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New insights into the phylogeny and relationships within the worldwide genus *Riccardia* (Aneuraceae, Marchantiophytina)

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Abstract. With 280 accepted species, the genus *Riccardia* S.F.Gray (Aneuraceae) is one of the most speciose genera of simple thalloid liverworts. The current classification of this genus is based on morphological and limited-sampling molecular studies. Very few molecular data are available and a comprehensive view of evolutionary relationships within the genus is still lacking. A phylogeny focusing on relationships within the large genus *Riccardia* has not been conducted. Here we propose the first worldwide molecular phylogeny of the genus *Riccardia*, based on Bayesian inference and parsimony ratchet analyses of sequences from three plastid regions (*psb*A-*trn*H, *rps*4, *trn*L-F). The results support the monophyly of *Riccardia* and a new monospecific genus, *Afroriccardia* Reeb & Gradst. gen. nov., is described based on molecular and morphological evidence. The results indicate that several currently recognized infrageneric divisions and a few species are not monophyletic, suggesting that further analyses are needed to arrive at a proper understanding of the phylogeny of the genus. Although evidence for an Andean clade was found, most of the species appear scattered in different clades without clear geographical segregation. Broader sampling and further analyses are necessary in order to improve our understanding of the phylogeny of this poorly known liverwort genus.

Keywords. *Afroriccardia* gen. nov., Aneuraceae, liverworts, phylogeny, *Riccardia*.

Introduction

Among simple thalloid liverworts (Marchantiophytina subclass Metzgeriidae s. lat.), Aneuraceae is the largest family in terms of species number, but remains the least known (Furuki 1991; Preuβing *et al.* 2010). Four genera are accepted so far: *Aneura* Dumort., *Lobatiriccardia* (Mizut. & S.Hatt.) Furuki, *Riccardia* S.F.Gray and *Verdoornia* R.M.Schust. (Preuβing *et al.* 2010). A fifth genus, *Cryptothallus* Malmb., has recently fallen into synonymy under *Aneura* (Wickett & Goffinet 2008). *Riccardia* is the largest genus of the Aneuraceae, with 280 accepted taxa at species level in the last world checklist (Nebel 2016). However, several authors assert that a worldwide taxonomical revision would reduce this number to about a hundred (Schuster 1992; Gradstein 2001; Preuβing *et al.* 2010). On the other hand, a recent study on African *Riccardia* shows the existence of several cryptic or undescribed species in the genus (Reeb unpubl. res.).

Riccardia species exhibit a simple, pinnate to multi-pinnate thallus, generally without internal differentiation. The development of the gametangia in two rows on short, specialized branches is a synapomorphy of the genus (vs gametangia in several rows in *Aneura*). The species present great phenotypic plasticity and the lack of constant diagnosis characters has led to difficulties in defining and identifying them. It is widely accepted that *Riccardia* is one of the most challenging genera of the Marchantiophytina (Hewson 1970; Meenks & Pócs 1985; Schuster 1989; Furuki 1991).

Riccardia has a cosmopolitan distribution but is predominant in the southern hemisphere; only seven species are encountered in the temperate regions of Europe and North America (Schuster 1992; Paton 1999; Grolle & Long 2000). The species colonize moist to rather wet substrates (soil, rock, rotten log, bark) in a wide range of habitats, usually under high atmospheric humidity, and do not tolerate desiccation (Schuster 1992).

The genus *Riccardia* has been divided into ten subgenera: *Arceoneura* Hässel, *Corioneura* Furuki, *Hyaloneura* R.M.Schuster, *Lophoneura* Hässel, *Neoneura* Furuki, *Phycaneura* R.M.Schuster, *Riccardia*, *Spinella* (Schiffn.) Hässel, *Thornoneura* Furuki and *Trichotallia* Hässel (Mizutani & Hattori 1957; Hässel de Menendez 1972; Schuster 1985, 1992; Furuki 1991; Nebel 2016). Subgenus *Riccardia* is the largest and is divided into four sections: *Alcicornia* Hässel, *Crassantia* Hässel, *Pallidevirida* Hässel and *Riccardia* (Hässel de Menendez 1972; Nebel 2016). The infrageneric divisions are based on morpho-anatomical characters and are usually studied at a regional or continental scale. Hewson (1970) arranged the Australasian *Riccardia* species in five informal groups based on multivariate analysis of qualitative and quantitative traits and did not recognize formal supraspecific entities.

The monophyly of Aneuraceae has been well supported in large-scale liverwort phylogenies (e.g., Qiu & Palmer 1999; Forrest *et al.* 2006; He-Nygrén *et al.* 2006). The first assumptions on relationships among Aneuraceae were proposed in an investigation of symbiosis between *Aneura* and fungi (Kottke *et al.* 2003; Wickett & Goffinet 2008; Bidartondo & Duckett 2010; Krause *et al.* 2011). The first phylogeny of the family focused on the genus *Lobatiriccardia*, including a few *Riccardia* sequences from Ecuador and Europe (Preuβing *et al.* 2010). However, a phylogeny focusing on the relationships within the large genus *Riccardia* has not been conducted.

The aim of the present work is to explore the relationships among *Riccardia* species using a large geographical sampling. This paper is part of a larger study addressing species delimitation, phylogenetic relationships and character evolution within the genus *Riccardia*, with special reference to Africa.

In this study, we provide support for the monophyly of the genus and highlight several well-supported clades. We also describe a new monospecific genus, *Afroriccardia*, based on morphological and molecular evidence.

Material and methods

Sampling

We studied 98 samples from Europe, southern South America, Tropical America, Africa, Asia and Oceania (Appendix). Specimens were kindly provided by collectors from all around the world and by several herbaria (AK, BORH, CONN, E, HSNU, KLU, PC; see Acknowledgments). Dates of collections spread from 1973 to 2013. Due to low amplification success, specimens older than about 30 years were not selected for molecular work.

Identification

Each sample was studied in the light microscope after treatment of the thallus with bleach (20%), degrading the cellular content, and followed by coloration with methylene blue (Rico 2011; Reeb & Bardat 2014). This greatly enhanced observations of the anatomical structure of the thallus and facilitated identification. Traceability of observations has been insured by numerous photographs of the plants *in toto* and in transverse section, taken with a Nikon CoolPix P5000 camera.

Identifications were conducted using keys and descriptions published by Arnell (1952, 1963), Hewson (1970), Hässel de Menendez (1972), Meenks & Pócs (1985), Meenks (1987), Brown & Braggins (1989), Furuki (1991, 1994, 1995), Schuster (1992), Perold (2001a, 2001b, 2002a, 2002b), Gradstein & Costa (2003), Wigginton (2004), Gradstein (2011) and Furuki & Tan (2013).

Phylogenetic reconstruction

Choice of markers

The plastid markers *rps*4, *trn*L-F and *psb*A-*trn*H, classically used in phylogenetic reconstructions among bryophytes (Quandt & Stech 2004; Preußing *et al.* 2010; Carter 2012), were selected because of their small size (< 1000 bp) allowing good amplification success and their potential informative variability at the infra-generic level (Liu *et al.* 2010; Stech & Quandt 2010). In total, 291 new sequences generated from 98 samples were used in this study: 95 *rps*4 sequences, 98 *trn*L-F sequences and 98 *psb*A-*trn*H sequences. GenBank accession numbers are given in the Appendix, together with voucher details.

DNA isolation, amplification and sequencing

Prior to extraction, samples were cleaned under the binocular microscope (dry or humidified with distilled water) using tweezers to remove micro-epiphytic leafy liverwort and debris. A few thalli, preferably green, chlorophyll-rich terminal thallus branches, were selected and placed in a 2 ml Eppendorf tube. Two tungsten beads (2 mm) and one volume of pure silica sand (*sable de Fontainebleau*) were added to the disrupted tissues, which were crushed in 2–3 iterations at 30 Hz for 1 min using Quiagen TissueLyser. DNA was extracted from the resulting powder. For older herbarium specimens (> 10 years), a supplementary CTAB procedure was applied beforehand: 400 µl AP1 lyse buffer + 30 µl CTAB buffer + 30 µl proteinase K were added to each specimen and tubes were placed for 20–24 hours in a thermocycler at 42°C. 460 µl of CIA (96 : 4 chloroform : isoamyl alcohol) was added to purify and solubilize remaining impurities. Tubes were gently mixed by inversion and centrifuged for 15 min at 13000 rpm and 4°C. DNA was then extracted following an adapted protocol of Dneasy® Plant Mini Kit Quiagen. Elution was performed in 50 µl of AE buffer and deposited a second time on the membrane of the spin column.

Polymerase Chain Reactions (PCR) were performed in a 20 µl reaction mixture, containing 2 µl of the DNA solution (or 2–3 µl of ¹⁄₁₀ diluted DNA solution) and PCR buffer with 1 mM MgCl$_2$, 1 µl DMSO (dymethyl sulfoxide), 1 µl BSA (bovine serum albumine), 0.26 mM of dNTP mix, 0.25 mM of each primer (0.5 mM for diluted DNA solution) and 0.06 mM (0.12 mM for diluted DNA) of QbioTaq®

Table 1. List of different primers used for amplification of the three markers.

Region	Primer names	Orientation	Sequence	Length	Reference
*psb*A-*trn*H	trnH-psbA-F	F	5' GTTATGC ATGAACGTA ATGCTC 3'	22	Sang *et al.* 1997
	trnH-psbA-R	R	5' CGCGCAT GGTGGATTC ACAATCC 3'	23	Chen *et al.* 2010
	trnK-psbA trnH 501 F	F	5' TTTCTCA GACGGTATG CC 3'	18	Forrest & Crandall-Stotler 2004
	trnK-psbA-trnH-TRNHT-R	R	5' GAACGAC GGGAATTGA AC 3'	18	Forrest & Crandall-Stotler 2004
*trn*L-F	trnL-C-Mosses	F	5' CGAAATT GGTAGACGC TACG 3'	20	Quandt & Stech 2004
	trnL-F-F	R	5' ATTTGAA CTGGTGACA CGAG 3'	20	Taberlet *et al.* 1991
	trnL-E-b49873	F	5' GGTTCAA GTCCCTCTA TCCC 3'	20	Taberlet *et al.* 1991
	trnL-E-ric1	F	5' GGTTCAA GTCCCTCCA CCCC 3'	20	designed for this study
	trnL-E-ric2	F	5' GGTTCAAGT CCCTCYAYC CC 3'	20	designed for this study
	trnL-D-a50272	R	5' GGGGGTAGA GGGACTTGA AC 3'	20	Taberlet *et al.* 1991
*rps*4	trnS-F	R	5' TACCGAG GGTTCGAAT C 3'	17	Souza-Chies *et al.* 1997
	rsp5rev	F	5' ATGTCCC GTTATCGAG GACCT 3'	21	Nadot *et al.* 1994
	rps4F3	F	5' TTTTTCG KTTRGGTAT RGTTCC 3'	22	designed for this study

(Quiagen) Polymerase. In case of samples with very low amplification signals on gels, 1 µl betaine and 1–2 µl MgCl$_2$ were added.

Amplification was performed using primers and programs presented in Tables 1 and 2. Six additional internal primers were designed using PhyDE v. 0.0997 (Müller *et al.* 2010) to amplify *rps*4 but only one, associated with *rps*4 *trn*S-F, gave significant results (Tables 1–2).

Table 2. List of PCR protocols used for each primer couple.

Region	Primers couples	PCR conditions
*psb*A-*trn*H	trnH-psbA-F & trnH-psbA-R trnK-psbA	4'94°; 40×[45"94°; 45"54°; 1'72°]; 5'72°
	trnH 501 F & trnK-psbA-trnH-TRNHT-R	4'94°; 40×[45"94°; 45"54°; 1'72°]; 5'72°
*trn*L-F	trnL-C-Mosses & trnL-F-F	4'94°; 40×[1'94°; 1'55°; 1'72°]; 5'72°
	trnL-C-Mosses & trnL-D-a50272	4'94°; 40×[1'94°; 45"55°; 1'72°]; 5'72°
	trnL-E-b49873 & trnL-F-F	4'94°; 40×[1'94°; 45"55°; 1'72°]; 5'72°
*rps*4	rsp5rev & trnS-F	4'94°; 45×[1'94°; 45"53°; 1'72°]; 5'72°
	rsp4F3 & trnS-F	4'94°; 45×[15"94°; 30"53°; 1'72°]; 7'72°

PCR products were revealed by migration of a 2 µl deposit on agarose gel. PCR revealing positives stripes were sent to Genoscope (supported by the MNHN project BDV– Bibliothèque du Vivant).

Choice of outgroups

Based on the multi-gene, multi-taxa studies of Crandall-Stotler *et al.* (2005), Forrest *et al.* (2006), He-Nygrén *et al.* (2006) and Preußing *et al.* (2010), sequences of 11 taxa of Aneuraceae, 3 of Metzgeriaceae and 2 of Pleuroziaceae were integrated as outgroups in the molecular matrix: *Aneura latissima* Spruce, *A. pinguis* (L.) Dumort., *A. mirabilis* (Malmb.) Wickett & Goffinet, *Lobatiriccardia alterniloba* (Hook.f. & Taylor) Furuki, *L. coronopus* (De Not. ex Steph.) Furuki, *L. oberwinkleri* Nebel, Preussing, Schäf.-Verw. & D.Quandt, *L. verdoornioides* Nebel, Preussing, Schäf.-Verw. & D.Quandt, *L.* "*yakusimensis*", *L.* sp. A1, *L.* sp. B2 and *Verdoornia succulenta* R.M.Schust. (Aneuraceae); *Apometzgeria pubescens* (Schrank.) Kuwah., *Metzgeria furcata* (L.) Corda and *M. myriopoda* Lindb. (Metzgeriaceae); and *Pleurozia gigantea* (F.Weber) Lindb. and *P. paradoxa* Schiffn. (Pleuroziaceae).

Sequences alignments

Sequence assembly and elimination of primer annealing sites were conducted using PhyDE v. 0.9971 and Geneious v. 6 (Kearse *et al.* 2012). The whole data set was aligned manually in PhyDE using the data set of Preußing *et al.* (2010) as a scaffold and applying the criteria laid out in Kelchner (2000). We identified two hairpin associated inversions in the *psb*A-*trn*H intergenic spacer (inversion 1: 14 nt stem, 22 nt loop; inversion 2: 23 nt stem, 5 nt loop) both of which were positionally separated in the alignment. Both inversions were included as reverse complemented in the phylogenetic analyses, as discussed in Quandt *et al.* (2003) and Borsch & Quandt (2009). Variable and parsimony-informative sites were estimated using MEGA v. 5.2 (Tamura *et al.* 2011).

Molecular species delimitation

Identification of *Riccardia* species is a challenging exercise and misidentifications are very common among herbarium materials (Reeb & Bardat 2014). Our sampling contained multiple accessions of several morphological species, e.g., *R. chamedryfolia* (With.) Grolle (13 specimens), *R. longispica* (Steph.) Pearson (8) and *R. fucoidea* (Sw.) Schiffn. (6); others were represented by singletons only (*R. diminuta* Schiffn., *R. crenulata* Schiffn., etc.; see Appendix). We used molecular species delimitation tools to check the congruence of morphological identifications with genetic signals and to clarify the initial dataset for the phylogenetic analyses while keeping the largest sampling of potential species.

We first used a non-tree based method, ABGD (Automatic Barcode Gap Discovery; Puillandre *et al.* 2012), not requiring monophyly to propose species delineation, and a tree-based method (Fontaneto *et al.* 2015), here the Poisson Tree Processes model (PTP; Zhang *et al.* 2013).

We analysed the initial dataset with ABGD in order to test morphological species delimitation, especially for samples identified as the same taxon. The following parameters were selected: distance Kimura-Nei, *P* = 0.0057 (*psb*A-*trn*H and *trn*L-F) and *P* = 0.0037 (*rps*4). Each gene was analysed independently. We also ran PTP on the bPTP server (http://species.h-its.org/ptp/), with 500 000 MCMC generations, thinning set to 100, burn-in 0.25 and the "remove out-groups" option selected. Input trees were RAxML trees calculated on CIPRES Science Gateway (Miller *et al.* 2010) under default parameters. Two analyses are provided: PTP_ML (maximum likelihood solution) which gives the most likely solution among the dataset, and PTP_sh (Bayesian solution) which considers the frequency of the nodes across the sampling (Lang *et al.* 2015). We only retained molecular species with posterior delimitation probabilities higher than 0.91 (Zhang *et al*. 2013).

All PTP retained species were congruent with ABGD results. Three strategies were selected to keep the largest number of species hypotheses: (1) if two samples assigned to the same morpho-species were considered as separate species with ABGD, we kept the two accessions; (2) if several samples assigned to the same morpho-species were considered as one species with ABGD, we selected the most informative accession (length and sequence quality); (3) if several samples assigned to different morpho-species were considered as the same species with ABGD, identifications were checked; when the initial species hypotheses were confirmed, the accessions of the different morpho-species were retained. Finally, we built a concatenated alignment with the reduced dataset (Appendix) based on ABGD / PTP analyses.

Phylogenetic analyses

File commands for the parsimony ratchet analysis (Nixon 1999) were generated by PRAP2 (Müller 2007) and run in PAUP.4.0 (Swofford 2002) with the following parameters: 10 cycles of 200 iterations each, with 25% positions chosen randomly and overweight to 2. Gaps were coded as missing data. Branches of minimum size of 0 were automatically collapsed.

The dataset was partitioned *a priori* on the basis of gene identity, i.e., *rps*4, *psb*A-*trn*H and *trn*L-F. For each alignment, the best partitioning scheme and the best nucleotide substitution model were defined using Partition Finder v. 1.1.1 (Lanfear *et al.* 2012) based on the Akaike Information Criterion. The GTR + Γ + I model of sequence evolution and the restriction site model (F81) for binary data were selected. Bayesian analysis was performed using MrBayes v. 3.2.6 (Huelsenbeck *et al.* 2001) on CIPRES Science Gateway (Miller *et al.* 2010) and 10 Markov Chain Monte Carlo (MCMC) runs with 4 chains (1.5×10^6 generations each) were run simultaneously. Chains were sampled every 1000 generations with the respective trees written to a tree file. We visualized the results with Tracer v. 1.6 (Rambaut *et al.* 2014) to verify the convergence of the runs. Calculation of the consensus tree and the posterior probabilities of clades were performed after removing the burn-in samples (25%). Finally, a 50% majority-rule consensus tree was built in MrBayes.

Consensus topologies and support values were compiled using Inkscape v. 0.91 (The Inkscape Team: https:// inkscape.org). Each name on the tree is formed by the name of the taxon followed by its voucher number.

Following recent studies, trees were rooted with *Pleurozia paradoxa* MPE02211 (Davis 2004; Forrest & Crandall-Stotler 2004, 2005; Forrest *et al.* 2006; Preuβing *et al.* 2010).

Matrices and obtained trees are available on TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19453).

Table 3. Number of sequences obtained and number of informative sites for each marker. All the numbers refer to the aligned matrix, except "Range of amplicon size", which refers to unaligned sequences. *rps*4F3 refers to the part of *rps*4 obtained with the newly designed primers. *trn*L-F information is detailed for the two internal portions often sequenced and concatenated for older samples. Percentage values are indicated in brackets next to absolute values for conserved, variable, parsim-info and singleton sites.

Loci		No. of sites	Conserved sites	Variable sites	Parsim-info sites	Singleton sites	Range of amplicon size
*rps*4	rsp5rev & trnS-F (partial)	680	264 (39%)	362 (53%)	301 (44%)	53 (8%)	426–504
	rsp4F3 & trnS-F	614	229 (37%)	331 (54%)	274 (45%)	49 (8%)	660–726
*trn*L-F	trnL-C-Mosses & trnL-F-F (total)	878	303 (35%)	373 (43%)	294 (33%)	52 (6%)	441–512
	trnL-C-Mosses & trnL-D-a50272	502	186 (37%)	239 (48%)	200 (40%)	35 (7%)	316–351
	trnL-E-b49873 & trnL-F-F	351	98 (28%)	132 (38%)	92 (26%)	17 (5%)	83–165
*psb*A-*trn*H		324	139 (42%)	157 (48%)	133 (41%)	24 (7%)	241–268
Total for matrix		1882	706 (38%)	892 (47%)	728 (39%)	129 (7%)	–

Results

Sequences and alignments

The final concatenated plastid matrix contained 1882 positions, including 706 conserved sites, 892 variable sites, and 728 informative sites (Table 3). Length variation for each marker is also given in this table. As we did not get full *rps*4 sequences for all specimens, we excluded the terminal part in order to avoid a high level of missing data. A homo-polynucleotide stretch (position 350 to 356) within P8 of the *trn*L group I intron (compare with Quandt & Stech 2005) wa been excluded from the phylogenetic analysis due to its ambiguous homology assessment (compare with Kelchner 2000).

Species delimitation

According to ABGD analyses, 48 molecular clusters were detected with *rps*4, 40 with *psb*A-*trn*H and 46 with *trn*L-F (Table 4). With PTP_Ph analyses, only 13 molecular species hypotheses showed more than 0.90 support for *trn*L-F, 10 for *psb*A-*trn*H; with PTP_ML it was 11 for *trn*L-F and 10 for *psb*A-*trn*H. These well-supported clusters support ABGD results. However, some clusters found with ABGD were not statistically confirmed with PTP (Table 4). Based on the smallest well-supported clusters, 55 species hypotheses were retained (Appendix). For example, *Riccardia chamedryfolia* was reduced from 13 samples to 2, *R. longispica* from 8 to 2, and *Afroriccardia comosa* (Steph.) Reeb & Gradst. comb. nov. from 4 samples to one (Appendix).

Phylogenetic analyses

Five main lineages were detected by both Bayesian inference (Fig. 1) and parsimony ratchet (Fig. 2) within the Aneuraceae: *Lobatiriccardia*, *Verdoornia*, *Aneura*, *Riccardia* and a fifth lineage proposed as a new genus, *Afroriccardia* gen. nov. (see below). *Verdoornia* is sister to all other genera. The clade

Table 4. Comparison of results given by three different methods of species delimitation used for our study in terms of number of species hypotheses: ABGD, PTP and morphological delimitation.

	Loci	No. of groups (*Riccardia* + outgroups)	No. of groups (*Riccardia* only)
ABGD	*rps*4	53	46
	*psb*A-*trn*H	44	43
	*trn*L-F	65	49
	Total matrix	44	36
PTP (> 0.91 posterior delimitation probability)	*rps*4	13	8
	*psb*A-*trn*H	10	10
	*trn*L-F	10	7
	Total matrix	14	10
Morphology		58	41
Final no. retained		–	59

including *Aneura* and *Lobatiriccardia* is sister to the clade formed by *Afroriccardia* and *Riccardia*. The monophyly of each genus was strongly supported in all analyses.

Within the genus *Riccardia*, clades were usually very well supported, with few exceptions. In the case of conserved species hypotheses with the same name, two cases were observed: (1) all hypotheses formed a clade (*R. alcicornis* (Hook.f. & Taylor) Trevis, *R. conimitra* (Steph.) A.Evans, *R. elata* (Steph.) Schiffn., *R. pallida* (Spruce) Meenks & C.De Jong, *R. stipatiflora* (Steph.) Pagan); (2) species hypotheses were scattered through the tree, not forming a monophyletic group (*R. aeruginosa* Furuki, *R.* sp8), although this might also due to identification problems.

The origin of our identified samples was congruent with the published distribution of the taxa except for the New Caledonian sample of *Riccardia* cf. *nagasakiensis* (Steph.) S.Hatt. The latter species is considered endemic to Japan (Fig. 2).

Description of the new genus *Afroriccardia*

In the consensus tree *Afroriccardia comosa* is a strongly supported lineage sister to the genus *Riccardia* (Figs 1–2). The four samples of this taxon in the initial dataset (Appendix) were confirmed by all species delimitation analyses as a single molecular species. It resembles *Lobaticcardia* in the broad, pinnately branched thallus and the wide expansion of rhizoids on the ventral face (Furuki 1991; Preußing *et al.* 2010), and was therefore initially assigned to the latter genus by Reeb & Bardat (2014). Hence *Aneura comosa* Steph. is not cited in the world checklist (Nebel 2016). However, the species clearly differs from *Lobatiriccardia* by having long female branches (to 1 cm long) and two regular rows of gametangia. The latter two characters are shared with *Riccardia* but the dense clusters of rhizoids covering the archegonia clearly separate *Afroriccardia comosa* from *Riccardia*. Since the morphological differences with *Riccardia* are subtle, *Afroriccardia comosa* could be considered a separate subgenus of the latter. Based on the topology of the tree (Figs 1–2) and its early divergence from *Riccardia*, however, this taxon is placed here in the new genus *Afroriccardia*. The genus contains one species, *Afroriccardia comosa*, restricted to East Africa and the western Indian Ocean. The hierarchy below follows Crandall-Stotler *et al.* (2009) and Ruggiero *et al.* (2015).
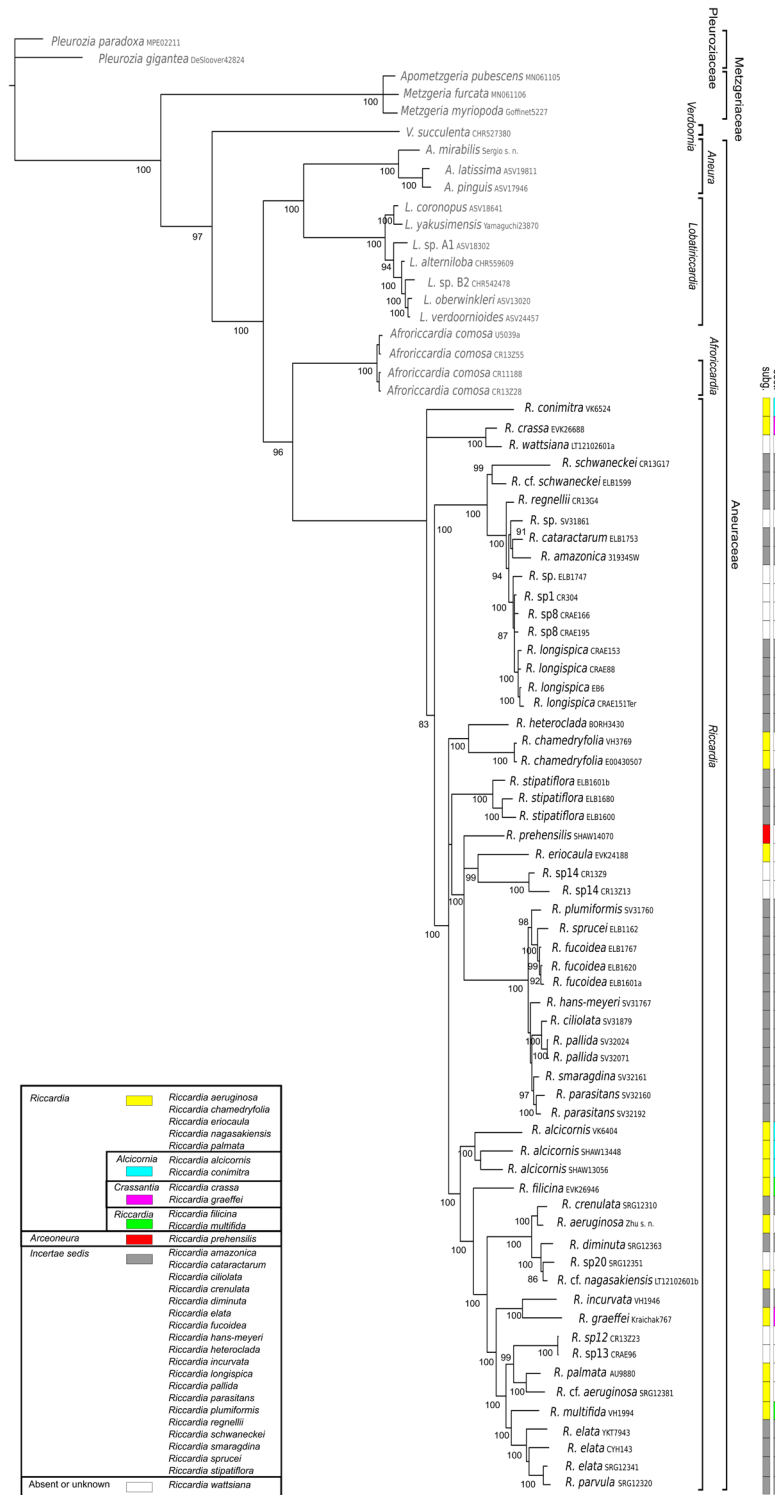
Fig. 1. The 50% majority-rule consensus of the trees generated by Bayesian Inference (BI) of the combined *psb*A-*trn*H, *rps*4 and *trn*L-F dataset. Posterior probability percentages above 80% are indicated under each node. Species names appear in italics, followed by the corresponding voucher number. Outgroup labels appear in light grey. Infra-generic divisions (sub-genera and sections) of *Riccardia* according to Nebel (2016) are reported in front of each name. The corresponding colour scheme for each sub-generic division is shown in the bottom left proportion of the figure.
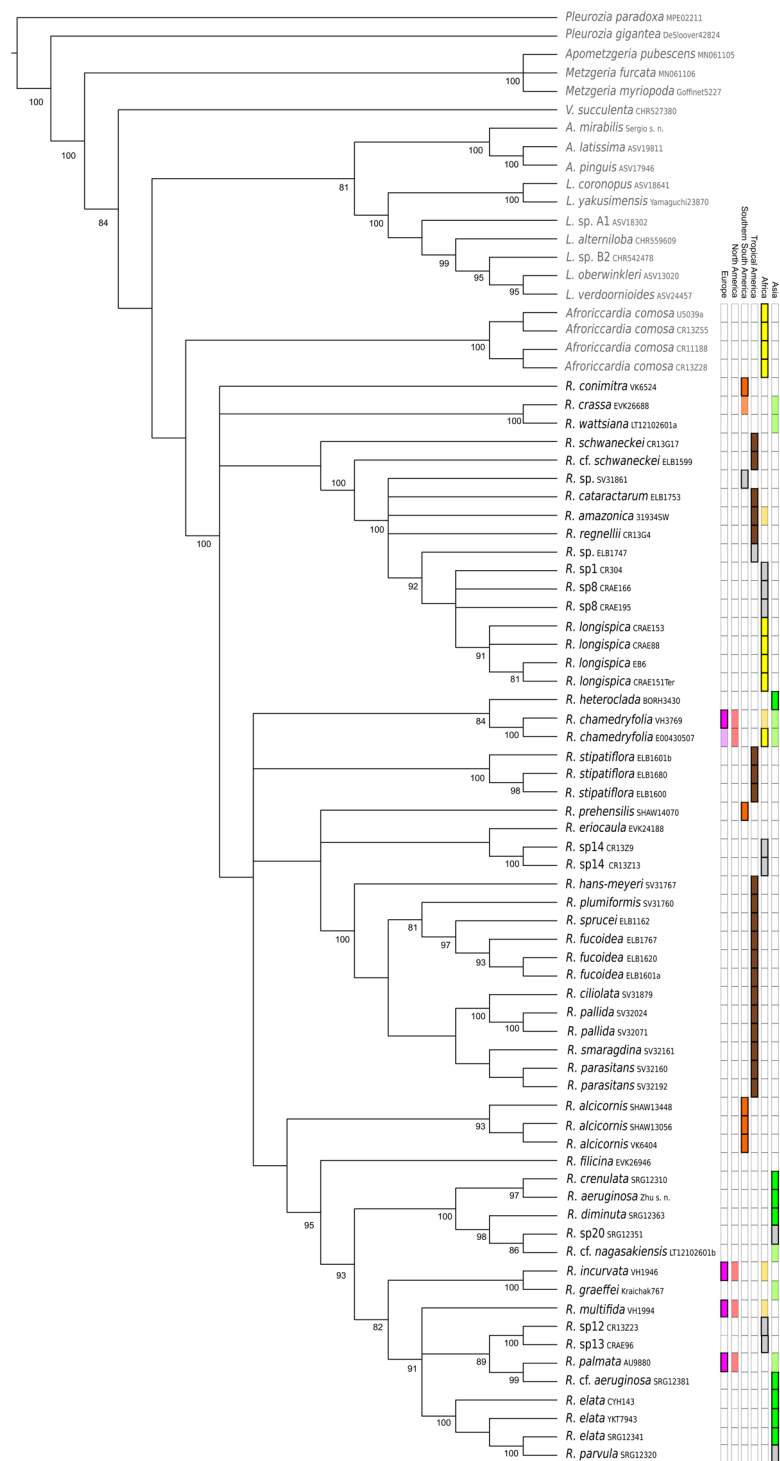
Fig. 2. This topology shows the result of the maximum parsimony ratchet. Bootstrap proportions above 80% are indicated under each node. Species names appear in italics, followed by the corresponding voucher number. Outgroup labels appear in light grey. The geographical origin of each *Riccardia* or *Afroriccardia* sample is marked by bold rectangles. The continental/geographical repartition of each species according to the literature is indicated with rectangles coloured by continents: Europe (purple), North America (red), southern South America (orange), tropical America (brown), Africa (yellow), Asia (green) and Australasia (blue). A grey rectangle indicates new continental records for the species.

Phylum Streptophyta Bremer (Bremer 1985)
Subdivision Marchantiophytina Doweld (Doweld 2001)
Class Jungermanniopsida Stotler & Crand.-Stotl. (Stotler & Crandall-Stotler 1977)
Order Metzgeriales Chalaud (Chalaud 1930)
Family Aneuraceae Klinggr. (Klinggräff 1858)

Genus *Afroriccardia* Reeb & Gradst. gen. nov.

Type species

Afroriccardia comosa (Steph.) Reeb & Gradst. (≡ *Aneura comosa* Steph.).

Diagnosis

Thallus (bi)pinnate. Main axis of thallus 2.5–4.0 mm wide. Rhizoids present over the whole width of the ventral thallus surface. Female branches to 1 mm long, archegonia in pairs, covered by a dense cluster of rhizoids with strongly thick-walled tips originating from beneath the apex of the female branch. Paraphyses lacking.

Remarks

Monospecific, contains only *A. comosa* from Madagascar, the Mascarene Islands and Uganda.

Afroriccardia comosa (Steph.) Reeb & Gradst. comb. nov.
Figs 3–4

Aneura comosa Steph., *Botanical Gazette* 15 (11): 281. (Stephani 1890). – *Riccardia comosa* (Steph.) E.W.Jones, *Transactions of the British Bryological Society* 3: 74. (Jones 1956, nom. inval.). – Type: France, La Réunion, 1889, *Rodriguez s.n.* (holo- : G-00045027!; iso- : PC-0103522!).

Material examined

MADAGASCAR: Angavokely Forest, humid rocks in caves, 18°55′16″ S, 47°44′30″ E, 1600 m, 2 Feb. 2011, *Reeb CR11188* (PC, TAN); Zahamena National Park, river crossing the camp, on rocks, 17°38′19″ S, 48°36′46″ E, 1156 m, 28 Dec. 2013, *Reeb & Andriamanantena, CR13Z28, CR13Z32* (PC, TAN); Zahamena National Park, on seeping rocks, highest part of the river crossing the camp, 17°38′22″ S, 48°38′45.3″ E, 1294 m, 30 Dec. 2013, *Reeb & Andriamanantena CR13Z55* (PC, TAN).

FRANCE, LA RÉUNION: "Sur les mousses, source pétrifiante de Hell-Bourge", *G. de l'Isle 220* (PC-0716023); "sous Piton de la Fournaise, le long GR2, Réserve de Mare Longue", 21°20′30″ S, 55°44′30″ E, 175–300 m, *Vojko 9435B* (EGR); without details, De Lisle, *De Lisle 570bis* (PC-0716024-G 00264057); *Rodrigues* s.d., s.n. (G-00264058); "plaine des palmistes", s.d., s.col. 56 (PC-0716026).

MAURITIUS: Without details, *Rodrigues* s.d. s.n. (PC-0716025).

UGANDA: Bwindi National Park, Rukungiri, "Kitahurira bridge. Damp rock surface by stream, shaded site in forest", 1480 m, 30 Jan. 1996, *Wigginton U5039A* (E-00430553).

Description

Dioicous. Thallus green, to 7 cm long, main axes 2.5–4.0 mm wide, creeping, ± regularly (bi-)pinnate, with 1–2 reiterations, branches alternate to subopposite, stolons not observed. Rhizoids developing over the whole width of the ventral surface of the thallus. Main axes plano-convex to biconvex, 6–8(–10) cells thick, margin entire, acute to rounded, un-winged, epidermal cells in cross section 1.5–2.0 × smaller than medullary cells, all cells thin-walled. Terminal branches to 8 mm long, 0.8–2.0 mm wide, 4–5 cells thick, with a conspicuous, 3–4(–6) cells wide wing, branch margins parallel, crenulate, thallus surface cells becoming smaller towards the margin, not or slightly bulging; branch apex rounded to truncate and

Fig. 3. *Afroriccardia comosa* (Steph.) Reeb & Gradst. comb. nov. Population close to sample *CR13Z28*, Zahamena National Park, Alaotra-Mangoro, Madagascar, 17°37′19″ S, 48°37′46″ E, altitude 1196 m. **A**. Photographs of the thallus. **B**. Magnified view of the thallus showing solitary female branches (red arrows). Scale bars = 1 cm.

usually narrowly incised (to 130 μm deep). Mucilage papillae on branches ca 20, present below the apex and in four rows on the ventral branch surface.

Female branches solitary or grouped on main axes and primary branches, 0.5–1.0 mm long, archegonia (unfertilized ones seen only) in pairs, covered by a dense cluster of rhizoids originating from beneath the apex of the female branches, rhizoids up to 0.7 mm long, with strongly thick-walled tips. Multicellular paraphyses lacking. Male branches, calyptra and sporophyte not seen. Vegetative reproduction not observed.

Distribution

Afroriccardia comosa is a rare species that was known only from a few old, 19th century collections from La Réunion and Mauritius; the species is newly reported here from Madagascar and Uganda. The species occurs in evergreen humid forest at mid-montane elevations in Uganda and Madagascar (1100–
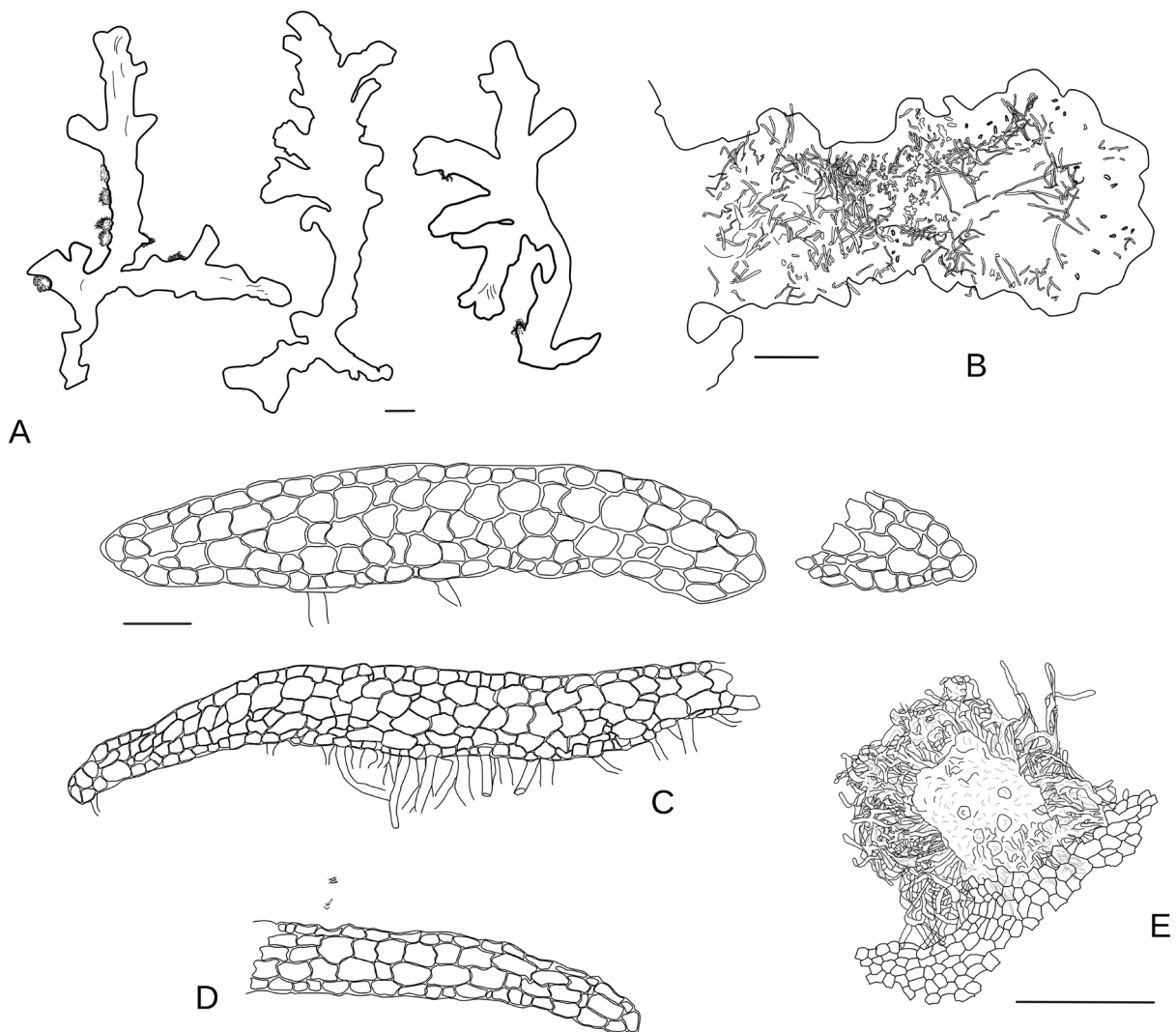

Fig. 4. *Afroriccardia comosa* (Steph.) Reeb & Gradst. comb. nov. **A**. Habit of the thallus, *Wigginton U5039a, Reeb & Andriamanantena CR13Z28*. **B**. Ventral face showing the wide insertion of rhizoids, *DeLisle 220*. **C**. Cross section of main axis showing variability in the thickening of cell walls, *Reeb & Andriamanantena CR13Z28*, holotype G0045027. **D**. Cross section of ultimate branch, holotype G0045027. **E**. Detail of female branch with dense cluster of rhizoids, holotype G0045027. Scale bars: A–B = 1 mm; C = 100 μm.

1600 m), and at lower elevations in La Réunion (175–300 m). Where habitat information is available, it was always collected on damp rock surfaces, in shaded places, close to water beds (shaded rivers, entrance of caves with water).

Discussion

Differences in success of amplification for the three markers

We observed a significant difference of the PCR success between the three plastid markers (Table 5). The matching of primers on *rps*4 sequences allowed us to detect variability near the 3' end of the two primers (F and R). This variability was only found in *Riccardia* and might affect primer annealing and thus reduce the amplification success. New primers were designed and tested in order to enhance *rps*4 PCR success (Tables 1–2). Only a part of *rps*4 could be amplified, for 40% of our final dataset. Improving this marker amplification for classical PCR still remains a challenge.

Most of the samples were herbarium specimens of various ages, dried under unknown conditions. Since these poikilohydrous plants are quite sensitive to humidity variation in their storage area, the DNA of these herbarium specimens can easily degrade. Internal primers generate shorter sequences, allowing amplification of deteriorated DNA. However, it implies an increase of experimentation time and budget, especially in the case of a large dataset.

Species delimitation

Molecular species delimitation using ABGD and PTP is sensitive to balanced sampling, especially in number of replicates per taxon (Puillandre *et al.* 2012; Zhang *et al.* 2013; Lang *et al.* 2015). The initial dataset (Appendix) contained singletons and some taxa with more than ten samples (*Riccardia chamedryfolia*). Some specimens of the latter species, from the Atlantic islands, Africa and Guadeloupe, were not initially identified as *R. chamedryfolia* but their proximity was revealed by the molecular analyses. Even if *psb*A-*trn*H, *trn*L-F and *rps*4 are located in the same area of the plastid genome (Wicke *et al.* 2011), they have not necessarily evolved in the same way or at the same speed (Preußing *et al.* 2010). ABGD was initially a unilocus tool based on calculated barcode gap outside the confidence interval (at 95%) of the population mutation rate θ, given a prior P of maximum intraspecific divergence (Puillandre *et al.* 2012; Fontaneto *et al.* 2015). We therefore analysed each gene independently. Although the results of the analysis of each of the three markers were rather similar, the dataset was most finely split with *psb*A-*trn*H. On the other hand, analysis with the concatenated markers did not separate all species. However, the latter has to be considered carefully. If the inversions that might occur at the population level (compare with Quandt *et al.* 2003), as, e.g., observed here in the *psb*A-*trn*H intergenic spacer, are not detected, the molecular species delimitation using ABGD and PTP will return a wrong concept (data not shown).

With PTP, even though the convergence of the runs was moderate to good, only singleton species were highly supported (> 0.91). Changing of parameters did not improve the results. It is possible that the PTP results were affected by unbalanced sampling and missing data.

Our phylogenetic results show that at least two species, *Riccardia aeruginosa* and *R.* sp8, are not monophyletic (Figs 1–2). These results may indicate that (1) samples were misidentified, (2) samples may represent undescribed species, (3) PCR contamination occurred, or (4) the species is paraphyletic. The latter case may be verified with tools such as Haplowebs (Fontaneto *et al.* 2015). Non-monophyly of species is frequently detected in bryophytes and molecular analyses are an important tool to reveal the existence of morphologically distinct species that would otherwise have remained undetected (e.g., Sukkharak *et al.* 2011; Hutsemékers *et al.* 2012; Aranda *et al.* 2014; Hedenäs *et al.* 2014; Heinrichs *et al.* 2015).

Table 5. Overview of PCR success depending on primer couples employed.

Region	Primers	Total PCR number	Successful PCR	Failed PCR	% success
*rps*4	rps5rev & trnS-F	139	46	93	33%
	rps4F3 & trnS-F	45	44	1	98.00%
	rps4 internal primers	32	0	32	0%
	total	216	90	126	41.70%
*trn*L-F	trnL-C & trnL-F	61	46	15	75.40%
	trnL-C & trnL-D	106	71	35	70.00%
	trnL-E & trnL-F	76	61	15	80.00%
	total	243	178	65	73.25%
*psb*A-*trn*H	*psb*A-*trn*H	81	79	2	97.50%

Some samples morphologically identified as the same species (*Riccardia alcicornis*, *R. elata*, *R.* sp14, *R. chamedryfolia*, *R. longispica*, *R. conimitra*, *R. stipatiflora*) were separated by at least one ABGD analysis but appeared to form a monophyletic group in all phylogenetic analyses. This could be due to (1) genetic variations among species and/or (2) high sensibility of the marker *psb*A-*trn*H, on which these delimitations were based.

The results indicate that the holarctic *Riccardia incurvata* Lindb., *R. multifida* (L.) Gray and *R. palmata* (Hedw.) Carruth. form a clade together with several African, Asian and Australasian species, but that the largely holarctic *R. chamedryfolia* is not a member of this clade (see also Preußing *et al.* 2010). *Riccardia chamedryfolia* is more widespread in the tropics and it seems to often be overlooked or misidentified (Schäfer-Verwimp *et al.* 2013; Nebel unpubl. res.).The results also provide robust evidence for an Andean clade (*R. fucoidea*, *R. parasitans* (Steph.) Meenks & C.De Jong, *R. pallida*, *R. ciliolata* (Spruce) Gradst., *R. smaragdina* Meenks & C.De Jong, *R. plumiformis* (Spruce) Hässel ex Meenks, *R. sprucei* (Steph.) Meenks & C.De Jong), earlier hinted at by Preußing *et al.* (2010).

The clade containing *Riccardia amazonica* (Spruce) Schiffn. ex Gradst. & Hekking, *R. longispica*, *R.* sp8 and *R. cataractarum* (Spruce) Schiffn. includes species from warm, low elevation areas of tropical Africa and tropical America. It is an interesting and somewhat puzzling group because of the strong polymorphy of some of the species in this group, contrasting with close genetic distances (Reeb unpubl. res.). All authors agree that *R. amazonica* is an Afro-American species (e.g., Meenks & Pócs 1985; Wigginton 2004; Perold 2003; but see Gradstein 2013). However, this is not supported by the experiment, showing that the spores of *R. amazonica* lose their capacity of germination after a few hours (Van Zanten & Gradstein 1988; Gradstein 2013), making successful long-distance-dispersal by spores unlikely. Some authors suggest polyploïdy as a possible explanation of the large range of morphological variability in *R. amazonica* (Berrie 1966). A closer look at this species is needed to improve our understanding of the delimitation and biogeography of this widely distributed and highly variable taxon.

Infrageneric placement

Subgeneric and sectional attribution of the species, following Nebel (2016) is shown on the consensus tree (Fig. 1). It appears that only two subgenera, subg. *Arceoneura* (*R. prehensilis* (Hook.f. & Taylor) C.Massal.) and subg. *Riccardia* (11 spp.) and three sections of subg. *Riccardia* (sects *Alcicornia*, *Crassantia* and *Riccardia*, each with 2 spp.) are represented in this study. The subgeneric or sectional placement of the great majority of *Riccardia* species analysed in this study is uncertain ("*incertae sedis*").

Therefore, only limited conclusions can be drawn here on the infrageneric placement of *Riccardia* species. The data indicate that the southern temperate sect. *Alcicornia*, represented here by *R. alcicornis* and *R. conomitra*, and the circumpacific sect. *Crassantia* (*R. crassa* (Schwägr.) Carrington & Pearson and *R. graeffii* (Steph.) Hewson) are polyphyletic because the species of these sections are placed in different lineages in the phylogeny. The two species in sect. *Riccardia*, *R. multifida* (type of the genus *Riccardia*) and *R. filicina* (Colenso) E.A.Hodgs., are nested in a clade together with three unclassified members of subg. *Riccardia* (*R. aeruginosa*, *R. nagasakiensis* and *R. palmata*) and four members of the *incertae sedis* group (*R. crenulata*, *R. diminuta*, *R. elata* and *R. incurvata*). This suggests that the latter four species belong in subg. *Riccardia*. The placement of *R. eriocaula* (Hook.) Besch. & C.Massal. and *R. chamedryfolia* in subg. *Riccardia* (Nebel 2016), is not supported by our phylogeny. *Riccardia eriocaula* is a morphologically highly unusual species that was placed in subg. *Arceoneura* by Brown & Braggins (1989).

Although our sampling has been insufficient to evaluate the infrageneric classification of *Riccardia*, these first results are suggestive of the very incomplete state of knowledge of the relationships of species within this large genus. A broader sampling of the genus, including representatives of the subgenera not analysed here, is needed to arrive at a better understanding of its phylogeny. In addition, revisions of species at continental and worldwide scales should be carried out, using an integrative taxonomy approach. In future, we plan to extend our sampling and use additional markers, including nuclear ones, in order to produce a more complete phylogeny, including reconstruction of character evolution in the worldwide genus *Riccardia*.

Acknowledgments

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Appendix. List of specimens, with country of origin (norm ISO 3166-1 alpha-3), family, ABGD partition, bPTP partition, species based on morphological identification, voucher number, herbarium acronym (see Thiers continuously updated) and GenBank accession number (* = missing data). Numbers in the ABGD columns represent the original number of the group given by ABGD for each sample. "Y" in the PTP column indicates retained PTP species. Taxon names in bold indicate samples that appear in Figs 1–2. The full dataset was used in ABGD and bPTP. ASV = Herbarium Alfons Schäfer-Verwimp. Numbered columns: 1, ABGD *rps4*; 2, ABGD *psbA-trn*H; 3, ABGD *trn*L-F; 4, ABGD total; 5, bPTP *rps4*; 6, bPTP *psbA-trn*H; 7, bPTP *trn*L-F; 8, PTP total.

Country	Family	1	2	3	4	5	6	7	8	Taxon	Voucher number	Herbarium	GenBank accession number *trn*L-F	GenBank accession number *rps4*	GenBank accession number *psbA-trn*H
NZL	Aneuraceae	2	4	2	2					**Riccardia crassa**	EVK26688	AK, PC	KX512012	KX512202	KX512124
NCL	Aneuraceae	2	4	3	2					**Riccardia wattsiana**	LT12102601a	PC	KX512013	KX512203	KX512125
GLP	Aneuraceae	3	3	4	3	Y	Y	Y	Y	**Riccardia schwaneckei**	CR13G17	PC	KX512014	KX512204	KX512123
CHL	Aneuraceae	4	11	5	4					**Riccardia conimitra**	VK6524	PC	KX512015	KX512205	KX512170
CHL	Aneuraceae	4	11	5	4					Riccardia conimitra	Goff. 11048	CONN, PC	KX512016	KX512206	KX512168
CHL	Aneuraceae	4	11	5	4					Riccardia conimitra	VK6473	PC	KX512017	KX512207	KX512169
ECU	Aneuraceae	5	1	6	5					**Riccardia sp.**	SV31861	PC	KX512018	KX512208	KX512105
FRA	Aneuraceae	6	17	7	6					Riccardia chamedryfolia	AU9728	PC	KX512019	KX512209	KX512178
FRA	Aneuraceae	6	17	7	6					Riccardia chamedryfolia	VH2060	PC	KX512020	KX512210	KX512179
FRA	Aneuraceae	6	17	7	6					**Riccardia chamedryfolia**	VH3769	PC	KX512021	KX512211	KX512177
GLP	Aneuraceae	7	18	8	7					**Riccardia stipatiflora**	ELB1680	PC	KX512022	KX512212	KX512191
GLP	Aneuraceae	7	18	8	7					Riccardia stipatiflora	CR13G33	PC	*	KX512213	KX512190
ECU	Aneuraceae	8	5	9	8					**Riccardia ciliolata**	SV31879	ASV, PC	KX512024	KX512214	KX512126
IDN	Aneuraceae	9	7	10	9					Riccardia sp20	SRG12311	PC	KX512025	KX512215	KX512147
IDN	Aneuraceae	9	7	10	9					**Riccardia sp20**	SRG12351	PC	KX512026	KX512216	KX512149
IDN	Aneuraceae	9	7	10	9					Riccardia sp20	SRG12314	PC	KX512027	KX512217	KX512148
CHL	Aneuraceae	10	6	11	10	Y	Y		Y	**Riccardia alcicornis**	SHAW13448	PC	KX512028	KX512218	KX512143
CHL	Aneuraceae	11	14	12	11	Y	Y	Y	Y	**Riccardia prehensilis**	SHAW14070	PC	KX512029	KX512219	KX512173
NZL	Aneuraceae	12	10	13	12					Riccardia filicina	EVK26948	AK, PC	KX512030	KX512220	KX512165
NZL	Aneuraceae	12	10	13	12					**Riccardia filicina**	EVK26946	AK, PC	KX512031	KX512221	KX512166
MDG	Aneuraceae	13	15	14	13					**Riccardia sp14**	CR13Z9	PC	KX512032	KX512222	KX512174
MDG	Aneuraceae	13	15	15	13					**Riccardia sp14**	CR13Z13	PC	KX512033	KX512223	KX512175
FRA	Aneuraceae	14	19	16	14					**Riccardia incurvata**	VH1946	PC	KX512034	KX512224	KX512195
FRA	Aneuraceae	14	19	16	14					Riccardia incurvata	VH2457	PC	KX512035	KX512225	KX512196

Country	Family	1	2	3	4	5	6	7	8	Taxon	Voucher number	Herbarium	GenBank accession number *trnL–F*	GenBank accession number *rps4*	GenBank accession number *psbA-trnH*
PYF	Aneuraceae	15	20	17	15	Y	Y	Y	Y	*Riccardia graeffei*	Kraichak767	PC	KX512036	KX512226	KX512197
MDG	Aneuraceae	16	8	18	16					*Riccardia* **sp12**	CR13Z23	PC	KX512037	KX512227	KX512151
MDG	Aneuraceae	16	8	18	16					*Riccardia* **sp13**	CRAE96	PC	KX512038	KX512228	KX512152
MDG	Aneuraceae	22	23	19	32					*Riccardia longispica*	CRAE44	PC	KX512039	KX512229	KX512108
MDG	Aneuraceae	22	27	19	32					*Riccardia longispica*	CRAE153	PC	KX512040	KX512230	KX512113
MDG	Aneuraceae	22	29	19	32					*Riccardia longispica*	EB6	PC	KX512041	KX512231	KX512115
MDG	Aneuraceae	22	23	19	32					*Riccardia longispica*	CRAE101	PC	KX512042	KX512232	KX512106
MDG	Aneuraceae	22	23	19	32					*Riccardia longispica*	PF2	PC	KX512043	KX512233	KX512112
REU	Aneuraceae	22	23	19	32					*Riccardia longispica*	CRAE88	PC	KX512044	KX512234	KX512120
MDG	Aneuraceae	22	23	19	32					*Riccardia longispica*	CRAE84	PC	KX512045	KX512235	KX512107
MDG	Aneuraceae	22	26	19	32					*Riccardia* **sp1**	CR304	PC	KX512046	KX512236	KX512111
MDG	Aneuraceae	22	30	19	32					*Riccardia* **sp8**	CRAE166	PC	KX512047	KX512237	KX512116
MDG	Aneuraceae	22	28	19	32					*Riccardia* **sp8**	CRAE195	PC	KX512048	KX512238	KX512114
MDG	Aneuraceae	22	32	19	32					*Riccardia longispica*	CRAE151Ter	PC	KX512049	KX512239	KX512119
GLP	Aneuraceae	23	24	20	33					*Riccardia cataractarum*	CR13G9	PC	KX512050	KX512240	KX512117
MTQ	Aneuraceae	23	24	20	33					*Riccardia cataractarum*	ELB1753	PC	KX512051	KX512241	KX512109
ECU	Aneuraceae	24	25	21	34					*Riccardia amazonica*	31934SW	ASV, PC	KX512052	KX512242	KX512110
GLP	Aneuraceae	25	2	22	17		Y	Y	Y	*Riccardia* cf. *schwaneckei*	ELB1599	PC	KX512053	KX512243	KX512122
MYS	Aneuraceae	26	41	23	18	Y				*Riccardia elata*	CYH143	KLU, PC	KX512054	KX512244	KX512159
IDN	Aneuraceae	27	42	24	18					*Riccardia elata*	SRG12341	PC	KX512055	KX512245	KX512161
IDN	Aneuraceae	27	42	25	18					*Riccardia parvula*	SRG12320	PC	KX512056	KX512246	KX512160
MYS	Aneuraceae	28	41	26	18					*Riccardia elata*	YKT7943	KLU, PC	KX512057	KX512247	KX512163
IDN	Aneuraceae	28	41	26	18					*Riccardia elata*	SRG12367	PC	KX512058	KX512248	KX512162
FRA	Aneuraceae	29	39	27	19					*Riccardia* **multifida**	VH1994	PC	KX512059	KX512249	KX512164
FRA	Aneuraceae	29	39	27	19					*Riccardia multifida*	VH934	PC	KX512060	KX512250	KX512157
FRA	Aneuraceae	30	9	28	20					*Riccardia* **palmata**	AU9880	PC	KX512061	KX512251	KX512155
FRA	Aneuraceae	30	9	28	20					*Riccardia palmata*	AU9877	PC	KX512062	KX512252	KX512156
FRA	Aneuraceae	30	9	28	20					*Riccardia palmata*	VH924	PC	KX512063	KX512253	KX512153
FRA	Aneuraceae	30	9	28	20					*Riccardia palmata*	VH2533	PC	KX512064	KX512254	KX512154
IDN	Aneuraceae	31	12	29	21	Y	Y	Y	Y	*Riccardia heteroclada*	BORH3430	BORH, PC	KX512065	KX512255	KX512171

Country	Family	1	2	3	4	5	6	7	8	Taxon	Voucher number	Herbarium	GenBank accession number *trnL–F*	GenBank accession number *rps4*	GenBank accession number *psbA–trnH*
CHL	Aneuraceae	32	13	30	22				Y	*Riccardia alcicornis*	SHAW13056	PC	KX512066	KX512256	KX512172
CHN	Aneuraceae	33	40	31	44		Y		Y	*Riccardia* cf. *aeruginosa*	SRG12381	HSNU, PC	KX512067	KX512257	KX512158
NZL	Aneuraceae	34	16	32	23	Y	Y	Y		*Riccardia eriocaula*	EVK24188	PC	KX512068	KX512258	KX512176
CHL	Aneuraceae	35	21	33	24	Y	Y	Y	Y	*Riccardia alcicornis*	VK6404	PC	KX512069	KX512259	*
GLP	Aneuraceae	36	18	34	7					*Riccardia stipatiflora*	ELB1600	PC	KX512070	KX512260	KX512192
GLP	Aneuraceae	37	44	35	7					*Riccardia stipatiflora*	ELB1730	PC	KX512071	KX512261	KX512194
GLP	Aneuraceae	37	44	35	7					*Riccardia stipatiflora*	ELB1601b	PC	KX512072	KX512262	KX512193
ECU	Aneuraceae	38	5	36	36					*Riccardia pallida*	SV32024	ASV, PC	KX512073	KX512263	*
ECU	Aneuraceae	38	37	36	36					*Riccardia pallida*	SV32071	ASV, PC	KX512074	KX512264	KX512135
ECU	Aneuraceae	39	36	37	37					*Riccardia parasitans*	SV32160	ASV, PC	KX512075	KX512265	KX512142
ECU	Aneuraceae	39	35	37	37					*Riccardia parasitans*	MB6654	PC	KX512076	KX512266	KX512130
ECU	Aneuraceae	39	35	37	37					*Riccardia parasitans*	MB6673	PC	KX512077	KX512267	KX512141
GLP	Aneuraceae	40	34	38	38					*Riccardia fucoidea*	ELB1620	PC	KX512078	KX512268	KX512133
GLP	Aneuraceae	40	34	39	38					*Riccardia fucoidea*	ELB1601a	PC	KX512079	KX512269	KX512134
BES	Aneuraceae	40	34	38	38					*Riccardia fucoidea*	WB51391	PC	KX512080	KX512270	KX512129
GLP	Aneuraceae	40	34	38	38					*Riccardia fucoidea*	CR13G29	PC	KX512081	KX512271	KX512137
MTQ	Aneuraceae	40	34	38	38					*Riccardia fucoidea*	ELB1757	PC	KX512082	KX512272	KX512139
MTQ	Aneuraceae	40	34	65	38					*Riccardia fucoidea*	ELB1767	PC	KX512083	KX512273	KX512128
ECU	Aneuraceae	41	5	40	39					*Riccardia hans-meyeri*	SV31767	ASV, PC	KX512084	KX512274	KX512131
ECU	Aneuraceae	42	38	41	40					*Riccardia parasitans*	SV32192	ASV, PC	KX512085	KX512275	KX512132
ECU	Aneuraceae	43	5	42	41					*Riccardia plumiformis*	SV31760	ASV, PC	KX512086	KX512276	KX512167
ECU	Aneuraceae	43	5	42	41					*Riccardia plumiformis*	MB7011	HSNU, PC	KX512087	KX512277	KX512136
GLP	Aneuraceae	44	34	43	42					*Riccardia sprucei*	ELB1162	PC	KX512088	KX512278	KX512138
ECU	Aneuraceae	45	5	44	43					*Riccardia smaragdina*	SV32161	ASV, PC	KX512089	KX512279	KX512140
IDN	Aneuraceae	46	7	45	9					*Riccardia diminuta*	SRG12363	PC	KX512090	KX512280	KX512144
IDN	Aneuraceae	47	7	46	9					*Riccardia crenulata*	SRG12310	PC	KX512091	KX512281	KX512145
CHN	Aneuraceae	48	7	47	9					*Riccardia aeruginosa*	Zhu. S. n.	HSNU, PC	KX512092	KX512282	KX512146
NCL	Aneuraceae	49	7	48	9					*Riccardia* cf. *nagasakiensis*	LT12102601b	PC	KX512093	KX512283	KX512150
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	05/339-Hb	E	KX512094	KX512284	KX512186
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	Wigg 05/168A	E	KX512095	KX512285	KX512182

Country	Family	1	2	3	4	5	6	7	8	Taxon	Voucher number	Herbarium	GenBank accession number *trn*L–F	GenBank accession number *rps4*	GenBank accession number *psb*A-*trn*H
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430505	E	KX512096	KX512286	KX512183
SHN	Aneuraceae	51	43	7	6					***Riccardia chamedryfolia***	E00430507	E	KX512097	KX512287	KX512189
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430526	E	KX512098	KX512288	KX512185
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430518	E	KX512099	KX512289	KX512180
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430517	E	KX512100	KX512290	KX512188
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430510	E	KX512101	KX512291	KX512187
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	E00430513	E	KX512102	KX512292	KX512181
SHN	Aneuraceae	51	43	7	6					*Riccardia chamedryfolia*	05/383-Hb	E	KX512103	KX512293	KX512184
MTQ	Aneuraceae	52	33	19	32					***Riccardia* sp.**	ELB1747	E	KX512104	KX512294	KX512121
GLP	Aneuraceae	53	31	49	35					***Riccardia regnellii***	CR13G4	PC	KX512023	KX512295	KX512118
MDG	Aneuraceae	1	22	1	1	Y				***Afroriccardia comosa***	CR13Z28	PC	KX512011	KX512201	KX512200
UGA	Aneuraceae	–	22	1	1					*Afroriccardia comosa*	U5039a	E	KX512008	–	KX512127
MDG	Aneuraceae	–	22	1	1					*Afroriccardia comosa*	CR11188	PC	KX512009	–	KX512199
MDG	Aneuraceae	–	22	1	1					*Afroriccardia comosa*	CR13Z55	PC	KX512010	–	KX512198
REU	Aneuraceae	17	–	50	25					***Aneura latissima***	ASV19811	ASV	FM210482.1	FM210626	–
GBR	Aneuraceae	17	–	51	26	Y			Y	***Aneura mirabilis***	Wickett 276	CONN	FM210481.1	DQ983846	–
DOM	Aneuraceae	17	–	52	25					***Aneura pinguis***	ASV17946	STU	FM210488.1	FM210632	–
NZL	Aneuraceae	19	–	54	28					***Lobatiriccardia alterniloba***	CHR559609	CHR	FM210493.1	FM210637	–
–	Aneuraceae	19	–	56	28					***Lobatiriccardia oberwinkleri***	ASV13020	ASV	FM210495.1	FM210639.1	–
AUS	Aneuraceae	19	–	57	28					***Lobatiriccardia* spec. A1**	ASV18302	ASV	FM210498.1	FM210642	–
NZL	Aneuraceae	19	–	58	28					***Lobatiriccardia* spec. B2**	CHR542478	CHR	FM210502.1	FM210645	–
ECU	Aneuraceae	19	–	56	28					***Lobatiriccardia verdoornioides***	ASV24457	ASV	FM210503.1	FM210646	–
MYS	Aneuraceae	50	–	55	28					***Lobatiriccardia coronopus***	ASV18641	ASV	FM210494.1	FM210638	–
JPN	Aneuraceae	50	–	59	28					***Lobatiriccardia yakusimensis***	Yamaguchi 23870	ASV	FM210506.1	FM210649	–
NZL	Aneuraceae	21	–	64	31	Y		Y	Y	***Verdoornia succulenta***	CHR527380	CHR	FM210522.1	FM210663	–
ITA	Metzgeriaceae	18	–	53	27					***Apometzgeria pubescens***	MN061105	STU	FM210491.1	FM210635	–
ITA	Metzgeriaceae	18	–	60	27					***Metzgeria furcata***	MN061106	STU	FM210507.1	FM210650	–
USA	Metzgeriaceae	18	–	61	27					***Metzgeria myriopoda***	Goffinet 5227	CONN	FM210508.1	DQ979339	–
PNG	Pleuroziaceae	20	–	62	29	Y		Y	Y	***Pleurozia gigantea***	De Sloover 42824	H	AY463582.1	AY462386	–
ECU	Pleuroziaceae	20	–	63	30	Y		Y	Y	***Pleurozia paradoxa***	MPE02211	STU	FM210509.1	FM210651	–