM992213
<i>Radula strangulata</i>	ND_099	HM992348	HM992090	HM992434	HM992259	HM991997	HM992180
<i>Radula subinflata</i>	ND_030	HM992328	HM992067	HM992414	HM992236	HM991974	HM992157
<i>Radula sullivantii</i>	ND_321	-	HM992121	HM992457	HM992289	HM992028	HM992203
<i>Radula tasmanica</i>	ND_101	HM992349	HM992091	HM992435	HM992260	HM991998	-
<i>Radula tenax</i>	ND_129	HM992360	HM992102	-	HM992271	HM992009	HM992186
<i>Radula tenera</i>	ND_022	HM992326	HM992065	HM992412	HM992234	HM991972	HM992156
<i>Radula thiersiae</i>	NSW897007	-	KX827465	-	KX827563	KX827492	KX827503
<i>Radula tibodensis</i>	ND_055	-	HM992073	HM992418	HM992242	HM991980	HM992163
<i>Radula tokiensis</i>	ND_003	HM992316	HM992055	-	HM992224	HM991962	-
<i>Radula voluta</i>	ND_014	HM992321	HM992060	HM992407	HM992229	HM991967	HM992151
<i>Radula weymouthiana</i>	NSW895586	KF495340	-	KX827526	KX827555	KF495278	KF495396
<i>Radula wichurae</i>	ND_057	HM992333	HM992074	HM992419	HM992243	HM991981	HM992164

**Table S2.** Coding matrix used for the ancestral character state reconstruction.

Taxa	Lobule	Leaf	Gemmae	Female bract
	insertion	apex		pairs
	0=transverse 1=longitudinal	0=round 1=acute	0=absent 1=present	0=1 1=2
<i>Porella navicularis</i>	0	0	0	0
<i>Radula acuminata</i>	1	0	1	0
<i>R. acuta</i>	1	1	1	1
<i>R. acutiloba</i>	1	0	1	0
<i>R. allisonii</i>	1	0	0	0
<i>R. aneurismalis</i>	1	0	0	0
<i>R. ankefinensis</i>	1	0	0	0
<i>R. antilleana</i>	1	0	0	0
<i>R. apiculata</i>	1	1	0	1
<i>R. appressa</i>	1	0	0	0
<i>R. aquilegia</i>	1	0	0	0
<i>R. australiana</i>	1	0	0	0
<i>R. australis</i>	1	0	0	0
<i>R. boryana</i>	0	0	0	0
<i>R. brunnea</i>	0	0	0	0
<i>R. buccinifera</i>	1	0	0	0
<i>R. campanigera</i>	0	0	0	0
<i>R. carringtonii</i>	1	0	0	0
<i>R. complanata</i>	1	0	1	0
<i>R. constricta</i>	1	0	1	0
<i>R. cubensis</i>	1	0	0	0
<i>R. cuspidata</i>	1	1	0	1
<i>R. decora</i>	1	0	0	0
<i>R. demissa</i>	1	0	0	0
<i>R. eggertii</i>	1	0	0	0
<i>R. episcia</i>	1	0	0	0
<i>R. fendleri</i>	1	0	0	0
<i>R. floridana</i>	1	0	0	0
<i>R. forficata</i>	1	0	0	0
<i>R. formosa</i>	1	0	0	0
<i>R. frondescens</i>	1	0	0	0
<i>R. fulvifolia</i>	1	0	0	0
<i>R. gottscheana</i>	0	0	0	0
<i>R. grandis</i>	1	0	1	0
<i>R. hicksiae</i>	1	0	0	0
<i>R. holtii</i>	1	0	0	0
<i>R. husnoti</i>	1	0	0	0
<i>R. imposita</i>	1	0	0	0



<i>R. inflexa</i>	1	0	0	0
<i>R. iwatsukii</i>	1	0	0	0
<i>R. japonica</i>	1	0	0	0
<i>R. javanica I</i>	1	0	0	0
<i>R. javanica II</i>	1	0	0	0
<i>R. jonesii</i>	1	0	0	0
<i>R. jovetiana</i>	1	0	0	0
<i>R. kegelii</i>	1	0	0	0
<i>R. kojana</i>	1	1	1	1
<i>R. lindenbergiana</i>	1	0	1	0
<i>R. loriana</i>	1	0	0	0
<i>R. macrostachya</i>	1	0	0	0
<i>R. madagascariensis</i>	1	0	0	0
<i>R. marojezica</i>	1	0	0	0
<i>R. mazarunensis</i>	1	0	0	0
<i>R. mittenii</i>	1	0	0	0
<i>R. multiamentula</i>	1	0	0	0
<i>R. myriopoda</i>	1	0	0	0
<i>R. neotropica</i>	1	0	0	0
<i>R. notabilis</i>	1	0	0	0
<i>R. novaehollandiae</i>	1	1	1	1
<i>R. nudicaulis</i>	1	0	0	0
<i>R. nymanii</i>	1	0	1	0
<i>R. obtusiloba subsp.</i>				
<i>polyclada</i>	1	0	0	0
<i>R. ocellata</i>	1	1	0	0
<i>R. oreopsis</i>	1	0	0	0
<i>R. physoloba</i>	1	0	0	0
<i>R. plicata</i>	1	0	0	0
<i>R. plumosa</i>	1	0	0	0
<i>R. pocsii</i>	1	0	0	0
<i>R. pseudoscripta</i>	1	0	0	0
<i>R. psychosis</i>	1	0	0	0
<i>R. pugioniformis</i>	1	0	0	1
<i>R. pulchella</i>	1	1	0	0
<i>R. quadrata</i>	1	0	0	0
<i>R. queenslandica</i>	1	1	0	0
<i>R. ratkowskiana</i>	1	0	0	0
<i>R. recubans</i>	1	0	0	0
<i>R. retroflexa</i>	1	0	0	0
<i>R. robinsonii</i>	1	0	0	0
<i>R. rottetii</i>	0	0	0	0
<i>R. saccatiloba</i>	1	0	0	0
<i>R. sainsburiana</i>	1	0	0	0
<i>R. scariosa</i>	1	0	0	0
<i>R. schaefer-verwimpii</i>	1	0	0	0

<i>R. sp. I</i>	1	0	0	0
<i>R. sp. II</i>	1	0	0	0
<i>R. sp. III</i>	1	0	0	0
<i>R. spicata</i>	1	0	0	0
<i>R. squarrosa</i>	1	0	0	0
<i>R. stenocalyx</i>	1	0	1	0
<i>R. strangulata</i>	1	0	0	0
<i>R. subinflata</i>	1	0	0	0
<i>R. sullivantii</i>	1	0	0	0
<i>R. tasmanica</i>	1	0	0	0
<i>R. tenax</i>	0	0	0	0
<i>R. tenera</i>	1	0	0	0
<i>R. thiersiae</i>	1	0	0	0
<i>R. tjibodensis</i>	1	0	1	0
<i>R. tokiensis</i>	1	0	0	0
<i>R. voluta</i>	1	0	1	0
<i>R. weymouthiana</i>	1	0	0	0
<i>R. wichurae</i>	1	0	0	0

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**Table S3.** Summary of molecular dating studies including *Radula*.

Reference	Study system	Stem age estimates of <i>Radula</i> in Ma [95%HPDI]	Calibration approach	Program used: BEAST (Drummond et al., 2012), r8s (Sanderson, 2003)
<b>Heinrichs et al. (2007)</b>	Dataset I: liverworts (Marchantiophyta)		<ul style="list-style-type: none"> <li>- minimum age constraints: 8 fossil calibrations</li> <li>- fixed age constraint: earliest split of vascular plants (430 Ma)</li> <li>- maximum age constraint: oldest fossil of land plants (475 Ma)</li> </ul>	penalized likelihood r8s
	Dataset II: leafy liverworts (Jungermanniopsida)	139.4 (typing error) $\pm$ 13.1 (MAX) 185.9 $\pm$ 12.1 (MIN)	<ul style="list-style-type: none"> <li>- minimum age constraints: 10 fossil calibrations</li> <li>- split Metzgeriidae and Jungermanniidae obtained in dataset I</li> <li>- minimum (MIN) and maximum (MAX) boarders of confidence intervals (obtained with dataset I) used to calibrate split Metzgeriidae and Jungermanniidae</li> </ul>	
<b>Fiz-Palacios et al. (2011)</b>	Land plants	230 [not given]	<ul style="list-style-type: none"> <li>- minimum constraint: age of eudicots, tricolpate pollen grain (121 Ma)</li> <li>- 16 additional minimum age constraints</li> <li>- maximum age for root of tree (725 Ma)</li> </ul>	penalized likelihood r8s
<b>Cooper et al. (2012)</b>	Liverworts (Marchantiophyta)	247 [204-285]	<ul style="list-style-type: none"> <li>- 9 fossil calibrations following Heinrichs et al. (2007) using a uniform distribution with minimum age of fossil and maximum age of root (475 Ma)</li> <li>- maximum age constraint on root: normal</li> </ul>	uncorrelated relaxed clock, BEAST

			distribution, mean of 475 Ma (SD 12.5 Ma)	
<b>Feldberg <i>et al.</i> (2014)</b>	Liverworts (Marchantiophyta)	264.76 [not given]	<ul style="list-style-type: none"> <li>– 20 fossil calibrations: uniform distribution with minimum age of fossil and maximum age of root (475 Ma)</li> <li>– maximum age constraint: liverworts, oldest known cryptospores (475 Ma)</li> </ul>	uncorrelated relaxed clock, BEAST
<b>Laenen <i>et al.</i> (2014)</b>	Liverworts (Marchantiophyta)	97.34 [52.55-150.94] (I) 98.33 [47.3-174.6] (II) 122.44 [51.56-200.22] (III)	<ul style="list-style-type: none"> <li>25 fossil calibrations: log-normal distribution using 3 different approaches:               <ul style="list-style-type: none"> <li>(I) timespan of the geological era attributed to the fossil</li> <li>(II) fossil age to age of oldest non-ambiguous fossil of land plants (475 Ma)</li> <li>(III) fossil age to upper limit of most ancient inferred estimate of the origin of land plants (815 Ma)</li> </ul> </li> </ul>	uncorrelated relaxed clock, BEAST
<b>Patino <i>et al.</i> (2017)</b>	<i>Radula</i>	175 [not given]	nucleotide substitution rate based on Laenen <i>et al.</i> (2014) using log-uniform prior distribution: $5.273 \times 10^{-4}$ (SD $6.463 \times 10^{-6}$ ) subst./site/My	uncorrelated relaxed clock, BEAST

## References

Cooper ED, Henwood MJ, Brown EA. 2012. Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution. *Biological Journal of the Linnean Society* **107**: 425–441.

- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012.** Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.
- Feldberg K, Schneider H, Stadler T, Schäfer-Verwimp A, Schmidt AR, Heinrichs J. 2014.** Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Scientific Reports* **4**: 5974.
- Fiz-Palacios O, Schneider H, Heinrichs J, Savolainen V. 2011.** Diversification of land plants. Insights from a family-level phylogenetic analysis. *BMC Evolutionary Biology* **11**: 341.
- Heinrichs J, Hentschel J, Wilson R, Feldberg K, Schneider H. 2007.** Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon* **56**: 31–44.
- Laenen B, Shaw B, Schneider H, et al. 2014.** Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications* **5**: 6134.
- Patiño J, Wang J, Renner MAM et al. 2017.** Range size heritability and diversification patterns in the liverwort *Radula*. *Molecular*

*Phylogenetics and Evolution* **106**: 73–85.

**Sanderson, M.J. 2003.** r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock.

*Bioinformatics* **19**: 301–302.

## Chapter 6

### GENERAL DISCUSSION





## 6.1 Phylogeny and classification of Lejeuneae (Lejeuneaceae)

Since the last classification of Lejeuneaceae by Gradstein (2013), a lot of progress has been made regarding the generic sampling of this family (Czumay *et al.* 2013; Heinrichs *et al.* 2014a, b, c; Schäfer-Verwimp *et al.* 2014; Shi *et al.* 2015; Wang *et al.* 2016). Still, many genera had never been studied molecularly when I started my research in 2014. I focused on the relationships within the largest tribe of Lejeuneaceae, the Lejeuneae, and due to available herbarium material, I could investigate the phylogenetic position of four not previously included genera. Therefore, in a first dataset, I was able to include sequences of 27 genera, representing all known subtribes of Lejeuneae, importantly also species of the Afro-Madagascan/Brazilian genus *Haplolejeunea*, the Asian and Fijian species of the genus *Metalejeunea* (3 species), and the Brazilian type species of *Vitalianthus* (another species, that I could not sample, has been described from China). Of the genus *Otolejeunea* (11 species, type species from Madagascar), I sequenced further material from Australia and Indonesia, representing the predominantly Asian and Australasian distribution of the genus. Another geographically important addition was Brazilian material of *Microlejeunea aphanella*. I also analyzed and scored morphological information on ocelli, sex system (monoicous or dioicous), outer lobule tooth, and underleaves.

The phylogenetic hypotheses (Chapter 2) obtained under maximum parsimony and maximum likelihood optimization showed, that *Metalejeunea* is a member of Lepidolejeuneinae and *Haplolejeunea* belongs to Echinolejeuneinae. Furthermore, the genus *Vitalianthus* needed to be transferred from Drepanolejeuneinae to Lepidolejeuneinae. The two specimens of *O. semperiana* did not group with the type species of this genus in the Lepidolejeuneinae, but instead with other Echinolejeuneinae. Morphological evidence coming from the reduced leaf lobules, the “*Allorgella*-type” marginal leaf denticulations, and the absence of ocelli (Chapter 2, Fig. 1) in this species supported this new grouping. Ocelli are gametophytical cells containing a single, large oil body (Fig. 2) and are a taxonomically important character in Lejeuneaceae (Schuster 1992a, b; He & Piippo 1999; Heinrichs *et al.* 2015a). Due to my results, I reinstated *Allorgella* at the genus rank and transferred *O. semperiana* to *Allorgella*. In addition, *Microlejeunea aphanella* turned out to be more closely related to the type species of the genus *Vitalianthus* than to the type species of *Microlejeunea* (Chapter 2, Figs. 2, 4) and therefore I transferred that species to *Vitalianthus*. This species possesses a linear row of ocelli in the leaf lobe that can be seen even in the isolectotype (in the Munich herbarium) of this species, which

was collected in Brazil in 1884. These ocelli resemble those seen in other species of *Vitalianthus* but not in other species of *Microlejeunea*.

In Chapter 3 of this thesis, I dealt with the phylogenetic position of the Neotropical – Asian genus *Pictolejeunea* (6 species; Söderström *et al.* 2016). I further increased my Lejeuneaceae molecular phylogenetic dataset by generating sequences of two Brazilian specimens of the Neotropical type species *Pictolejeunea picta* and other newly available sequences. The results revealed a sister relationship of *Pictolejeunea* to the remaining Lejeuneaceae genera (Chapter 3, Fig. 1), and I therefore placed this genus in a new subtribe, Pictolejeuneinae. Except for the subtribe Ceratolejeuneinae, the remaining subtribes are well supported in my phylogenetic trees.

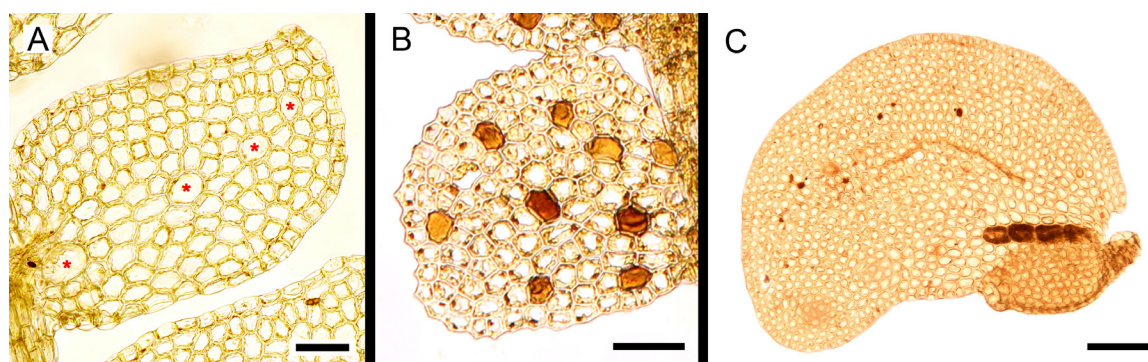


Figure 2. Diversity of ocelli in Lejeuneaceae. A. *Leptolejeunea epiphylla*, stars indicate broken row of ocelli, B. *Pictolejeunea picta* with reddish brown ocelli, C. *Otolejeunea moniliata* with a moniliate row of four ocelli; A from Schäfer-Verwimp 16245 (herbarium Schäfer-Verwimp), B from Lopes 202 (SP), C from Pócs *et al.* 901131/EA (EGR); All photographs by J. Bechteler; scale bars A, B = 50  $\mu\text{m}$ , C = 100  $\mu\text{m}$ .

## 6.2 Evolution of epiphyllous liverworts: The case of *Leptolejeunea*

My study on *Leptolejeunea* represents the first biogeographic and molecular clock-dated analysis of an epiphyllous liverwort genus. The geographic ranges of the supposedly pantropical species *L. elliptica* and *L. maculata*, and the paleotropical *L. epiphylla* (Grolle 1976; Schuster 1980; Pócs & Lye 1999; Zhu & So 2001; Chapter 1, Table 1) are biologically unlikely because trans-oceanic populations obviously do not engage in gene flow. For each of these species, I obtained sequences from multiple herbarium specimens

representing their range in South America, Africa, and Asia. Thus, I included 16 specimens of *L. maculata* from the Dominican Republic, Ecuador, Panama, Mayotte, Malaysia, and Sumatra, 14 specimens of *L. elliptica* from the Neotropics and Asia (African specimens are still missing), and eight specimens of *L. epiphylla* from Cambodia, Malaysia, Sumatra, Thailand, Mayotte, and Príncipe Island. Additionally, my dataset comprised five species restricted to Asia and Australasia (15% of 33 species), four species from the Neotropics (40% of 10 species), and the single species having its distribution only in Africa, *L. astroidea*. The results refute the assumed pantropical distributions of the species *L. elliptica* and *L. maculata*, which each split into three, well-supported geographically defined clades (Chapter 4, Fig. 2).

To translate these results into an improved classification, I resurrected several formerly synonymized names, each of which had originally been based on geographical and morphological differences, such as leaf shape, lobule size, and distribution of ocelli.

The taxonomy of *Leptolejeunea* mainly relies on the presence/absence and size and distribution of ocelli (Herzog 1942; Bischler 1969; Fig. 1 in Chapter 4 of this thesis). Ocelli can best be studied in fresh material since they usually disappear in dried specimens, making the use of herbarium material challenging. Leaf cells that are larger or smaller than the surrounding cells may indicate ocelli (Chapter 6.1, Fig. 2; Chapter 4, Fig. 1).

To obtain a chronogram showing absolute ages, I used plastid and nuclear standard bryophyte substitution rates for calibration (Villarreal & Renner 2014). *Leptolejeunea* diverged from *Pycnolejeunea* and *Xylolejeunea* during the Late Cretaceous to Early Eocene at around 68 [48-94] Ma (Chapter 4, Figs. 3, 4), which is in line with the results of Wilson *et al.* (2007b), who included one representative of *Leptolejeunea* in a chronogram for Lejeuneaceae that was constrained by a secondary calibration point obtained from Heinrichs *et al.* (2007) and reported a stem age of this genus of 56-63 Ma. In my analyses, the crown group diversification of *Leptolejeunea* took place in the Eocene to Early Oligocene at around 38 [27-53] Ma, with most extant species establishing during the Miocene (Chapter 4, Figs. 3, 4). Based on the geographic ranges of the 17 species in my phylogeny, I assigned each of them to either the Neotropics, the Afro-Madagascan region, or Asia including Australasia. The supposed pantropical species *L. elliptica*, *L. epiphylla*, and *L. maculata* were coded according to voucher label information to accommodate the results of my phylogenetic analyses, which had shown that these species are artificial (above). The results of these coarse biogeographic analyses show that the paleotropical

species, *L. epiphylla* and *L. schiffneri*, probably originated in Asia and colonized Africa during the Pliocene to Pleistocene (Chapter 4, Figs. 3, 4).

Important caveats in the above analyses are incomplete species sampling and extinction and recolonization. The latter two factors have undoubtedly occurred during the past 68 million years, and my species sampling is only 35% of c. 48 species. Extant species of *Leptolejeunea* can disperse via spores or asexually via gametophyte fragments and cladia, which are specialized, very fragile branches that break off the main stem easily and develop large rhizoid discs to facilitate substrate adherence (Bischler 1969; Zhu & So 2001). The long-held hypothesis of asexual propagules being mainly involved in population maintenance and dispersal over short distances has recently been challenged, and it is now assumed that spores and asexual propagules are both important for long-distance dispersal (LDD) of bryophytes (Laenen *et al.* 2016b). Floating islands that consist of huge trunks carried out from the mouths of such rivers as the Amazon and the Congo and that then follow ocean currents for many weeks are well-documented by ship sightings (Van Duzer 2004). Such islands transport microbes, cryptogams, birds and other animals, and of course also plant fragments (Houle 1998; Renner 2004). Circum-Antarctic wind currents have also been correlated with extant bryophyte distributions on Antarctic islands (Muñoz *et al.* 2004). Spores of liverworts have a low resistance to high levels of UV radiation, drought, and frost, and their successful dispersal may occur mostly by air currents below jet stream altitudes (Van Zanten & Gradstein 1988).

The Asian – African disjunctions may be due to migratory birds millions of which migrate twice each year between Africa, Madagascar, and India (Berthold 2001; Newton 2008). Birds can carry bryophyte propagules in their plumage (Lewis *et al.* 2014).

A further factor that likely influenced the evolution of *Leptolejeunea* are the megathermal forests that dominate much of the Earth during the Paleocene and Eocene (Morley 2011). These forests were dominated by angiosperms and provided a humid atmosphere, which is an important factor for the growth of epiphyllous liverworts (Winkler 1967; Olarinmoye 1974; Coley *et al.* 1993; Sonnleitner *et al.* 2009). The Eocene and Oligocene Baltic and Bitterfeld ambers in northern regions of Europe have yielded inclusions of related genera (Grolle & Meister 2004; Heinrichs *et al.* 2018) and entire communities analogous to extant East Asian humid warm-temperate forests (Sadowski *et al.* 2017).

### 6.3 A Cretaceous amber fossil belonging to the genus *Radula* (Radulaceae)

Cretaceous amber fossils of plants and animals are numerous (Grimaldi *et al.* 2002; Azar *et al.* 2010), but when I began my research, few species of liverworts had been documented from such amber inclusions (Heinrichs *et al.* 2018), one from a deposit close to Wainwright, Alaska (Langenheim *et al.* 1960) and six from Tanai in Kachin State, Myanmar (Grimaldi *et al.* 2002). One of these Cretaceous inclusions, from the Burmese amber of Myanmar (Shi *et al.* 2012: late Albian to earliest Cenomanian; minimum age 98 Ma), was available to me for morphological study. It has *Radula*-type branching, bilobed leaves, numerous archegonia per gynoecium, and lacks underleaves, allowing the reliable assignment to the genus *Radula*. In Chapter 5, I described this fossil as *Radula cretacea*.

I then used the newly assigned fossil to calibrate my molecular phylogeny of *Radula*, which is based on a sampling of 99 species (40% of 245 *Radula* species), representing all subgenera. I also carried out ancestral state reconstructions for four potentially informative characters of the fossil, namely shape of the leaf apex (Fig. 3), number of female bract pairs, gemmae development, type of lobule insertion. It turned out, that these characters are derived features within subgenus *Odontoradula* (Chapter 5, Figs. 1, 2), which comprises 18 species (Söderström *et al.* 2016) that are mainly distributed in Oceania and Asia (Yamada & Piippo 1989; Patiño *et al.* 2017), in line with the Burmese origin of the fossil.

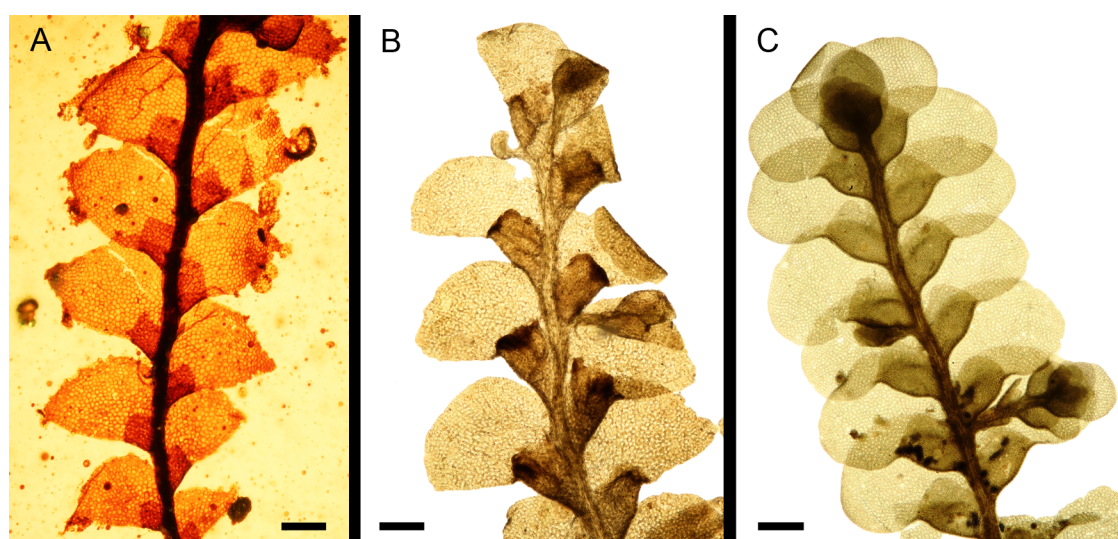


Figure 3. Members of *Radula* subgenus *Odontoradula*. A. Fossil of *Radula cretacea*, B. *Radula novae-hollandiae*, C. *Radula decora*; Note the acute leaf apex in A and B, and the round leaf apex in C; B from M-0088183 (M), C from Schäfer-Verwimp & Verwimp 8106

(M); Photograph A by A.R. Schmidt, B and C by J. Bechteler; scale bars A, B, C = 200  $\mu\text{m}$ .

For the clock models, I first used the fossil to constrain the age of the clade sharing the same morphological features as the fossil to 98.0 ( $\pm$  1.0) Ma. In a second model, I used the fossil to instead constrain the stem node of subgenus *Odontoradula* to 98.0 ( $\pm$  1.0) Ma. In a third model, I applied a standard plastid substitution rate of  $5 \times 10^{-4}$  subst./sites/Myr as secondary calibration (Palmer 1991; Villarreal & Renner 2014). The first approach yielded a stem age for *Radula* of 508 [341-714] Ma, which is unrealistically old. The other two approaches resulted in estimates of, respectively, 228 [165-307] Ma or 236 [143-369] Ma for the stem age of *Radula*, which is in accordance with other molecular clock studies that have used different taxon samples and different fossil or rate calibrations (Chapter 5, Supplement Table S3). A stem age estimate for subgenus *Odontoradula* of 101.2 [78.5-129.2] Ma obtained by Patiño *et al.* (2017) is in agreement with the minimum age of my Burmese *Radula* fossil (98 Ma) and provides further support for the fossil being a stem group member of this subgenus.

Fossils provide insights into the evolution of morphological traits. The c. 100 million years old amber inclusion of *Radula cretacea* so far is the oldest fossil of Radulaceae and also one of the oldest amber inclusion of leafy liverworts in general (Heinrichs *et al.* 2018). Unfortunately, *Radula cretacea* is preserved without fragments of the substrate it was growing on. Nevertheless, the fossil shows many other important traits, such as an acute leaf apex, two female bract pairs, the development of gemmae, and a longitudinal lobule insertion, all of which occur in extant *Radula* species (Fig. 3; Chapter 5, Fig. 1). On this basis, as well as the morphology of Cenozoic *Radula* fossils from the Baltic and Bitterfeld amber (Heinrichs *et al.* 2016) and Dominican amber (Grolle 1987; Kaasalainen *et al.* 2017a), it appears that the morphology of *Radula* has not changed much since the Paleocene and Eocene. This is similar to Cretaceous amber inclusions of the leafy liverwort family Frullaniaceae (Heinrichs *et al.* 2012) and Cretaceous fossils of ferns (Regalado *et al.* 2017), mushrooms (Hibbett *et al.* 1997), and bees (Poinar & Danforth 2006). Other Cretaceous amber fossils of the leafy liverworts *Kaolakia borealis* (Heinrichs *et al.* 2011) and *Protofrullania cornigera* (Heinrichs *et al.* 2017), and the moss *Eupolytrichum antiquum* (Konopka *et al.* 1997), however, do possess morphological traits no longer seen today, such as a strap-shaped underleaf and rhizoids originating from the uppermost sector of the underleaf.

#### 6.4 General conclusion and perspective

My studies on Lejeuneaceae resulted in a phylogeny that now includes species from 30 of the 43 genera of Lejeuneaceae and that provides new insights into relationships in this family. Especially interesting was my discovery that *Pictolejeunea* is sister to the remaining 29 genera (Chapter 2, 3). The phylogenetic positions of the ‘missing’ ten genera still remain to be investigated, and the backbone of the phylogeny is still poorly resolved. More of the 1,700 species of Lejeuneaceae will need to be sampled to achieve even deeper insights into the evolution of this largest family of liverworts.

My research on the pantropical genus *Leptolejeunea* (Chapter 4) revealed the unnaturalness of two supposedly pantropical species. Other supposedly widely distributed species, for example *L. epiphylla*, still need to be studied in more detail with microsatellite markers and a denser geographical sampling. Geometric morphometrics (Adams *et al.* 2004) has been used in other leafy liverworts (Renner *et al.* 2013a) and might be tried for subgroups of *Leptolejeunea* for which sufficient well-preserved material is available. The predominant dioicous sexual system of *Leptolejeunea* species (Chapter 1.2, Table 1) raises questions about how reproduction is ensured, given that the majority of its species live on leaves, which persist for only a few months.

Last not least, my analyses greatly increased the future utility of *Radula cretacea* for the calibration of liverwort molecular-clock models.





## REFERENCES

- Adams, D.C., Rohlf, F.J., Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71, 5–16.
- Anthony, P.A., Holtum, J.A.M., Jackes, B.R. 2002. Shade acclimation of rainforest leaves to colonization by lichens. *Functional Ecology* 16, 808–816.
- Azar, D., Gèze, R., Acra, F., Penney, D. 2010. Lebanese amber. In: Penny, D. (Ed.), *Biodiversity of fossils in amber from the major world deposits*, Siri Scientific Press, Sofia, pp. 271–298.
- Bentley, B.L. 1979. Longevity of individual leaves in a tropical rainforest under-story. *Annals of Botany*, 43, 119–121.
- Bentley, B.L., Carpenter, E.J. 1984. Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. *Oecologia*, 63, 52–56.
- Bentley, B.L. 1987. Nitrogen fixation by epiphylls in a tropical rainforest. *Annals of the Missouri Botanical Garden*, 74, 234–241.
- Benton, M. 2010. The origins of modern biodiversity on land. *Philosophical Transactions of the Royal Society of London, Biology*, 365, 3667–3679.
- Berrie, G.K., Eze, J.M.O. 1975. The relationship between an epiphyllous liverwort and host leaves. *Annals of Botany*, 39, 955–963.
- Berthold, P. 2001. *Bird migration: a general survey*. Oxford University Press.
- Bischler, H. 1969. Le genre *Leptolejeunea* (Spruce) Steph. en Amérique. *Nova Hedwigia*, 17, 265–350.
- Boyce, C.K., Lee, J.E. 2010. An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20100485.
- Boyce, C.K., Lee, J.E., Feild, T.S., Brodribb, T.J., Zwieniecki, M.A. 2010. Angiosperms helped put the rain in the rainforests: The impact of plant physiological evolution on tropical biodiversity. *Annals of the Missouri Botanical Garden*, 97, 527–540.
- Boyce, C.K., Leslie, A.B. 2012. The paleontological context of angiosperm vegetative evolution. *International Journal of Plant Sciences*, 173, 561–568.
- Carter, B.E., Larraín, J., Manukjanová, A., Shaw, B., Shaw, A.J., Heinrichs, J., de Lange, P., Suleiman, M., Thouvenot, L., von Konrat, M. 2017. Species delimitation and biogeography of a southern hemisphere liverwort clade, *Frullania* subgenus

- Microfrullania* (Frullaniaceae, Marchantiophyta). *Molecular Phylogenetics and Evolution*, 107, 16–26.
- Chen, P.C. 1955. Bryophyta nova sinica. *Feddes Repertorium Specierum Novarum Regni Vegetabilis*, 58, 23–52.
- Coley, P.D., Kursar, T.A., Machado, J.L. 1993. Colonization of tropical rain forest leaves by epiphylls: effects of site and host plant leaf lifetime. *Ecology*, 74, 619–623.
- Cooper, E.D., Henwood, M.J., Brown, E.A. 2012. Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution. *Biological Journal of the Linnean Society*, 107, 425–441.
- Cornelissen, J.T., Ter Steege, H. 1989. Distribution and ecology of epiphytic bryophytes and lichens in dry evergreen forest of Guyana. *Journal of Tropical Ecology*, 5, 131–150.
- Cox, C.J., Li, B., Foster, P.G., Embley, T.M., Civián, P. 2014. Conflicting phylogenies for early land plants are caused by composition biases among synonymous substitutions. *Systematic Biology*, 63, 272–279.
- Crosby, M.R., Magill, R.E., Allen, B., He, S. 1999. *A checklist of the mosses*. Missouri Botanical Garden, St. Louis.
- Crum, H.A. 1972. The geographic origins of the mosses of North America's eastern deciduous forest. *Journal of the Hattori Botanical Laboratory*, 35, 269–298.
- Czumay, A., Dong, S., Scheben, A., Schäfer-Verwimp, A., Feldberg, K., Heinrichs, J. 2013. Transfer of *Lejeunea huctumalcensis* to *Physantholejeunea* (Lejeuneaceae, Porellales). *Australian Systematic Botany*, 26, 386–392.
- DeLuca, T.H., Zackrisson, O., Nilsson, M.C., Sellstedt, A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature*, 419, 917–920.
- Devos, N., Vanderpoorten, A. 2009. Range disjunctions, speciation, and morphological transformation rates in the liverwort genus *Leptoscyphus*. *Evolution*, 63, 779–792.
- Devos, N., Renner, M.A.M., Gradstein, S.R., Shaw, A.J., Vanderpoorten, A. 2011. Molecular data challenge traditional subgeneric divisions in the leafy liverwort *Radula*. *Taxon*, 60, 1623–1632.
- Dey, M., Singh, D.K. 2010. Two new epiphyllous *Leptolejeunea* (Hepaticae: Lejeuneaceae) from Eastern Himalaya, India. *Taiwania*, 55, 355–362.

- Dong, S., Schäfer-Verwimp, A., Meinecke, P., Feldberg, K., Bombosch, A., Pócs, T., Schmidt, A.R., Reitner, J., Schneider, H., Heinrichs, J. 2012. Tramps, narrow endemics and morphologically cryptic species in the epiphyllous liverwort *Diplasiolejeunea*. *Molecular Phylogenetics and Evolution*, 65, 582–594.
- Feldberg, K., Hentschel, J., Wilson, R., Rycroft, D.S., Glenny, D., Heinrichs, J. 2007. Phylogenetic biogeography of the leafy liverwort *Herbertus* (Jungermanniales, Herbertaceae) based on nuclear and chloroplast DNA sequence data: correlation between genetic variation and geographical distribution. *Journal of Biogeography*, 34, 688–698.
- Feldberg, K., Schneider, H., Stadler, T., Schäfer-Verwimp, A., Schmidt, A.R., Heinrichs, J. 2014. Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Scientific Reports*, 4.
- Frahm, J.-P., 2010. *Die Laubmoosflora des baltischen Bernsteinwaldes*. Weissdorn, Jena.
- Fürnkranz, M., Wanek, W., Richter, A., Abell, G., Rasche, F., Sessitsch, A. 2008. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *The ISME Journal*, 2, 561–570.
- Gradstein, S.R., Equihua, C. 1995. An epizoic bryophyte and algae growing on the lizard *Corythophanes cristatus* in Mexican rain forest. *Biotropica*, 27, 265–268.
- Gradstein, S.R., Churchill, S.P., Salazar-Allen, N. 2001. Guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden*, 86, 1–577.
- Gradstein, S.R., Costa, D.P. 2003a. The Hepaticae and Anthocerotae of Brazil. *Memoirs of the New York Botanical Garden*, 87, 1–318.
- Gradstein, S.R., Reiner-Drehwald, M.E., Schneider, H. 2003b. A phylogenetic analysis of the genera of Lejeuneaceae (Hepaticae). *Botanical Journal of the Linnean Society*, 143, 391–410.
- Gradstein, S.R., Ilkiu-Borges, A.L. 2009. Guide to the vascular plants of Central French Guiana. Part IV. Liverworts and Hornworts. *Memoirs of the New York Botanical Garden*, 76, 1–140.
- Gradstein, S.R. 2013. A classification of Lejeuneaceae (Marchantiophyta) based on molecular and morphological evidence. *Phytotaxa*, 100, 6–20.
- Grimaldi, D.A., Engel, M.S., Nascimbene, P.C. 2002. Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates*, 3361, 1–71.

- Grolle, R. 1976. *Drepanolejeunea* subgen. *Kolpolejeunea* – eine neue Untergattung aus der Paläotropis. *Journal of the Hattori Botanical Laboratory*, 40, 191–216.
- Grolle, R. 1987. *Radula steerei* sp. nov. – A further hepatic in Dominican amber. *Memoirs of the New York Botanical Garden*, 45, 259–263.
- Grolle, R., Braune, W. 1988. *Bazzania oleosa*, ein Lebermoos im Dominikanischen Bernstein mit erhaltenen Ölkörpern. *Beihefte zur Nova Hedwigia*, 90, 101–108.
- Grolle, R., Meister, K. 2004. *The liverworts in Baltic and Bitterfeld amber*. Weissdorn, Jena.
- Hartmann, F.A., Wilson, R., Gradstein, S.R., Schneider, H., Heinrichs, J. 2006. Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *International Journal of Plant Sciences*, 167, 1205–1214.
- He, X.-L. 1997. Type studies on *Pycnolejeunea* (Lejeuneaceae, Hepaticae). V. On the identity of *Pycnolejeunea spinistipula* Mizut. and *Lepidolejeunea queenslandica* Thiers. *Annales Botanici Fennici*, 34, 127–132.
- He, X.-L., Piippo, S. 1999. On the taxonomic significance and classification of ocelli characters in the hepatic family Lejeuneaceae. *Bryobrothera*, 5, 93–97.
- He, Q., Zhu, R.-L. 2011. Spore output in selected species of Lejeuneaceae (Marchantiophyta) from China. *Cryptogamie, Bryologie*, 32, 107–112.
- Heinrichs, J., Hentschel, J., Wilson, R., Feldberg, K., Schneider, H. 2007. Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon*, 56, 31–44.
- Heinrichs, J., Klugmann, F., Hentschel, J., Schneider, H. 2009. DNA taxonomy, cryptic speciation and diversification of the Neotropical-African liverwort, *Marchesinia brachiata* (Lejeuneaceae, Porellales). *Molecular Phylogenetics and Evolution*, 53, 113–121.
- Heinrichs, J., Reiner-Drehwald, M.E., Feldberg, K., Grimaldi, D.A., Nascimbene, P.C., von Konrat, M., Schmidt, A.R. 2011. *Kaolakia borealis* nov. gen. et sp. (Porellales, Jungermanniopsida): a leafy liverwort from the Cretaceous of Alaska. *Review of Palaeobotany and Palynology*, 165, 235–240.
- Heinrichs, J., Reiner-Drehwald, M.E., Feldberg, K., von Konrat, M., Hentschel, J., Váňa, J., Nascimbene, P., Grimaldi, D., Schmidt, A.R. 2012. The leafy liverwort

- Frullania* (Jungermanniopsida) in the Cretaceous amber forest of Myanmar. *Review of Palaeobotany and Palynology*, 169, 21–28.
- Heinrichs, J., Czumaj, A., Dong, S., Scheben, A., Schäfer-Verwimp, A., Peralta, D.F., Feldberg, K., Schmidt, A.R., Schneider, H. 2014a. The Bromeliaceae tank dweller *Bromeliophila* (Lejeuneaceae, Porellales) is a member of the *Cyclolejeunea-Prionolejeunea* clade. *Plant Systematics and Evolution*, 300, 63–73.
- Heinrichs, J., Dong, S., Schaefer-Verwimp, A., Peralta, D.F., Feldberg, K., Schmidt, A.R., Schneider, H. 2014b. Towards a monophyletic classification of Lejeuneaceae II: subtribes Pycnolejeuneinae and Xylolejeuneinae subtr. nov., transfer of *Otolejeunea* to Lepidolejeuninae, and generic refinements. *Phytotaxa*, 163, 61–76.
- Heinrichs, J., Schäfer-Verwimp, A., Czumaj, A., Dong, S., Scheben, A., Feldberg, K., Schneider, H. 2014c. Towards a monophyletic classification of Lejeuneaceae I: subtribe Leptolejeuneinae subtr. nov. *Phytotaxa*, 156, 165–174.
- Heinrichs, J., Feldberg, K., Bechteler, J., Scheben, A., Czumaj, A., Pócs, T., Schneider, H., Schäfer-Verwimp, A. 2015a. Integrative taxonomy of *Lepidolejeunea* (Jungermanniopsida: Porellales): Ocelli allow the recognition of two neglected species. *Taxon*, 64, 216–228.
- Heinrichs, J., Kettunen, E., Lee, G.E., Marzaro, G., Pócs, T., Ragazzi, E., Renner, M.A.M., Rikkinen, J., Sass-Gyarmati, A., Schäfer-Verwimp, A., Scheben, A., Solórzano Kraemer, M., Svojtka, M., Schmidt, A.R. 2015b. Lejeuneaceae (Marchantiophyta) from a species-rich taphocoenosis in Miocene Mexican amber, with a review of liverworts fossilised in amber. *Review of Palaeobotany and Palynology*, 221, 59–70.
- Heinrichs, J., Schmidt, A.R., Schäfer-Verwimp, A., Bauerschmidt, L., Neumann, C., Gröhn, C., Krings, M., Renner, M.A.M. 2016. Revision of the leafy liverwort genus *Radula* (Porellales, Jungermanniopsida) in Baltic and Bitterfeld amber. *Review of Palaeobotany and Palynology*, 235, 157–164.
- Heinrichs, J., Feldberg, K., Bechteler, J., Müller, P., Renner, M.A.M., Vána, J., Schäfer-Verwimp, A., Schmidt, A.R. 2017. A fossil genus of the Frullaniaceae (Porellales, Jungermanniopsida) from the mid-Cretaceous of Myanmar. *Cretaceous Research*, 74, 223–226.

- Heinrichs, J., Feldberg, K., Bechteler, J., Regalado, L., Renner, M.A.M., Schäfer-Verwimp, A., Gröhn, C., Müller, P., Schneider, H., Krings, M. 2018. A comprehensive assessment of the fossil record of liverworts in amber. In: Krings, M., Cúneo, N.R., Harper C.J., Rothwell, G.W. (Eds.), *Transformative Paleobotany: Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. Burlington MA, London, San Diego CA, New York NY, Elsevier/Academic Press Inc., in press.
- Hernick, L.V., Landing, E., Bartowski, K.E. 2008. Earth's oldest liverworts – *Metzgeriothallus sharonae* sp. nov. from the Middle Devonian (Givetian) of eastern New York, USA. *Review of Palaeobotany and Palynology*, 148, 154–162.
- Herzog, T. 1942. Revision der Lebermoosgattung *Leptolejeunea* Spr. in der Indomalaya. *Flora oder Allgemeine Botanische Zeitung*, 135, 377–434.
- Herzog, T. 1950. Miscellanea Bryologica. II. Paleotropica. *Memoranda Societatis pro Fauna et Flora Fennica*, 26, 37–66.
- Hibbett, D., Grimaldi, D., Donoghue, M. 1997. Fossil mushrooms from Miocene and Cretaceous ambers and the evolution of Homobasidiomycetes. *American Journal of Botany*, 84, 981–981.
- Houle, A. 1998. Floating islands: a mode of long-distance dispersal for small and medium-sized terrestrial vertebrates. *Diversity and Distributions*, 4, 201–216.
- Inoue, H., Miller, H.A. 1965. Hepaticae from Kusaie, Caroline Islands. *Bulletin of the National Science Museum Tokyo*, 8, 139–160.
- Iturralde-Vinent, M.A., MacPhee, R.D.E. 1996. Age and paleogeographical origin of Dominican amber. *Science*, 273, 1850–1852.
- Iturralde-Vinent, M.A. 2001. Geology of the amber-bearing deposits of the Greater Antilles. *Caribbean Journal of Science*, 37, 141–167.
- Ivey, C.T., DeSilva, N. 2001. A test of the function of drip tips. *Biotropica*, 33, 188–191.
- Jongmans, A.G., van Breemen, N., Gradstein, S.R., van Oort, F. 2001. How liverworts build hanging gardens from volcanic ash in Costa Rica. *Catena*, 44, 13–22.
- Kaasalainen, U., Heinrichs, J., Renner, M.A.M., Hedenäs, L., Schäfer-Verwimp, A., Lee, G.E., Ignatov, M.S., Rikkinen, J., Schmidt, A.R. 2017a. A Caribbean epiphyte community preserved in Miocene Dominican amber. *Earth and Environmental Science Transactions of The Royal Society of Edinburgh*, 107, 1–11 (in press).

- Kaasalainen, U., Schmidt, A.R., Rikkinen, J. 2017b. Diversity and ecological adaptations in Palaeogene lichens. *Nature Plants*, 3, 17049.
- Köhler, L., Tobón, C., Frumau, K. A., Bruijnzeel, L.S. 2007. Biomass and water storage dynamics of epiphytes in old-growth and secondary montane cloud forest stands in Costa Rica. *Plant Ecology*, 193, 171–184.
- Konopka, A.S., Herendeen, P.S., Merrill, G.L.S., Crane, P.R. 1997. Sporophytes and gametophytes of Polytrichaceae from the Campanian (Late Cretaceous) of Georgia, USA. *International Journal of Plant Sciences*, 158, 489–499.
- Krassilov, V.A., Schuster, R.M. 1984. Paleozoic and Mesozoic fossils. In: Schuster, R.M. (Ed.), *New manual of bryology, Vol. 2*. The Hattori Botanical Laboratory, Nichinan, Japan, pp. 1172–1193.
- Laenen, B., Shaw, B., Schneider, H., Goffinet, B., Paradis, E., Désamoré, A., Heinrichs, J.; Villarreal, J.C., Gradstein, S.R., McDaniel, S.F., Long, D.G., Forrest, L.L., Hollingsworth, M.L., Crandall-Stotler, B., Davis, E.C., Engel, J., Konrat von, M., Cooper, E.D., Patiño, J., Cox, C.J., Vanderpoorten, A., Shaw, A.J. 2014. Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications*, 5, 5134.
- Laenen, B., Machac, A., Gradstein, S.R., Shaw, B., Patiño, J., Désamoré, A., Goffinet, B., Cox, C.J., Shaw, A.J., Vanderpoorten, A. 2016. Geographical range in liverworts: does sex really matter? *Journal of Biogeography*, 43, 627–635.
- Langenheim, R.L., Smiley, C.J., Gray, J. 1960. Cretaceous amber from the Arctic Coastal Plain of Alaska. *Bulletin of the Geological Society of America*, 71, 1345–1356.
- Langenheim, J.H. 1990. Plant resins. *American Scientist*, 78, 16–24.
- Langenheim, J.H. 1995. The biology of amber-producing trees: Focus on case studies of *Hymenaea* and *Agathis*. *ACS Symposium Series*, 617, 1–31.
- Lewis, L.R., Behling, E., Gousse, H., Qian, E., Elphick, C.S., Lamarre, J.F., Bety, J., Liebezeit, J., Rozzi, R., Goffinet, B. 2014. First evidence of bryophyte diaspores in the plumage of transequatorial migrant birds. *PeerJ*, 2, e424.
- Liu, Y., Cox, C.J., Wang, W., Goffinet, B. 2014. Mitochondrial phylogenomics of early land plants: mitigating the effects of saturation, compositional heterogeneity, and codon-usage bias. *Systematic Biology*, 63, 862–878.
- Longton, R.E. 1988. *Biology of polar bryophytes and lichens*. Cambridge University Press, Cambridge.

- Lücking, A. 1997. Diversity and distribution of epiphyllous bryophytes in a tropical rainforest in Costa Rica. *Abstracta Botanica*, 21, 79–87.
- Lücking, R., Bernecker-Lücking, A. 2005. Drip-tips do not impair the development of epiphyllous rain-forest lichen communities. *Journal of Tropical Ecology*, 21, 171–177.
- Lücking, R., Mata-Lorenzen, J., Dauphin L.G. 2010. Epizoic liverworts, lichens and fungi growing on Costa Rican Shield Mantis (Mantodea: *Choeradodis*). *Studies on Neotropical Fauna and Environment*, 45, 175–186.
- Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist*, 207, 437–453.
- Miller, N.G. 1984. Tertiary and Quaternary fossils. In: Schuster, R.M. (Ed.), *New manual of bryology*, Vol. 2. The Hattori Botanical Laboratory, Nichinan, Japan, pp. 1194–1232.
- Monge-Nájera, J. 1989. The relationship of epiphyllous liverworts with leaf characteristics and light in Monte Verde, Costa Rica. *Cryptogamie, Bryologie*, 10, 345–352.
- Monge-Nájera, J., Blanco, M.A. 1995. The influence of leaf characteristics on epiphyllous cover: a test of hypotheses with artificial leaves. *Tropical Bryology*, 11, 5–9.
- Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., Pierce, N.E. 2006. Phylogeny of the ants: diversification in the age of angiosperms. *Science*, 312, 101–104.
- Morley, R.J. 2011. Cretaceous and Tertiary climate change and the past distribution of megathermal rainforests. In: Bush, M.B., Flenley, J.R., Gosling, W.D. (Eds.). *Tropical rainforest responses to climatic change*, Springer Berlin, pp. 1–34.
- Mueller, U.G., Wolf-Mueller, B. 1991. Epiphyll deterrence to the leafcutter ant *Atta cephalotes*. *Oecologia*, 86, 36–39.
- Muñoz, J., Felicísimo, Á.M., Cabezas, F., Burgaz, A.R., Martínez, I. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science*, 304, 1144–1147.
- Newton, I. 2008. *The migration ecology of birds*. Elsevier/ Academic Press.
- Olarinmoye, S.O. 1974. Ecology of epiphyllous liverworts: growth in three natural habitats in Western Nigeria. *Journal of Bryology*, 8, 275–289.



- Palmer, J.D. 1991. Plastid chromosome, structure and evolution. In: Bogorad, L., Vasil, I. K. (Eds.), *The Molecular Biology of Plastids*. Academic Press San Diego, CA, pp. 5–53
- Patiño, J., Wang, J., Renner, M.A., Gradstein, S.R., Laenen, B., Devos, N., Shaw, A.J., Vanderpoorten, A. 2017. Range size heritability and diversification patterns in the liverwort genus *Radula*. *Molecular Phylogenetics and Evolution*, 106, 73–85.
- Pearson, W.H. 1922. Hepaticae. *Journal of the Linnean Society, Botany*, 46, 13–44.
- Pearson, J., Wells, D.M., Seller, K.J., Bennett, A., Soares, A., Woodall, J., Ingrouille, M.J. 2000. Traffic exposure increases natural <sup>15</sup>N and heavy metal concentrations in mosses. *New Phytologist*, 147, 317–326.
- Peñalver, E., Grimaldi, D.A., Delclòs, X. 2006. Early Cretaceous spider web with its prey. *Science*, 312, 1761–1761.
- Peralta-Medina, E., Falcon-Lang, H.J. 2012. Cretaceous forest composition and productivity inferred from a global fossil wood database. *Geology*, 40, 219–222.
- Perkovsky, E.E., Rasnitsyn, A.P., Vlaskin, A.P., Tatarchuk, M.V. 2007. A comparative analysis of Baltic and Rovno amber arthropod faunas: perspective samples. *African Invertebrates*, 48, 229–245.
- Perkovsky, E.E., Zosimovich, V.Y., Vlaskin, A.P. 2010. Rovno amber. In: Penney, D. (Ed.), *Biodiversity of fossils in amber from the major world deposits*. Siri Scientific Press, Sofia, pp. 116–136.
- Pócs, T. 1996. Epiphyllous liverwort diversity at worldwide level and its threat and conservation. *Anales del Instituto de Biología Serie Botánica*, 67, 109–127.
- Pócs, T., Lye, K.A. 1999. New records and additions to the hepatic flora of Uganda. 2. *Tropical Bryology*, 17, 23–33.
- Poinar, G.O. 1993. Insects in amber. *Annual Review of Entomology*, 38, 145–159.
- Poinar, H.N., Melzer, R.R., Poinar, G.O. 1996. Ultrastructure of 30–40 million year old leaflets from Dominican amber (*Hymenaea protera*, Fabaceae: Angiospermae). *Cellular and Molecular Life Sciences*, 52, 387–390
- Poinar, G.O., Brown, A.E. 2002. *Hymenaea mexicana* sp. nov. (Leguminosae: Caesalpinioideae) from Mexican amber indicates Old World connections. *Botanical Journal of the Linnean Society*, 139, 125–132.
- Poinar, G.O., Danforth, B.N. 2006. A fossil bee from Early Cretaceous Burmese amber. *Science*, 314, 614–614.

- Proctor, M.C.F. 2009. Physiological ecology. In: Goffinet, B., Shaw, A.J. (Eds.) *Bryophyte Biology*, 2<sup>nd</sup> Edition. Cambridge University Press, Cambridge, pp. 237–268.
- Qiu, Y.-L., Lia, L., Wanga, B., Chen, Z., Knoop, V., Groth-Malonek, M., Dombrowska, O., Lee, J., Kent, L., Rest, J., Estabrook, G.F., Hendry, T.A., Taylor, D.W., Testa, C.M., Ambros, M., Crandall-Stotler, B., Duff, R.J., Stech, M., Frey, W., Quandt, D., Davis, C.C. 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 15511–15516.
- Regalado, L., Schmidt, A.R., Appelhans, M.S., Ilsemann, B., Schneider, H., Krings, M., Heinrichs, J. 2017. A fossil species of the enigmatic early polypod fern genus *Cystodium* (Cystodiaceae) in Cretaceous amber from Myanmar. *Scientific Reports*, 7, 14615.
- Renner, S. 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences*, 165, S23–S33.
- Renner, M.A.M., Brown, E.A., Wardle, G.M. 2013a. Averaging v. outlier removal. Decrypting variance among cryptic *Lejeunea* species (Lejeuneaceae: Jungermanniopsida) using geometric morphometrics. *Australian Systematic Botany*, 26, 13–30.
- Renner, M.A.M., Devos, N., Patiño, J., Brown, E.A., Orme, A., Elgey, M., Wilson, T.C., Gray, L.J., von Konrat, M.J. 2013b. Integrative taxonomy resolves the cryptic and pseudo-cryptic *Radula buccinifera* complex (Porellales, Jungermanniopsida), including two reinstated and five new species. *PhytoKeys*, 27, 1–113.
- Renner, M.A.M. 2015. Lobule shape evolution in *Radula* (Jungermanniopsida): one rate fits all? *Botanical Journal of the Linnean Society*, 178, 222–242.
- Renzaglia, K.S., Duff, R.J., Nickrent, D.L., Garbary, D.J. 2000. Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. *Philosophical Transactions of the Royal Society of London B*, 355, 769–793.
- Richards, P.W. 1984. The ecology of tropical forest bryophytes. In: Schuster, R.M. (Ed.), *New manual of bryology*, Vol. 2. The Hattori Botanical Laboratory, Nichinan, Japan, pp.1233–1271.

- Ronquist, F., Sanmartín, I. 2011. Phylogenetic methods in biogeography. *Annual Review of Ecology, Evolution, and Systematics*, 42, 441–464
- Roskoski, J.P. 1981. Epiphyll dynamics of a tropical understory. *Oikos*, 37, 252–256.
- Ruinen, J. 1961. The phyllosphere. *Plant and Soil*, 15, 81–109.
- Rust, J., Singh, H., Rana, R.S., McCann, T., Singh, L., Anderson, K., Sarkar, N., Nascimbene, P.C., Stebner, F., Thomas, J.C., Kraemer, M.S., Williams, C.J., Engel, M.S., Sahni, A., Grimaldi, D. 2010. Biogeographic and evolutionary implications of a diverse paleobiota in amber from the Early Eocene of India. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 18360–18365.
- Sadowski, E.-M., Schmidt, A.R., Kunzmann, L., Gröhn, C., Seyfullah, L. 2016. *Sciadopitys cladodes* from Eocene Baltic amber. *Botanical Journal of the Linnean Society*, 180, 258–268.
- Sadowski, E.-M., Schmidt, A.R., Seyfullah, L.J., Kunzmann, L. 2017. Conifers of the ‘Baltic amber forest’ and their paleoecological significance. *Stapfia*, 106, 1–73.
- Sanmartín, I. 2012. Historical biogeography: evolution in time and space. *Evolution: Education and Outreach*, 5, 555–568.
- Schäfer-Verwimp, A., Feldberg, K., Dong, S., Van Melick, H., Peralta, D.F., Schmidt, A.R., Schneider, H., Heinrichs, J. 2014. Towards a monophyletic classification of Lejeuneaceae III: the systematic position of *Leiolejeunea*. *Phytotaxa*, 170, 187–198.
- Scheben, A., Bechteler, J., Lee, G.E., Pócs, T., Schäfer-Verwimp, A., Heinrichs, J. 2016. Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *Journal of Biogeography*, 43, 1739–1749.
- Schmidt, A.R., Perrichot, V., Svojtka, M., Anderson, K.B., Belet, K.H., Bussert, R., Dörfelt, H., Jancke, S., Mohr, B., Mohrmann, E., Nascimbene, P.C., Nel, A., Nel, P., Ragazzi, E., Roghi, G., Saupe, E.E., Schmidt, K., Schneider, H., Selden, P.A., Vávra, N. 2010. Cretaceous African life captured in amber. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 7329–7334.
- Schmidt, A.R., Jancke, S., Lindquist, E.E., Ragazzi, E., Roghi, G., Nascimbene, P.C., Schmidt, K., Wappler, T., Grimaldi, D.A. 2012. Arthropods in amber from the

- Triassic Period. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 14796–14801.
- Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R. 2004. Ferns diversified in the shadow of angiosperms. *Nature*, 428, 553.
- Schuster, R.M. 1967. North American Lejeuneaceae. X. *Harpalejeunea*, *Drepanolejeunea*, and *Leptolejeunea*. *The Journal of the Mitchell Society*, 83, 192–229.
- Schuster, R.M. 1980. *The Hepaticae and Anthocerotae of North America east of the Hundredth Meridian, Vol. 4.*, Columbia University Press, New York.
- Schuster, R.M. 1992a. The oil-bodies of the Hepaticae. I. Introduction. *Journal of the Hattori Botanical Laboratory*, 72, 151–162.
- Schuster, R.M. 1992b. The oil-bodies of the Hepaticae. II. Lejeuneaceae (Part 2). *Journal of the Hattori Botanical Laboratory*, 72, 163–359.
- Shaw, J. 2001. Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography*, 28, 253–261.
- Shi, G., Grimaldi, D.A., Harlow, G.E., Wang, J., Wang, J., Yang, M., Lei, W., Li, Q., Li, X. 2012. Age constraint on Burmese amber based on U–Pb dating of zircons. *Cretaceous Research*, 37, 155–163.
- Shi, X.Q., Gradstein, S.R., Zhu, R.-L. 2015. Phylogeny and taxonomy of *Archilejeunea* (Marchantiophyta: Lejeuneaceae) based on molecular markers and morphology. *Taxon*, 64, 881–892.
- Shu, L., Zhu, R.-L., Pócs, T. 2016. A new species of *Leptolejeunea* (Lejeuneaceae, Marchantiophyta) from Fiji with special reference to *Leptolejeunea tripuncta*. *Cryptogamie, Bryologie*, 37, 157–165.
- Söderström, L., Hagborg, A., von Konrat, M., Bartholomew-Began, S., Bell, D., Briscoe, L., Brown, E., Cargill, D.C., Costa, D.P., Crandall-Stotler, B.J., Cooper, E.D., Dauphin, G., Engel, J.J., Feldberg, K., Glenney, D., Gradstein, S.R., He, X., Heinrichs, J., Hentschel, J., Ilkiu-Borges, A.L., Katagiri, T., Konstantinova, N.A., Larraín, J., Long, D.G., Nebel, M., Pócs, T., Puche, F., Reiner-Drehwald, E., Renner, M.A.M., Sass-Gyarmati, A., Schäfer-Verwimp, A., Segarra Moragues, J.G., Stotler, R.E., Sukkharak, P., Thiers, B.M., Uribe, J., Váña, J., Villarreal, J.C., Wigginton, M., Zhu, R.-L. 2016. World checklist of hornworts and liverworts. *PhytoKeys*, 59, 1–828.

- Solórzano Kraemer, M.M. 2007. Systematic, palaeoecology, and palaeobiogeography of the insect fauna from Mexican amber. *Palaeontographica Abt. A*, 282, 1–133.
- Sonnleitner, M., Dullinger, S., Wanek, W., Zechmeister, H. 2009. Microclimatic patterns correlate with the distribution of epiphyllous bryophytes in a tropical lowland rain forest in Costa Rica. *Journal of Tropical Ecology*, 25, 321–330.
- Standke, G. 2008. Bitterfelder Bernstein gleich Baltischer Bernstein? – Eine geologische Raum-Zeit-Betrachtung und genetische Schlußfolgerungen. *Exkursionsführer und Veröffentlichungen der Deutschen Gesellschaft für Geowissenschaften*, 236, 11–33.
- Stech, M., Wagner, D. 2005. Molecular relationships, biogeography, and evolution of Gondwanan *Campylopus* species (Dicranaceae, Bryopsida). *Taxon*, 54, 377–382.
- Stephani, F. 1913. Species Hepaticarum (Vol. 5), Georg & Cie, Geneva.
- Stephani, F. 1923. Species Hepaticarum (Vol. 6), Georg & Cie, Geneva.
- Stotler, R.E., Crandall-Stotler, B.J. 1974. A monograph of the genus *Bryopteris* (Swartz) Nees von Esenbeck. *Bryophytorum Bibliotheca*, 3, 1–159.
- Sukkharak, P., Gradstein, S.R. 2017. Phylogenetic study of *Mastigolejeunea* (Marchantiophyta: Lejeuneaceae) and an amended circumscription of the genus *Thysananthus*. *Phytotaxa*, 326, 91–107.
- Swartz, O. 1788. *Nova Genera & Species Plantarum*. Stockholm.
- Taylor, E.L., Taylor, T.N., Krings, M. 2009. *Paleobotany: the biology and evolution of fossil plants*. 2<sup>nd</sup> edition, Elsevier/Academic Press.
- Turetsky, M.R. 2003. The role of bryophytes in carbon and nitrogen cycling. *The Bryologist*, 106, 395–409.
- Vanderpoorten, A., Goffinet, B. 2009. *Introduction to bryophytes*. Cambridge University Press, Cambridge.
- Van Duzer, C. 2004. *Floating islands: A global bibliography*. Los Altos Hills, California: Cantor Press.
- Van Zanten, B.O., Gradstein, S.R. 1988. Experimental dispersal geography of neotropical liverworts. *Beiheft zur Nova Hedwigia*, 90, 41–94.
- Vermeij, G.J. 2011. The energetics of modernization: the last one hundred million years of biotic evolution. *Paleontological Research*, 15, 54–61.
- Villarreal, J.C., Renner, S.S. 2014. A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a

- taxonomically cleaned-up genus *Nothoceros*. *Molecular Phylogenetics and Evolution*, 78, 25–35.
- Von Konrat, M., Söderström, L., Renner, M.A.M., Hagborg, A., Briscoe, L., Engel, J.J. 2010. Early Land Plants Today (ELPT): How many liverwort species are there? *Phytotaxa*, 9, 22–40.
- Wanek, W., Pörtl, K. 2005. Phyllosphere nitrogen relations: reciprocal transfer of nitrogen between epiphyllous liverworts and host plants in the understorey of a lowland tropical wet forest in Costa Rica. *New Phytologist*, 166, 577–588.
- Wang, J., Zhu, R.-L., Gradstein, S.R. 2016. Taxonomic Revision of Lejeuneaceae subfamily Ptychanthoideae (Marchantiophyta) in China. *Bryophytorum Bibliotheca*, 65, 1–141.
- Wellman, C.H., Osterloff, P.L., Mohiuddin, U. 2003. Fragments of the earliest land plants. *Nature*, 425, 282–285.
- Wickett, N.J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., Matasci, N., Ayyampalayam, S., Baker, M.S., Burleigh, J.G., Gitzendanner, M.A., Ruhfel, B.R., Wafula, E., Der, J.P., Graham, S.W., Mathews, S., Melkonian, M., Soltis, D.E., Soltis, P.S., Miles, N.W., Rothfels, C.J., Pokorny, L., Shaw, A.J., DeGironimo, L., Stevenson, D.W., Surek, B., Villarreal, J.C., Roure, B., Philippe, H., dePamphilis, C.W., Chen, T., Deyholos, M.K., Baucom, R.S., Kutchan, T.M., Augustin, M.M., Wang, J., Zhang, Y., Tian, Z., Yan, Z., Wu, X., Sun, X., Wong, G.K.-S., Leebens-Mack, J. 2014. Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E4859–E4868.
- Wilf, P., Labandeira, C.C., Kress, W.J., Staines, C.L., Windsor, D.M., Allen, A.L., Johnson, K.R. 2000. Timing the radiations of leaf beetles: hispines on gingers from latest Cretaceous to recent. *Science*, 289, 291–294.
- Wilson, R., Gradstein, S.R., Schneider, H., Heinrichs, J. 2007a. Unravelling the phylogeny of Lejeuneaceae (Jungermannioptida): evidence for four main lineages. *Molecular Phylogenetics and Evolution*, 43, 270–282.
- Wilson, R., Heinrichs, J., Hentschel, J., Gradstein, S.R., Schneider, H. 2007b. Steady diversification of derived liverworts under Tertiary climatic fluctuations. *Biology Letters*, 3, 566–569.
- Winkler, S. 1967. Die epiphyllen Moose der Nebelwälder von El Salvador. *Revue Bryologique et Lichénologique*, 35, 303–369.

- Wolfe, A., McKellar, R.C., Tappert, R., Sodhi, R.N.S., Muehlenbachs, K. 2016. Bitterfeld amber is not Baltic amber: three geochemical tests and further constraints on the botanical affinities of succinite. *Review of Palaeobotany and Palynology*, 225, 21–32.
- Yamada, K., Piippo, S. 1989. Bryophyte flora of the Huon Peninsula, Papua New Guinea. XXXII. *Radula* (Radulaceae, Hepaticae). *Annales Botanici Fennici*, 26, 349–387.
- Yang, X., Wu, X. 2010. *Sinolejeunea yimaensis* gen. et sp. nov. (Hepaticae) from the Middle Jurassic Yima Formation in Henan Province of China. *Science China, Earth Sciences*, 54, 228–232.
- Yang, J.D. 2014. Taxonomic Studies of Lejeuneaceae subfamily Lejeuneoideae in Taiwan (Doctoral dissertation).
- Ye, W., Gradstein, S.R., Shaw, A.J., Shaw, B., Ho, B.C., Schäfer-Verwimp, A., Pócs, T., Heinrichs, J., Zhu, R.L. 2015. Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers. *Cryptogamie, Bryologie*, 36, 313–333.
- Yu, Y., Pócs, T., Schäfer-Verwimp, A., Heinrichs, J., Zhu, R.-L., & Schneider, H. 2013. Evidence for rampant homoplasy in the phylogeny of the epiphyllous liverwort genus *Cololejeunea* (Lejeuneaceae). *Systematic Botany*, 38, 553–563.
- Zartman, C.E., Nascimento, H.E. 2006. Are habitat-tracking metacommunities dispersal limited? Inferences from abundance-occupancy patterns of epiphylls in Amazonian forest fragments. *Biological Conservation*, 127, 46–54.
- Zhu, R.-L., So, M.L. 2001. Epiphyllous liverworts of China. *Beiheft zur Nova Hedwigia*, 121, 1–418.





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## CURRICULUM VITAE

Name: Julia Maria Theresa Bechteler

### EDUCATION

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- 08/2014 – 02/2018 Ph.D.: “Phylogeny, biogeography, classification, and amber fossils of the liverwort families Lejeuneaceae and Radulaceae”  
Advisor: Prof. Dr. J. Heinrichs
- 10/2008 – 12/2013 First State Examination in Biology, Chemistry, and Educational Science at the LMU for a teaching career at the Gymnasium  
Thesis: “DNA-Barcoding an Flechtensymbionten – Voruntersuchungen an potentiellen Klimawandelzeigern”  
Advisors: Prof. Dr. R. Agerer, Dr. A. Beck
- 06/2008 University-entrance diploma at “Gymnasium Füssen”, Germany  
Specialized courses: Biology and French  
“Bio Zukunftspreis 2008”

### PEER-REVIEWED PUBLICATIONS

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- Regalado, L., Lóriga, J., **Bechteler, J.**, Beck, A., Schneider, H., Heinrichs, J. Accepted. Phylogenetic biogeography reveals the timing and source areas of the *Adiantum* species (Pteridaceae) in the West Indies, with a special focus on Cuba. *Journal of Biogeography*.
- Heinrichs, J., Feldberg, K., **Bechteler, J.**, Regalado, L., Renner, M.A.M., Schäfer-Verwimp, A., Gröhn, C., Müller, P., Schneider, H., Krings, M. 2018. A comprehensive assessment of the fossil record of liverworts in amber. In: Krings, M., Cúneo, N.R., Harper C.J., Rothwell, G.W. (Eds.), *Transformative Paleobotany: Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. Burlington MA, London, San Diego CA, New York NY, Elsevier/Academic Press Inc., in press.
- Schäfer-Verwimp, A., **Bechteler, J.**, van Melick, H., Renner, M.A.M., Heinrichs, J. 2017. *Lepidolejeunea grandiocellata* sp. nov. (Lejeuneaceae, Porellales), a new leafy liverwort from the West Indies based on morphological and molecular evidence. *Cryptogamie, Bryologie*, 38, 253-263.
- Bechteler, J.**, Schmidt, A.R., Renner, M.A.M., Wang, B., Pérez-Escobar, O.A., Schäfer-Verwimp, A., Feldberg, K., Heinrichs, J. 2017. A Burmese amber fossil of *Radula* (Porellales, Jungermanniopsida) provides insights into the Cretaceous evolution of epiphytic lineages of leafy liverworts. *Fossil Record*, 20, 201-213.
- Regalado, L., Schmidt, A.R., Krings, M., **Bechteler, J.**, Schneider, H., Heinrichs, J. 2017. Fossil evidence of eupolypod ferns in the mid-Cretaceous of Myanmar. *Plant Systematics and Evolution*, DOI 10.1007/s00606-017-1439-2.

- Heinrichs, J., Feldberg, K., **Bechteler, J.**, Müller, P., Renner, M.A.M., Vána, J., Schäfer-Verwimp, A., Schmidt, A.R. 2017. A fossil genus of Frullaniaceae (Porellales, Jungermanniopsida) from the mid-Cretaceous of Myanmar. *Cretaceous Research*, 74, 223-226.
- Vána, J., **Bechteler, J.**, Schäfer-Verwimp, A., Renner, M.A.M., Heinrichs, J. 2017. Integrative Taxonomy Substantiates the Presence of Three *Radula* Species in Austria: *Radula complanata*, *R. lindenberiana*, and *R. visianica* (Porellales, Jungermanniopsida). *Cryptogamie, Bryologie*, 38, 125-135.
- Lee, G.E., Kolberg, L., **Bechteler, J.**, Schäfer-Verwimp, A., Renner, M.A.M., Schmidt, A.R., Heinrichs, J. 2017. The leafy liverwort genus *Lejeunea* (Porellales, Jungermanniopsida) in Miocene Dominican amber. *Review of Palaeobotany and Palynology*, 238, 144-150.
- Bechteler, J.**, Schäfer-Verwimp, A., Lee, G.E., Feldberg, K., Perez Escobar, O.A., Pócs, T., Peralta, D.F., Renner, M.A.M., Heinrichs, J. 2017. Geographical structure, narrow species ranges and Cenozoic diversification in a pantropical clade of epiphyllous leafy liverworts. *Ecology and Evolution*, 7, 638-653.
- Bechteler, J.**, Lee, G.E., Schäfer-Verwimp, A., Renner, M.A.M., Peralta, D.F., Heinrichs, J. 2016. Towards a monophyletic classification of Lejeuneaceae V: the systematic position of *Pictolejeunea*. *Phytotaxa*, 280, 259-270.
- Heinrichs, J., Scheben, A., **Bechteler, J.**, Lee, G.E., Schäfer-Verwimp, A., Hedenäs, L., Singh, H., Pócs, T., Nascimbene, P.C., Peralta, D.F. 2016. Crown Group Lejeuneaceae and Pleurocarpous Mosses in Early Eocene (Ypresian) Indian Amber. *PLoS One*, 11:e0156301.
- Scheben, A., **Bechteler, J.**, Lee, G.E., Pócs, T., Schäfer-Verwimp, A., Heinrichs, J. 2016. Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *Journal of Biogeography*, 43, 1739-1749.
- Bechteler, J.**, Lee, G.E., Schäfer-Verwimp, A., Pócs, T., Peralta, D.F., Renner, M.A.M., Schneider, H., Heinrichs, J. 2016. Towards a monophyletic classification of Lejeuneaceae IV: reinstatement of *Allorgella*, transfer of *Microlejeunea aphanella* to *Vitalianthus* and refinements of the subtribal classification. *Plant Systematics and Evolution*, 302, 187-201.
- Lee, G.E., **Bechteler, J.**, Pócs, T., Schäfer-Verwimp, A., Heinrichs, J. 2016. Molecular and morphological evidence for an intercontinental range of the liverwort *Lejeunea pulchriflora* (Marchantiophyta: Lejeuneaceae). *Organisms Diversity & Evolution*, 16, 13-21.
- Lee, G.E., **Bechteler, J.**, Schäfer-Verwimp, A., Heinrichs, J. 2015. *Microlejeunea miocenica* sp. nov. (Porellales, Jungermanniopsida) in Dominican amber, the first fossil of a subcosmopolitan genus of leafy liverworts. *Review of Palaeobotany and Palynology*, 222, 16-21.
- Vána, J., Schäfer-Verwimp, A., **Bechteler, J.**, Schmidt, A.R., Heinrichs, J. 2015. *Notoscyphus grollei* sp. nov. in Bitterfeld amber rather than the extant *Notoscyphus lutescens* (Lehm. & Lindenb.) Mitt. *Phytotaxa*, 222, 151-154.

- Váňa, J., Schäfer-Verwimp, A., **Bechteler, J.**, Schmidt, A.R., Heinrichs, J. 2015. Transfer of the Eocene *Jungermannia berendtii* Grolle to *Solenostoma*. *Cryptogamie, Bryologie*, 36, 285-288.
- Heinrichs, J., Feldberg, K., **Bechteler, J.**, Scheben, A., Czumaj, A., Pócs, T., Schneider, H., Schäfer-Verwimp, A. 2015. Integrative taxonomy of *Lepidolejeunea* (Jungermanniopsida: Porellales): Ocelli allow the recognition of two neglected species. *Taxon*, 64, 216-228.

#### **RESEARCH PRESENTATIONS (\* speaker)**

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- Bechteler J.\*** 2016. Phylogeny and evolutionary history of the epiphyllous liverwort *Leptolejeunea*. Institute Seminar, Systematic Botany and Mycology, LMU Munich, Germany.
- Heinrichs J.\*, Schneider, H., Scheben, A., Lee, G.E., **Bechteler, J.**, Schmidt, A.R. 2015. A new look on old fossils – amber inclusions of bryophytes and ferns in the molecular age. Botanikertagung 2015: From Molecules to the Field, Freising, Germany.
- Beck A.\*, Kuhn, V., Eisenreich, W., Divakar, P.K., Rojas, C., Cuellar, M., Quilhot, W., **Bechteler, J.**, Rocha, G.G., Casanova-Katny, A. 2015. Metabolic profile and antibacterial activity of extracts from *Himantormia lugubris*. 26<sup>th</sup> International Congress on Polar Research, Munich, Germany.

#### **POSTER PRESENTATION**

- Beck A., Kuhn, V., Eisenreich, W., Divakar, P.K., Rojas, C., Cuellar, M., Quilhot, W., **Bechteler, J.**, Rocha, G.G., Casanova-Katny, A. 2016. Phylogenetic position, metabolic profile, and antibacterial extracts of the Antarctic lichen *Himantormia lugubris*. 8<sup>th</sup> International Lichenological Symposium IAL8, Helsinki, Finland.

#### **RESEARCH EXPERIENCE**

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03/2014 – 12/2015: Research assistant, Dr. A. Beck, Botanische Staatssammlung Munich. Involved in several projects regarding mycobiont and photobiont diversity, and ecophysiological studies using stable isotope analyses of Antarctic lichen genera. Responsibilities: conducting laboratory work and teaching laboratory skills, data analysis.

#### **RELEVANT SKILLS**

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Laboratory skills (bryophytes and lichen): genomic DNA extraction, PCR amplification, sanger-sequencing, isolation of lichen myco- and photobionts using gradient techniques. Phylogenetic and dating analyses (RAxML, MrBayes, BEAST, R), alignment building. Liverwort morphology, also as fossils in amber inclusions, particularly Lejeuneaceae.

## **ADVISING (FORMALLY WITH PROF. DR. J. HEINRICHS) AND TEACHING**

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2017: Melina Licht (Bachelor thesis, research course) and Markus Egg (teacher's thesis)

2015: Teaching assistant in "Botanik und Mykologie" for 5<sup>th</sup> semester students

2015: Teaching assistant in "Artenvielfalt Botanik" for 2<sup>nd</sup> semester students

2014: Teaching assistant in "Artenvielfalt Botanik" for 2<sup>nd</sup> semester students