

Yanoella (Marchantiophyta: Lejeuneaceae), a new genus from the Brazilian Atlantic Forest

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ABSTRACT. The Brazilian Atlantic Forest is one of 25 biodiversity hotspots with a rich liverwort diversity, especially of Lejeuneaceae. *Rectolejeunea truncatilobula* C.J.Bastos endemic to Brazil's Atlantic Forest was one member of the subtribe Lepidolejeuneinae of Lejeuneaceae. Recent a field trip to the area permitted us to study living samples of the plant, and we found that the species is characterized by the granular oil bodies in median and basal chlorophyllose cells of the leaf lobe and presence of *Allorgella*-type marginal denticulations, which are striking characters of the subtribe Echinolejeuneinae. Our phylogenetic analyses based on the nuclear ribosomal ITS region and two chloroplast regions (*rbcL*, *trnL-trnF*) also suggest that this species belongs in the subtribe Echinolejeuneinae rather than the subtribe Lepidolejeuneinae. On the basis of the combined molecular phylogenetic and morphological data, a new genus *Yanoella* is proposed for accommodating the remarkable Brazilian species. Molecular evidence of *Stenolejeunea thallophora* (Eifrig) R.M.Schust. (type species of *Stenolejeunea* R.M.Schust.) supports the earlier reduction of *Stenolejeunea* to synonymy under *Lejeunea* Lib.

KEYWORDS. Liverworts, new combination, ocelli, oil bodies, *Rectolejeunea*, *Stenolejeunea*.



Lejeuneaceae, the largest family of liverworts with over 1800 species in about 70 genera, exhibits richest biodiversity in tropical rain forests where several new genera have continuously been discovered recently (Gradstein et al. 2018; Wang et al. 2016; Ye & Zhu 2018; Zhu et al. 2017; Zhu & Shu 2018). Brazil's Atlantic Forest is one of 25 biodiversity hotspots (Myers et al. 2000), and considered a priority area for biodiversity conservation in Brazil and worldwide (Brasil - MMA 2002; Martini et al. 2007).

Rectolejeunea truncatilobula C.J.Bastos was originally described by Schuster (2000a) as *R. monoica* R.M.Schust., an invalid name owing to the lack of the herbarium cited (Art. 37.7) (Bastos 2012; Passos Bastos & Yano 2003). *Rectolejeunea truncatilobula* is considered an extraordinary species endemic to Brazil's Atlantic Forest (Bastos 2012; Reiner-Dreh-

wald & Grolle 2012; Schuster 2000a). Although the molecular phylogenetic analysis based on one accession of this species revealed that this extraordinary plant was nested within the subtribe Lepidolejeuneinae together with the two typical species of *Rectolejeunea* A.Evans (Heinrichs et al. 2014), such a result seems to be unconvincing because the species bears several interesting characters not occurring in typical *Rectolejeunea*, including the inconspicuous apical lobular tooth, usually 3–4 basal ocelli in an unbroken transverse row, usual presence of *Allorgella*-type marginal denticulations of the leaf lobe, distal and ental hyaline papilla of the leaf lobule, and lack of specialized caducous flagelliform branches. Recent molecular-phylogenetic studies have revealed that oil bodies and ocelli as well as their distribution patterns are indispensable in lejeuneaceous taxonomy (Ye & Zhu 2018; Zhu et al. 2017; Zhu & Shu 2018). Oil bodies, however, have so far not been known from living samples of this

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Table 1. Sequences newly generated in the study, including taxa, vouchers and GenBank accession numbers. “—” missing sequences.

Taxon	Voucher	<i>rbcL</i>	<i>trnL</i> –F	nrITS
<i>Lejeunea thallophora</i> (Eifrig) Gradst.	Malaysia, Zhu et al. 20160916-85 (HSNU)	MH431603	MH431611	MH431614
<i>Rectolejeunea truncatilobula</i> C.J.Bastos I	Brazil, Reis 1611 (ALCB 97007)	MH431609	—	MH431613
<i>Rectolejeunea truncatilobula</i> C.J.Bastos II	Brazil, Reis 1610 (ALCB 97008)	MH431610	MH431617	MH431618
<i>Rectolejeunea truncatilobula</i> C.J.Bastos III	Brazil, Reis 1251 (ALCB 97050)	MH431608	MH431605	MH431607
<i>Rectolejeunea truncatilobula</i> C.J.Bastos IV	Brazil, Zhu & Shu 20171203-27 (HSNU)	MH431615	MH431616	MH431619
<i>Rectolejeunea versifolia</i> (Schiffn.) L.Söderstr. et A.Hagborg III	Brazil, Zhu & Shu 20171203-110 (HSNU)	MH431606	MH431604	MH431612

species (Reiner-Drehwald & Grolle 2012; Schuster 2000a).

In order to reassess the systematic position of *Rectolejeunea truncatilobula* and elucidate phylogenetic relationships, we examined oil bodies and ocelli in living samples, and found that this species has granular oil bodies distinct from typical *Rectolejeunea*. A further molecular phylogenetic analysis based on an extended taxon sampling also revealed that *R. truncatilobula* is nested within the lineage of Echinolejeuneinae Gradst. rather than Lepidolejeuneinae Gradst. Furthermore, the earlier unusual phylogenetic relationship with the typical *Rectolejeunea* (Heinrichs et al. 2014 as *Rectolejeunea monoica* nom. inval.) was proved to be a mistake caused by an erroneous identification of DNA samples. On the basis of the combined molecular phylogenetic and morphological evidence, we propose a new genus, *Yanoella*, to accommodate the Brazilian plant.

MATERIALS AND METHODS

Taxon sampling. We included 117 species of Lejeuneaceae in our study, representing 37 genera stratified across all known 13 subtribes of subfamily Lejeuneoideae. Five extremely rare genera in Lejeuneoideae, *Aphanotropis* Herzog, *Calatholejeunea* K.I.Goebel, *Dactylophorella* R.M.Schust., *Hattoriolejeunea* Mizut. and *Schusterolejeunea* Grolle, could not be included in the present study because no samples for DNA extraction are available. The outgroups consisted of 13 species in 13 genera of subfamily Ptychanthoideae (Lejeuneaceae). In the most recent classification of Lejeuneaceae, *Rectolejeunea* including *R. truncatilobula* was placed in the subtribe Lepidolejeuneinae (Söderström et al. 2016). We accordingly included 21 accessions representing all genera recognized for Lepidolejeuneinae. *Rectolejeunea truncatilobula* is morphologically similar to *Haplolejeunea* Grolle of the subtribe Echinolejeunei-

nae; therefore, we also included all known genera of Echinolejeuneinae. All generic type species in Echinolejeuneinae were involved in our study except for *Allorgella hoana* Tixier (the type species of *Allorgella* Tixier) known only from the type locality in southern Vietnam (Shu et al. 2017; Tixier 1980). *Allorgella semperiana* (Steph.) Bechteler et al. most similar to *A. hoana* thus was selected as a representative of *Allorgella* in our analysis (Table 1). Our sampling represented both morphological and geographic variations in both Echinolejeuneinae and Lepidolejeuneinae. The sequences of *Lejeunea thallophora* (Eifrig) Gradst. (*Stenolejeunea thallophora* (Eifrig) Gradst., type species of *Stenolejeunea* R.M.Schust.) and *Rectolejeunea truncatilobula* were newly generated in the present study, and the remaining sequences were downloaded from GenBank. The sequences of *Rectolejeunea truncatilobula* (Heinrichs et al. 2014 as *R. monoica*) available in GenBank (KJ408380, KJ408331) proved to be erroneous because the voucher specimen of this sampling was assignable to *Rectolejeunea versifolia* (Schiffn.) L.Söderstr. et A.Hagborg (Supplementary Table S1). The taxa, voucher information and GenBank accession numbers are presented in Table 1 and Supplementary Table S1.

Morphological observations. The field pictures were photographed with a digital camera (Sony ILCE-6000) in the Ecological Station of Wenceslau Guimarães, Nova Esperança Village, Wenceslau Guimarães County, Bahia, Brazil. Morphological and anatomical characters were observed and photographed using an Olympus BX43 microscope equipped with a DP71 digital camera. The partial photos were treated using Zerene Stacker (<http://www.zerenesystems.com>). The terminology used for the descriptions in this paper mainly follows Gradstein et al. (2001) and Zhu & So (2001). Habitat data were gathered based on the study of collections of herbarium specimens including our recent collections in December 2017.

DNA extraction and sequencing. Total genomic DNA was extracted from silica-dried samples of shoots using the DNeasy Plant Mini Kits (Qiagen, Hilden, Germany) and eluted in 100 µL AE buffer and kept at -80°C until used. Three short PCR fragments including nrITS, *rbcL*, *trnL*-F were amplified as in previous publications (Zhu et al. 2017). PCR products were purified and sequenced bidirectionally by Jie Li Biology Inc., China (<http://www.geneseq.com>).

Phylogenetic analyses. Sequences were aligned separately for each fragment with MAFFT version 7 (Katoh & Standley 2013) and then manually aligned in PhyDE v.0.9971 (<http://www.phyde.de/index.html>). Phylogenetic trees were inferred using maximum parsimony (MP) as implemented in PAUP* version 4.0b10 (Swofford 2003). Maximum parsimony heuristic searches were conducted with the following options implemented: heuristic searches mode 1000 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. Nonparametric bootstrapping values (BS) were generated as heuristic searches with 500 replicates, each with ten random-addition replicates. The number of rearrangements was restricted to ten million per replicate. Where more than one most parsimonious tree was found, trees were summarized in a strict consensus trees. Congruence among the trees was inferred by inspecting bootstrap scores above 70% in bootstrap consensus trees obtained in independent analyses of the nrITS, *trnL*-F, and *rbcL* data sets. The trees gave no evidence of incongruence, so the datasets were combined.

Maximum-likelihood (ML) analyses were conducted using RAxML-HPG V.8.2.6 (Stamatakis 2014) on the Cipres Science Gateway (Miller et al. 2010, <http://www.phylo.org>). GTR+G+I was used for concatenated dataset. The node support under ML is based on 1000 bootstrap replicates.

Bayesian inference was undertaken with MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003) using a partition for each marker and a GTR substitution model with the rate of invariable sites and gamma rate heterogeneity as recommended by MrModeltest 2.3 (Nylander 2004). Two metropolis-coupled Markov chain Monte Carlo (MCMC) analyses, including three heated chains and one cold

chain, were run for 50 million generations, samples 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 (SD) of split frequency below 0.01 indicated a sufficiently long run. The initial 25% of sampled trees were discarded as burn-in. The resulting maximum clade credibility (MCC) tree was visualized using FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). PP_{BI} values ≥ 0.95 were regarded as good support.

RESULTS

The nrITS dataset included 745 aligned character sites, 230 of which were constant, 515 autapomorphic, and 442 parsimony informative. Out of 934 aligned character sites of the *rbcL* dataset, 626 were constant, 308 autapomorphic, and 225 parsimony informative. The *trnL*-*trnF* dataset contained 567 aligned character sites (274 constant, 293 autapomorphic, and 74 parsimony informative). The maximum parsimony analysis of the concatenated dataset resulted in 196 equally parsimonious trees of 7958 steps, consistency index (CI) 0.254, and retention index (RI) 0.614.

The MP, ML and BI analyses recovered highly concordant phylogenetic hypotheses (Fig. 1). Four accessions of *Rectolejeunea truncatilobula* formed a clade with high support values ($\text{BS}_{\text{MP}}=100$, $\text{BS}_{\text{ML}}=100$, $\text{PP}_{\text{BI}}=1.00$), which was nested in the well-supported clade of the subtribe Echinolejeuneinae ($\text{BS}_{\text{MP}}=100$, $\text{BS}_{\text{ML}}=100$, $\text{PP}_{\text{BI}}=1.00$). *Rectolejeunea truncatilobula* clade was unambiguously retrieved as sister to a clade composed of *Kymato-lejeunea* Grolle, *Echinolejeunea* R.M.Schust. and *Allorgella* Tixier ($\text{BS}_{\text{MP}}=93$, $\text{BS}_{\text{ML}}=95$, $\text{PP}_{\text{BI}}=1.00$). The other representatives of *Rectolejeunea* were placed in the subtribe Lepidolejeuneinae ($\text{BS}_{\text{MP}}=76$, $\text{BS}_{\text{ML}}=97$, $\text{PP}_{\text{BI}}=1.00$) and sister to a highly supported clade consisted of *Otolejeunea* Grolle et Tixier, *Capillolejeunea* S.W.Arnell and *Vitalianthus* R.M.Schust. et Giancotti ($\text{BS}_{\text{MP}}=100$, $\text{BS}_{\text{ML}}=100$, $\text{PP}_{\text{BI}}=1.00$).

An examination of fresh samples of *Rectolejeunea truncatilobula* was made for the first time. Our results showed that oil bodies in median and basal cells of leaf lobes are finely segmented (Fig. 2K-M). Such an oil body character never occurs in *Rectolejeunea* s.str. Oil bodies, however, are lacking or usually replaced by minute oil droplets in

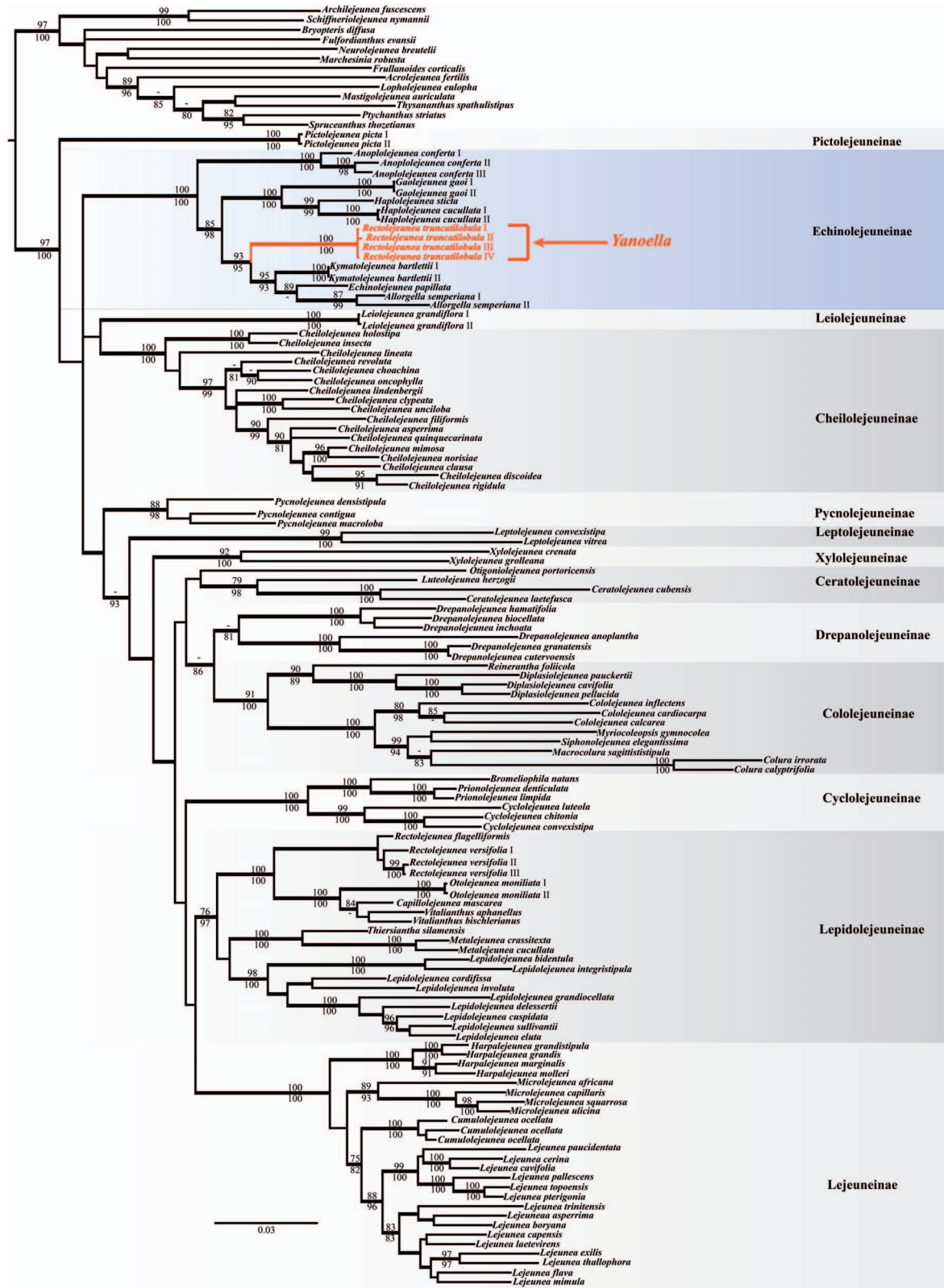


Figure 1. Phylogeny of Lejeuneaceae illustrating position of *Rectolejeunea truncatilobula*, inferred from combined dataset (*rbcl*, *trnL-trnF* and *nrITS*) and topology displayed as majority rule consensus tree of trees recovered in stationary phase of Bayesian search with branches thickened when $PP_{BI} \geq 0.95$. $BS_{ML} \geq 80$, $BS_{MP} \geq 70$ are displayed besides the nodes with the former above the branch and the later below the branch.

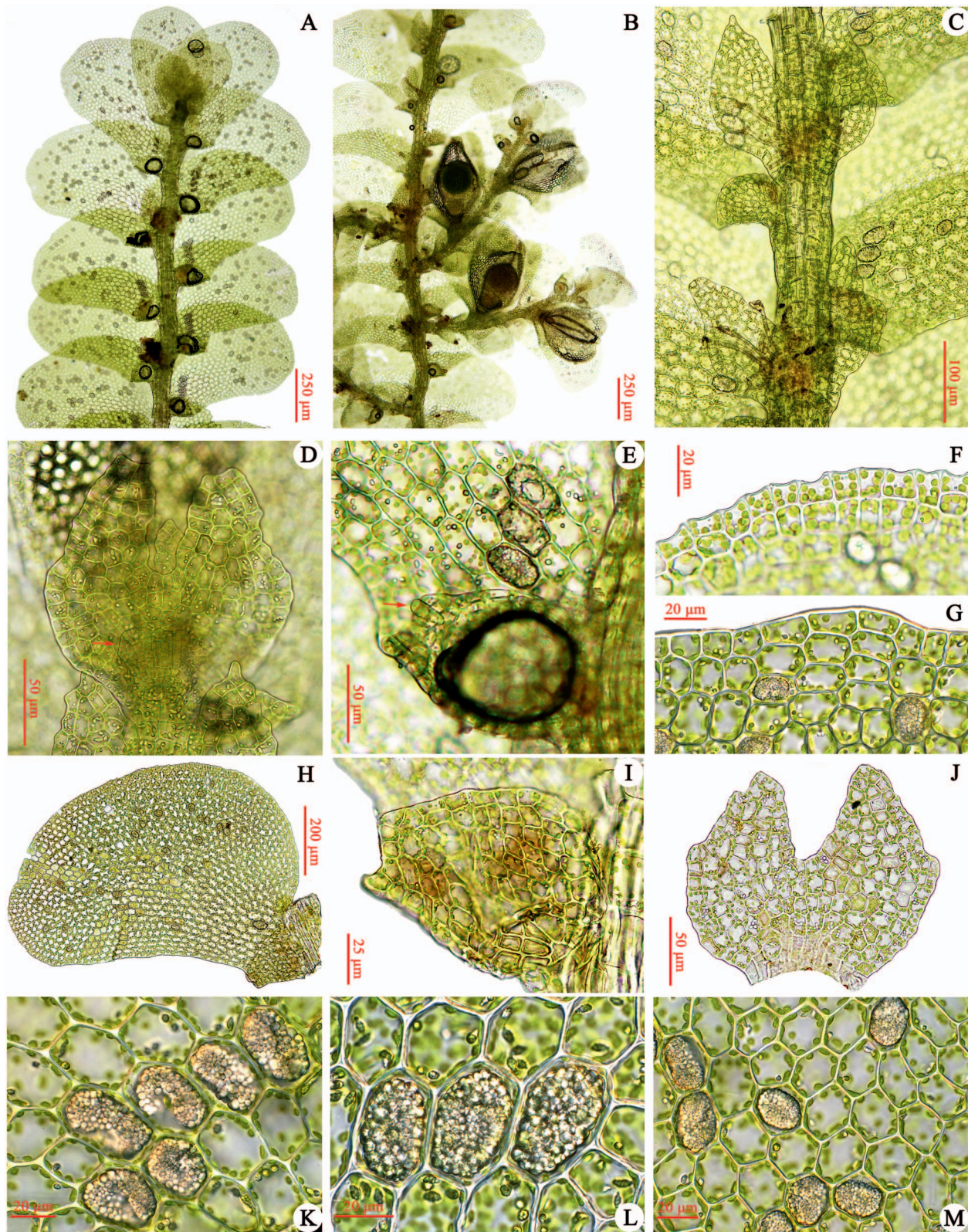


Figure 2. *Yanoella truncatilobula*. A. Plant, ventral view. B. Portion of shoot with gynoecia, ventral view. C. Portion of shoot showing underleaves and leaf lobules, ventral view. D. Underleaf showing rhizoid disc (red arrow). E. Leaf lobule showing hyaline papilla (red arrow). F–G. Marginal cells of leaf lobe, F showing *Allorgella*-type marginal denticulations. H. Leaf, ventral view. I. Leaf lobule and portion of stem, showing lobular apex and free lateral margin. J. Underleaf. K–L. Basal ocelli and non-ocellate cells in leaf lobe. M. Median cells of leaf lobe, showing ocelli and oil bodies. B, and H–J from R.-L. Zhu & L. Shu 20171203-49, the others from R.-L. Zhu & L. Shu 20171203-27.

Rectolejeunea s.str. On the other hand, as in most taxa of the subtribe Echinolejeuneinae, a further character, *Allorgella*-type marginal denticulations of leaf lobes (**Fig. 2F**) was newly found in *Rectolejeunea truncatilobula*, but lacking in *Rectolejeunea* s.str. and other members of Lepidolejeuneinae.

Rectolejeunea truncatilobula, *Kymatolejeunea*, *Echinolejeunea* and *Allorgella* share many similarities, e.g. the minute size of plants, pycnolejeuneoid gynoecial innovations, no distinct lobular teeth, thin-walled leaf cells without large trigones, granular oil bodies, *Lejeunea*-type branching, remote bilobed leaves with erect lobes, distal and ental hyaline papilla at lobular apex, stem with seven rows of cortical cells and over four rows of medullary cells in transverse section, autoicous condition, and lack of vegetative reproductive organs. *Rectolejeunea truncatilobula*, however, is immediately separated from *Kymatolejeunea*, *Echinolejeunea* R.M.Schust., and *Allorgella* by the ocellate leaves.

DISCUSSION

The earlier phylogenetic analysis based on the sequences of *trnL*-F and nrITS from a specimen collected in São Paulo, Brazil revealed that *Rectolejeunea truncatilobula* was nested in a clade consisted of other *Rectolejeunea* species, including *R. flagelliformis* A.Evans (type species of *Rectolejeunea*) and *R. versifolia* (Bechteler et al. 2016a,b; Heinrichs et al. 2014; Ye & Zhu 2018; Zhu et al. 2017). The sequences of *trnL*-F and nrITS provided by Heinrichs et al. (2014) belong to those of *Rectolejeunea versifolia* because the voucher specimen of this sampling (Peralta 8083 in SP as *Rectolejeunea monoica*) was assignable to *Rectolejeunea versifolia* (**Supplementary Table S1**). Our new phylogenetic analysis based on 11 newly generated sequences clearly shows that *Rectolejeunea truncatilobula* is nested within the lineage of the subtribe Echinolejeuneinae, and amazingly sister to *Kymatolejeunea*, *Allorgella* and *Echinolejeunea* (**Fig. 1**), but not a member of the subtribe Lepidolejeuneinae, which includes the type species of *Rectolejeunea*, *R. flagelliformis* A.Evans. *Kymatolejeunea* is a monospecific and highly specialized genus endemic to New Zealand (Grolle 1984). It is immediately recognized and separated from *Rectolejeunea truncatilobula* by the deeply lobed, partially recurved and crispatus margin of leaf lobes, strongly involute lobular margin, lack of ocelli, and epiphyllous habitat.

Echinolejeunea, also a monospecific genus endemic to New Zealand (Schuster 2001), is readily distinguished from *Rectolejeunea truncatilobula* by the leaf surfaces beset with 1-celled, tapered projections, lack of ocelli, and strongly involute lobular margin. *Allorgella* is a small pantropical genus which comprises eight species, i.e., *A. australiensis* (B.M.Thiers) Bechteler et al., *A. hoana* Tixier, *A. rabenorii* (Tixier) Bechteler et al., *A. schmidii* Tixier, *A. schnellii* Tixier, *A. semperiana* (Steph.) Bechteler et al., *A. subana* (Pócs) Pócs, and *A. zantenii* (Grolle) Bechteler et al. Bechteler et al. (2016a) transferred the Philippine *Otolejeunea philippinensis* R.L.Zhu et M.L.So to *Allorgella* and proposed a new combination: *A. philippinensis* (R.L.Zhu & M.L.So) Bechteler et al. *Otolejeunea philippinensis*, however, lacks *Allorgella*-type marginal denticulations as in *Otolejeunea streimannii* Grolle, the type species of *Otolejeunea* subg. *Phoxolejeunea* Grolle (Grolle 1985; Zhu & So 1998). In our view, *Otolejeunea philippinensis* may belong in a group together with *O. streimannii*. As discussed in Bechteler et al. (2016a), the status of *Otolejeunea* subg. *Phoxolejeunea* is not clear before the molecular data are available. *Allorgella* differs from *Rectolejeunea truncatilobula* in the lack of ocelli, strongly compressed perianths with two auriculate keels, and epiphyllous habitat.

In our phylogenetic analysis four extremely rare genera known only from tropical Asian islands (*Aphanotropis*, *Calatholejeunea*, *Dactylophorella* and *Hattoriolejeunea*) and one Neotropical monospecific genus (*Schusterolejeunea*) in Lejeuneoideae could not be included owing to a lack of samples for DNA extraction. The five small genera are morphologically very different from *Rectolejeunea truncatilobula* especially in the non ocellate leaves. The existence of ocelli and ocellus patterns usually serve as one of most useful characters in species and generic delimitation in recent phylogenetic studies on Lejeuneaceae (Bechteler et al. 2016a; Czumay et al. 2013; Dong et al. 2013; Schäfer-Verwimp et al. 2017; Ye & Zhu 2018; Zhu et al. 2017, 2018; Zhu & Shu 2018). The monospecific rheophytic *Aphanotropis* and *Schusterolejeunea* clearly belong in the subtribe Cololejeuneinae Gradst. while epiphytic *Hattoriolejeunea* belongs in the subtribe Lejeuneinae Gradst. (Gradstein 2013; Söderström et al. 2016), and may even be a member of *Lejeunea* Lib. (Zhu & Shu 2018). *Calatholejeunea* known only from Ambon and New Guinea was placed in the subtribe Cololejeune-

neinae (Gradstein 2013), but it is more reasonable to assign it to Cheilolejeuneinae Gradst. owing to the long inserted leaf leaves (Gradstein et al. 2018; Mizutani 1984). *Dactylophorella* is a monospecific genus whose plants bear densely spinose teeth, and thus far has not been classified in any subtribe of the tribe Lejeuneae (Gradstein et al. 2013, Söderström et al. 2016). *Dactylophorella* and *Lejeunea* share many striking characters including the lejeuneoid gynoecial innovation, bilobed underleaves, over 5 rows of medullary cells in transverse section of stem, bilobed underleaves, single non-curved lobular tooth, proximal hyaline papilla, large leaf cells, and lack of ocelli and *Allorgella*-type marginal denticulations. Morphologically *Dactylophorella* fits well into the subtribe Lejeuneinae, but does not belong in Echinolejeuneinae.

Rectolejeunea truncatilobula therefore merits the recognition as a new genus, *Yanoella* which is dedicated to Dr. Olga Yano in recognition of her important contributions to the Brazilian bryology.

Out of six species in *Rectolejeunea* currently accepted in the world checklist of liverworts and hornworts (Söderström et al. 2016), *R. colombiana* R.M.Schust. and *R. queenslandica* (B.M.Thiers) Xiao L.He have to be excluded from *Rectolejeunea* because the former has the lejeuneoid gynoecial innovation while in the latter the lobular tooth is non curved and the lack of specialized caducous flagelliform branches. *Rectolejeunea colombiana* may be one member of *Lejeunea* while *R. queenslandica* may belong in *Thiersianthus*, an ocellate genus known only from Beoneo (Zhu et al. 2017).

Yanoella is well characterized and easily recognized by the pycnolejeuneoid gynoecial innovation, usually 3–4 basal ocelli in an unbroken transverse row in leaf lobes, numerous scattered ocelli in leaf lobes, usual presence of *Allorgella*-type marginal denticulations of the leaf lobe, distal and ental hyaline papilla, granular oil bodies in median and basal chlorophyllous cells in leaf lobes (oil bodies usually almost homogeneous in marginal cells of leaf lobe), truncate apex of the leaf lobule without a distinct apical tooth, stem with 7–9-seriate hyaloderm and (6–)9–13 rows of smaller medullary cells, usual presence of rhizoid discs, and specialized caducous flagelliform branches lacking (Fig. 2). With the addition of *Yanoella*, the subtribe Echinolejeuneinae now contains seven genera: *Allorgella*, *Anoplolejeunea* (Spruce) Schiffn., *Echinolejeunea*, *Gaolejeunea* R.L.Zhu & W.Ye, *Haplolejeunea* Grolle, *Kymatolejeunea* and *Yanoella*. The

last two genera always bear scattered ocelli in leaf lobes which can distinguish them from the remaining genera. *Haplolejeunea*, an Afro-American genus with four species (Gradstein & Ilkiu-Borges 2018), is very similar to *Yanoella* in the ocellate leaves, small leaf cells without large trigones, distal and ental hyaline papilla, granular oil bodies, lack of curved lobular tooth, pycnolejeuneoid gynoecial innovation, usual presence of *Allorgella*-type marginal denticulations and presence of rhizoid discs. In *Haplolejeunea*, however, basal ocelli never form an unbroken transverse row in leaf lobes. In addition, the leaf insertion to stem is short, only 2–3 lobular cells long (4–7 lobular cells long in *Yanoella*). The neotropical *Anoplolejeunea* is a monospecific genus whose leaf lobes also usually have 1–3 basal ocelli (Gradstein et al. 2001, present study). However, unlobed underleaves and granular oil bodies in marginal cells of leaf lobes in *Anoplolejeunea* are rather different from *Yanoella*. Although *Pictolejeunea* Grolle (*Pictolejeuneinae*), a small genus with six species (Pócs 2007), is phylogenetically distantly related to *Yanoella* (Fig. 1), but morphologically the two genera share many similarities, including the minute size of plants, ocellate leaves, thin-walled leaf cells without large trigones, granular oil bodies (if present), remote bilobed leaves with erect lobes, distal and ental hyaline papilla at lobular apex, and stem with seven rows of cortical cells and over five rows of medullary cells in transverse section. *Pictolejeunea*, however, differs in the lejeuneoid gynoecial innovations (if present) (Grolle & Reiner-Drehwald 2005; Ilkiu-Borges 2003; Pócs 2007), lack of *Allorgella*-type marginal denticulations, and vegetative reproduction usually via marginal, linear gemmae. In addition, in herbarium materials of *Pictolejeunea* the ocelli are usually brown to black and easily detected, but in *Yanoella* ocelli are fully disintegrated and not easily distinguished from surrounding cells in herbarium materials.

Stenolejeunea was a small genus of Lejeuneaceae subfamily Lejeuneoideae containing seven described species. It was reduced to synonymy under *Lejeunea* based on no single morphological character separating the two genera (Söderström et al. 2010). Our molecular phylogenetic analyses first unambiguously placed *Lejeunea thallophora* (*Stenolejeunea thallophora*, type species of *Stenolejeunea*) in the subtribe Lejeuneinae, and sister to *Lejeunea exilis* (Reinw., Blume et Nees) Grolle (Fig. 1). With the inclusion of molecular data of the type species of *Stenolejeunea* in phylogenetic analyses, both morphological and

molecular evidence supports the earlier reduction of *Stenolejeunea* to synonymy under *Lejeunea* Lib. *Lejeunea thallophora* was recorded only in Indonesia (Java, Sulawesi) and Papua New Guinea (Schuster 2000b as *Stenolejeunea thallophora*). It is newly reported for Malaysia (voucher specimen: Malaysia, Sabah, Lahad Datu, Sepagaya Forest Reserve, Mount Silam, along trail to Silam water spring, 04°58'35.60"N, 118°01'50.13"E, 240 m, on rock, 16 Sept. 2016, R.-L. Zhu et al 20160916-85 (BORH, HSN, SAN)).

TAXONOMY

Yanoella R.L.Zhu, L.Shu, C.J.Bastos et S.Vilas Bôas-Bastos, *gen. nov.*

Plants epiphytic, whitish green to yellowish green, autoicous. Shoots irregularly pinnately branched, branches Lejeunea-type. Stems in transverse section with 7–9 cortical cells and (6–)9–13 smaller medullary cells, ventral merophyte 2 cells wide. Leaves imbricate. Leaf lobes oblong-ovate, falcate, margins slightly denticulate (denticulations Allorgella-type) to entire, apex obtusely rounded to rounded, cells thin-walled, trigones small, intermediate thickenings absent to weakly nodulous, laminar ocelli (15–) 20–50, scattered in leaf lobe, almost as large as non-ocellate cells, basal ocelli usually (2–)3–4 in a transverse row, oil bodies small, finely segmented, (–)3)4–7(–9) per median cell, cuticle smooth or sometimes very weakly striate. Leaf lobules ovoid-rectangular, apex truncate, hyaline papilla distal and ental. Underleaf 1 per pair of leaves, bifid, rhizoid disc usually present. Androecia terminal or proliferating vegetatively from apex, with bracteoles present only at base. Gynoecial with one pycnolejeuneoid innovation, female bract lobe almost entire, female bracteole bilobed to 1/3 its length. Perianth emergent, 4–5-keeled. Vegetative reproduction by caducous leaves.

TYPE SPECIES: *Yanoella truncatilobula* (C.J.Bastos) R.L.Zhu, L.Shu, C.J.Bastos et S.Vilas Bôas-Bastos (≡ *Rectolejeunea truncatilobula* C.J.Bastos, J. Bryol. 34(2): 144. 2012).

Yanoella truncatilobula (C.J.Bastos) R.L.Zhu, L.Shu, C.J.Bastos et S.Vilas Bôas-Bastos, *comb. nov.* **Figs. 2–3**

≡ *Rectolejeunea truncatilobula* C.J.Bastos, J. Bryol. 34(2): 144. 2012. TYPE: BRAZIL. BAHIA: Eunápolis, Estação Veracruz, 16°22'S, 39°10'W, 10 June 1999, S.B. Vilas Bôas-Bastos & C. Bastos 475 (holotype: ALCB!; isotype: SP!).

Description. Autoicous. Plants green, whitish green to yellowish green, minute, delicate, 3–15 mm long. Shoots 0.80–1.25 mm wide, irregularly pinnately branched, branches *Lejeunea*-type, leaf sequence of vegetative branches lejeuneoid. Stems 65–90 µm in diam., in transverse section with 7–9 cortical cells surrounding (6–)9–13 medullary cells, cortical cells subquadrate to oblong, 22–33 × 12–19 µm, much larger than medullary cells, medullary ones ± isodiametric, 9–22 × 6–13 µm; ventral merophyte two cells wide. Rhizoids at base of underleaves, rhizoid disc usually developed. Leaves imbricate, flattened, insertion shortly J-shaped along 6–13 lobe cells and 4–7 lobular cells, diverging from stem at an angle of 45–90°. Leaf lobes asymmetrically oblong-ovate, falcate, 0.45–0.62 mm long, 0.40–0.48 mm wide, margins with *Allorgella*-type denticulations or almost entire, apex rounded to obtusely rounded, ventral margin sinuated to straight, dorsal margin arched. Leaf lobules ovate to subquadrate, strongly inflated, 1/6–1/4 as long as the lobes (sometimes strongly reduced), attached to the stem by 4–7 cells, lateral free margin slightly arched, usually slightly incurved (except for apex), bordered by 7–10 subquadrate to rectangular marginal cells, apex usually truncate, without tooth, keel straight to slightly arched, smooth, hyaline papilla narrowly oblong or clavate, 24–30 × 8–12 µm, distal, attached to the inner surface of the margin between the two apical cells. Stylus not seen. Cells of leaf lobe with thin walls and small trigones, intermediate thickenings sometimes developed, cuticle smooth or sometimes very weakly striate, at margin quadrate to rectangular, 11–16 × 8–14 µm, in the middle ± hexagonal, 22–27 × 15–20 µm, near base similar to median ones in shape, slightly larger than median cells, 24–38 × 15–23 µm. Oil bodies almost homogenous to weakly granular in marginal cells, but granular in median and basal non-ocellate cells. Scattered ocelli present in leaf lobes, female bract lobes and perianths, 20–40 per leaf lobe, almost as large as non-ocellate cells, basal ocelli (0–)2–4 (–6), usually slightly larger than or almost as large as non-ocellate cells, 30–45 × 22–28 µm, usually (2–)3–4 in a transverse row, the lowermost ocellus separated by



Figure 3. A. The lowland ombrophious forest in Ecological Station of Wenceslau Guimarães where *Yanoella truncatilobula* was found. B. Habitat, showing tree trunk with *Y. truncatilobula* (white arrow). C–D. Close-ups of *Y. truncatilobula* on tree trunks. B–D from R.-L. Zhu & L. Shu 20171203–27.

2–3 non-ocellate leaf cells from stem cells (of the suprabasal type, cf. Zhu & So 2001). Underleaves remote, longer than wide, 2–3 times as wide as stem, bilobed to ca. 1/2 their length, sinus V-shaped, lobes narrowly triangular, erect, acute at apex, 5–11 cells long, 4–9 cells wide at base, inner lateral margin nearly entire, outer lateral one with *Allorgella*-type denticulations or almost entire, sometimes with a weak tooth, ocelli absent, insertion line subtransverse, base cuneate (never cordate). Androecia frequent, terminal or intercalary, usually on long lateral branches or on main shoots, bracts in 2–4 pairs, hypostatic, strongly concave and inflated, shortly and subequally bifid, lobes 0.20–0.24 mm long, 0.16–0.18 mm wide, apex rounded, nearly entire, lobule slightly shorter, keels strongly arched, almost entire, bracteoles 1–2, borne only at the basal portion of androecium, similar to ordinary under-

leaves, antheridia 2 per bract, subspherical, 75–100 μm in diameter. Gynoecia usually on short or long branches, with one pycnolejeuneoid innovation; bracts obovate, 0.40–0.57 mm long, 0.20–0.30 mm wide, deeply and unequally bifid, the lobe oblong-obovate, apex rounded, margin almost entire, with numerous ocelli as in leaf lobe, lobule oblong or lingulate, 2/5–1/2 as long as the bract lobe, margin almost entire, apex obtuse to rounded-obtuse, keel almost straight, over 4/5 as long as the lobule; bracteole connate with bracts on both sides at base, ovate to oblong, 0.25–0.36 mm long, 0.16–0.19 mm wide at middle, margin nearly entire, apex bilobed to 1/3 its length, sinus V-shaped, lobes triangular. Perianths immerse to 1/3 exserted, obovoid, 0.48–0.52 mm long, 0.29–0.31 mm wide at middle, inflated, with 4–5 entire keels, antical keel a little lower than the others and usually indistinct, surface

of perianth smooth, beak short, 1 cell long, ocelli in perianth several to numerous, scattered. Capsule spherical, 0.21–0.25 mm in diam., capsule walls 2-stratose, seta 7–9 cells long, with 4 inner and 12 outer rows of cells in transverse section, foot with 3 transversal cell rings, elaters 22 per capsule (5–6 per valve), 170–230 µm long, 7.5–11 µm wide; elateroids rudimentary. Spores elongate, 30–45 × 14–22 µm, spore surface irregularly papillose, rosettes indistinct. Asexual reproduction by caduceus leaves.

Habitats and distribution. *Yanoella truncatilobula* is known from Eastern Brazil, including Bahia, Parana, and São Paulo. It was found on tree trunks in the ombrophious forest at an altitude of 0–900 m (Fig. 3).

Representative specimens examined. BRAZIL. BAHIA: Jaguaripe, 13°11'37.6"S, 39°00'41.4"W, Floresta Atlântica, corticícola, 21 Feb. 2011, L. C. Reis 1255 (ALCB 97048 as *Rectolejeunea truncatilobula*), *ibid.*, L. C. Reis 1251 (ALCB 97050 as *R. truncatilobula*); BAHIA: Jaguaripe, 13°11'21.5"S, 39°01'20.6"W, 26 Feb. 2011, L. C. Reis 1610 (ALCB 97008 as *R. truncatilobula*), L. C. Reis 1611 (ALCB 97007 as *R. truncatilobula*); BAHIA: Igrapiúna, Reserva Ecológica da Michelin, 13°48'S, 39°10'W, 92–383 m, corticícola, 11 Aug. 2013, C. Bastos 4672 (ALCB 72117 as *R. truncatilobula*); BAHIA: Wenceslau Guimarães County, Nova Esperança Village, Ecological Station of Wenceslau Guimarães, Trilha Água Vermelha (Red water trail), 13°34'44.76"S, 39°42'27.88"W, 473 m, on tree trunks, 3 Dec. 2017, R.-L. Zhu & L. Shu 20171203-27 (HSNU), *ibid.*, 13°34'43.85"S, 39°42'30.08"W, 504 m, 3 Dec. 2017, R.-L. Zhu & L. Shu 20171203-49 (HSNU), *ibid.*, 13°34'43.81"S, 39°42'30.08"W, 504 m, 3 Dec. 2017, R.-L. Zhu & L. Shu 20171203-50B (HSNU).

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Supplementary document online:

Supplementary Table S1. Sequences from GenBank used in this study, including taxa, locations, vouchers, and GenBank accession numbers. Missing sequences indicated by a dash (—).