



Towards a monophyletic classification of Lejeuneaceae I: subtribe Leptolejeuneinae subtr. nov.

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Abstract

We employed sequences of two chloroplast regions (*trnL-trnF*, *rbcL*) and the nuclear ribosomal ITS region of seven accessions of *Leptolejeunea* to explore its phylogenetic position. Maximum parsimony and maximum likelihood analyses led to similar topologies but deeper nodes received good bootstrap support only with maximum likelihood methods. *Leptolejeunea* formed an early diverging, robust monophyletic lineage within Lejeuneaceae tribe Lejeuneaeae. Contrary to earlier belief, it is not closely related to *Drepanolejeunea*. To amend the current classification of Lejeuneaeae into subtribes, we propose Leptolejeuneinae, subtr. nov.

Key words: epiphyll, Lejeuneaeae, *Leptolejeunea*, liverwort, Porellales, taxonomy

Introduction

Lejeuneaceae are a nearly cosmopolitan family with the majority of species in the humid tropics, where they form a major component of the epiphytic, especially epiphyllous diversity (Pócs 1996). Species numbers are still unclear, with recent estimates ranging from 750 (Wilson *et al.* 2007a) to 1700 species (He & Zhu 2011). An equally controversial topic is the supraspecific classification, especially when considering exclusively morphological and chemical evidence (Gradstein *et al.* 2003). These challenges are now increasingly addressed in studies using DNA sequence data to reconstruct relationships. The application of this molecular phylogenetic approach has improved our understanding of evolutionary patterns within Lejeuneaceae (e.g., Wilson *et al.* 2004, 2007b, Dong *et al.* 2012, 2013, Heinrichs *et al.* 2013, 2014, Yu *et al.* 2013), and has led to a considerable reduction of genera from some 90 to 68 (Gradstein *et al.* 2006, Heinrichs *et al.* 2012a, 2012b, Ye *et al.* 2013).

Based on the hitherto published molecular phylogenies of Lejeuneaceae, Gradstein (2013) proposed a new classification of Lejeuneaceae. However, he deemed this classification “somewhat preliminary” because not all genera of Lejeuneaceae have been studied using DNA sequence data, and many are represented only by a single or a few species in published molecular datasets. This holds especially true for Lejeuneaeae, the most diverse tribe of Lejeuneaceae. Gradstein (2013) classified tribe Lejeuneaeae into eight subtribes but felt unable to assign seven of its 40 genera to any of them due to a lack or paucity of molecular data. One of these “unassigned” genera is the largely epiphyllous genus *Leptolejeunea* (Spruce 1884: 193) Schiffner (1893: 126) (Pócs 1996), of which a single species was included in previous molecular phylogenetic analyses of Lejeuneaceae (Wilson *et al.* 2007b). In that study, it was resolved in an early diverging clade of Lejeuneaeae.

Here, we test the monophyly of *Leptolejeunea* by sequencing chloroplast and nuclear markers of seven Neotropical or Paleotropical accessions and including them in a large alignment of published Lejeuneaeae sequences. Based on maximum parsimony and maximum likelihood analyses of this dataset we confirm the monophyly of *Leptolejeunea* and propose a new subtribe, Leptolejeuneinae Heinrichs et Schäf.-Verw.

explore the evolution of ecological preferences within Lejeuneaceae, and to further amend the current classification, an extension of the molecular data sets, and inclusion of genera such as *Metalejeunea* Grolle (1995: 17), *Schusterolejeunea* Grolle (1980: 105), *Tuyamaella* Hattori (1951: 60), and *Vitalianthus* Schuster & Giancotti (1993: 447) is required. Work to obtain these data is under way.

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References

- Bischler, H. (1969) Le genre *Leptolejeunea* (Spruce) Steph. en Amérique. *Nova Hedwigia* 17: 255–350.
- 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., Meinecke, P., Feldberg, K., Bombois, 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.
<http://dx.doi.org/10.1016/j.ympev.2012.07.009>
- Dong, S., Schäfer-Verwimp, A., Pócs, T., Feldberg, K., Czumaj, A., Schmidt, A.R., Schneider, H. & Heinrichs, J. (2013) Size doesn't matter – recircumscription of *Microlejeunea* based on molecular and morphological evidence. *Phytotaxa* 85: 41–55.
<http://dx.doi.org/10.11646/phytotaxa.85.2.2>
- Dumortier, B.C.J. (1831) *Sylloge Jungermannidearum Europae indigenarum: earum genera et species systematicae 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.
- Gradstein, S.R. (2013) A classification of Lejeuneaceae based on molecular and morphological evidence. *Phytotaxa* 100: 6–20.
<http://dx.doi.org/10.11646/phytotaxa.100.1.2>
- 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., Ilku-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.
- Grolle, R. (1976) *Drepanolejeunea* subgen. *Kolpolejeunea* – eine neue Untergattung aus der Paläotropis. *Journal of the Hattori Botanical Laboratory* 40: 191–216.
- Grolle, R. (1980) *Schusterolejeunea* Grolle nom. nov. statt *Cladocolea* Schust. 1963, non Van Tieghem 1895. *Journal of Bryology* 11: 105–106.
<http://dx.doi.org/10.1179/jbr.1980.11.1.105>
- Grolle, R. (1995) The Hepaticae and Anthocerotae of the East African Islands. *Bryophytorum Bibliotheca* 48: 1–178.
<http://dx.doi.org/10.2307/3244116>
- Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
- Hall, T.A. (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposia Series* 41: 95–98.
- 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.
<http://dx.doi.org/10.1086/508023>
- Hattori, S. (1951) Contributio ad Floram Hepaticarum Yakusimensem, V. *Journal of the Hattori 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., Czumaj, A., Dong, S., Scheben, A., Schäfer-Verwimp, A., Peralta, D.F., Feldberg, K., Schmidt, A.R. & Schneider, H. (2014) 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., Feldberg, K., Schäfer-Verwimp, A. & Schmidt, A.R. (2012a) *Sphaerolejeunea* (Lejeuneaceae, Porellales) is a synonym of *Lejeunea*. *Phytotaxa* 69: 7–15.
- 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(12): e82547.
- http://dx.doi.org/10.1371/journal.pone.0082547
- Heinrichs, J., Dong, S., Yu, Y., Schäfer-Verwimp, A., Pócs, T., Feldberg, K., Hentschel, J., Schmidt, A.R. & Schneider, H. (2012b) A 150 year old mystery solved: Transfer of the rheophytic liverwort *Myriocolea irrorata* to *Colura*. *Phytotaxa* 66: 55–64.
- Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing the confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
- Mason-Gamer, R.J. & Kellogg, E.A. (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology* 45: 524–545.
- Mitten, W. (1861) Hepaticae Indiae Orientalis: an enumeration of the Hepaticae of the East Indies. *Journal of the Proceedings of the Linnean Society, Botany* 5: 89–128.
- 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.
- Schiffner, V. (1893) Hepaticae. In: Engler, A. & Prantl, K., *Die natürlichen Pflanzenfamilien* 1. Engelmann, Leipzig, pp. 97–141.
- Schiffner, V. (1898) *Conspectus Hepaticarum Archipelagi Indici*. Staatsdruckerei, Batavia, 425 pp.
- Schuster, R.M. (1963) An annotated synopsis of the genera and subgenera of Lejeuneaceae. *Beihefte zur Nova Hedwigia* 9: 1–203.
- Schuster, R.M. & Giancotti, C. (1993) On *Vitalianthus* Schust. & Giancotti, a new genus of Lejeuneaceae. *Nova Hedwigia* 57: 445–456.
- Spruce, R.M. (1884) Hepaticae Amazonicae et Andinae. Tribus I: Jubuleae. *Transactions and Proceedings of the Botanical Society of Edinburgh* 15: 1–308.
- Swofford, D.L. (2000) PAUP*, phylogenetic analyses using parsimony (* and other methods), version 4.01b10. Sinauer Associates, Sunderland, Massachusetts.
- Wilson, R., Gradstein, S.R., Heinrichs, J., Groth, H., Ilkiu-Borges, A.L. & Hartmann, F.A. (2004) Phylogeny of Lejeuneaceae: A cladistic analysis of chloroplast gene *rbcL* and morphology with preliminary comments on the mitochondrial *nad4*-2 spacer region. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 189–202.
- Wilson, R., Gradstein, S.R., Schneider, H. & Heinrichs, J. (2007a) 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.ymp.2006.10.017
- 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.
- http://dx.doi.org/10.1098/rsbl.2007.0287
- 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
- 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: 553–564.
- http://dx.doi.org/10.1600/036364413x670304
- Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. GARLI version 2.0 available online at <https://code.google.com/p/garli/>.