# **Research Article**

# Phylogenetic position of *Oryzolejeunea* (Lejeuneaceae, Marchantiophyta): Evidence from molecular markers and morphology

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Abstract The systematic position of the small neotropical genus *Oryzolejeunea* (three spp.) has long been controversial. Phylogenetic analyses of molecular data for the present study using DNA markers (*trnL*, *psbA*, and a nuclear ribosomal internal transcribed spacer [nrITS] region) shows that the genus is nested in *Lejeunea*. The results not only reveal the phylogenetic position of *Oryzolejeunea* for the first time, but also challenge the taxonomic value of the proximal hyaline papilla as a key feature in *Lejeunea*. The present study shows the urgent need for a reassessment of the perimeters of the genus *Lejeunea* and its infrageneric classification. Three new combinations, namely *Lejeunea* saccatiloba, *Lejeunea* grolleana, and *Lejeunea* venezuelana, are proposed.

Key words Cheilolejeunea, Hepaticae, Lejeuneaceae, liverworts, oil body.

The large liverwort family Lejeuneaceae comprises approximately 1 700 species in 81 genera (He & Zhu, 2011), a diversity that is estimated to have originated in the mid-Cretaceous (Heinrichs et al., 2007). The species are abundant in moist, tropical forests where they inhabit a diversity of epiphytic habitats ranging from large trunk bases to tiny twigs in tree crowns and the surfaces of living leaves. More than 70% of the liverwort species in tropical lowland rainforests are Lejeuneaceae (Gradstein, 1994). The systematics of the family is considered a major challenge, although considerable progress has been made in recent years (e.g. Gradstein et al., 2003; Wilson et al., 2007). Several recent molecular phylogenetic studies of Lejeuneaceae have dealt with small and enigmatic taxa for which the systematic positions have been unknown or contentious; for example, Dendrolejeunea (Spruce) Lacout. (Sukkharak et al., 2011), Metzgeriopsis K. I. Goebel (Gradstein et al., 2006), Myriocolea Spruce (Heinrichs et al., 2012a), and Sphaerolejeunea Herzog (Heinrichs et al., 2012b). The backbone of the molecular phylogeny of the family was established by Wilson et al. (2007). However, most genera have not been studied in detail using modern molecular methods. The present study focused on the small neotropical genus *Oryzolejeunea* (R. M. Schust.) R. M. Schust.

Oryzolejeunea was established by Schuster (1970) as a subgenus of Cyrtolejeunea A. Evans (= Cheilolejeunea (Spruce) Schiffn.) based on Cyrtolejeunea antillana R. M. Schust. (= Orvzolejeunea saccatiloba (Steph.) Gradst.), and elevated to generic rank by the same author in 1992 (Schuster, 1992). Orvzolejeunea is found on tree bark or leaves in montane forest areas, at elevations of 460-2830 m. The genus currently contains three species: the type species O. saccatiloba (Steph.) Gradst., which is widespread in tropical America, and two further species that are very rare and are only known from type specimens, namely Oryzolejeunea grolleana Bern.-Lück. from Costa Rica and Oryzolejeunea venezuelana (R. M. Schust.) R. M. Schust. from northern Venezuela (Gradstein et al., 2001). There is also an undescribed species from Colombia with a "roughened cuticle" and "homogeneous oil-bodies" (Schuster, 1992), although, unfortunately, this has note been validly published. Characteristics for Oryzolejeunea are: a large leaf lobule with a truncate apex and a short, blunt apical tooth; thin-walled cells with glistening homogeneous or faintly segmented oil bodies; and distal hyaline papilla (Schuster, 1970; Bernecker-Lücking, 1999; Gradstein et al., 2001). The systematic position of the genus is unclear and affinities to the genera Cheilolejeunea and Lejeunea Libert. have been suggested (Schuster, 1992,

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2001; Bernecker-Lücking, 1999). Gradstein et al. (2001) emphasized the close resemblance between *O. saccatiloba* and *Amblyolejeunea fulfordiae* Ast, the only species in the genus *Amblyolejeunea* Ast and currently considered a member of *Lejeunea* (Zhu & Cheng, 2008). In a broad morphological–phylogenetic analysis of the genera of Lejeuneaceae (Gradstein et al., 2003), *Oryzolejeunea* was recovered as a member of the Lejeuneae clade together with 42 other genera, but its closer affinities remained unresolved. No molecular studies have been undertaken as yet to clarify the position of the genus.

In the present study, we explored the systematic position of *Oryzolejeunea* using evidence from three molecular markers (*trnL*, *psbA*, and nuclear ribosomal internal transcribed spacers [ITS] 1 and 2, including the intervening 5.8S ribosomal subunit [ITS1–5.8S–ITS2; collectively called the ITS region]), as well as morphology.

# 1 Material and methods

# 1.1 Taxon sampling and molecular data

To reveal the phylogenetic position of Orvzolejeunea we sequenced the type species, Oryzolejeunea saccatiloba based on material from the Dominican Republic collected by A. Schäfer-Verwimp (two accessions) and M. Pérez (one accession), and Lejeunea bidentula Herzog from China. The two further species of Orvzolejeunea, both known only from type material, could not be sequenced. The type of Oryzolejeunea venezuelana (hb. R. M. Schuster, collected in 1976) was not available for study. The sequences of a further 12 species of Lejeunea, seven species of Cheilolejeunea, and representative members from Ceratolejeunea (Spruce) Schiffn., Cololejeunea (Spruce) Schiffn., Colura (Dumort.) Dumort., Cyclolejeunea A. Evans, Diplasiolejeunea (Spruce) Schiffn., Drepanolejeunea (Spruce) Schiffn., Lepidolejeunea R. M. Schust., Leptolejeunea (Spruce) Schiffn., Luteolejeunea Piippo, and Pycnolejeunea (Spruce) Schiffn. were downloaded from GenBank, representing the subfamily Lejeuneoideae. Lopholejeunea subfusca (Nees) Schiffn. and Thysananthus comosus Lindenb. ex Lehm. were chosen as outgroup representatives based on Wilson et al. (2007). Specimen data and GenBank accession numbers are presented in Appendix I.

# 1.2 DNA extraction, amplification, and sequencing

DNA was extracted using Qiagen's DNeasy Plant Mini Kit (Qiagen Inc., Valencia, USA). The *trnL*, *psbA*, and nrITS regions were amplified using previously published protocols, specifically those of Taberlet et al. (1991) for the *trnL* region, those of Forrest and Crandall-Stotler (2004) for the *psbA* region, and those of Hartmann et al. (2006) for the nrITS region. In cases where no clear amplification products were seen, additional polymerase chain reactions (PCR) were run using 5 min at 80°C followed by 33 cycles of 1 min at 95°C, 1 min at 51°C, 4 min at 65°C, and a final extension step of 5 min at 65° before being held at 4°C. Both strands of the PCR products were sequenced.

# 1.3 Sequence alignment and phylogenetic analysis

All sequences were edited and aligned using PhyDE 0.997 (Müller et al., 2008). Regions of ambiguous alignment in the dataset were determined and excluded from phylogenetic analysis.

Maximum parsimony (MP) analyses were performed using command files with the parsimony ratchet (Nixon, 1999) generated from the program PRAP2 (Müller, 2007) applying the default settings, and executed in PAUP 4.0b10 (Swofford, 2002). Heuristic bootstrap searches under parsimony were performed with 10 000 replicates.

The three regions were first analyzed separately to check possible incongruence among them. The Akaike information criterion was used to decide the best-fit substitution models for maximum likelihood (ML) analysis and Bayesian inference (BI) analysis with MrModeltest v.2.3 (Nylander, 2004). A general time reversible (GTR) model with y-distributed rate variation across sites and a proportion of invariable sites was selected for all three regions. The ML trees were generated using GARLI version 2.0 (Zwickl, 2006) with models implemented. Bayesian searches were performed using four simultaneous Markov chain Monte Carlo (MCMC) with a heterogeneous model for 1 000 000 generations, and sampled every 1000th generation. The burn-in point was confirmed and the log likelihoods examined using Tracer v1.5 (Rambaut & Drummond, 2009). The outputs from the four runs were combined for final inference of posterior probabilities of both trees and model parameters after discarding a suitable burn-in.

Strict consensus trees from these separate analyses were compared to identify conflicting nodes supported by at least 70% (Mason-Gamer & Kellogg, 1996). They gave no evidence of incongruence. Thus, the three datasets were combined into a single matrix as partitions. The combined data were submitted to Bayesian analysis with the best-fit model detailed above in MrBayes (Huelsenbeck & Ronquist, 2001). Four chains of the MCMC were run for 4 000 000 generations and sampled every 1000th generation. The ML analysis was performed in GARLI with the models designated by MrModeltest implemented; bootstrap values were calculated based on 1200 bootstrap replicates.

#### 1.4 Morphological study

Morphological data were obtained from a study of herbarium specimens of two species of *Oryzolejeunea* (*O. grolleana*, *O. saccatiloba*) and of various species of the related genera *Cheilolejeunea* and *Lejeunea*. Cuticular structure from air-dried herbarium specimens was examined under a light microscope (Zeiss Imager A1; Carl Zeiss AG, Oberkochen, Germany), as well as using scanning electron microscopy (Hitachi S-4800; Hitachhi, Tokyo, Japan).

#### 2 Results

#### 2.1 Molecular dataset

Alignment of the analyzed dataset included nrITS (1090 sites), *psbA* (1274 sites), and *trnL* (415 sites) for 39 taxa. The combined data included 2779 characters,

of which 1729 were constant, 731 were parsimony informative, and 319 were autapomorphic. Parsimony analyses generated 201 maximally parsimonious trees (MPTs) of 3169 steps, with a consistency index (CI) of 0.532 and a retention index (RI) of 0.615. All ML searches yielded consistent topologies. The topology of the ML searches ( $\ln = -18435.09427$ ; Fig. 1) and the majority rule consensus tree derived from BI was congruent with the MPTs from MP analysis except for a few nodes towards the tips of the topology that lacked support. The three accessions of Oryzolejeunea saccatiloba and Orvzolejeunea + Lejeunea catinulifera were recovered as strongly supported monophyletic groups nested within Lejeunea, with 100% bootstrap support from ML and MP analysis and a posterior probability (PP) of 1. Accessions of Cheilolejeunea and Lejeunea formed two robust separate clades (maximum likelihood bootstrap support (MLBS) = 100, PP = 1, maximum parsimony bootstrap support (MPBS) = 100; and MLBS = 100, PP = 1, MPBS = 100, respectively). All taxa from Lejeuneoideae formed a well-supported clade (MLBS = 100, PP = 1, MPBS = 100).



Fig. 1. A single most likely phylogram ( $\ln = -18\ 435.09427$ ) resulting from maximum likelihood analysis of a combined molecular dataset. Values above the branches (from left to right) indicate maximum parsimony bootstrap support and Bayesian posterior probability, respectively, whereas values below the branches show maximum likelihood bootstrap support.

# 2.2 Morphology

Morphological examination of specimens of *Oryzolejeunea*, *Lejeunea*, and *Cheilolejeunea* revealed a striking cytological similarity between *Oryzolejeunea* species (*O. saccatiloba*, *O. grolleana*) and many *Lejeunea* species in having relatively large, thin-walled leaf cells with a finely punctate cuticle (Fig. 2). Conversely, in *Cheilolejeunea* the leaf cells were often thick walled and mammillose or papillose, but they never had a finely punctuate cuticle.

# **3** Discussion

Although previous authors have proposed distant relationships between Oryzolejeunea and either Lejeunea (Bernecker-Lücking, 1999; Gradstein et al., 2001) or Cheilolejeunea (e.g., Schuster, 1992), the molecular evidence presented in the present study clearly shows that Oryzolejeunea is nested within Lejeunea and does not deserve generic status. Recently, based on morphological evidence, many other genera, such as Amblvolejeunea (Zhu & Cheng, 2008), Amphilejeunea R. M. Schust., Cryptogynolejeunea R. M. Schust. (Reiner-Drehwald, 2005), Cladolejeunea Zwickel (Pócs, 2010), Crossotolejeunea (Spruce) Schiffn. (Reiner-Drehwald & Goda, 2000), Dactylolejeunea R. M. Schust. (Reiner-Drehwald & Goda, 2000), Echinocolea R. M. Schust. (Ilkiu-Borges, 2005), Neopotamolejeunea E. Reiner (as a subgenus of Lejeunea; Gradstein & Reiner-Drehwald, 2007), Otigoniolejeunea (Spruce) Schiffn. (Reiner-Drehwald & Ilkiu-Borges, 2007; as a subgenus), Stenolejeunea R. M. Schust. (Söderström et al., 2010), and possibly Papillolejeunea Pócs (Schuster, 1998; as a subgenus), have been shown to be synonyms of *Lejeunea*, which, together with *Cololejeunea*, has become the largest and most diversified genus within the family. Consequently, the number of accepted genera in Lejeuneaceae has now been reduced to approximately 75.

By including *Oryzolejeunea* in *Lejeunea*, the definition of *Lejeunea* has broadened. Importantly, the entire underleaves are rare in *Lejeunea* and are furthermore found in the neotropical *Lejeunea catinulifera* Spruce (Reiner-Drehwald, 2005), *Lejeunea fulfordiae* (Ast) R. L. Zhu, *Lejeunea reflexistipula* (Lehm. & Lindenb.) Gottsche, Lindenb. and Nees, and the Asiatic *L. mimula* Hürl. (Zhu & Cheng, 2008). The results of our molecular analysis indicate a close relationship between the type species of *Oryzolejeunea* and the neotropical *L. catinulifera*. The latter not only has entire underleaves, but also has two lobule teeth, as discussed below.

The distal hyaline papilla at the lobule is generally considered the most significant morphological character separating the Cheilolejeunea clade from the Lejeunea clade and other clades within the family (Gradstein et al., 2003; Wilson et al., 2007). As for the position of the hyaline papilla in Oryzolejeunea, Schuster (1970, 1978, 1992, 2001) described it as distal to the apical tooth. Our examinations reveal that the leaf lobule of Oryzolejeunea in fact has two teeth at the lobular apex, both of which are well developed in Orvzolejeunea grolleana (Bernecker-Lücking, 1999), whereas one is reduced in Oryzolejeunea saccatiloba. Although most Lejeunea species have only one welldeveloped lobule tooth, two teeth are found in Lejeunea boliviensis (Steph.) R. L. Zhu & E. Reiner from Bolivia (Zhu & Reiner-Drehwald, 2004), L. catinulifera Spruce from the Neotropics (Reiner-Drehwald, 2005),



Fig. 2. Finely punctuate cuticle of leaf cells of *Oryzolejeunea saccatiloba*. A, Scanning electron microscopy. B, Light microscopy. Both images are based on *Schäfer-Verwimp & Verwimp 26633*.

Lejeunea norrisii Grolle from Tasmania (Grolle, 1982), Lejeunea bidentula Herzog from Bhutan, China, India, Japan, and Nepal (Mizutani, 1971; Asthana & Saxena, 2011), and L. latilobula (Herzog) R. L. Zhu & M. L. So from China (Zhu & So, 2002). In all these species, the hyaline papilla is proximal to the first tooth (interpreted as the apical tooth in Lejeunea) and distal to the second tooth, which is usually reduced in most Lejeunea species. In O. saccatiloba, however, the first tooth is reduced and, consequently, the second tooth is usually considered the "apical" tooth in this species, the hyaline papilla being distal to this tooth. The same condition is found in Cheilolejeunea, which also has a reduced first lobular tooth. Thus, the different positions of the hyaline papilla relative to the apical tooth in Cheilolejeunea and Lejeunea are due to the fact that the apical tooth concepts in these two genera, as described by, for example, Mizutani (1961), He (1996), and Schuster (1970, 1978, 1992, 2001), are not homologous. In both Lejeunea and Cheilolejeunea, the hyaline papilla is, in fact, proximal to the first tooth of the leaf lobule. As emphasized by He (1996), the terms "proximal hyaline papilla" and "distal hyaline papilla" traditionally used in descriptions of Lejeuneaceae are misleading.

The oil bodies of two Oryzolejeunea species (O. saccatiloba, O. venezuelana) are small, usually glistening and homogeneous, with approximately 20-40 per median cells in O. saccatiloba (Schuster, 1992, as O. antillana) and "numerous" in O. venezuelana (Schuster, 1978, p. 426, as Cyrtolejeunea venezuelana), a condition also found in L. cavifolia (Ehrh.) Lindb., the type species of Lejeunea (Smith, 1990). The third species of Oryzolejeunea, O. grolleana, has finely granular and glistening oil bodies (six to 10 per leaf cell). Although finely granular oil bodies also occur in several species of Cheilolejeunea, for example C. holostipa (Spruce) Grolle & R. L. Zhu, C. insecta Grolle & Gradst., and C. obtusifolia (Steph.) S. Hatt., the number of oil bodies per leaf cell in these Cheilolejeunea species is always fewer (only one to three, except for species from the Crvtolejeunea clade (four to five); Grolle et al., 2001).

The rough cuticle of leaf cells found in every species of *Oryzolejeunea* has been considered a "trademark" of this group (Schuster, 1992; Bernecker-Lücking, 1999). However, our examination of *Lejeunea* specimens reveals that such an asperulate cuticle occurs widely in *Lejeunea* (Zhu & So, 1999, 2001; Heinrichs & Reiner-Drehwald, 2012).

The inclusion of the genus *Oryzolejeunea* in *Lejeunea* shows that a reduction of the first lobule tooth, traditionally considered the main character to separate

Lejeunea and its allies from *Cheilolejeunea*, may also occur in *Lejeunea* and does not serve to sharply separate the two. Other diagnostic features of *Lejeunea*, such as the lejeuneoid innovations and the absence of ocelli (Gradstein et al., 2001), have recently been shown not to be constant in this genus by the inclusion of *Otigoniolejeunea* (Spruce) Schiffn. (*L. huctumalcensis* Lindenb. & Gottsche) in *Lejeunea* (Reiner-Drehwald & Ilkiu-Borges, 2007), a taxon characterized by pycnolejeuneoid innovations and the presence of ocelli in leaf lobes.

Thus, recent studies have led to a broader morphological definition of *Lejeunea* and less clear separation of *Lejeunea* from the neighboring genera of Lejeuneaceae. Thus, the clarification of the systematic relationships of *Lejeunea* (Wei et al., 2012, unpublished data) and related genera has become one of the main challenges for future Lejeuneaceae research.

# 4 Taxonomic conclusion

**Lejeunea** Lib., Ann. Gén. Sci. Phys. 6: 372. 1820. *"Lejeunia"*. corr. Hampe 1837, nom. et orth. cons. Type: *L. serpyllifolia* (Dicks.) Libert (= *L. cavifolia* (Ehrh.) Lindb.).

Oryzolejeunea (R. M. Schust.) R. M. Schust., J. Hattori Bot. Lab. 72: 249. 1992. syn. nov. Type: O. antillana (R. M. Schust.) R. M. Schust. (= O. saccatiloba (Steph.) Gradst.).

# 1. Lejeunea saccatiloba (Steph.) R. L. Zhu & W. Ye, comb. nov.

Archilejeunea saccatiloba Steph., Hedwigia 34: 61. 1895. —*Cyrtolejeunea saccatiloba* (Steph.) Gradst., Beih. Nova Hedwigia 80: 110. 1985. —*Oryzolejeunea saccatiloba* (Steph.) Gradst. Lindbergia 23: 77. 1998. Type: Brazil: Sine loc., *Beyrich s.n.* (holotype, G 20795!).

*Cyrtolejeunea antillana* R. M. Schust., Bull. Torrey Bot. Club 97: 336. 1970. —*Oryzolejeunea antillana* (R. M. Schust.) R. M. Schust., J. Hattori Bot. Lab. 72: 249. 1992. Type: Dominica: Freshwater Lake near Morne Micotrin, ca. 790 m, in wet rainforest, *R. M. Schuster 66619a* (holotype, hb. Schuster, n.v.), syn. fide R. Grolle in Dauphin et al., 1998.

2. Lejeunea grolleana (Bern.-Lück.) R. L. Zhu & W. Ye, comb. nov.

*Oryzolejeunea grolleana* Bern.-Lück., Haussknechtia Beih. 9: 37. 1999. Type: Costa Rica: Limon Province, Botarrama-Trail in the Braulio Carrillo National Park, section Quebrada Gonzales, 40 km NNE of San José, 10°12'N, 83°55'W, approximately 460–480 m, on leaves of *Rhodospatha wendlandii* Schott. ex Engl., Jan.–July 1992, *A. Bernecker 92-147* (holotype, JE!).

3. Lejeunea venezuelana (R. M. Schust.) R. L. Zhu & W. Ye, comb. nov.

*Cyrtolejeunea venezuelana* R. M. Schust., Phytologia 39: 426. 1978. —*Oryzolejeunea venezuelana* (R. M. Schust.) R. M. Schust., J. Hattori Bot. Lab. 72: 251. 1992. Type: Venezuela: Estado Tachira, Villa Paez, 2450 m, *R. M. Schuster & L. Ruíz-Terán 76-2708a* (holotype, hb. Schuster, n.v.).

# Key to former Oryzolejeunea species

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# Appendix I

Taxa included in the present study with voucher information and GenBank accession numbers for the sequences of nuclear ribosomal internal transcribed spacers (ITS) 1 and 2, including the intervening 5.8S ribosomal subunit (ITS1–5.8S–ITS2), *trnL*, and *psbA* from chloroplast DNA. An asterisk indicates sequences generated by the present study; a dash indicates a missing sequence.

| Taxon  | Voucher  | GenB                 | enBank accession No. |               |
|--|--|----------------------|----------------------|---------------|
|  |  | ITS1-5.8S-ITS2       | trnL                 | psbA          |
| Ceratolejeunea cornuta (Lindenb.) Steph.<br>Ceratolejeunea grandiloba J. B. Jack &<br>Steph. | Drehwald 4739 (GOET), Bolivia<br>Gradstein 7055 (GOET), Bolivia                | DQ987257<br>DQ987274 | DQ238570<br>_        | AM396280      |
| Cheilolejeunea acutangula (Nees) Grolle  | Gradstein & Velasquez s.n. (GOET),<br>Mexico                                   | DQ987270             | DQ987386             | EF011755      |
| Cheilolejeunea beyrichii (Lindenb.)<br>E. Reiner   | Allen 17393 (GOET), Honduras   | DQ987271             | DQ987387             | EF011756      |
| Cheilolejeunea clausa (Nees & Mont.)<br>R. M. Schust.  | Schäfer-Verwimp 13212 (GOET), Brazil   | DQ987293             | DQ987399             | EF011773      |
| Cheilolejeunea lineata A. Evans  | Schäfer-Verwimp 22183 (GOET),<br>Guadeloupe                                    | DQ987295             | DQ987401             | EF011775      |
| Cheilolejeunea meyeniana (Gottsche,<br>Lindenb. & Nees) R. M. Schust. &<br>Kachroo           | Schäfer-Verwimp 24836/C (GOET),<br>Sumatra                                     | DQ987370             | _                    | EF011857      |
| Cheilolejeunea revoluta (Herzog) Gradst. & Grolle  | Dauphin 1990 (GOET), Coasta Rica   | DQ987354             | DQ987454             | EF011838      |
| Cheilolejeunea rigidula (Mont.) R. M. Schust.  | Muñoz 98-62 (GOET), Suriname   | DQ987353             | DQ987453             | EF011837      |
| Cololejeunea peculiaris (Herzog) Benedix   | Schäfer-Verwimp 18861/A (GOET),<br>Malaysia                                    | DQ987280             | DQ238572             | EF011761      |
| Colura imperfecta Steph.<br>Cyclolejeunea convexistipa (Lehm. &<br>Lindenb) A Evans          | Pócs 03261/BA (GOET), Fiji<br>Salazar Allen & Gradstein 9406 (GOET),<br>Panama | DQ987305<br>DQ207882 | DQ238585<br>DQ207905 | EF011785<br>- |
| Diplasiolejeunea involuta S. Winkl.  | Schäfer-Verwimp 23508 (GOET), Ecuador  | DQ987288             | DQ238582             | EF011769      |
| Drepanolejeunea biocellata A. Evans  | Gradstein et al. 10053 (GOET), Ecuador   | DQ987276             | DQ238578             | EF011/59      |
| Drepanolejeunea vesiculosa (Mitt.) Steph.  | Gradstein et al. 10371 (GOET), Malaysia  | DQ987317             | DQ987421             | EF011797      |
| Lejeunea bidentula Herzog  | Yu 20100924-3 (HSNU), China  | KC136346*            | KC136350*            | KC136347*     |
| Lejeunea cancellata Nees & Mont.   | Wilson et al. 04-02 (GOET), Ecuador  | DQ987329             | DQ987433             | EF011810      |
| Lejeunea catinulifera Spruce (accession 1)   | Wilson et al. 04-01 (GOET), Ecuador  | DQ987328             | DQ987432             | EF011809      |
| Leieunea catinulifera Spruce (accession 2)   | Gradstein & Mandl 10141 (GOET), Ecuador  | DO987307             | DO987411             | EF011787      |
| Leieunea cavifolia (Ehrh.) Lindh   | Heinrichs 3695 (GOFT) Germany  | DO987259             | DO238581             | _             |
| Lejeunea cerina (Lehm. & Lindenb.)<br>Gottsche, Lindenb & Nees                               | Wilson et al. 04-13 (GOET), Ecuador  | DQ987339             | DQ987441             | EF011820      |
| Lejeunea eckloniana Lindenb.   | Schäfer-Verwimp 24788 (GOET), Canary<br>Islands                                | DQ987357             | DQ987457             | -             |
| Leieunea exilis (Reinw, et al.) Grolle   | Gradstein 10336 (GOET), Malaysia   | _                    | DO987472             | EF011863      |
| Leieunea flava (Sw.) Nees (accession 1)  | Gradstein s.n. (GOET), Brazil  | DO987309             | DO987413             | EF011789      |
| Lejeunea flava (Sw.) Nees (accession 2)  | Schäfer-Verwimp 24780 (GOET), Canary<br>Islands                                | DQ987363             | -                    | EF011846      |
| Leieunea laetevirens Nees & Mont   | Schäfer-Verwimp 17899 (GOET) Dominica  | DO987296             | DO987402             | EF011776      |
| Lejeunea lamacerina (Steph.) Schiffn.  | Schäfer-Verwimp 24616 (GOET), Canary<br>Islands                                | DQ987358             | -                    | EF011842      |
| Leieunea mimula Hürl   | Schäfer-Verwimp 20930 (GOET) Bali  | DO987261             | _                    | EF011747      |
| Lejeunea pallescens Mitt   | Schäfer-Verwimp 23533 (GOET) Ecuador   | DO987292             | _                    | EF011772      |
| Lejeunea paucidentata (Steph) Grolle   | Schäfer-Verwimp 17737 (GOET) Dominica  | DO987345             | DO987447             | EF011826      |
| Lepidolejeunea eluta (Nees) R. M. Schust.  | Churchill & Vasquez 21800 (GOET),<br>Bolivia                                   | DQ987266             | DQ238579             | EF011752      |
| Leptolejeunea elliptica (Lehm. & Lindenb.)<br>Schiffn.                                       | Wilson et al. 04-18 (GOET), Ecuador  | DQ987375             | -                    | EF011862      |
| Lopholejeunea subfusca (Nees) Schiffn.   | Gradstein et al., 10368 (GOET), Malaysia                                       | DQ987326             | DQ987430             | EF011807      |
| Luteolejeunea herzogii (Buchloh) Piippo  | Schäfer-Verwimp & Holz 0294/B (GOET),<br>Costa Rica                            | DQ987368             | DQ987467             | EF011854      |
| Oryzolejeunea saccatiloba (Steph.) Gradst.<br>(accession 1)                                  | M.Pérez 2614 (USF), Dominican Republic   | KC136345*            | KC136351*            | -             |
| Oryzolejeunea saccatiloba (Steph.) Gradst.<br>(accession 2)                                  | Schäfer-Verwimp26633 (herb. Schäfer-<br>Verwimp), Dominican Republic           | KC136343*            | -                    | KC136348*     |
| Oryzolejeunea saccatiloba (Steph.) Gradst.<br>(accession 3)                                  | Schäfer-Verwimp 26641 (herb. Schäfer-<br>Verwimp), Dominican Republic          | KC136344*            | -                    | KC136349*     |
| Pycnolejeunea densistipula (Lehm. & Lindenb.) Steph.   | Schafer-Verwimp 23368 (GOET), Ecuador  | DQ987294             | DQ987400             | EF011774      |
| Thysananthus comosus Lindenb. ex Lehm.   | Sukkharak 730 (BKF, GOET), Malaysia  | JN184607             | JN184563             | JN184476      |