

***Lepidolejeunea grandiocellata* sp. nov.
(Lejeuneaceae, Porellales), a new leafy liverwort
from the West Indies based on morphological
and molecular evidence**

Alfons SCHÄFER-VERWIMPA*, Julia BECHTELER^b, Huub VAN MELICK^c,
Matthew A. M. RENNER^d & Jochen HEINRICH^b

^aMittlere Letten 11, 88634 Herdwangen-Schönach, Germany

^bSystematic Botany and Mycology, GeoBio-Center, Ludwig Maximilian University,
Menzinger Str. 67, 80638 Munich, Germany

^cMerellaan 13, 5552 BZ Valkenswaard, The Netherlands

^dRoyal Botanic Gardens and Domain Trust, Mrs Macquaries Road, Sydney,
NSW 2000, Australia

Abstract – *Lepidolejeunea grandiocellata*, sp. nov. is described for two specimens from the Blue Mountains of Jamaica. This delicate species is characterized by ocelli in basal parts of leaves, underleaves, bracteoles and bracts often being conspicuously larger than the surrounding cells, whereas ocelli in central and upper parts of these structures are more or less the same size as surrounding cells. Further defining characters include sharply 5-keeled perianths without inflated horns, and orbicular underleaves with a sharp, v-shaped notch. In a molecular phylogeny based on the nrITS region and the cp DNA markers *rbcL* and *trnL-F*, *L. grandiocellata* is placed sister to a clade including accessions of *L. delessertii* from Réunion and accessions of *L. cuspidata*, *L. eluta*, and *L. sullivanii* from the Neotropics [*L.* subg. *Perilejeunea*]. Based on the phylogeny and the characters of the ocelli, *L. grandiocellata* is placed in a new subgenus *Caribeolejeunea*.

Bryophyte / classification / integrative taxonomy / Jamaica / Jungermanniopsida / ocelli

INTRODUCTION

Recent taxonomic research on liverworts provided evidence that documentation of extant species is still incomplete. On one hand, integrative approaches have identified numerous morphologically cryptic or near cryptic species that were not accepted based solely on morphology (Bakalin & Vilnet, 2014; Buczkowska *et al.*, 2015; Renner *et al.*, 2017). On the other hand, morphologically well-circumscribed species are still being discovered through revision of herbarium material or fieldwork (Potemkin *et al.*, 2015; Bastos *et al.*, 2016; Szabó & Pócs, 2016).

* Corresponding author: moos.alfons@kabelbw.de

In 2013, ASV and HvM collected bryophytes in Jamaica and have so far identified some 200 liverwort species (Schäfer-Verwimp & van Melick, 2016). However, among their collections were several species that could not yet be identified or were new to science, including distinctive plants belonging to the pantropical genus *Lepidolejeunea* R.M.Schust. *Lepidolejeunea* is well characterized by gynoeical innovations with a pycnolejeuneoid leaf arrangement, pyriform perianths with 5 smooth to crenulate keels, presence of scattered ocelli in leaves, underleaves, bracts, bracteoles, and perianths, lack of well-developed oil bodies in cells other than ocelli, and a marginal hyaline papilla at the proximal base of the first tooth of the leaf lobule. *Lepidolejeunea* was monographed by Piippo (1986) who accepted 12 species based on morphological evidence. A recent integrative study (Heinrichs *et al.*, 2015) led to the recognition of 14 species and corroborated the importance of ocelli characters for the classification of *Lepidolejeunea* species. Ocelli – modified cells containing only a single large oil body – are of prime importance for the classification of Lejeuneaceae yet are difficult to study in older herbarium specimens because the oil bodies usually have disappeared (He & Piippo, 1999; Dong *et al.*, 2013). Fortunately, the *Lepidolejeunea* specimens were investigated only a few weeks after collecting, and the ocelli were still in nearly perfect condition.

Here we describe this plant as *Lepidolejeunea grandiocellata* sp. nov. based on morphological evidence and the outcome of a molecular phylogenetic study.

MATERIALS AND METHODS

Herbarium material of *Lepidolejeunea grandiocellata* sp. nov. was investigated using an Olympus CH2 microscope and its morphology was documented using a drawing tube (Figs 1-17). Morphological distinctness was confirmed by comparing *L. grandiocellata* to *Lepidolejeunea* specimens from the herbarium Schäfer-Verwimp and using the *Lepidolejeunea* treatments of Piippo (1986) and Heinrichs *et al.* (2015).

Gametophytical tissue was isolated from the holotype of *Lepidolejeunea grandiocellata*. Total genomic DNA was extracted, and PCR and sequencing reactions were performed as described in Bechteler *et al.* (2016a). The chloroplast *rbcL* gene and *trnL-trnF* region, as well as the nuclear ribosomal ITS region (ITS1-5.8S-ITS2) were amplified, and 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; Altschul *et al.*, 1990). In a second step the sequences were aligned with published sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Representatives of the genera *Metalejeunea* Grolle, *Otolejeunea* Grolle & Tixier, *Rectolejeunea* A.Evans and *Vitalianthus* R.M.Schust. & Giancotti were chosen as outgroup based on the phylogenetic hypotheses of Bechteler *et al.* (2016a, b) (Appendix 1). Sequences were aligned manually in PhyDE v.0.9971 (<http://www.phyde.de/index.html>) and ambiguous positions were excluded for phylogenetic analyses.

Phylogenetic analyses

The best fit models of evolution determined under the Akaike Information Criterion (AIC; Akaike, 1973) in jModelTest2 (Darriba *et al.*, 2012) were TIM3+I+G

for *rbcL*, TPM1uf+I for *trnL-trnF*, and TrN+I+G for ITS. RAxML 8.2.4 (Stamatakis, 2014) was used for maximum likelihood (ML) inference. Clades with bootstrap percentage values (BP) of 70-94 % were regarded as moderately supported and those with BP \geq 95 % as strongly supported (Erixon *et al.*, 2003). Since no incongruence among the single marker datasets was evident, they were concatenated. For the combined analysis, the dataset was partitioned by marker and a GTR+G model was used, following the recommendation given by Stamatakis (2016). Multi-parametric bootstrapping using the autoMRE function (Pattengale *et al.*, 2010) and ten thorough ML searches were executed.

MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003) was used for Bayesian inference (BI) of the partitioned dataset. The GTR+I+G model as suggested by jModelTest and two Metropolis-coupled Markov Chain Monte Carlo (MCMC) analyses, including three heated chains and one cold chain running for 10 million generations, sampling every 1,000 generations were employed. An average standard deviation of split frequencies below 0.01 indicated a sufficiently long run and TRACER 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) was used to check for convergence and stationarity. Burn-in was set to the first 25 % of sampled trees and the remainder was summarized with TreeAnnotator 1.8.2 (Drummond *et al.*, 2012). FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to visualize the resulting maximum clade credibility (MCC) tree. Bayesian Posterior Probability (PP) values \geq 0.95 were regarded as significant (Larget & Simon, 1999).

RESULTS

Taxonomy

Lepidolejeunea subg. *Caribeolejeunea* Schäf.-Verw., Bechteler, van Melick, M.A.M.Renner & Heinrichs **subg. nov.**

Type – *Lepidolejeunea grandiocellata* Schäf.-Verw., Bechteler, van Melick, M.A.M.Renner & Heinrichs

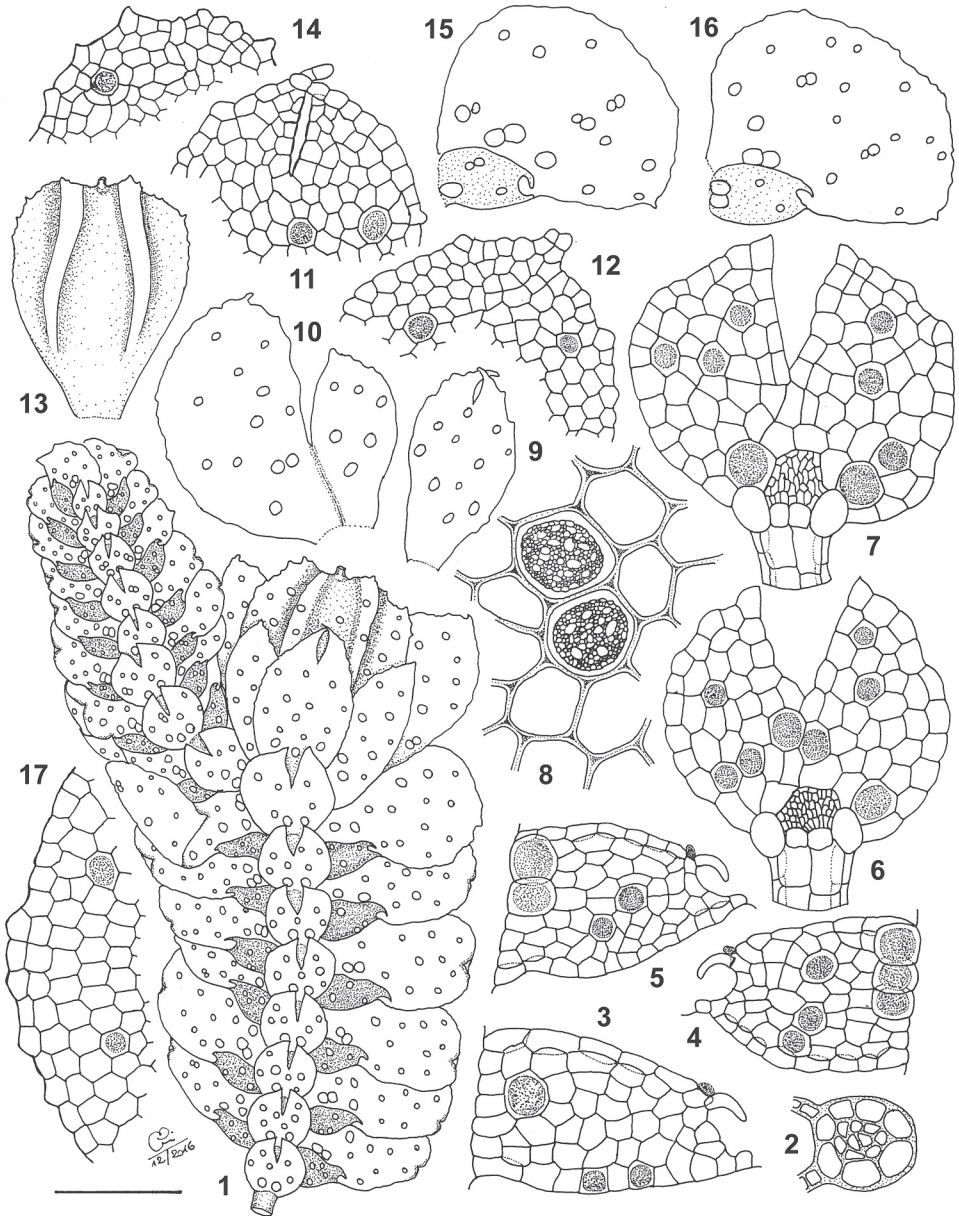
This subgenus includes *Lepidolejeunea* species with ventral merophytes two cells wide, a short underleaf insertion comprising two inferior cells and two enlarged basal cells, and ocelli in basal parts of leaves, underleaves, bracteoles and bracts being often conspicuously larger than the surrounding cells whereas the ocelli in central and upper parts of these structures are more or less the same size as surrounding cells.

Lepidolejeunea grandiocellata Schäf.-Verw., Bechteler, van Melick, M.A.M.Renner & Heinrichs, **sp. nov.**

Figs 1-17

Type – JAMAICA: St. Andrew, Blue Mountains, military road from Newcastle to Catherines Peak, secondary forest near summit, on bark of young hardwood tree, 1525 m, WGS84: 18°04,7' N, 76°42,2' W, 4. Dez. 2013, leg. A. Schäfer-Verwimp 35071 & H. van Melick (Holotype: JE, isotypes: M, FR). Paratype collection from the same site, leg. A. Schäfer-Verwimp 35058/A (JE).

Plants delicate, shoots up to 8 mm long and 0.9-1.1 mm wide, three year old herbarium material light green, greyish green or greyish, sparsely to irregularly pinnately branched, branches 1-1.5(-2) mm long, slightly to strongly adhering to substrate. **Stem** (60-)65-75(-80) μ m in diameter, in cross section with 7 cortical cell



Figs 1-17. *Lepidolejeunea grandiocellata*. **1.** Upper portion of shoot with perianth, ventral view. **2.** Cross section of stem. **3-5.** Leaf lobules. **6-7.** Underleaves. **8.** Leaf cells above leaf base with two ocelli. **9.** Female bracteole. **10.** Female bract. **11.** Upper part of female bracteole. **12.** Upper part of female bract lobe. **13.** Perianth. **14.** Upper part of perianth keel. **15-16.** Two leaf lobes with lobules. **17.** Part of dorsal leaf lobe (all from holotype). Scales: 400 μm for 1, 9-10, 13, 17; 100 μm for 2-7, 11-12, 14, 17; 60 μm for 8; 270 μm for 15-16.

rows and 9-12 medullary cells, cortical cells up to $22 \times 30 \mu\text{m}$, medullary cells up to $7 \times 14 \mu\text{m}$; stem cells on ventral side of stem in top view subquadrate to rectangular to irregularly polygonal, rarely wider than long, $32\text{-}36 \times 40\text{-}65 \mu\text{m}$. **Leaves** imbricate, spreading widely to slightly obliquely upward, insertion nearly straight, the lobe (480-)490-520(-550) μm long, 400-420(-440) μm wide, asymmetrically broadly ovate (Figs 15-16), slightly apiculate, slightly incurved at apex, dorsal margin strongly arched, ventral margin nearly straight with keel of lobule slightly convex and junction of lobule and free margin of lobe concave, margin irregularly crenulate-denticulate (Fig. 17). Leaf lobe cells (sub-)quadrate to irregularly polygonal in outline, irregular in size, walls thin, trigones small (Fig. 8), intermediate thickenings absent, cuticle smooth. Apical marginal leaf cells $16\text{-}20 \times 18\text{-}24 \mu\text{m}$, central cells from $20 \times 24 \mu\text{m}$ up to $26 \times 30 \mu\text{m}$, basal cells from $20 \times 24 \mu\text{m}$ and $16 \times 30 \mu\text{m}$ up to $40 \times 45 \mu\text{m}$; oil bodies seen only in ocelli. **Ocelli** present in all examined parts of gametophyte except stem, ca. 15-25(-30) per leaf lobe, scattered, in distal part of leaf lobe more or less of the same size as surrounding cells, in proximal half of leaf lobe often conspicuously larger than surrounding cells, the largest ones up to $40 \times 45 \mu\text{m}$, consisting of numerous small oil droplets of different size and filling almost the whole cell lumen, chloroplasts absent. **Leaf lobules** ovate, reaching 0.33-0.4 of length of lobes, basal portion covered by underleaves (Fig. 1), inflated, oriented at an angle of $70^\circ\text{-}90^\circ$ to stem, attached to stem by 3-(4)-5 elongated cells; lobule cells \pm isodiametric to subrectangular to polygonal, similar in size and shape as in leaf lobes; 3-6(-7) scattered ocelli present per lobule, those along and near lobule insertion line conspicuously larger (up to $40 \times 40 \mu\text{m}$) than surrounding cells, others \pm of the same size as surrounding cells (Figs 3-5); free lobule margin entire, 6-8 cells long, slightly incurved, apex semicircular, consisting of (4)-5-6 cells (incl. apical tooth); keel slightly to moderately arched, apical tooth falcate, $10\text{-}12 \times (26\text{-})30\text{-}35 \mu\text{m}$, hyaline papilla conspicuously smaller than apical tooth, proximal between first lobule tooth and first cell of free margin, ca. $7 \times 15 \mu\text{m}$; stylus not seen.

Ventral merophytes two cells wide; **underleaves** (Figs 1, 6-7) contiguous to imbricate, $3\text{-}4.5 \times$ stem width, rounded, often slightly wider than long, (200-)220-280 μm wide, 200-250(-280) μm long (subgynoecial underleaves somewhat larger), bases cuneate, lobed to 0.5 of their length, lobes acute, sinus v-shaped, margins entire or slightly and irregularly crenulate-denticulate, line of insertion straight, attached to stem by two inferior central cells and two enlarged elliptical cells (Figs 6-7), underleaf cells mostly isodiametric to short rectangular, variable in size, from $15 \times 16 \mu\text{m}$ up to $38 \times 40 \mu\text{m}$ (incl. ocelli); ocelli scattered, 7-8(-10), those near underleaf base usually conspicuously larger than surrounding cells, others more or less of the same size as surrounding cells. Rhizoid initial disc small, rhizoids not seen.

Dioicous. Androecia usually intercalary on short or long branches, bracts in 3-7 pairs, imbricate, saccate, mostly smaller than ordinary leaves, lobules slightly to considerably smaller than lobes. Bracteoles present throughout the androecium, similar to underleaves but sometimes less deeply incised. Scattered ocelli present in bracts and bracteoles. **Gynoecia** on main shoots with one or two *Pycnolejeunea* type innovations; bracts longer than vegetative leaves, their lobes 720-750 μm long, 420-450 μm wide, asymmetrically ovate-oblong, apiculate, margin irregularly crenulate-denticulate; lobules extending 0.7-0.75 of length of lobes, subrectangular to \pm rhombic; apex apiculate, margin irregularly crenulate-denticulate, keel about 1/3 of length of bract lobe, occasionally narrowly winged. Bracteole connate with bracts on both sides, ovate-oblong, narrowed towards base, ca. 600 μm long and 315 μm wide, apex incised, sinus sharp and narrow, lobe acute-acuminate, ending in 1-2(-3)

superposed cells, margin slightly and irregularly crenulate-denticulate. Bracts and bracteoles with 10-20 scattered ocelli, those near base often conspicuously larger than surrounding cells, others \pm of same size as surrounding cells; cells and ocelli of bracts and bracteoles similar to those of leaf lobes. **Perianth** \pm as long as bracts, 675-750 μm long, 520-540 μm wide, pyriform, not inflated distally, sharply 5-keeled, upper portions of keels irregularly denticulate; beak small, often inconspicuous, 1(-2) cells high; perianth cells resembling those of bracts, bracteoles and leaves, scattered ocelli present. **Mature sporophyte** not observed (only a single immature one observed inside of a perianth). **Vegetative reproduction** not observed.

Molecular Phylogeny

The ML phylogram (Fig. 18) largely resembles the MCC tree of the Bayesian analysis. All species with multiple accessions were monophyletic, with strong BP and significant PP. An Asian clade (BP = 100, PP = 1.0) with specimens of *L. integristipula* (J.B.Jack. & Steph.) R.M.Schust. and *L. bidentula* (J.B.Jack & Steph.) R.M.Schust. [*L.* subg. *Lepidolejeunea*] is placed sister to a clade (BP = 94, PP = 1.0) containing a Neotropical lineage (BP = 91, PP = 1.0) with accessions of *L. cordifissa*, *L. auriculata* Schäf.-Verw. & Heinrichs, *L. grossepapulosa* (Steph.) Piippo and *L. involuta* (Gottsche) Grolle [*L.* subg. *Kingiolejeunea* (H.Rob.) R.M.Schust.], and a lineage (BP = 100, PP = 1.0) with the Jamaican *L.* (subg. *Caribeolejeunea*) *grandiocellata* sp. nov. in a sister relationship to an Afromadagascan-Neotropical clade (BP = 94, PP = 1.0) with accessions of *L. delessertii* (Nees & Mont.) Grolle, *L. sullivanii* (Gottsche) M.E.Reiner, *L. eluta* (Nees) R.M.Schust. and *L. cuspidata* (Gottsche) Heinrichs & Schäf.-Verw. [*L.* subg. *Perilejeunea* (Kachroo & R.M.Schust.) Schust.]

DISCUSSION

Distinction of *Lepidolejeunea* (subg. *Caribeolejeunea*) *grandiocellata*

Lepidolejeunea grandiocellata was initially recognized by its unusual morphology, particularly the ocelli larger than the surrounding leaf cells in the proximal half of the leaf and lobule. *Lepidolejeunea grandiocellata* was also well separated from the other *Lepidolejeunea* species included in the molecular phylogeny (Fig. 18), indicating congruence of morphology-based taxonomy and molecular data. Based on morphology, *L. grandiocellata* can be recognized by the following combination of characters: (1) delicate plants being less than 1 cm long and ca. 1 mm wide, (2) underleaves orbicular, being 3-4.5 \times the stem width and having a sharp, v-shaped notch of $\frac{1}{2}$ underleaf length, (3) perianths sharply 5-keeled and without inflated horns, and (4) ocelli of variable size of which those in basal parts of leaves, underleaves, bracteoles and bracts are conspicuously larger than surrounding cells and thus recognisable in older herbarium material. The latter character separates *L. grandiocellata* from nearly all other *Lepidolejeunea* taxa. Similar ocelli have been reported for only a single *Lepidolejeunea* taxon, the Australasian *L.* (subg. *Lepidolejeunea*) *novae-caledoniae* (Piippo) R.L.Zhu & Frank Müll. (Piippo, 1986, as *L. bidentula* var. *novae-caledoniae*; Shu *et al.*, 2016) but its ocelli are almost all larger than the surrounding cells. Further differences include the deviant underleaf insertion and the more robust stature of the gametophytes.

surrounding leaf cells or partly as large as the surrounding cells. The very large basal leaf ocelli of *L. grandiozellata* and the considerable variation in ocellus size do not correspond to this circumscription. Accordingly, we place *L. grandiozellata* in a new subgenus *Caribeolejeunea*.

Distribution and Ecology

Currently, *Lepidolejeunea grandiozellata* is known only from the type locality in the Blue Mountains of Jamaica. The plants were growing on bark of two young hardwood trees in an open secondary forest just below the summit of Catherines Peak, at 1525 m. Söderström *et al.* (2011) consider knowledge of the Caribbean, and especially the Jamaican, liverwort flora too poor to reliably comment about range-restricted or endemic species. Local endemics are rare in *Lepidolejeunea* (Piippo, 1986), however, plants with the morphology of *L. grandiozellata* have neither been observed by Piippo (1986) nor Heinrichs *et al.* (2015), and were also not present in other collections made by SV in Jamaica.

The plants of the *Lepidolejeunea grandiozellata*-paratype grew intermingled with *Rectolejeunea emarginuliflora* (Gottsche ex Schiffn.) A.Evans. Further liverworts at the type locality include *Cheilolejeunea unciloba* (Lindenb.) Malombe, *Cheilolejeunea adnata* (Kunze) Grolle, *Cryptolophocolea martiana* (Nees) L.Söderstr., Crand.-Stotl. & Stotler, *Calypogeia peruviana* Nees & Mont., *Telaranea nematodes* (Gottsche ex Aust.) M.A.Howe, *Radula pallens* (Sw.) Nees & Mont., *Riccardia fucoidea* (Sw.) Schiffn., *Bazzania gracilis* (Hampe & Gottsche) Steph., *Trichocolea elliotii* Steph., *Mnioloma cyclostipum* (Spruce) R.M.Schust., and *Odontoschisma longiflorum* (Taylor) Trevis. Mosses at the type locality were *Leskeodon auratus* (Müll. Hal.) Broth., *Rhynchostegiopsis flexuosa* (Sull.) Müll. Hal. and *Isodrepanium lentulum* (Wils.) Britt.

Perspectives

Lepidolejeunea grandiozellata adds to growing evidence that the species inventory of Lejeuneaceae is still incomplete (e.g., Heinrichs *et al.*, 2015; Reiner-Drehwald, 2015; Shi & Zhu, 2015; Bastos *et al.*, 2016; Dey & Sing, 2016), a perspective facilitated by the considerable recent efforts to record the global diversity of liverworts (Söderström *et al.*, 2016). Only extended fieldwork, revision of available herbarium material, and completion of the taxonomic sampling in molecular phylogenies will allow for deeper insights into the diversity and evolution of bryophytes.

Acknowledgements. Financial support by the German Research Foundation (grant HE 3584/6 to JH) is gratefully acknowledged.

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.
- 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*, Budapest, Akadémiai Kiadó, pp. 267-281.

- BAKALIN V. & VILNET A.A., 2014 — Two new species of the liverwort genus *Hygrobriella* Spruce (Marchantiophyta) described from the North Pacific based on integrative taxonomy. *Plant systematics and evolution* 300: 2277-2291.
- BASTOS C.J.P., SIERRA A.M. & ZARTMANN C.E., 2016 — Three new species of *Cheilolejeunea* (Spruce) Steph. (Marchantiophyta, Lejeuneaceae) from Northern Brazil. *Phytotaxa* 277: 36-46.
- BECHTELER J., LEE G.E., SCHÄFER-VERWIMP A., PÓCS T., PERALTA D.F., RENNER M.A.M., SCHNEIDER H. & HEINRICHS J., 2016a — 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., 2016b — Towards a monophyletic classification of Lejeuneaceae V: the systematic position of *Pictolejeunea*. *Phytotaxa* 280: 259-270.
- BUCKOWSKA K., HORNİK B. & CZOLPIŃSKA M., 2015 — Two ploidy levels of genetically delimited groups of the *Calypogeia fissa* complex (Jungermanniopsida, Calypogeiaceae). *Biodiversity research and conservation* 39: 1-6.
- DARRIBA D., TABOADA G.L., DOALLO R. & POSADA D., 2012 — JModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772.
- DEY M. & SINGH D.K., 2016 — A new species and a new record of *Cololejeunea* (Lejeuneaceae, Marchantiophyta) from Andaman & Nicobar Islands, India. *Cryptogamie Bryologie* 37: 149-156.
- 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.
- 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., SVENBLAD B., BRITTON T. & OXELMAN B., 2003 — Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic biology* 52: 665-673.
- HE X.-L. & PIIPPO S., 1999 — On the taxonomic significance and classification of ocelli characters in the hepatic family Lejeuneaceae. *Bryobrothera* 5: 93-97.
- 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.
- 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.
- SHU L., ZHANG L.-N., PROMMA C., MÜLLER F. & ZHU R.-L., 2016 — *Lepidolejeunea novae-caledoniae* (Piippo) R.L.Zhu & Frank Müll. (Marchantiophyta, Lejeuneaceae), *stat. nov.* from New Caledonia. *Phytotaxa* 253: 232-234.
- 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.
- PIIPPO S., 1986 — A monograph of the genera *Lepidolejeunea* and *Luteolejeunea* (Lejeuneaceae: Hepaticae). *Acta botanica Fennica* 132: 1-69.
- POTEMKIN A.D., HENTSCHEL J., SOFRONOVA E.V. & MAMONTOV Y.S., 2015 — *Frullania dorsimamillosa*, a unique new species from Central China and the resurrection of *Frullania chinlingensis* (Frullaniaceae, Marchantiophyta). *Phytotaxa* 227: 1-12.
- REINER-DREHWALD M.E., 2015 — *Lejeunea tunquiniensis* (Lejeuneaceae, Marchantiophyta), a new species from a humid montane forest in the Yungas region, Bolivia. *Nova Hedwigia* 100: 583-588.
- RENNER M.A.M., HESLEWOOD M., PATZAK S.D.F., SCHÄFER-VERWIMP A. & HEINRICHS J., 2017 — By how much do we underestimate species diversity of liverworts using morphological evidence? An example from Australasian *Plagiochila* (Plagiochilaceae: Jungermanniopsida). *Molecular phylogenetics and evolution* 107: 576-593.
- RONQUIST F. & HUELSENBECK J.P., 2003 — MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- SCHÄFER-VERWIMP A. & VAN MELICK H.M.H., 2016 — A contribution to the bryophyte flora of Jamaica. *Cryptogamie, Bryologie* 37: 305-348.
- SHI X.Q. & ZHU R.L., 2015 — A revision of *Archilejeunea* s.str. (Lejeuneaceae, Marchantiophyta). *Nova Hedwigia* 100: 589-601.

- SÖDERSTRÖM L., HAGBORG A., VÁÑA J. & VON KONRAT M., 2011 — Land of wood and water: A checklist of liverworts and hornworts of Jamaica. *The bryologist* 114: 67-91.
- 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., DAUPHING, ENGEL J.J., FELDBERG K., GLENNY D., GRADSTEIN S.R., HE X., HEINRICHS J., HENTSCHERL J., ILKIU-BORGES A.L., KATAGIRI T., KONSTANTINOVA N.A., LARRAÍN 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.
- STAMATAKIS A., 2014 — RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.
- 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)
- SZABÓ A. & PÓCS T., 2016 — New or little known epiphyllous liverworts, XX *Cololejeunea nosykombae* A.Szabó & Pócs sp. nov. from Madagascar. *Journal of bryology* 38: 302-307.

APPENDIX 1

Taxa, voucher information, and GenBank accession numbers for specimens of *Lepidolejeunea* and outgroup species used in this study, ‘xx’ indicates missing sequence.

Species, voucher, *rbcl*, *trnL-trnF*, nrITS

OUTGROUP: *Metalejeunea crassitexta* (J.B.Jack & Steph.) Pócs, Fiji, *Pócs & Pócs 03303/R* (EGR), KT626918, KT626934, KT626899; *M. cucullata* (Reinw., Blume & Nees) Grolle, Fiji, *Pócs & Pócs 03305/Q* (EGR), KT626913, KT626929, KT626894; *Otolejeunea moniliata* Grolle, Madagascar (I), *9448/L* (EGR), KT626923, KT626937, KT626904; *O. moniliata*, Madagascar (II), *Pócs et al. 90113/EA* (EGR), KT626922, KT626936, KT626903; *Rectolejeunea flagelliformis* A.Evans, Panama, *Schäfer-Verwimp & Verwimp 34286* (JE), KT626924, KT626938, KT626905; *R. versifolia* (Schiffn.) L.Söderstr. & A.Hagborg, Guadeloupe, *Schäfer-Verwimp & Verwimp 22245/A* (GOET), DQ983724, DQ987444, DQ987342; *Vitalianthus aphanellus* (Spruce) Bechteler *et al.*, Brazil, *Yano & Zartman 32771* (SP), KT626920, xx, KT626900; *V. bischlerianus* (K.C. Pôrto & Grolle) R.M.Schust. & Giancotti, Brazil, *Schäfer-Verwimp & Verwimp 9505* (M), KT626921, xx, KT626901; – INGROUP: *Lepidolejeunea auriculata* Schäf.-Verw. & Heinrichs, Colombia, *Wolf 1595* (GOET), KP635310, xx, KP635281; *L. auriculata* Ecuador, *Schäfer-Verwimp & Preussing 23265* (JE), KP635309, KP635335, KP635280; *L. bidentula* (J.B.Jack & Steph.) R.M.Schust., China, *Koponen et al. 51525* (H) AY125936, AY144476, AY125340; *L. bidentula*, Thailand, *Schäfer-Verwimp & Verwimp 16266* (JE), xx, KP635355, KP635303; *L. cordifissa* (Taylor) M.E.Reiner, Costa Rica, *Schäfer-Verwimp & Holz SV/H-0284/A* (JE), KP635306, KP635332, KP635277; *L. cordifissa*, Ecuador (I), *Schäfer-Verwimp & Nebel 31716/B* (JE), KP635305, KP635331, KP635276; *L. cordifissa*, Ecuador (II), *Schäfer-Verwimp & Nebel 33219* (M), KP635307, KP635333, KP635278; *L. cuspidata* (Gottsche)

Heinrichs & Schäf.-Verw., Brazil (I), *Schäfer-Verwimp & Verwimp 12659/B* (JE), KP635318, KP635343, KP635289; *L. cuspidata*, Brazil (II), *Schäfer-Verwimp & Verwimp 14363* (M), KP635319, KP635344, KP635290; *L. cuspidata*, Costa Rica, *Dauphin & Morales* s.n. (GOET), xx, KP635347, KP635294; *L. cuspidata*, Dominica, *Schäfer-Verwimp & Verwimp 17905/B* (JE), KP635324, KP635349, KP635296; *L. cuspidata*, Ecuador (I), *Schäfer-Verwimp & Nebel 32123/C* (JE), KP635320, KP635345, KP635291; *L. cuspidata*, Ecuador (II), *Gradstein & al. 10054* (GOET), KP635322, xx, KP635293; *L. cuspidata*, Guadeloupe, *Schäfer-Verwimp & Verwimp 22193* (M), KP635323, KP635348, KP635295; *L. cuspidata*, Panama, *Schäfer-Verwimp & Verwimp 30835* (JE), KP635321, KP635346, KP635292; *L. delessertii* (Nees & Mont.) Grolle, Réunion (I), *Schäfer-Verwimp & Verwimp 19985/A* (JE), KP635316, KP635341, KP635287; *L. delessertii*, Réunion (II), *Schäfer-Verwimp & Verwimp 20276/A* (JE), KP635317, KP635342, KP635288; *L. delessertii*, Réunion (III), *Gyarmati et al. 9651/CB* (JE), KP635315, KP635340, KP635286; *L. delessertii*, Réunion (IV), *Schäfer-Verwimp & Verwimp 20355/B* (M), KF039819, KF039853, KF039781; *L. eluta* (Nees) R.M.Schust., Bolivia (I), *Gradstein 9937* (GOET), KP635329, KP635354, KP635302; *L. eluta*, Bolivia (II), *Acebey & Krömer 855* (GOET), KP635328, KP635353, KP635301; *L. eluta*, Bolivia (III), *Drehwald 4833* (GOET), DQ983696, DQ987379, DQ987257; *L. eluta*, Bolivia (IV), *Churchill & Vásquez 21800* (GOET), AY548066, DQ238579, DQ987266; *L. eluta*, Ecuador, *Schäfer-Verwimp & Nebel 33212/B* (JE), KP635327, xx, KP635300; *L. grandiocellata*, Jamaica, *Schäfer-Verwimp 35071* (JE), KY550264, KY550265, KY550263; *L. grossepapulosa* (Steph.) Piippo, Colombia, *Gradstein & Varon 11065* (GOET), KP635308, KP635334, KP635279; *L. integristipula* (J.B.Jack. & Steph.) R.M.Schust., Fiji Isls., *Pócs & Pócs 03307/AC* (GOET), DQ983697, DQ987417, DQ987313; *L. involuta* (Gottsche) Grolle, Colombia, *Gradstein 8626* (GOET), xx, KP635351, KP635298; *L. involuta*, Costa Rica, *Gradstein & Dauphin 9347* (GOET), KP635311, KP635336, KP635282; *L. involuta*, Dominica, *Schäfer-Verwimp & Verwimp 17855* (JE), KP635314, KP635339, KP635285; *L. involuta*, Guadeloupe (I), *Schäfer-Verwimp 22178* (M), KP635312, KP635337, KP635283; *L. involuta*, Guadeloupe (II), *Schäfer-Verwimp & Verwimp 22242* (JE), KP635326, KP635352, KP635299; *L. involuta*, Guadeloupe (III), *Schäfer-Verwimp & Verwimp 22309* (M), KP635313, KP635338, KP635284; *L. sullivanii* (Gottsche) M.E.Reiner, Ecuador, *Schäfer-Verwimp & Preussing 23373* (JE), KP635325, KP635350, KP635297.