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Towards a monophyletic classification of Lejeuneaceae II: subtribes Pycnolejeuneinae and Xylolejeuneinae subtr. nov., transfer of *Otolejeunea* to Lepidolejeuninae, and generic refinements

JOCHEN HEINRICHS[1], SHANSHAN DONG[2], ALFONS SCHÄFER-VERWIMP[3], DENILSON F. PERALTA[4], KATHRIN FELDBERG[1], ALEXANDER R. SCHMIDT[5] & HARALD SCHNEIDER[6]

[1] *Systematic Botany, Faculty of Biology, University of Munich (LMU), Menzinger Str. 67, 80638 Munich, Germany. Email: jheinrichs@lmu.de, k.feldberg@biologie.uni-muenchen.de*

[2] *Department of Systematic Botany, Albrecht von Haller Institute of Plant Sciences, Georg August University, Untere Karspüle 2, 37073 Göttingen, Germany. Email: shanshan.dong@stud.uni-goettingen.de*

[3] *Mittlere Letten 11, 88634 Herdwangen-Schönach, Germany. Email: moos.alfons@kabelbw.de*

[4] *Instituto de Botânica, Caixa Postal 68041, 04045-972 São Paulo, SP, Brazil. Email: denilsonfp@yahoo.com.br*

[5] *Courant Research Centre Geobiology, Georg August University, Goldschmidtstraße 3, 37077 Göttingen, Germany. Email: alexander.schmidt@geo.uni-goettingen.de*

[6] *Department of Life Science, Natural History Museum, London, SW7, 5BD, UK. Email: h.schneider@nhm.ac.uk*

Abstract

Lejeuneaceae are the most species rich family of leafy liverworts and arguably the most difficult group in terms of generic delimitation. Although much progress has been made in recent years, the generic classification of Lejeuneaceae has not yet been satisfactorily solved. Here, we present phylogenetic analyses of a three marker dataset (nrITS, cp DNA *rbc*L and *trn*L-*trn*F) derived from 113 accessions of Lejeuneeae and 13 outgroup species. Based on maximum parsimony analyses and Bayesian inference of phylogeny, we propose the new subtribes Pycnolejeuneinae and Xylolejeuneinae, reinstate the genera *Cystolejeunea* and *Cyrtolejeunea*, and transfer *Otolejeunea* from Cyclolejeuneinae to Lepidolejeuneinae.

Key words: liverwort, Lejeuneeae, molecular phylogeny, Porellales, taxonomy

Introduction

Leafy liverworts (Jungermanniidae) basically split into two clades, Jungermanniales and Porellales (Heinrichs *et al.* 2005, Forrest *et al.* 2006, He-Nygrén *et al.* 2006). Porellales include predominantly epiphytes and are characterized by bundled rhizoids, exclusively lateral branching, complicate-bilobed leaves with a lobule often forming a water-sac or pocket, frequent occurrence of endosporous protonemata, and a complete lack of mycorrhiza-like mutualisms. Their largest family is the Lejeuneaceae with an estimated species number of about 1700 species (He & Zhu 2011). This family is very abundant in the humid tropics (Cornelissen & Ter Steege 1989, Pócs 1996, Lücking 1997) and includes plants with a ventral leaf lobule attached to the lobe along a keel, the presence of underleaves and/or rhizoid tufts, attachment of underleaves by means of U-shaped cells, *Lejeunea*-type branching, the nearly complete lack of reddish pigmentation, and the presence of only one archegonium per perianth (Ahonen *et al.* 2003). Classification of Lejeuneaceae is notoriously difficult and subject to controversy, as elucidated by systematic treatments using exclusively morphological evidence (Gradstein *et al.* 2003). Much progress has been made since molecular phylogenies of Lejeuneaceae became available (e.g., Ahonen *et al.* 2005, Wilson *et al.* 2007, Czumay *et al.* 2013, Dong *et al.* 2009, 2013, Sukkharak *et al.* 2011, Heinrichs *et al.* 2012, 2013, Ye *et al.* 2013, Yu *et al.* 2013a) but only a small fraction of the extant diversity has been so far included in these studies. Gradstein (2013a) used the available phyologenies to outline a revised classification of Lejeuneaceae in which he introduced a system of subfamilies, tribes and subtribes. He deemed this system somewhat preliminary

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entire leaf margins, and thin-walled leaf cells (Grolle 1985). Grolle (l.c.: 47) proposed a relationship with *Pycnolejeunea* and *Cyclolejeunea* Evans (1904: 193); Gradstein (2013a) placed the genus in the subtribe Cyclolejeuneinae. We were able to include an accession of the type species in our study and resolved it nested in Lepidolejeuneoideae rather than Cyclolejeuneoideae, in a robust sister relationship with *Rectolejeunea*. This position requires confirmation with additional accessions, but unfortunately no further material of *Otolejeunea* suitable for DNA extraction was available. *Rectolejeunea* and *Otolejeunea* share the proximal position of the hyaline papilla, as well as the presence of ocelli and flattened perianths (Grolle 1985, Reiner-Drehwald & Grolle 2012) but a close relationship has not yet been assumed based on morphological evidence.

Perspectives

He (1999: 2) considered Lejeuneaceae "the most difficult group in terms of generic delimitation". Our study confirms this statement and provides further evidence that we need densely sampled molecular datasets to arrive at a classification of Lejeuneaceae into monophyletic entities. Although the molecular datasets of Lejeuneaceae are rapidly growing, much effort is still needed to reach a satisfactory reconstruction of the evolution of this speciose, largely epiphytic lineage in time and space, and to establish a comprehensive classification from species to family level.

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