# **Research Article**

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# A molecular approach to the phylogeny of the moss genus *Pseudocrossidium* (Pottiaceae, Bryopsida) and its taxonomic implications

María J. Cano<sup>\*</sup>, Juan A. Jiménez, M. Teresa Gallego, and Juan Guerra

Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, Campus de Espinardo, Murcia 30100, Spain \*Author for correspondence. E-mail: mcano@um.es

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**Abstract** *Pseudocrossidium* is a genus of 21 species belonging to the Pottiaceae with the highest concentration of taxa and morphological variation found in South America. To investigate the evolutionary relationships among the species of *Pseudocrossidium* and other members of the Pottioideae, molecular phylogenetic reconstructions, using the nuclear ITS1-5.8S-ITS2, and the plastid *atpB-rbcL*, *trnG*, and *trnL-F*, has been performed because this genus has only been partially tested using molecular markers. Bayesian and maximum likelihood topologies show that the genus, as presently circumscribed, is polyphyletic. Consequently, the circumscription of *Pseudocrossidium* is amended and numerous taxonomic changes resulting from the molecular, morphological, and nomenclatural studies are proposed. The phylogenetic and morphologically divergent *Pseudocrossidium mendozense* is renamed as *Gertrudiella mendozensis*. *Pseudocrossidium linearifolium* and *P. porphyreoneurum* are representatives of the new genera *Barbulastrum* and *Helicobarbula*, respectively. *Pseudocrossidium carinatum* and *P. santiagense* are accommodated in a new genus Austrobarbula. Aloinella, nested in a paraphyletic *Pseudocrossidium*, is maintained at generic rank, apparently derived from *Pseudocrossidium*. *Barbula integrifolia*, *B. riograndensis*, and Tortula *jaffuelii* are transferred to *Pseudocrossidium*. The remaining species of *Pseudocrossidium* are maintained in this genus, pending further studies. Conflicts of the trees observed could be evidence of interspecific or intergeneric gene flow in various lineages in the Pottioideae.

Key words: Aloinella, atpB-rbcL, Barbula, Gertrudiella, molecular systematics, nrITS, Pottioideae, trnG, trnL-F.

# 1 Introduction

The Pottiaceae are one of the most taxonomically problematic families of mosses and by far the most species-rich moss lineage (Saito, 1975; Zander, 1993; Ochyra et al., 2008). More than 1400 species of this family are widely distributed in the world and they are characteristic of variable or harsh environments, forming a conspicuous portion of the vegetation of ruderal, arid land, alpine, or arctic areas (Zander, 1993). Due to its high diversity, the taxonomy of their genera has been complicated and its generic circumscription has been the subject of continued debate since the late 19th century and well into the 20th century. The main reason for these uncertainties is the considerable variability of taxonomically important characteristics, and their convergence and morphological reductions, as well as the reticulating nature of character variation (Ochyra et al., 2008). The latest intensive treatment of genera in this family was establishment by Zander (1993), who recognized 76 genera based on a morphological and cladistic analysis of the whole family at that level. A few years later, this same author reevaluated the evolutionary scheme of the family, readjusting the genera to 79 (Zander, 2006). Many small or monotypic genera are relatively well defined; however, for genera with large numbers of species and high morphological diversity, such as Barbula Hedw. or Trichostomum Bruch, taxonomists have not been able to properly delimit these taxa. The advent of molecular phylogenetics has considerably advanced our understanding of the evolution in bryophytes; however, most genera included in the Pottiaceae have not been the subject of extensive study using molecular data. Some of the genera studied, such as Didymodon Hedw., Erythrophyllopsis Broth., Hennediella Paris, Pleurochaete Lindb., or Tortula Hedw., have been resolved as paraphyletic or polyphyletic molecular entities (Werner et al., 2002; Grundmann et al., 2006; Cano et al., 2009, 2010b; Jiménez et al., 2021). In many cases, the polyphyly caused by molecular homoplasy and the use of plesiomorphic characters in taxon circumscriptions have required the reestablishment or description of new genera such as in

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Barbula (Kučera et al., 2013). This tendency has also been followed using macroevolutionary systematics studies applied to the Pottiaceae, where essentially different sections of broad genera, such as *Leptodontium* (Müll. Hal.) Lindb. or *Didymodon*, have been raised to the generic level (Zander, 2013, 2017, 2019a). On the contrary, *Chionoloma* Dixon, *Oxystegus* (Limpr.) Hilp., and *Pseudosymblepharis* Broth., were merged into a single genus based on molecular phylogenetic inference and morphology, for which the oldest name *Chionoloma* has priority (Alonso et al., 2016).

*Pseudocrossidium* R.S. Williams is a dioicous genus of 21 species (Zander, 1993; Cano, 2011; Cano et al., 2011, 2015, 2016), with the highest concentration of taxa and morphological variation in South America (Fig. 1). In fact, 12 endemic species occur in this area and only four species presently included in this genus have not been reported in this territory. Since the genus was erected

by Williams (1915) to include three South American species (P. apiculatum R.S. Williams, P. chilense R.S. Williams, and P. excavatum (Mitt.) R.S. Williams), many species traditionally included in other genera, such as Barbula, Crossidium Jur., Desmatodon Brid., or Tortula, have been transferred to Pseudocrossidium (Thériot, 1920, 1921; Brotherus, 1924; Delgadillo, 1975; Zander, 1979; Crum & Anderson, 1989). Zander (1993) expanded the concept of the genus by including species previously included mainly in Barbula. According to this author, important characteristics for this genus are the usual small size, absence of a ventral stereid band, and the broadly crescentshaped dorsal stereid band of the costa, with a clearly differentiated dorsal epidermis of cells often with semicircular lumina through differential thickening of the walls. Other important characteristics often found are leaves ending in a short or long awn, differentiation of photosynthetic tissue either



Fig. 1. Morphological diversity in Pseudocrossidium and allied genera. A, Aloinella galeata var. andina (Cano 217, MUB).
B, Pseudocrossidium linearifolium (Cano & Alonso 8473a, MUB).
C, Pseudocrossidium mendozense (Cano et al. 4381a, MUB).
D, Pseudocrossidium santiagense (Cano 372, MUB).
E, Pseudocrossidium porphyreoneurum (Perold & Koekemoer 3597, PRE).
F, Pseudocrossidium chilense (Cano 136, MUB). Photos: C. Aedo.

as a ventral pad of costal filaments or within rolled margins or both, medial paracostal cells more papillose than the marginal ones, and perichaetial leaves abruptly enlarged with a sheathing base (Cano et al., 2016). However, many of the characteristics mentioned above such as broadly crescent-shaped dorsal stereid band of the costa, with a clearly differentiated dorsal epidermis of cells and sheathing perichaetial leaves, can also be found in other closely related genera, such as Barbula. In fact, the limits between many Pottioideae genera are not vet clear (Zander, 1993, 2006; Spagnuolo et al., 1999; Werner et al., 2004). Zander's (1993) delimitation of Pseudocrossidium included 16 species, which has been widely accepted in most of the checklists and world floras (e.g., Zander, 1994, 2007; Churchill & Linares, 1995; Churchill et al., 2000, 2009; Cortini Pedrotti, 2001; Li et al., 2001; Allen, 2002; Ignatov & Ignatova, 2003; Smith, 2004; Guerra et al., 2006). Since the publication of Zander (1993), two species recognized in Pseudocrossidium have been transferred to Andinella J.A. Jiménez & M.J. Cano (Jiménez et al., 2012; Jiménez & Cano, 2020), recently considered as a synonym of Gertrudiella Broth. (Jiménez et al., 2021), two taxa have been elevated or considered again at the rank of species (Eckel et al., 1997; Zander, 2007; Jiménez et al., 2009), three species have been resurrected from synonymy (Cano et al., 2016), and two have been newly described (Cano et al., 2011, 2015). Therefore, currently, 21 species are recognized in Pseudocrossidium. However, the genus has only been partially tested using molecular markers. Only a very few representatives have been included as outgroups in more general phylogenetic studies of the Pottiaceae. For example, one accession of Pseudocrossidium hornschuchianum (Schultz) R.H. Zander and another of P. porphyreoneurum (Müll. Hal.) R.H. Zander were used in a preliminary reconstruction of the phylogeny for the Pottiaceae based on rps4 (Werner et al., 2004). Shaw et al. (2005) sequenced rps4 and nad5 of Pseudocrossidium aureum (E.B. Bartram) R.H. Zander (presently as P. arenicola (Dusén) M.J. Cano). Werner et al. (2005), in their preliminary reconstruction of the phylogeny of the genus Didymodon, included nrITS sequences of P. hornschuchianum and P. revolutum (Brid.) R.H. Zander. The same species were evaluated by Jiménez et al. (2012) based on two plastid markers in their phylogenetic study of the genus Andinella, by Jiménez et al. (2021), using nrITS, atpBrbcL, trnG, and trnL-F in the phylogenetic study of Didymodon and allied genera, and by Kučera et al. (2013), based on nrITS, rps4, and trnM-trnV sequences in their partial phylogenetic approach of the genus Barbula. In addition, a specimen of Pseudocrossidium denticulatum (Dusén) M.J. Cano was used to investigate the phylogenetic relationships of Didymodon tophaceus (Brid.) Lisa and allied species based on nrITS, rps4, and trnM-trnV (Kučera et al., 2018). Finally, the complete chloroplast and mitochondria genome sequences for one specimen of Pseudocrossidium replicatum (Taylor) R.H. Zander have been recently published (Cevallos et al., 2019, 2020).

The only molecular phylogenetic study on representatives of this genus was performed by Cano et al. (2016). They conducted an approach focused on *Pseudocrossidium crinitum* (Schultz) R.H. Zander, a dioicous moss distributed mainly in the Southern Hemisphere, which had been treated as a single species, in spite of its phenotypical variability. To place *P. crinitum* in a wider phylogenetic context, five additional species of *Pseudocrossidium* (*P. apiculatum, P. excavatum, P. hornschu-* chianum, P. replicatum, and P. revolutum) were included in this study. Morphometric methods (PCA; DA) based on quantitative characteristics and molecular variation using the plastid regions *trnL-F* and *trnG* suggested that this putative taxon consists of at least five different species, which could belong to different lineages in the Pottiaceae. Thus, P. carinatum (Gillies ex Grev.) R.H. Zander and P. santiagense (Broth.) M.J. Cano showed a considerable phylogenetic distance with respect to the rest of the species of Pseudocrossidium included in this study and suggested the possible polyphyly of this genus, although the molecular results were unsatisfactory with regard to the relationship between the different species.

Within the framework of a current taxonomic revision of *Pseudocrossidum*, the present study aims to achieve the following goals: (i) investigate the suspected polyphyly of *Pseudocrossidium*; (ii) reconstruct a complete phylogeny for the genus based on extensive sampling, including other members of the Pottioideae; and (iii) to identify any evidence for cryptic speciation or reticulate evolutionary processes within *Pseudocrossidium* s.l.

# 2 Material and Methods

### 2.1 Taxon sampling

For morphological delimitation and characterization of the species, we studied approximately 2200 specimens of Pseudocrossidium from 37 herbaria (B, BCB, BM, BOL, CANM, DR, E, EGR, F, FH, FI, FLAS, FT, GDA, H, JE, LISU, M, MA, MEL, MEXU, MGC, MICH, MO, MUB, NY, PAMP, PC, PRE, RO, S, SGO, SP, U, US, VAL, VIT), including type specimens of all currently recognized species. For molecular studies, we used 88 specimens of Pseudocrossidium belonging to the 21 currently recognized species (Zander, 1993; Cano, 2011; Cano et al., 2011, 2015, 2016). The phylogenetic reconstruction includes samples from field collections made in Argentina, Bolivia, Chile, Ecuador, Peru, and Venezuela, as well as herbarium material from BOL, F, MEL, MO, MUB, NY, PRE, and SP. We have tried, when available, to represent the morphological diversity observed and the distribution range of the species. Due to the fact that many species presently combined in Pseudocrossidium have been previously included in Barbula and Tortula, we also included some South American species of these genera that could potentially belong to Pseudocrossidium, such as Barbula integrifolia (R.S. Williams) R.H. Zander, Barbula riograndensis E.B. Bartram, and Tortula jaffuelii Thér. Other members of the Pottioideae were included to cover the variation of the subfamily as outgroups: Acaulon Müll. Hal. (1 sp.), Aloina Kindb. (2 spp.), Aloinella Cardot (2 spp.), Barbula (1 sp.), Bryoerythrophyllum P.C. Chen (2 spp.), Bulbibarbula (Müll. Hal.) R.H. Zander (1 sp.), Cinclidotus P. Beauv. (1 sp.), Chenia R.H. Zander (1 sp.), Crossidium (1 sp.), Didymodon (2 spp.), Dolotortula R.H. Zander (1 sp.), Erythrophyllopsis (1 sp.), Geheebia Schimp. (1 sp.), Gertrudiella (6 spp.), Guerramontesia M.J. Cano, J.A. Jiménez, M.T. Gallego & J.F. Jiménez (1 sp.), Hennediella (2 spp.), Hilpertia R.H. Zander (1 sp.), Husnotiella Cardot (1 sp.), Leptodontium (1 sp.), Microbryum Schimp. (2 spp.), Pararhexophyllum Jan Kučera (1 sp.), Pterygoneurum Jur. (1 sp.), Rhexophyllum Herzog (1 sp.), Sagenotortula R.H.

Zander (1 sp.), Stegonia Venturi (1 sp.), Syntrichia Brid. (1 sp.), Tortula (3 spp.), Trichostomopsis Cardot (1 sp.), Tridontium Hook. f. (1 sp.), Triquetrella Müll. Hal. (1 sp.), and Vinealobryum R.H. Zander (1 sp.). Guerramontesia was used to root the phylogeny, given that this species is supported as sister to the rest of the subfamily (Cano et al., 2010a, 2016; Gallego et al., 2014; Jiménez et al., 2021).

Specimen data are listed in Appendix S1, including information on locality, herbarium references, and GenBank accession numbers. All sequences were newly generated for these analyses, except for 213 sequences that were downloaded from GenBank, prioritizing those identified or published previously by us. Moreover, DNA of 31 specimens already sequenced for one or two of the selected loci in Cano et al. (2016) and submitted to GenBank were amplified for the remaining markers.

### 2.2 DNA extraction, amplification, and sequencing

Plant tissue from the distal portions of gametophore shoots was isolated from herbarium specimens or freshly collected material. Whole-genome DNA was extracted either using the CTAB method (Doyle & Doyle, 1987) with some minor modifications or with the DNeasy Plant Mini kit (QIAGEN, Madrid, Spain) following the manufacturer's instructions. We selected four loci; three from the chloroplast genome, the *atpB-rbcL* intergenic spacer region (*atpB-rbcL*), the  $trnG_{UCC}$  G2 intron (trnG), and the trnL<sub>UAA</sub> exon trnF<sub>GAA</sub> region (trnL-F), as well as the nuclear internal transcribed spacers 1 and 2 (ITS1-5.8S-ITS2). The ITS1 and ITS2 were either amplified and sequenced separately or in a single amplification. All loci have been shown to be useful for phylogenetic reconstruction in the Pottiaceae (Cano et al., 2009; Cano et al., 2010a, 2010b; Jiménez et al., 2012, 2021; Alonso et al., 2016). The primer pairs used for each locus were atpB-1/rbcL-1 (Chiang et al., 1998), trnG-F/trnG-R (Pacak & Szweykowska-Kulińska, 2000), trnC/trnF (Taberlet et al., 1991), ITS5-bryo/ ITS4-bryo (Stech & Frahm, 1999), ITS1-F/ITS1-R (Sawicki & Szczecińska, 2011), and seqITS2 (Olsson et al., 2009).

Amplification reactions were performed using the Eppendorf Mastercycler in a 25  $\mu$ l volume containing 1  $\mu$ l of Tag DNA Polymerase (1 U/ $\mu$ l; Biotools, Madrid, Spain), 2.5  $\mu$ l of Mg<sup>2+</sup> buffer provided by the manufacturer, 2  $\mu$ l of 2.5 mM dNTP mix, 1.5  $\mu$ l of each primer (10  $\mu$ M), and 1  $\mu$ l of the DNA extract. Thermocycling conditions for *atpB-rbcL*, trnG, and trnL-F were as follows: 95 °C for 4 min linked to 38 cycles at 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 40 s, with a final extension of  $72 \degree C$  for 10 min. For the amplification of nrITS, the cycling conditions were identical, with the exception of the elongation step, which had a duration of 1 min instead of 40 s. PCR products were visualized on a 1% agarose gel. Successful amplifications were purified using the GenElute PCR Clean-Up kit (Sigma-Aldrich, St. Louis, Missouri) and sequenced at Macrogen Spain (Madrid, Spain).

### 2.3 Sequencing editing and model testing

Nucleotide sequence contigs were edited and assembled for each DNA region using Geneious 9.1.8 (Kearse et al., 2012). Consensus sequences were aligned using default parameters of MUSCLE (Edgar, 2004) implemented in Geneious with subsequent manual adjustments. Regions of partially incomplete data at the beginning and end of the sequences were identified and excluded from subsequent analyses. Insertions and deletions (indels) were coded using SeqState v.1.4.1 (Müller, 2005) using a simple coding model as suggested by Simmons & Ochoterena (2000). The indels provided additional phylogenetic evidence and we present the analyses with these included.

Each gene partition was tested for the best-fit substitution model using jModelTest v.2.1.6 (Darriba et al., 2012) under the Akaike information criterion (AIC). The selected models were TVM+1+G (Posada, 2003) for trnG, TPM1uf+1+G (Kimura, 1981) for trnL-F, TIM+1+G (Posada, 2003) for nrITS, and GTR+1+G (Tavaré, 1986) for *atpB-rbcL*.

# 2.4 Phylogenetic analyses

Molecular phylogenetic relationships were examined using both maximum likelihood (ML) and Bayesian inference (BI). Analyses were performed separately on each data set and the chloroplast data were combined afterward. To check for incongruence among the plastid data sets and between plastid versus ITS partitions, phylogenetic reconstructions under ML and BI were visually compared. The node bootstrap support of  $\geq$ 70 in the ML analysis and posterior probability  $\geq$ 0.95 were chosen as values for supported incongruence.

Maximum likelihood analyses were performed using RAxML (Stamatakis, 2014) through the graphical front-end raxmlGUI v.2.0 (Edler et al., 2019). A rapid bootstrap option with 1000 replicates and search for the best-scoring ML tree were conducted under the GTRCAT model for all concatenated and individual gene data sets. Nodes with bootstrap (BS) values equal to or above 70% were treated as well supported.

Bayesian inference (BI) analyses were performed using MrBayes v.3.2.6 (Ronquist et al., 2012) on the CIPRES Gateway v.3.3 (Miller et al., 2012), running a partitioned analysis and specifying a substitution model for each block. The data were analyzed using Markov chain Monte Carlo (MCMC), running two parallel analyses with four chains each for 30 million generations, sampling trees, and parameters every 1000 generations. Chain convergence and stationarity were checked in Tracer v.1.7 (Rambaut et al., 2018), making sure that the average standard deviation of split frequencies remained below 0.01. Twenty-five percent of the trees were discarded as burn-in, and a 50% majority-rule consensus tree was constructed. The resulting trees for both ML and BI analyses were visualized and partially edited in FigTree v.1.4.4 (Rambaut, 2012). Posterior probabilities (PP) of 0.95–1.00 were considered to indicate strong support.

# 3 Results

# 3.1 Phylogenetic analyses

We generated a total of 262 new sequences: 93 of ITS, 56 of *trnL-F*, 50 of *trnG*, and 63 of *atpB-rbcL*. We combined them with sequences available from GenBank and generated three data sets: the combined plastid database, the nrITS database, and the concatenated plastid-ITS database. The

combined alignment of three plastid loci consists of 127 specimens with 1789 characteristics. The locus atpB-rbcL is represented by 626 nucleotides, of which 111 (17.7%) are parsimony informative, trnG by 693 nucleotides, of which 134 (19.3%) are parsimony informative, trnL-F by 470 nucleotides, of which 89 (18.9%) are parsimony informative. The nrITS is represented by 1621 nucleotides, of which 768 are parsimony informative (47.3%). As expected, the chloroplast loci contributed less phylogenetic information than ITS. The BI and ML analyses of the single marker data sets (trnL-F, trnG, atpB-rbcL, nrITS) had nearly identical topologies, and only the Bayesian topologies are shown (Figs. 2-4), with bootstrap support (BS) as well as posterior probabilities (PP) indicated where applicable. The congruence between nuclear and plastid loci was assessed by comparing individual trees and indicating disagreements.

The topologies of trees inferred from each individual region as well as from the combined plastid, nuclear data, and combined plastid and nuclear data confirm that *Pseudocrossidium* as understood by Zander (1993) is polyphyletic. Thus, the species traditionally placed in this genus are distributed in multiple major clades, labeled as clades A–E, G–K in Figs. 2–4. In general, the topology was rather well resolved, but support for the deep nodes was often missing or low, which does not allow us to discuss the position of the genera in the Pottioideae.

# 3.2 Analysis of ITS sequences

The phylogenetic tree based on ITS sequences (Fig. 2) shows a polytomy comprised of a clade with Leptodontium excelsum (Sull.) E. Britton and a well-supported clade of Triquetrella arapilensis Luisier plus Bulbibarbula eubryum (Müll. Hal.) R.H. Zander (PP = 0.99; BS = 99) and another clade with the remaining accessions currently considered as Pottioideae (PP = 1; BS = 100). At the next level, relationships are generally poorly resolved and only a few lineages appear strongly supported in the Pottioideae: Erythrophyllopsis and Bryoerythrophyllum (PP = 0.99; BS = 93); species of Aloina (PP = 1; BS = 100); accessions of Barbula unguiculata Hedw. (PP = 1; BS = 100); a supported clade by BI (PP = 0.98)divided into two subclades: Dolotortula sister to Microbryum plus Acaulon (BS = 80), and Chenia leptophylla (Müll. Hal.) R.H. Zander sister to Hennediella, Tortula, and the related genera, including Crossidium, Pterygoneurum, Stegonia, and Hilpertia (PP = 1; BS = 98); Syntrichia ruralis (Hedw.) F. Weber & D. Mohr and Sagenotortula quitoensis (Taylor) R.H. Zander (PP = 1; BS = 100) sister to a clade consisting of Didymodon and allied genera (Geheebia, Gertrudiella, Husnotiella, Trichostomopsis, Tridontium, Vinealobryum) and part of Pseudocrossidium (PP = 1; BS = 97); Pseudocrossidium porphyreoneurum and an apparently undescribed species (PP = 1; BS = 100) and Pseudocrossidium-core clade (PP = 1;BS = 78). Pseudocrossidium linearifolium (Müll. Hal.) J.A. Jiménez & M.J. Cano (clade A) forms a separate lineage from the rest of specimens of Pseudocrossidium, sister to one accession of Cinclidotus riparius (Host ex Brid.) Arn. in a clade that also includes Rhexophyllum and Pararhexophyllum, but all these relationships are not supported. In the clade formed by Didymodon and allied genera, the only accession of Pseudocrossidium mendozense (Mitt.) R.H. Zander is merged in Gertrudiella (clade B), specifically sister to Gertrudiella oedocostata (J.A. Jiménez & M.J. Cano) J.A. Jiménez & M.J. Cano (PP = 1; BS = 100) and the accessions of Pseudocrossidium santiagense (Broth.) M.J. Cano and P. carinatum form a supported clade C (PP = 1; BS = 100) sister to Geheebia and Vinealobryum, without any significant support values. The strongly supported clade D (BS = 100; PP = 1) is formed by accessions of Pseudocrossidium porphyreoneurum and one apparently undescribed species from Ecuador, as mentioned above. This clade is recovered sister to the remaining species of Pseudocrossidium, although this relationship is only supported by BI (PP = 0.97). The bulk of Pseudocrossidium species are included in a strongly supported Pseudocrossidium core clade (PP = 1; BS = 78). However, it is only monophyletic if Aloinella and some species morphologically similar to Pseudocrossidium, such as Tortula jaffueli, Barbula riograndensis, and Barbula integrifolia, are included. Within the Pseudocrossidium core, two clades can be identified supported by BI: one (PP = 0.98) comprises clade E (PP = 1; BS = 100), clade F (PP = 1; BS = 100), and clade G (PP = 1; BS = 87) and the other (PP = 1) includes clade H plus a maximally supported clade I (PP = 1; BS = 100) sister to clade J (PP = 1; BS = 98), plus clade K (PP = 1; BS = 92). Clades J and K are supported as sister lineages (PP = 0.98). In clade E, specimens of Pseudocrossidium chilense R.S. Williams are sister to the species of Aloinella (PP = 1; BS = 99), which are in turn sister to P. adustum (PP = 1; BS = 100). Clade F is composed only by the accessions of Tortula jaffuelii and sister to a well-supported clade G, which comprises P. excavatum, P. hornschuchianum, P. obtusulum (Lindb.) H.A. Crum & L.E. Anderson, and P. revolutum. However, this presumed sister relation receives no significant support. In clade G, P. excavatum is sister to P. hornschuchianum, P. obtusulum, and P. revolutum (PP = 1; BS = 87). Within this latter subclade, P. revolutum is resolved as monophyletic, being sister to a clade comprising the accessions of P. hornschuchianum and P. obtusulum nested (PP = 1; BS = 93). Clade H is composed of the accessions of P. apiculatum and P. pachygastrellum (Herzog) Broth. but the relationship with the remaining species of Pseudocrossidium is unresolved. The strongly supported clade I (PP = 1; BS = 100) accommodates samples of P. perpapillosum M.J. Cano & J.A. Jiménez nested with accessions of P. leucocalyx (Mont.) Thér. The highly supported clade J (PP = 1; BS = 98) accommodates the accessions of P. exiguum M.J. Cano & J.A. Jiménez, P. arenicola, P. replicatum, and P. austrorevolutum (Besch.) R.H. Zander. Within this latter group, some subclades such as the accessions of P. exiguum or P. austrorevolutum are supported. Finally, in the strongly supported clade K (PP = 1; BS = 92), there is successive branching of supported clades, with P. denticulatum (Dusén) M.J. Cano branching off first, followed in succession by B. integrifolia, B. riograndensis, and P. crinitum.

# 3.3 Analysis of combined plastid sequences

The phylogenetic tree derived from combined plastid sequences (Fig. 3) shows a well-supported clade with *Leptodontium excelsum* and *Triquetrella arapilensis* recovered at the base (PP = 1; BS = 97). After that, a clade consisting of *Didymodon* and allied genera plus *Pseudocrossidium mendo*zense is well supported (PP = 1; BS = 88), with *P. mendozense* 



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merged in Gertrudiella (clade B). The next split places the accessions of Pseudocrossidium linearifolium (Clade A) in an isolated lineage (PP = 1; BS = 100). The remaining species of Pottioideae are resolved in a well-supported clade (PP = 1; BS = 89), forming a polytomy with Rhexophyllum and Pararhexophyllum (PP = 1; BS = 99) placed in a sister position. Clades moderately to strongly supported in this clade are Erythrophyllopsis and Bryoerythrophyllum (PP = 0.96); Microbryum and Acaulon (PP = 1; BS = 83); Chenia, Sagenotortula, Syntrichia, Hennediella, Tortula, and related genera, including Crossidium, Pterygoneurum, and Stegonia (PP = 0.98); clade D, formed by Pseudocrossidium porphyreoneurum and an undescribed species of Pseudocrossidium (PP = 1; BS = 100); clade E composed by Pseudocrossidium chilense and P. adustum (BS = 76) sister to Aloinella (PP = 1; BS = 100); clade C formed by the accessions of Pseudocrossidium santiagense sister to P. carinatum (PP = 1; BS = 93); and the remaining species of Pseudocrossidium plus Barbula unguiculata, Cinclidotus riparius, B. riograndensis, B. integrifolia, and Tortula jaffuelii in a well-supported clade by BI (PP = 0.97) and weakly supported by ML (BS = 69, data not shown). Within this latter clade, the following well-supported subclades can be identified: clade J comprises the accessions of P. exiguum, P. replicatum, P. austrorevolutum, and P. arenicola (PP = 1; BS = 100); a clade composed of Barbula unguiculata and Cinclidotus riparius (PP = 1; BS = 99); clade F comprises Tortula jaffuelii sister to clade K (PP = 0.99) with Pseudocrossidium denticulatum sister to a clade with Barbula integrifolia, B. riograndensis, and P. crinitum (PP = 0.98; BS = 70; clade I includes P. perpapillosum merged in P. leucocalyx (PP = 1; BS = 96); clade H is composed of P. apiculatum and P. pachygastrellum (PP = 0.99; BS = 99); and clade G includes P. excavatum sister to P. hornschuchianum, P. obtusulum, and P. revolutum (PP = 0.98; BS = 74), P. obtusulum being nested within P. hornschuchianum (PP = 1; BS = 100).

# 3.4 Analysis of combined plastid and ITS sequences

The overall topology of the combined phylogram (Fig. 4) is similar to that of the ITS-derived tree. Accessions of Pseudocrossidium linearifolium (Clade A) form a separate lineage sister to most of the Pottioideae species included in this study, although this relationship is not supported. Barbula unguiculata is sister to Cinclidotus riparius in an isolated position. Clade D (P. porphyreoneurum plus Pseudocrossidium sp.) is placed sister to the remaining species of Pseudocrossidium by BI and sister to Aloina, plus Bryoerythrophyllum and Erythrophyllopsis by ML, in both cases without statistical support. Clade C (P. santiagense plus P. carinatum) is placed sister to a clade composed by Geheebia and Gertrudiella, including P. mendozense (clade B), Didymodon, Husnotiella, Trichostomopsis, Tridontium, and Vinealobryum. The Pseudocrossidium core is clearly monophyletic (PP = 1; BS = 84), although the combined plastid and ITS data sets provide a novel hypothesis of a sisterclade relationship between Clade E (*P. adustum-P. chilense-Aloinella*) and the remaining species of *Pseudocrossidium* supported only by BI. The remaining species form a polytomy with the following clades: clade J (PP = 1; BS = 100), clade H (PP = 1; BS = 100) sister to clade I (PP = 1; BS = 100), clade K (PP = 1; BS = 100), and clade F (PP = 1; BS = 100) sister to clade G (PP = 1; BS = 93); however, these presumed sister relationships receive no significant support.

# 4 Discussion

The present study shows that *Pseudocrossidium* as traditionally conceived is polyphyletic and that the species currently accepted in this genus are distributed in multiple wellsupported clades. Therefore, our results support the redefinition of the genus *Pseudocrossidium*, with new combinations of some species previously included in *Barbula* or *Tortula*, combination of one species previously in *Pseudocrossidium* to *Gertrudiella*, and the recognition of new genera, which correspond to molecularly supported lineages.

The relationships among lineages within the Pottioideae are poorly resolved in our analyses. In addition, several cases of discrepancy between ITS and plastid relationships have been observed such as the position of *Barbula unguiculata* and *Cinclidotus riparius* or the placement of the *Syntrichia-Sagenotortula* clade. These data could suggest an exchange of genetic material between genera or perhaps the presence of paralogous loci, which has not been reported in the Trichostomoideae, but which is often seen in the Pottioideae (Hedenäs et al., 2019). Therefore, additional sampling to assess the relationship with the Pottioideae is required.

### 4.1 Clade A: Pseudocrossidium linearifolium

Pseudocrossidium linearifolium was described as a variety of Barbula subrevoluta Müll. Hal. by Müller (1879), an illegitimate later homonym of Barbula subrevoluta Hampe. Later, the homonymy was recognized by Müller (1882), who established a new name, Barbula perrevoluta Müll. Hal., and Paris (1884–1898) combined the variety (B. perrevoluta var. linearifolia (Müll. Hal.) Paris). Zander (1993) transferred this variety to Pseudocrossidium as P. perrevolutum var. linearifolium (Müll. Hal.) R.H. Zander, and later Jiménez et al. (2009) elevated it to the rank of species. Characteristics like the leaves appressed to contorted when dry, recurved margins (Fig. 1B), the crescent-shaped dorsal stereid band, the differentiated dorsal surface cells in cross-section, and yellow lamina in KOH probably led Zander (1993) to move this taxon to Pseudocrossidium. However, some characteristics such as the undifferentiated marginal laminal cells, nonrevolute leaf margins, costa with two bands of stereids, and undifferentiated perichaetial leaves resemble species of Barbula such as B. orizabensis Müll. Hal. or B. unguiculata

**Fig. 2.** Majority-rule consensus tree of the Bayesian inference analysis inferred from ITS sequences. Bayesian posterior probabilities (PP), followed by maximum likelihood bootstrap values (BS) are shown above the branches. Support values of BS < 70 and PP < 0.95 are not shown. The clades that include species considered in *Pseudocrossidium* or allies of this genus in this study are labeled A to K and referred to in the text.



more than they do *Pseudocrossidium*. Kučera et al. (2013), in a partial phylogenetic study of *Barbula*, resolved only the type of the genus, *B. unguiculata*, plus *B. orizabensis*, in the Pottioideae subfamily, while most of the species occurring in the Northern Hemisphere were part of the Trichostomoideae. Moreover, they found *B. unguiculata* and *B. orizabensis* in isolated lineages in their analyses, and only a sister-group relationship of *B. unguiculata* and *B. orizabensis* was suggested without bootstrap support in the BI of ITS data.

Accessions of P. linearifolium form one of the first diverging branch in our trees with high support. Unfortunately, no specimens of Central American Barbula orizabensis could be included in our analysis. Other South American species with superficial cells of the costa differentiated ventrally such as Barbula costesii Thér. could not be amplified either. Barbula orizabensis is similar to P. linearifolium because both species have leaf margins recurved to the apex or nearly so and a cross section of the costa with two stereid bands, but the latter species can be easily distinguished from B. orizabensis by its narrowly recurved leaf margins and the absence of gemmae in the leaf axils. Our phylogenetic study clearly established that Pseudocrossidium linearifolium should be excluded from Pseudocrossidium and Barbula. Therefore, a new genus, Barbulastrum, is proposed here to include P. linearifolium (see 5 Taxonomic implications). The genus exhibits remarkable characteristics such as ligulate to lingulate leaves (Fig. 1B), sometimes constricted with widely recurved margins, costa with superficial ventral cells differentiated and two bands of stereids, and perichaetial leaves scarcely differentiated. Pseudocrossidium linearifolium has been reported in Tucumano-Boliviano, Chaco, and Chaco Serrano forests, and dry inter-Andean valleys of Argentina, Bolivia, and Ecuador at 350-2150 m, usually on sandy, clayey soils (Jiménez et al., 2009). Species of Barbula with revolute to recurved margins and superficial cells of the costa differentiated ventrally should be molecularly and morphologically studied, because additional species may be added to this genus.

### 4.2 Clade B: Pseudocrossidium mendozense

Pseudocrossidium mendozense was described as Tortula mendozensis Mitt. from a specimen collected by J. Gillies in the Andes of Mendoza, Argentina (Mitten, 1869). No new reports and no updated description of this species have been provided since it was published, so it is presently considered an endemic species of the western central part of Argentina.

Recently, Jiménez et al. (2021) expanded the genus *Gertrudiella* to include *Andinella*. According to these authors, the genus is characterized by having stems with a central strand and without a hyalodermis, recurved to revolute leaf margins, unistratose lamina with a reddish to orange color in KOH, bulging upper and median laminal cells, costa usually with ventral costal outgrowths that is rather broad and adaxially protuberant and papillose, and in transverse section

usually with numerous guide cells in several layers and lacking ventral stereids. Our results confirm the relation of Pseudocrossidium mendozense with the Gertrudiella clade. Morphologically, Gertrudiella closely resembles Pseudocrossidium because both genera include plants with dioicous sexuality, ovate to lanceolate leaves (Fig. 1C) that are appressed and spiraled when dry, with recurved to revolute margins, ventral costal outgrowths usually differentiated and small distal laminal cells. In fact, Pseudocrossidium elatum (R.S. Williams) Delgad. and P. granulosum (Thér.) S.P. Churchill, included by Delgadillo (1975) and Churchill et al. (2000), respectively, in Pseudocrossidium, were later transferred to Andinella (as Andina nom. illeg., see Jiménez & Cano, 2020) and are presently accommodated in Getrudiella (Jiménez et al., 2021). Pseudocrossidium can be easily distinguished from Gertrudiella by the hyaline basal cells of the axillary hairs on the stem, the flat or very slightly convex surface of the leaf cells, and the yellow color of the lamina with KOH. Pseudocrossidium mendozense is characterized by axillary hairs with brown basal cells, strongly recurved to revolute leaves, lamina with red to orange KOH coloration, costa transverse section with numerous guide cells in several layers and lacking ventral stereids, ventral costal outgrowths differentiated as an unistratose pad of bulging and papillose cells, and upper and median laminal cells bulging. All these characteristics can be found in the generitype of Gertrudiella (G. validinervis (Herzog) Broth.). Therefore, according to the morphology and molecular data, Pseudocrossidium mendozense must be transferred to Gertrudiella and a new combination in this genus should be proposed (see section 5 Taxonomic implications).

## 4.3 Clade C: Pseudocrossidium carinatum-P. santiagense

Pseudocrossidium carinatum, known in southern Africa and South America, and the southern South America P. santiagense (Fig. 1D) appear as a clearly supported lineage in the Pottioideae in our analyses. However, the position of both species as a sister group to the Didymodon and allied genera clade in ITS and combined plastid/ITS is an unexpected result obtained in the molecular analyses. The study of Pseudocrossidium crinitum s.l. performed by Cano et al. (2016) already indicated a considerable phylogenetic distance of P. carinatum and P. santiagense with respect to the rest of the species of Pseudocrossidium included in that study. However, morphologically, they are very similar to the species included in the clade P. denticulatum-P. crinitum (clade K in the present study). Pseudocrossidium carinatum and P. santiagense can be distinguished from P. crinitum and P. denticulatum by the usually flat upper and median laminal cells on both surfaces (slightly convex in P. denticulatum and P. crinitum), and quadrate to short-rectangular surface cells of the costa dorsally in the distal part (linear in P. denticulatum and P. crinitum) and sometimes the bistratose leaf margins (unistratose in P. denticulatum and P. crinitum).

**Fig. 3.** Majority-rule consensus tree of the Bayesian inference analysis inferred from combined plastid (atpB-rbcL, trnG, and trnL-F) sequences. Bayesian posterior probabilities (PP), followed by maximum likelihood bootstrap values (BS) are shown above the branches. Support values of BS < 70 and PP < 0.95 are not shown. The clades that include species considered in *Pseudocrossidium* or allied of this genus in this study are labeled A to K and referred to in the text.



Fig. 4. Continued

According to Cano et al. (2016), Pseudocrossidium carinatum and P. santiagense share leaves with quadrate to shortrectangular surface cells of the costa dorsally, and both species are sympatric in central Chile. In addition, the basal cells of the axillary hairs are hvaline, unlike Didymodon and allied genera, which have axillary hairs with brown basal cells. Convergent evolution is especially common in plants that have independently adapted to the same extreme environments (Xu et al., 2020) and it has been widely reported in mosses (e.g., Olsson et al., 2009; Huttunen & Ignatov, 2010; Bell & Hyvönen, 2012). However, lateral gene transfer between distantly related species and hybridization between closely related species may also mislead the assessment of convergent evolution (Dunning & Christin, 2020). Our molecular results revealed that this clade is clearly separate from the bulk of Pseudocrossidium and would be better accommodated in a new genus, Austrobarbula (see section 5 Taxonomic implications). However, more studies should be carried out to better understand the relationship of this new genus with the rest of the genera of the Pottioideae.

# 4.4 Clade D: Pseudocrossidium porphyreoneurum

Pseudocrossidium porphyreoneurum is a tropical species known from Africa and southwestern and central Asia (Stern, 2000; O'Shea, 2006; Kürschner & Frey, 2020). It was described in the genus Barbula by Müller in Venturi (1872). Townsend (1979) transferred this species to Tortula because of the anatomy of the costa, and later Zander (1993) placed it in the genus Pseudocrossidium. In our molecular analysis, this species shows an isolated position among the genera of Pottioideae included together with one accession of an undescribed species from Ecuador. Only a sister-group relationship of P. porphyreoneurum and the Pseudocrossidium core is suggested in the BI of ITS data. Pseudocrossidium porphyreoneurum shares with the species traditionally placed in Pseudocrossidium the shape of the leaves (Fig. 1E), the strongly differentiated marginal laminal cells, and revolute leaf margins. However, it can be distinguished from Pseudocrossidium by the combination of undifferentiated perichaetial leaves, and section of the leaf costa that is more similar to that found in the genus Tortula with two guide cells, a single band of dorsal stereids that is semicircular in shape, hydroids, and differentiated superficial ventral and dorsal cells. The strongly singular differentiated marginal laminal cells rolled in a spiral tube, thin-walled with hollow-papillae of many species of Pseudocrossidium, seem to have evolved several times independently in the Pottioideae. This characteristic can be found in other genera of Pottioideae such as Tortula or Hilpertia. Pseudocrossidium porphyreoneurum shares with both genera the anatomy of the costa; however, it can be mainly distinguished from Tortula by the undifferentiated basal laminal area (differentiated by hyaline and inflated cells in Tortula). From Hilpertia, it differs in the yellow KOH laminal reaction (red in *Hilpertia*), equally thickened laminal cell walls (dorsally thickened laminal cell walls in *Hilpertia*), and differentiated ventral superficial costal cells (usually undifferentiated in *Hilpertia*). According to Olsson et al. (2011), compared with angiosperm, the lack of a sufficient amount of morphological characteristics in bryophytes makes it more difficult to reveal convergent evolution in this group based on morphology alone, but with well-resolved and highly supported phylogenies, this can be addressed. Therefore, based on the morphological and molecular differences, we accommodate here *P. porphyreoneurum* as a distinct genus, *Helicobarbula* (see section 5 Taxonomic implications section).

## 4.5 Clades E-K: Pseudocrossidium core

The ITS and combined ITS and plastid data sets suggest that the remaining species of *Pseudocrossidium* plus Tortula *jaffuelii*, Aloinella, Barbula riograndensis, and B. integrifolia form a monophyletic group. However, BI of plastid data (Fig. 3) includes Barbula and Cinclidotus in the *Pseudocrossidium* core and excludes clade E (*P. chilense-P. adustum* and *Aloinella*).

A surprising finding of our study is the strong support for the monophyly of the clade comprising the generitype of Pseudocrossidium, P. chilense, P. adustum, and Aloinella. The position of Aloinella in our analyses could be resolved in three different ways: (i) to merge Aloinella into Pseudocrossidium, (ii) to recognize three individual genera: Aloinella, Pseudocrossidium s. str., and a new genus for the remaining species of Pseudocrossidium, and (iii) to recognize Aloinella and Pseudocrossidium in a paraphyletic arrangement. Aloinella is a Neotropical, morphologically homogeneous genus of six species, which exhibits elongate stems with catenulate, ovate to lingulate leaves, with plane margins, a strongly cucullate apex (Fig. 1A), ventral superficial cells of the costa forming filaments, sheathing perichaetial leaves, and straight peristome with short teeth (Delgadillo, 1975). Most of the Pseudocrossidium species share with Aloinella strongly differentiated perichaetial leaves and only Pseudocrossidium apiculatum and P. pachygastrellum have the ventral superficial cells of the costa forming filaments. However, the latter can be distinguished from Aloinella species by its noncatenulate habit, not strongly cucullate leaf apex, revolute leaf margins, and different shape of the leaf. Unfortunately, other accessions of Aloinella sequenced for this study could not be amplified. Although Pseudocrossidium lacks putative synapomorphies, no morphological characteristic is known that would support the extended genus concept of Pseudocrossidium, comprising both Aloinella and Pseudocrossidum. Consequently, the option of merging Aloinella into Pseudocrossidium is strongly rejected here and the generic status of Aloinella is maintained, despite its paraphyletic nature, appearing to have been derived from within the genus Pseudocrossidium. According to Brummitt (2002),

**Fig. 4.** Majority-rule consensus tree of the Bayesian inference analysis inferred from combined plastid (atpB-rbcL, trnG, and trnL-F) and ITS sequences. Bayesian posterior probabilities (PP), followed by maximum likelihood bootstrap values (BS) are shown above the branches. Support values of BS < 70 and PP < 0.95 are not shown. The clades that include species considered in *Pseudocrossidium* or allies of this genus in this study are labeled A to K and referred to in the text.

paraphyletic taxa are products of the evolutionary process, which is left behind as evolution moves on to a new level of organization. It is quite possible that considerable information may be obtained on evolution if paraphyly were ultimately recognized by phylogenetic systematists as informative of evolutionary direction (Zander, 2019b). The Pseudocrossidium adustum-P. chilense lineage can be differentiated from the rest of the species of Pseudocrossidium by the slightly cucullate apex (Fig. 1F) and a tendency toward bistratification of the lamina. However, the interpretation of Pseudocrossidium as a genus only formed by P. chilense and P. adustum, and therefore, the combination of the remaining Pseudocrossidum species in a new genus is for the time being rejected. The weak support of the backbone phylogeny and the incongruences observed between ITS and plastid information require us to wait for new studies that clarify the relationship between the different groups established here in Pseudocrossidium and the Pottioideae. Thus, we tentatively opt for a paraphyletic disposition of Aloinella and Pseudocrossidium. A more thorough sampling could resolve the remaining questions related to the phylogenetic relationships of Pseudocrossidium, and therefore, it would be prudent to refrain from application of premature nomenclatural changes in this group at this stage.

Tortula jaffuelii is an endemic central Chilean species. According to Cano & Gallego (2008), common morphological characteristics of this species are lingulate leaves, yellow reaction with KOH, and a costal cross-section similar to that found in Tortula, with surface cells differentiated dorsal and ventrally, hydroids, and a band of developed semicircular ventral stereids, although in some cross-sections, the ventral band of stereids is slightly lunulate. However, the perichaetial leaves are long and slightly sheathing at the base, the basal cells are not strongly differentiated from the distal cells, which can be papillose (mainly, on the dorsal side of the leaf), and usually slightly thick-walled, the marginal laminal cells are undifferentiated, and some crosssections of the stem seem to show a weakly developed sclerodermis. This combination of characteristics is not found in Tortula. Maybe because of all these deviant characteristics, Zander (1993) transferred it to the genus Syntrichia, although the anatomy of the costa and the leaf reaction with KOH clearly rejected its inclusion in this genus. Cano & Gallego (2008) provisionally placed this species in Tortula, awaiting new data. Our present results place Tortula jaffuelii (clade F) in Pseudocrossidium with high support, specifically sister to clade K based on the chloroplast data set and sister to clade G, without support from ITS data and the combined plastid and ITS data. Tortula jaffuelii shares with Pseudocrossidium the sheathing perichaetial leaves, costa with 2-6 guide cells in 1-2 layers, slightly differentiated sclerodermis, undifferentiated marginal laminal cells, and basal cells not strongly differentiated from distal cells. The margins are only recurved, and the marginal laminal cells are undifferentiated, but these characteristics are also found in many other species of Pseudocrossidium. Therefore, here, this species is transferred to this latter genus.

Clade G accommodates the South American Pseudocrossidium excavatum, sister to the mainly holartic clade formed by P. revolutum, P. hornschuchianum, and P. obtusulum. Clade G can be characterized by plants twisted helicoidally when dry, ovate to oblong-ovate or ovate-lanceolate, sometimes triangular leaves, with percurrent or excurrent costa as a short mucro or apiculus, with 2-4 guide cells, revolute margins, scarcely to strongly differentiated marginal leaf cells, and differentiated perichaetial leaves. Our results show the accessions of P. obtusulum nested in P. hornschuchianum in a clade sister to P. revolutum. Pseudocrossidium obtusulum is very close to P. hornschuchianum and they can be distinguished mainly by the obtuse to broadly acute leaf apex (acute to acuminate, rarely broadly acute in P. hornschuchianum), and costa excurrent in a short mucro (long-mucronate or apiculate in P. hornschuchianum). Many intermediate specimens between both species have been found. In fact, P. obtusulum has already been combined as a variety of P. hornschuchianum (Barbula hornschuchiana var. obtusula (Lindb.) Podp.) and P. revolutum (Pseudocrossidium revolutum var. obtusulum (Lindb.) B.C. Tan, R.H. Zander & Ter.Taylor). According our data, the two taxa should be considered synonymous.

Pseudocrossidium apiculatum and P. pachygastrellum (clade H) can be distinguished from the rest of the species included in Pseudocrossidium by the ventral superficial cells of the costa forming filaments of (2-)3-4 cells high. Pseudocrossidium apiculatum was described by Williams (1915) and characterized by its apiculate, excurrent costa. Herzog (1916) described Barbula pachygastrella Herzog on the basis on a Bolivian specimen, which was transferred to Pseudocrossidium by Brotherus (1924). Zander (1979) distinguished the two species by mucronate leaves in P. pachygastrellum and short-awned leaves in P. apiculatum. Our molecular study shows that there are practically no molecular differences between both species. In addition, after studying the type of material of both species and numerous South American specimens, we agree with Hegewald & Hegewald (1977), who considered P. pachygastrellum as synonymous with P. apiculatum. On the other hand, our analysis does not resolve the relationship of *P. apiculatum* with the rest of the species included in the Pseudocrossidium core.

Our molecular analysis included two Southern America taxa, Pseudocrossidium leucocalyx and P. perpapillosum, in a monophyletic group (clade I); however, these species are not resolved as monophyletic. Both species share conspicuous papillae on the superficial ventral cells of the costa and on the distal paracostal cells of the leaf, which are long and bito quadrifurcate and often pedicellate. In addition, both species have differentiated perichaetial leaves. Specimens identified as P. leucocalyx are polyphyletic. The results obtained could be an indication of the presence of cryptic species. In fact, specimens with leaves similar to P. perpapillosum, but without an excurrent costa, and specimens with oblong-ovate leaves similar to P. leucocalyx, but without differentiated median marginal cells, have been found. Therefore, a more detailed taxonomic study at the species level is needed to identify characteristics that can be used for the identification of the species in this complex.

Cano et al. (2016), in their morphologic and phylogenetic study of *P. crinitum*, already showed the relation between *Pseudocrossidium replicatum* and *P. arenicola*. In fact, the former was only resolved as monophyletic by including *P. arenicola* in that study. Our molecular analysis clearly increases the number of species in this clade. Thus, *P.* 

austrorevolutum, and the more recently described *P.* exiguum, previously not associated with this group, are placed here (clade J). The taxa of this clade are mainly distinguished from the remaining species of *Pseudocrossi*dium by undifferentiated perichaetial leaves. The four entities molecularly recognized almost correspond with the morphologically defined species *P. exiguum, P. austrorevolutum, P. replicatum,* and *P. arenicola*; however, incongruences between plastid and ITS information at the species level have been observed. As in the case of the *P. leucocalyx-P. perpapillosum* complex, a more detailed taxonomic study at the species level is needed to identify specific characteristics that can be used for identification in the members of this clade and additional evidence is required to explain the incongruences observed.

Among the species earlier considered as *Barbula* included in this study, *Barbula riograndensis* and *B. integrifolia* clearly are nested in the *Pseudocrossidium* core, specifically in the clade formed by *P. crinitum* and *P. denticulatum* (clade K). *Barbula riograndensis* is known from a few localities in Brazil. Meanwhile, *Barbula integrifolia* is an endemic species of Andean Peru. In fact, both species are morphologically very similar to *Pseudocrossidium denticulatum* and *P. crinitum*. This entire group of species shares a similar leaf shape, usually more than 1.5 mm long, excurrent costa in a long mucro or awn, costa with dorsal and ventral stereid band developed, and perichaetial leaves differentiated. Both species are transferred here to *Pseudocrossidium* (see 5 Taxonomic implications).

We have also included a central to southern African species of *Barbula*, *B. eubryum* Müll. Hal. (*Barbula* sect. *Bulbibarbula* Müll. Hal.), in our analysis. According to Zander (2017), this species is similar to *Leptodontium*, but differs in the lack of leaf teeth, undifferentiated sheathing base, short excurrent costa, basal cells split, and large rhizoidal brood bodies. For this reason, this author transferred this species to the new monospecific genus *Bulbibarbula* and he considered this species as a relic of that mostly extinct group out of which both the Pottiaceae and Streptotrichaceae (tribe Leptodontiae) arose. Our results suggest its proximity to *Triquetrella*.

# **5** Taxonomic implications

**Austrobarbula** M.J. Cano, **gen. nov.**— Type: Austrobarbula santiagensis (Broth.) M.J. Cano  $[\equiv$  Barbula santiagensis Broth.]

**Diagnosis:** Differs from *Pseudocrossidium* in the costa excurrent as a hyaline hair point, with quadrate to short-rectangular surface cells dorsally in the distal part, and two bands of stereids, upper and middle laminal cells usually flat in both surfaces, and sometimes bistratose leaf margins.

**Description**: Plants small, forming dense turfs, yellowish to dull or brownish green. Stems branched; sclerodermis not or scarcely differentiated, central strand well developed. Leaves apressed, usually spirally twisted when dry, erect-patent to spreading when wet, lingulate to oblong-ovate, slightly keeled distally; lamina unistratose, yellow with KOH; apex rounded, obtuse or emarginate, rarely acute; margins recurved to revolute 1-2 turns from the near apex or upper third to near the base or below middle, papillose-crenulate from the apex to the below middle or base, uni- to bistratose: costa excurrent as a mucro or hair-point, hvaline. rarely yellowish, smooth to denticulate, rarely percurrent, surface cells dorsally quadrate to short-rectangular, oblate or rounded near apex, elongate in the rest, surface cells ventrally guadrate to short-rectangular in the distal part, the central rows occasionally long-rectangular or linear, usually papillose, in cross-section at midleaf semicircular to elliptical or slightly reniform, 4 guide cells in one layer, differentiated ventral stereids band, dorsal stereids band lunulate in shape, hydroids developed, dorsally differentiated surface cells, smooth or papillose, surface cells ventrally differentiated or not; upper and median paracostal cells hexagonal to quadrate, rectangular or rounded, usually thinwalled, flat in both surfaces, papillose; upper and median marginal cells slightly thicker-walled and less papillose than inner cells; basal cells rectangular, not inflated, thin-walled, rarely slightly thick-walled, smooth. Dioicous. Perichaetial leaves differentiated, sheathing. Setae yellowish to orange, twisted to the right in the upper part and slightly to the left in the lower part. Urns cylindrical, yellowish brown to reddish brown; exothecial cells rectangular to hexagonal-rectangular, thin-walled; peristome of 32 teeth, spirally twisted more than one turn, yellowish brown to orange, basal membrane short; operculum conical. Calyptrae cucullate. Spores spherical, light brown, finely papillose.

Etymology: From the Latin australis (southern) plus Barbula.

**Austrobarbula carinata** (Gillies ex Grev.) M.J. Cano, **comb. nov**.  $\equiv$  Tortula carinata Gillies ex Grev., Edinburgh J. Nat. Geogr. Sci. 2: 2. 1830  $\equiv$  Pseudocrossidium carinatum (Gillies ex Grev.) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 119. 1993 — Type: "South America", J. Gillies s.n. (**lectotype, designated here**: E–11966!; isolectotypes: E–9555615, NY–371639!).

Note: This species was described by Greville (1830) based on a single gathering collected by J. Gillies in Chile. We have found syntypes of this collection deposited in E and NY herbaria. Since there is no designation of a single specimen from this gathering in the protologue of *Tortula carinata*, all these specimens are syntypes. Long (2020) considered material from Greville's herbarium deposited at E (E–11966) as the holotype and the specimen from Arnott's herbarium (E–955615) as the isotype. However, this statement cannot be considered an inadvertent lectotypification, according to Art. 7.11 and 9.23 of the ICN (Turland et al., 2018). The specimen from herbarium Greville at E is designated here as lectotype to prioritize the author's herbarium.

Austrobarbula santiagensis (Broth.) M.J. Cano, comb. nov. ≡ Barbula santiagensis Broth., Ark. Bot. 6(10): 3, Taf. 1 figs. 5–7. 1907 ≡ Pseudocrossidium santiagense (Broth.) M.J. Cano, Nova Hedwigia 102: 101. 2016 — Type: CHILE. Sta Lucia, 17 Jun 1896, P. Dusén 108 (lectotype, designated here: S–B6291!).

Note: This species was described by Brotherus in Dusén (1907) from a collection made by Dusén (*Dusén 108*) in Chile. We have found two specimens deposited in S (S–B6291 and S–B6292) that agree with the date, locality, and collector provided in the protologue. However, the specimen S–B6292

corresponds to a *Bryum* specimen. The specimen S–B6291 is selected here as the lectotype.

**Barbulastrum** M.J. Cano & J.A. Jiménez, **gen. nov.** — Type: Barbulastrum linearifolium (Müll. Hal.) M.J. Cano & J.A. Jiménez [≡ Barbula subrevoluta var. linearifolia Müll. Hal.].

**Diagnosis:** Differs from *Pseudocrossidium* by its ligulate to lingulate leaves, with a rounded or obtuse apex, widely recurved margins of undifferentiated cells, costa with two stereid bands, and perichaetial leaves scarcely differentiated from vegetative leaves.

Description: Plants small, forming dense turfs, yellowish green to dark green. Stems branched or not; sclerodermis not or scarcely differentiated, central strand developed. Leaves appressed to contorted when dry, spreading to reflexed when wet, ligulate to lingulate, sometimes constricted at the middle; lamina unistratose, yellow with KOH, apex rounded, sometimes widely obtuse, rarely apiculate, not cucullate; margins widely recurved in the midleaf part, slightly recurved from the midleaf to the base, papillose-crenulate from the apex to below the mid-leaf; costa percurrent or excurrent as a short mucro, surface cells dorsally rectangular to linear, usually papillose, surface cells ventrally quadrate to rectangular, papillose, in cross-section at midleaf elliptic to slightly reniform, (3–)4 guide cells in one layer, ventral stereid band differentiated, dorsal stereid band lunulate in shape, hydroids usually undeveloped, dorsal surface cells differentiated at the margins, ventral surface cells quadrate-rounded to rectangular or oblate, disposed in one layer; upper and median paracostal cells quadrate to rectangular or oblate, thin-walled; upper and middle marginal cells undifferentiated; basal cells usually rectangular, not inflated, thin-walled, smooth. Dioicous. Perichaetial leaves scarcely differentiated, sometimes shorter and wider than vegetative leaves. Setae orange to reddish brown, slightly twisted to the left in the lower part and to the right in the upper part. Urns cylindrical to ovoid-cylindrical, yellowish brown to reddish brown; exothecial cells rectangular, thinwalled; peristome of 32 teeth, filiform, papillose, spirally twisted, usually less than one turn, basal membrane short; operculum conical. Calyptrae cucullate. Spores spherical, yellowish, apparently smooth.

**Etymology:** The name refers to the similarity to the genus *Barbula*.

**Barbulastrum linearifolium** (Müll. Hal.) M.J. Cano & J.A. Jiménez, **comb. nov.**  $\equiv$  Barbula subrevoluta var. linearifolia Müll. Hal., Linnaea 42: 335. 1879  $\equiv$  Barbula perrevoluta var. linearifolia (Müll. Hal.) Paris, Index. Bryol.: 88. 1894  $\equiv$  Pseudocrossidium perrevolutum var. linearifolium (Müll. Hal.) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 119. 1993  $\equiv$  Pseudocrossidium linearifolium (Müll. Hal.) J.A. Jiménez & M.J. Cano, Bryologist 112: 189. 2009 — Type: ARGENTINA. Córdoba: Barrancas, Nov 1871, Lorentz s.n. (lectotype, designated by Jiménez et al. (2009): NY!).

**Gertrudiella** Broth. in Engler, Nat. Pflanzenfam. (ed. 2), 11: 528. 1925 — Type: Gertrudiella validinervis (Herzog) Broth. = Andina J.A. Jiménez & M.J. Cano, Syst. Bot. 37: 296. 2012, nom. illeg., non Andinia (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 79: 5. 2000  $\equiv$  Andinella J.A. Jiménez & M.J. Cano, Phytotaxa 452: 243. 2020.

**Gertrudiella mendozensis** (Mitt.) J.A. Jiménez & M.J. Cano, **comb. nov.**  $\equiv$  Tortula mendozensis Mitt., J. Linn. Soc., Bot. 12: 154. 1869  $\equiv$  Barbula mendozensis (Mitt.) A. Jaeger, Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1871–72: 427. 1873  $\equiv$ Pseudocrossidium mendozense (Mitt.) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 119. 1993 — Type: ARGENTINA. Mendoza, J. Gillies s.n. (**lectotype, designated here**: NY–371777!; isolectotypes: H-BR–3300674, NY–371778!, NY–371779!, PC–0134821!, S–119039!).

Note: This species was described by Mitten (1869) based on a single gathering collected by J. Gillies in the province of Mendoza (Argentina). The gathering was represented by more than one specimen (syntypes) that are deposited in H-BR in Mitten's herbarium at NY (three syntypes), PC, and S. We designate here the best-preserved specimen deposited at NY (NY–371777) as the lectotype to prioritize the author's herbarium.

Helicobarbula M.J. Cano, gen. nov. — Type: Helicobarbula porphyreoneura (Müll. Hal.) M.J. Cano [≡ Barbula porphyreoneura Müll. Hal.]

**Diagnosis:** Differs from *Pseudocrossidium* by the combination of undifferentiated perichaetial leaves, section of the leaf costa with two guide cells, and dorsal stereid band semicircular in shape, and strongly revolute margins with more than 2–3 turns from the apex to the base.

Description: Plants small, forming loose to dense tufs, yellowish green. Stems simple or branched; sclerodermis undifferentiated or scarcely differentiated, and central strand developed. Leaves contorted to spirally twisted when dry, patent to spreading when wet, ovate-lanceolate or oblong-lanceolate to triangular, concave, slightly channaled near the apex; lamina unistratose, yellow with KOH, apex obtuse, occasionally acute, not cucullate; margins strongly revolute more than 2-3 turns from the apex to the base, entire; costa excurrent in an awn, reddish brown, surface cells dorsally linear, smooth, surface cells ventrally quadrate to rectangular, papillose, in cross-section at the midleaf circular to semicircular, with 2 guide cells in 1 layer, undifferentiated ventral stereid band, dorsal stereid band semicircular in shape, hydroids developed, differentiated dorsal surface cells, differentiated ventral surface cells, disposed in one layer; upper and median paracostal cells quadrate-hexagonal to rectangular or oblate, thin- to slightly thick-walled, papillose; upper and median marginal cells strongly differentiated, rounded, inflated, thin-walled, papillose; basal cells rectangular, quadrate or oblate, thin-walled, smooth. Dioicous. Perichaetial leaves not or scarcely differentiated from vegetative leaves. Setae orange to reddish brown, straight to slightly twisted to the left. Urns cylindrical, yellowish brown; exothecial cells rectangular; peristome of 32 teeth, straight to spirally twisted 1-turn, basal membrane differentiated; operculum conical. Calyptrae cucullate. Spores spherical, yellowish brown, apparently smooth.

**Etymology:** The name refers to helix, "anything assuming a spiral shape" by the strong spirally turned margins and its similarity to *Barbula*.

Helicobarbula porphyreoneura (Müll. Hal.) M.J. Cano.  $\equiv$  Barbula porphyreoneura Müll. Hal., Nuovo Giorn. Bot. Ital. 4: 13. 1872  $\equiv$  Tortula porphyreoneura (Müll. Hal.) C.C. Towns., J.

Bryol. 10: 576. 1979  $\equiv$  Pseudocrossidium porphyreoneurum (Müll. Hal.) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 119. 1993. — Type: ERITREA. Keren, 1500 m, Beccari s.n. (lectotype, designated here: BM–000661503!; isolectotypes: BM–000661504!, S–B4656!, FT!, NY–01128391).

Note: Barbula porphyreoneura was described by Müller in Venturi (1872) based on a material collected by O. Beccari in Eritrea ("regione Bogos circa Keren"). Müller's herbarium at B was destroyed, but five syntypes of this collection were found in BM, FT NY, and S. All duplicates coincide with the protologue and they do not contradict the guidelines in the ICN. The sample from BM–000661503 is chosen as the lectotype since it includes more material.

**Pseudocrossidium** R.S. Williams, Bull. Torrey Bot. Club 42: 396, pl. 23. 1915 — Type: Pseudocrossidium chilense R.S. Williams.

**Pseudocrossidium riograndense** (E.B. Bartram) M.J. Cano & J.A. Jiménez, **comb. nov.**  $\equiv$  Barbula riograndensis E.B. Bartram, J. Wash. Acad. Sci. 42(6): 179. 1952 — Type: BRAZIL. Rio Grande do Sul: Quilombo, 30 m, 10 Sep 1941, A. Sehnem 195 (holotype: FH–79982!).

**Pseudocrossidium integrifolium** (R.S. Williams) M.J. Cano & J.A. Jiménez, **comb. nov.**  $\equiv$  Leptodontium integrifolium R.S. Williams, Bull. Torrey Bot. Club. 43: 324, pl. 17, figs. 1–7. 1916  $\equiv$  Barbula integrifolia (R.S. Williams) R.H. Zander, Bryologist 75: 277. 1972 — Type: PERU. [Cuzco]: Ollantaytambo, about 3000 m, May 1915, O. F. Cook & G. B. Gilbert 704a (holotype: NY–944880!; isotype: PC–697175!).

**Pseudocrossidium jaffuelii** (Thér.) M.J. Cano & M.T. Gallego, **comb. nov.**  $\equiv$  Tortula jaffuelii Thér., Revista Chilena Hist. Nat. 27: 9, pl. 2, fig. 1. 1923  $\equiv$  Syntrichia jaffuelii (Thér.) R.H.Zander, Bull. Buffalo Soc. Nat. Sci. 32: 269. 1993 — Type: CHILE. [Valparaíso]: Los Perales de Marga Marga, Sep 1919, F. Jaffuel 23 (**lectotype, designated here**: PC-0052204!; isolectotypes: B!, FH-220103!, PC-0054274!, NY-371719, W-1931-0005435!).

Note: Cano & Gallego (2008) considered a specimen from PC as the holotype. However, this statement cannot be considered an inadvertent lectotypification (Art. 7.11 and Art. 9.23, Turland et al., 2018) and lectotypfication is required. Here, material from PC–0052204 (author's herbarium) is selected as lectotype.

### Key to Pseudocrossidium and allied genera

We present a key to the new genera that have been segregated from *Pseudocrossidium* and the related genera of the subfamily Pottioideae according to this study.

 Axillary hairs with brownish basal cells; lamina red to orange color reaction in KOH......

.....Gertrudiella (G. mendozensis)

- 1b. Axillary hairs with all hyaline cells; lamina yellow to yellowish orange color reaction in KOH......2
- 2a. Central strand of the stem absent; leaves with multistratose margins......**Cinclidotus**

- 6a. Ventral surface cells of the costa undifferentiated as an epidermal layer in cross-section.....
- 6b. Ventral surface cells of the costa well-differentiated as an epidermal layer in cross-section......7
- 7a. Leaf margins widely recurved......Barbulastrum
- 8a. Costa with 2 guide cells at midleaf; leaf margins revolute 2–3 turns from the apex to the base; dorsal stereid band of the costa semicircular in shape....... Helicobarbula
- 8b. Costa with (3–)4–6 guide cells, rarely 2 at midleaf; leaf margins revolute 1–2 turns from the apex to the base; dorsal stereid band of the costa usually lunulate in shape......Pseudocrossidium

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# References

Allen BH. 2002. Moss flora of Central America, Part 2. Encalyptaceae-Orthotrichaceae. Monographs in Systematic Botany from the Missouri Botanical Garden 90: 1–699.

- Alonso M, Jiménez JA, Nylinder S, Hedenäs L, Cano MJ. 2016. Disentangling generic limits in Chionoloma, Oxystegus, Pachyneuropsis and Pseudosymblepharis (Bryophyta: Pottiaceae): An inquiry into their phylogenetic relationships. Taxon 65: 3–18.
- Bell N, Hyvönen J. 2012. Gametophytic simplicity in Laurasian and Gondwanan Polytrichopsida — the phylogeny and taxonomy of the Oligotrichum morphology. Journal of Bryology 34: 160–172.
- Brotherus VF. 1924. Musci (Laubmoose). 1 Hälfte. 10. In: Engler HGA ed. Die Natürlichen Pflanzenfamilien. 2nd ed. Berlin: Duncker & Humblot. 1–478.
- Brummitt RK. 2002. How to chop up a tree. Taxon 51: 31-41.
- Cano MJ. 2011. Pseudocrossidium adustum (Pottiaceae) an overlooked taxon in Southern Hemisphere. The Bryologist 114: 356–361.
- Cano MJ, Gallego MT. 2008. The genus Tortula (Pottiaceae, Bryophyta) in South America. Botanical Journal of the Linnean Society 156: 173–220.
- Cano MJ, Jiménez JA, Alonso M, Guerra J. 2015. Pseudocrossidium exiguum (Pottiaceae) a new species from South America. Journal of Bryology 37: 56–61.
- Cano MJ, Jiménez JF, Alonso M, Jiménez JA. 2016. Untangling Pseudocrossidium crinitum s.l. (Pottiaceae, Bryophyta) through molecular and morphometric analysis. Nova Hedwigia 102: 89–106.
- Cano MJ, Jiménez JF, Gallego MT, Jiménez JA, Guerra J. 2009. Phylogenetic relationships in the genus *Hennediella* (Pottiaceae, Bryophyta) inferred from nrITS sequence data. *Plant Systematics* and Evolution 281: 209–216.
- Cano MJ, Jiménez JA, Gallego MT, Jiménez JF. 2010a. Guerramontesia microdonta (Pottiaceae, Bryophyta) a new monotypic genus from South America. Systematic Botany 35: 453–460.
- Cano MJ, Jiménez JA, Guerra J. 2011. Pseudocrossidium perpapillosum (Pottiaceae) a new species from South America. Journal of Bryology 33: 300–303.
- Cano MJ, Jiménez JA, Jiménez JF. 2010b. A systematic revision of the genus Erythrophyllopsis (Pottiaceae, Bryophyta). Systematic Botany 35: 683–694.
- Cevallos MA, Guerrero G, Ríos S, Arroyo A, Villalobos MA, Porta H. 2019. The chloroplast genome of the desiccation-tolerant moss *Pseudocrossidium replicatum* (Taylor) R.H. Zander. *Genetics and Molecular Biology* 42: 488–493.
- Cevallos MA, Guerrero G, Ríos S, Arroyo A, Villalobos MA, Porta H. 2020. The mitogenome of *Pseudocrossidium replicatum*, a desiccation-tolerant moss. *Mitochondrial DNA Part B* 5: 2339–2341.
- Chiang TY, Schaal BA, Peng CI. 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. Botanical Bulletin of Academia Sinica 39: 245–250.
- Churchill SP, Griffin III D, Muñoz J. 2000. A checklist of the mosses of the tropical Andean countries. *Ruizia* 17: 1–203.
- Churchill SP, Linares EL. 1995. Prodromus Bryologiae Novo-Granatensis: Introducción a la flora de musgos de Colombia. Parte 1: Adelotheciaceae a Funariaceae. Biblioteca José Jerónimo Triana 12: 1–924.
- Churchill SP, Sanjines N, Aldana C. 2009. Catálogo de las Briofitas de Bolivia: Diversidad, Distribución y Ecología. Santa Cruz de la Sierra: Missouri Botanical Garden & Museo Noel Kempff Mercado.
- Cortini-Pedrotti C. 2001. Flora dei Muschi d'Italia. Sphagnopsida, Andreaeopsida, Bryopsida (I parte). Rome: Antonio Delfino Editore.

- Crum HA, Anderson LE. 1989. New names for some North American mosses. The Bryologist 92: 533.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Delgadillo C. 1975. Taxonomic revision of Aloina, Aloinella and Crossidium (Musci). The Bryologist 78: 245–303.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19: 11–15.
- Dunning LT, Christin PA. 2020. Reticulate evolution, lateral gene transfer, and innovation in plants. *American Journal of Botany* 107: 541–544.
- Dusén P. 1907. Beitrage zur Bryologie der Magellanslander von Westpatagonien und Südchile V. Arkiv for Botanik utgivet av K. Svenska Vetenskaps-Akademien 6: 1–32.
- Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Edler D, Klein J, Antonelli A, Silvestro D. 2019. raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. *bioRxiv* 800912.
- Eckel PM, Hoy JA, Elliot JC. 1997. Pseudocrossidium obtusulum (Pottiaceae, Bryopsida) new to Montana with a key to North American species in the genus. Great Basin Naturalist 57: 259–262.
- Gallego MT, Cano MJ, Jiménez JF, Jiménez JA, Guerra J. 2014. Morphological and molecular data support a new combination in the Neotropical complex of cucullate-leaved species of Syntrichia (Pottiaceae). Systematic Botany 39: 361–368.
- Greville RK. 1830. Descripiton of some new mosses discovered in South America by Dr. Gillies. Edinburgh Journal of Natural and Geographical Science 2: 1–4.
- Grundmann M, Schneider H, Russell SJ, Vogel JC. 2006. Phylogenetic relationships of the moss genus *Pleurochaete* Lindb. (Bryales: Pottiaceae) based on chloroplast and nuclear genomic markers. *Organisms, Diversity & Evolution* 6: 33–45.
- Guerra J, Cano MJ, Ros RM eds. 2006. Flora Briofítica Ibérica, volumen III. Pottiales, Encalyptales. Murcia: Universidad de Murcia, Sociedad Española de Briología.
- Hedenäs L, Heinrichs J, Gallego MT. 2019. The Scandinavian Syntrichia ruralis complex (Musci, Pottiaceae): a chaos of diversification. Plant Systematics and Evolution 305: 639–661.
- Hegewald E, Hegewald P. 1977. Eine Moossammlung aus Peru I. Nova Hedwigia 28: 731–758.
- Herzog T. 1916. Die Bryophyten meiner zweiten Reise durch Bolivia. Bibliotheca Botanica 87: 1–168.
- Huttunen S, Ignatov MS. 2010. Evolution and taxonomy of aquatic species in the genus Rhynchostegium (Brachytheciaceae, Bryophyta). Taxon 59: 791–808.
- Ignatov MS, Ignatova EA. 2003. Flora mkhov srednei chasti evropeiskoi Rossii. Tom 1. Sphagnaceae-Hedwigiaceae. Moss flora of the Middle European Russia. Volume 1: Sphagnaceae-Hedwigiaceae. Arctoa 11(Suppl 1): 1–608.
- Jiménez JA, Cano MJ. 2020. Andinella, a new name for Andina hom. illeg. (Pottiaceae, Bryophyta) non Andinia (Luer) Luer (Orchidaceae). Phytotaxa 452: 243–245.
- Jiménez JA, Cano MJ, Guerra J. 2021. A multilocus phylogeny of the moss genus Didymodon and allied genera (Pottiaceae): Generic delimitations and their implications for systematics. Journal of Systematics and Evolution https://doi.org/10.1111/jse.12735

- Jiménez JA, Cano MJ, Guerra J, Churchill SP, Suárez GM. 2009. Pseudocrossidium linearifolium (Potttiaceae) comb. et stat. nov. The Bryologist 112: 188–193.
- Jiménez JA, Cano MJ, Jiménez JF. 2012. Taxonomy and phylogeny of Andina (Pottiaceae, Bryophyta): a new moss genus from the tropical Andes. Systematic Botany 37: 293–306.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data (version 9.1.8). *Bioinformatics* 28: 1647–1649.
- Kimura M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proceedings of the National Academy of Sciences of the United States of America 78: 454–458.
- Kučera J, Blockeel TL, Erzberger P, Papp B, Soldán Z, Vellak K, Werner O, Ros RM. 2018. The Didymodon tophaceus complex (Pottiaceae, Bryophyta) revisited: new data support the subspecific rank of currently recognized species. Cryptogamie, Bryologie 39: 241–257.
- Kučera J, Košnar J, Werner O. 2013. Partial generic revision of Barbula (Musci: Pottiaceae): Re-establishment of Hydrogonium and Streblotrichum, and the new genus Gymnobarbula. Taxon 62: 21–39.
- Kürschner H, Frey W. 2020. Liverworts, mosses and hornworts of Southwest Asia (Marchantiophyta, Bryophyta, Anthocerotophyta: A systematic treatise on liverworts, mosses and hornworts with keys to genera and species occurring in Afghanistan, Bahrain, Iraq, Iran, Israel, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Sinai Peninsula, Syria, Turkey, United Arab Emirates and Yemen (incl. Socotra Island), 2 enlarged and revised edition. Nova Hedwigia Beiheft 139: 1–240.
- Li X-J, He S, Iwatsuki Z. 2001. Pottiaceae. In: Li X-J, Crosby MR eds. Moss Flora of China. English Version. Volume 2. Fissidentaceae–Ptychomitriaceae. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 114–249.
- Long DG. 2020. Anictangium orthotrichoides Gillies ex Grev., an overlooked name for Ptychomitrium chimborazense (Spruce ex Mitt.) A.Jaeger (Musci, Ptychomitriaceae). Journal of Bryology 42: 189–191.
- Miller MA, Pfeiffer W, Schwartz T. 2012. The CIPRES science gateway: Enabling high-impact science for phylogenetics researchers with limited resources. In: Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond, Association for Computing Machinery, USA. 1–8.
- Mitten W. 1869. Musci Austro-Americani. Journal of the Linnean Society, Botany 12: 1–659.
- Müller C. 1879. Prodromus bryologiae Argentinicae. I. Linnaea 42: 217–460.
- Müller C. 1882. Prodromus bryologiae Argentinicae II, seu musci Lorentziani Argentinici. *Linnaea* 43: 341–486.
- Müller K. 2005. SeqState: primer design and sequence statistics for phylogenetic DNA data sets. Applied Bioinformatics 4: 65–69.
- Ochyra R, Lewis Smith RI, Bednarek-Ochyra H. 2008. The illustrated moss flora of Antarctica. Cambridge: Cambridge University Press.
- Olsson S, Buchbender V, Enroth J, Hedenäs L, Huttunen S, Quandt D. 2009. Phylogenetic analyses reveal high levels of polyphyly among pleurocarpous lineages as well as novel clades. The Bryologist 112: 447–466.

- Olsson S, Enroth J, Buchbender V, Hedenäs L, Huttunen S, Quandt D. 2011. Neckera and Thamnobryum (Neckeraceae, Bryopsida): paraphyletic assemblages. Taxon 60: 36–50.
- O'Shea BJ. 2006. Checklist of the mosses of sub-Saharan Africa (version 5, 12/06). Tropical Bryology Research Reports 6: 1–252.
- Pacak A, Szweykowska-Kulińska Z. 2000. Molecular data concerning the allopolyploid character and the origin of chloroplast and mithochondrial genomes in the liverwort species *Pellia borealis*. *Journal of Plant Biotechnology* 2: 101–108.
- Paris JÉGN 1894-1898. Index Bryologicus. Paris: Author.
- Posada D. 2003. Using Modeltest and PAUP\* to select a model of nucleotide substitution. In: Baxevanis AD, Davison DB, Page RDM, Petsko GA, Stein LD, Stormo GD eds. Current protocols in bioinformatics. New York: John Wile & Sons. 6.5.1–6.5.14.
- Rambaut A. 2012. FigTree, version 1.4.4 [online]. Available from http:// tree.bio.ed.ac.uk/software/figtree/ [accessed 18 March 2021].
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in bayesian phylogenetics using tracer 1.7. Systematic Biology 67: 901–904.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Saito K. 1975. A monograph of Japanese Pottiaceae (Musci). Journal of the Hattori Botanical Laboratory 39: 373–537.
- Sawicki J, Szczecińska M. 2011. A comparison of PCR-based markers for molecular identification of Sphagnum species of the section Acutifolia. Acta Societatis Botanicorum Poloniae 80: 185–192.
- Shaw AJ, Cox CJ, Goffinet B. 2005. Global patterns of moss diversity: taxonomic and molecular inferences. *Taxon* 54: 337–352.
- Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence based phylogenetic analyses. Systematic Biology 49: 369–381.
- Smith AJE. 2004. The moss flora of Britain and Ireland. 2nd ed. Cambridge: Cambridge University Press.
- Spagnuolo V, Caputo P, Cozzolino S, Castaldo R, de Luca P. 1999. Patterns of relationships in Trichostomoideae (Pottiaceae, Musci). Plant Systematics and Evolution 216: 69–79.
- Stamatakis A. 2014. Raxml version 8: A tool for phylogenetic analysis andpost-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Stech M, Frahm JP. 1999. The status of *Platyhypnidium mutatum* Ochyra & Vanderpoorten and the systematic value of Donrichardsiaceae based on molecular data. *Journal of Bryology* 21: 191–195.
- Stern RC. 2000. Some bryophytes collected in Rajasthan. Journal of Bryology 22: 298–299.
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura RM ed. Some mathematical questions in biology: DNA sequence analysis. Providence: American Mathematical Society. 57–86.
- Thériot I. 1920. Notes bryologiques. Bulletin de la Société Botanique de Genève 14: 44–49.
- Thériot I. 1921. Contribution a la flore bryologique du Chili. Revista Chilena de Historia Natural 25: 389–312.
- Townsend CC. 1979. A new combination in African Tortula. Journal of Bryology 10: 576.

- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF. 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the nineteenth International botanical congress Shenzhen, China, July 2017. Glashütten: Koeltz Botanical Books.
- Venturi G. 1872. Muschi raccolti dal Signor Odoardo Beccari nella terra dei Bogos in Abissinia. Nuovo Giornale Botanico Italiano 4: 7–21.
- Werner O, Jiménez JA, Ros RM, Cano MJ, Guerra J. 2005. Preliminary investigation of the systematics of Didymodon (Pottiaceae, Musci) based on nrITS sequence data. Systematic Botany 30: 461–470.
- Werner O, Ros RM, Cano MJ, Guerra J. 2002. Tortula and some related genera (Pottiaceae, Musci): phylogenetic relationships based on chloroplast rps4 sequences. *Plant Systematics and Evolution* 235: 197–207.
- Werner O, Ros RM, Cano MJ, Guerra J. 2004. Molecular phylogeny of Pottiaceae (Musci) based on chloroplast rps4 sequence data. *Plant Systematics and Evolution* 243: 147–164.
- Williams RS. 1915. Mosses from the west coast of South America. Bulletin of the Torrey Botanical Club 42: 393–404.
- Xu S, Wang J, Guo Z, He Z, Shi S. 2020. Genomic convergence in the adaptation to extreme environments. *Plant Communications* 1: 100117.
- Zander RH. 1979. Notes on Barbula and Pseudocrossidium (Bryopsida) in North America and an annotated key to the taxa. Phytologia 44: 177–214.
- Zander RH. 1993. Genera of the Pottiaceae: Mosses of harsh environments. Bulletin of the Buffalo Society of Natural Sciences 32: 1–378.
- Zander RH. 1994. *Pseudocrossidium* R.S. Williams. In: Sharp AJ, Crum H, Eckel P eds. *The moss Flora of Mexico*. New York: Memoirs of the New York Botanical Garden 69: 296–299.

- Zander RH. 2006. The Pottiaceae s. str. as an evolutionary Lazarus taxon. Journal of the Hattori Botanical Laboratory 100: 581–602.
- Zander RH. 2007. Pseudocrossidium R.S. Williams. In: Flora of North America Editorial Committee eds. Flora of North America North of Mexico. Volume 27, Bryophyta, part 1. New York: Oxford University Press. 569–572.
- Zander RH. 2013. A Framework for Post-Phylogenetic Systematics. St. Louis: Zetetic Publications.
- Zander RH. 2017. Macroevolutionary Systematics of Streptotrichaceae of the Bryophyta and Application to Ecosystem Thermodynamic Stability. St. Louis: Zetetic Publications.
- Zander RH. 2019a. Macroevolutionary versus molecular analysis: Systematics of the *Didymodon* segregates *Aithobryum*, Exobryum and Fuscobryum (Pottiaceae). *Hattoria* 10: 1–38.
- Zander RH. 2019b. Infraspecific molecular trees are associated with serial macroevolution in Pottiaceae (Bryophyta). Ukrainian Botanical Journal 76: 390–405.

# Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/ jse.12801/suppinfo:

**Appendix S1.** Vouchers and GenBank accession numbers for taxa used in the molecular phylogenetic analysis. Taxon name, country and next division, voucher (collector, number, and where the specimen is housed), and GenBank accession number for DNA sequences ITS, *trn-LF*, *trnG*, and *atpB-rbcL*; a dash (–) indicates missing data. An asterisk (\*) is provided for sequences retrieved directly from GenBank.