Contributions to the phylogeny of the haplolepideous mosses

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Chapter 4

Phylogenetic inferences reveal deep polyphyly of Aongstroemiaceae and Dicranellaceae within the haplolepideous mosses (Dicranidae, Bryophyta)

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Introduction

The classification of mosses (Bryophyta) has changed considerably during the last two decades based on molecular phylogenetic inference. By identifying homology and convergence in morphological characters, molecular data has helped to tackle main challenges resulting from the traditional morphology-based moss classifications, such as different interpretations of the significance of gametophytic versus sporophytic traits, and the presence of morphologically ill-defined genera, families and orders that frequently changed their circumscription through time (Carvalho-Silva et al., 2017; Huttunen et al., 2018; and references therein). On the other hand, low molecular diversity and short branch lengths, probably resulting from rapid radiation, hampered assessing suprafamilial relationships, at least in the largest moss lineage, the pleurocarpous mosses (Huttunen et al., 2012; Shaw et al., 2003).

Haplolepideous mosses (subclass Dicranidae) form the second largest lineage of mosses with ca. 4000 species, corresponding to 30% of the currently recognised moss diversity (Frey & Stech, 2009). Over the last 20 years, molecular phylogenetic reconstructions have indicated the need for revising morphology-based classifications. Early molecular studies already resulted in significant rearrangements within the subclass at the suprafamilial level. Examples are the split of the Dicranaceae in its traditional sense (cf. Brotherus, 1909, 1924) into several families (La Farge et al., 2002; Stech, 1999b; Stech & Frey, 2008) and the transfer of several families and genera from other subclasses to the Dicranidae (Goffinet et al., 1998, 2001; Hedderson et al., 2004; La Farge et al., 2000; Stech, 1999a; Tsubota et al., 2003). Subsequent molecular analyses (Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a, 2016b; Goffinet et al., 2011; Ignatov et al., 2015; Inoue & Tsubota, 2014; Krug, 2017; Liu et al., 2019; Stech et al., 2012) added support to a division of Dicranidae into a paraphyletic assemblage of ‘protohaplolepideous’ taxa, an intermediate grade or differently supported clade, and a ‘core’ clade comprising the largest portion of the haplolepideous mosses, which only partially correspond to the existing ordinal classifications (cf. Stech et al., 2012).

Despite new insights from molecular data, analyses of a broader sampling from all major Dicranidae lineages, along with detailed studies of morphological and ecological evolution (Huttunen et al., 2018), are necessary for a revised classification. The importance of including understudied haplolepideous taxa in molecular analyses was recently exemplified by phylogenetic reconstructions of the morphologically diverse family Ditrichaceae, which turned out to be highly polyphyletic (Fedosov et al., 2015; Fedosov et al., 2016a). Three new families were established to accommodate part of the Ditrichaceae, namely Chrysoblastellaceae, Saelaniaceae, and Flexitrichaceae (Fedosov et al., 2016a). The latter study also shed new light on the evolution of the peristome as one of the main sporophytic characters for moss classification (Edwards, 1979). In addition to the different types of haplolepideous peristomes occurring in the Dicranidae (Frey & Stech, 2009; Ignatov et al., 2015; Shaw et al., 1989), with a single row of teeth (endostome) around the capsule mouth, Fedosov et al. (2016a) described the double-opposite peristome, with a developed exostome and endostome elements opposite the exostome teeth. The latter type occurs in the protohaplolepideous genus *Pseudoditrichum* and, albeit rather strongly reduced, in the likewise protohaplolepideous taxa *Catoscopium*, *Chrysoblastella* R.S. Williams, *Distichium* Bruch & Schimp. and *Flexitrichum flexicaule* (Schwägr.) Ignatov & Fedosov.

The circumscription of the Aongstroemiaceae and Dicranellaceae, and generic delimitations within them, are among the major problems remaining in the Dicranidae classification. The two families were resurrected or newly circumscribed, respectively, as segregates of the former Dicranaceae s.l. based on molecular data (see Stech & Frey, 2008). Aongstroemiaceae at present comprises five genera (*Aongstroemia*, *Aongstroemiopsis* M. Fleisch., *Dichodontium* Schimp., *Diobelonella* Ochyra, and *Polymerodon* Herzog) with 14 species, and Dicranellaceae comprises five genera (*Bryotestua* Thér. & P. de la Varde, *Campylopodium* (Müll.Hal.) Besch., *Dicranella*, *Leptotrichella* (Müll.Hal.) Lindb., and *Microcampylopus*) with about 230 species (Frey & Stech, 2009).

The families Aongstroemiaceae and Dicranellaceae, as well as their respective types *Aongstroemia* and *Dicranella*, have rather weak morphological circumscriptions (Frey & Stech, 2009). *Aongstroemia* currently has seven species (cf. Crosby et al., 1999; Frey & Stech, 2009; *Tropicos.org*) characterised by julaceous gametophytes with proximally concave leaves that are tightly appressed to the stem (in contrast to the more or less patent leaves in *Dicranella*). However, the species vary greatly in other morphological features (overall leaf shape, costa length, lamina cell shape, presence of gemmae in the leaf axils, presence of stomata on the capsule wall, presence of annulus, presence of peristome, shape of the peristome teeth; Allen, 1994; Crum, 1994; Drugova, 2010; Eckel, 2007), and some were considered to more closely resemble species from other genera (*Astomiopsis* Müll.Hal. and *Bryomanginia* Thér. from the Ditrichaceae; Allen, 1994).

One of the main problems concerning the circumscription of *Dicranella* started when Mitten (1869) and Bescherelle (1872) separated a number of species from *Dicranella* in the newly described genera *Anisothecium* Mitt. and *Microdus* Schimp. *Anisothecium* is distinguished from *Dicranella* by its peristome teeth that are attached to a somewhat higher basal membrane (Allen, 1994; Crum, 2007), while *Microdus* is considered to differ from *Dicranella* by its undivided, lightly papillose to nearly smooth and sometimes rudimentary peristome teeth (Ochyra, 1997). Different opinions about whether *Anisothecium* and *Microdus* should be kept separate, or included in *Dicranella*, have persisted until recently (*Anisothecium*: Allen, 1994; Crosby et al., 1999; Goffinet et al., 2008 *vs.* Crum, 2007; Frey & Stech, 2009; *Microdus*: Crosby et al., 1999; Frey & Stech, 2009; Goffinet et al., 2008 *vs.* Crum, 2007). *Microdus* was recognised as a synonym of *Leptotrichella*, and as the later name has priority (cf. Ochyra, 1997), the genus will be further referred to as *Leptotrichella* in this article. *Dicranella* s.l. comprises just under 220 accepted species, divided differently across the segregate genera in recent publications, e.g., 40 accepted species in *Anisothecium*, 162 in *Dicranella*, and 11 in *Leptotrichella* (as *Microdus*) in Crosby et al. (1999) vs. 158 in *Dicranella* (including *Anisothecium*), and 60 in *Leptotrichella* in Frey & Stech (2009). Not surprisingly, *Dicranella* s.l. comprises broad ranges in many morphological characters, e.g., rhizoidal gemmae (tubers), occurrence of sheathing leaf bases, differentiation of perichaetial leaves, color of the seta, inclination and shape of the capsule, presence of an annulus, and height of the basal membrane of the peristome (see, e.g., Nyholm, 1987; Risse, 1986; Smith, 2004).

So far, only two species of *Aongstroemia* and three of *Dicranella* have been included in molecular phylogenetic reconstructions, and already the analysis of such a small part of the (morphological) diversity of both genera indicates that they may not be monophyletic. Aongstroemiaceae and Dicranellaceae appeared as clearly separate in analyses that included the type species of *Aongstroemia*, *A. longipes* (Sommerf.) Bruch & Schimp., and of *Dicranella*, *D. heteromalla* (Hedw.) Schimp. (Bonfim Santos & Stech, 2017a; Stech, 1999b; Stech et al., 2012). In contrast, *Aongstroemia filiformis* (P. Beauv.) Wijk & Margad., occupied different phylogenetic positions, either together with genera of Ditrichaceae (Cox et al., 2010; Fedosov et al., 2015; as *A. jamaicensis* Müll.Hal.) or in the same clade as *D. heteromalla* (Fedosov & al., 2016a). *Dicranella cerviculata* (Hedw.) Schimp. was resolved as closely related to *D. heteromalla* in the Dicranellaceae, whereas *D. palustris* (Dicks.) Crundw. ex E.F. Warb. was resolved as more closely related to *Aongstroemia* and *Dichodontium* (La Farge et al., 2002; Stech, 1999b; Stech et al., 2012; Stech & Frey, 2008) and placed into Aongstroemiaceae based on molecular and morphological characters (cf. Frey & Stech, 2009; Ryszard Ochyra et al., 2003; Stech, 1999b, 1999c).

Furthermore, several genera presently classified in other families (cf. Frey & Stech, 2009) possibly belong to the Aongstroemiaceae or Dicranellaceae, namely *Bryowijkia* (Bryowijkiaceae Stech & W. Frey), *Cladophascum* Dixon (Bruchiaceae Schimp.), *Hygrodicranum*

Cardot and *Trichodontium* (Dixon) Fife (Dicranaceae), and three genera of Ditrichaceae (*Chrysoblastella* R.S. Williams, *Eccremidium* Wilson, *Garckea* Müll.Hal.) (Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a; Goffinet et al., 2011; Inoue & Tsubota, 2014; La Farge et al., 2002; Stech & Frey, 2008; Tsubota et al., 2003, 2004). *Chrysoblastella chilensis* (Mont.) Reimers was resolved in different positions in the phylogenetic trees based on different samples, either among the protohaplolepideous lineages (Cox et al., 2010; Fedosov et al., 2016a) or as sister to *Dicranella* (Inoue & Tsubota, 2014), indicating at least one misidentified specimen or contamination. *Microcampylopus* was erroneously resolved as part of the Leucobryaceae (Cox et al., 2010; Stech, 1999b) based on specimens later verified as belonging to *Campylopus* (see Bonfim Santos & Stech, 2017b) and *Pilopogon* (M. Stech pers. obs.).

The present study is intended to provide a baseline for future research on the phylogenetic relationships and circumscriptions of the Aongstroemiaceae and Dicranellaceae by summarizing the available knowledge and providing new phylogenetic analyses of published and newly generated molecular data. Specific goals of this study are to assess whether (i) Aongstroemiaceae and Dicranellaceae are molecularly distinct, (ii) *Aongstroemia* and *Dicranella* are monophyletic, and (iii) the current circumscriptions of the Aongstroemiaceae, Dicranellaceae, and their types are in line with their estimated phylogenetic relationships. Furthermore, the Dicranidae phylogeny will be reviewed by discussing the results of the present study in the context of earlier phylogenetic reconstructions.

Material and Methods

Taxon sampling, DNA extraction and sequencing

The sampling comprised DNA sequences of 168 specimens representing 117 species of haplolepideous mosses and all haplolepideous families except Viridivelleraceae, which has not yet been included in molecular analyses. Taxon and specimen selection were based on published phylogenetic reconstructions of the Dicranidae and the classification of Frey & Stech (2009). The latter listed species number per genus and adopted a narrower circumscription of the Dicranaceae, with the Aongstroemiaceae and Dicranellaceae regarded as separate families, in contrast to the most recent online classification (Goffinet & Buck, 2021). Thirteen species of *Dicranella*, namely *D. campylophylla* (Taylor) A. Jaeger, *D. cardotii* (R.Br.bis) Dixon, *D. cerviculata*, *D. crispa* (Hedw.) Schimp., *D. curvipes* (Lindb.) Ignatov, *D. grevilleana* (Brid.) Schimp., *D. heteromalla*, *D. howei* Renauld & Cardot, *D. rufescens* (With.) Schimp., *D. schreberiana* (Hedw.) Hilf. ex H.A. Crum & L.E. Anderson, *D. staphylina* H.Whitehouse, *D. subulata* (Hedw.) Schimp., and *D. varia*, as well as *Kiaeria riparia* (H. Lindb.) M.F.V.Corley (*Dicranella riparia* (H. Lindb.) Mårtensson & Nyholm) and three species of Aongstroemia, namely *A. filiformis*, *A. longipes*, and *A. orientalis* Mitt., were included.

Sequences of mitochondrial (*nad*5 G1 intron) and chloroplast markers (two parts of the *trn*S-*trn*F region: *trn*S-*rps*4 spacer/*rps*4 gene and *trn*L gene/*trn*L-*trn*F spacer; Hernández-Maqueda et al., 2008) were in part obtained from previous studies (Bonfim Santos & Stech, 2017a, 2017b; Cox et al., 2010; Fedosov et al., 2016a; La Farge et al., 2002; O'Brien, 2007; Stech, 1999b, 2004; Stech et al., 2012). The loci choice was based on the availability of sequences from these studies and considerations on marker variability. For example, the nuclear ribosomal ITS region was not included (following Bonfim Santos & Stech, 2017b) since the internal transcribed spacers are largely unalignable at family and suprafamilial levels in the Dicranidae.

Additionally, 208 new sequences of the target loci were generated either from specimens obtained from the herbaria L, MW and SP, either using DNA extracts from concluded (DNA barcoding of the Dutch bryophytes) or ongoing (Russian bryophyte flora) studies or from newly extracted DNA. These represented *Dicranella* (45 specimens), *Aongstroemia* (8), other taxa that are considered morphologically or phylogenetically close to Aongstroemiaceae or Dicranellaceae (7; of genera *Bryowijkia*, *Campylopodium*, *Campylopus*, *Dichodontium*, *Hygrodicranum*, *Kiaeria* I. Hagen, and *Microcampylopus*), and 11 representatives of other underrepresented haplolepideous lineages (*Blindia* Bruch & Schimp., *Dicranum*, *Distichium*, *Ditrichum* Hampe, *Erpodium* (Brid.) Brid., *Flexitrichum* Ignatov & Fedosov, *Platyneuron* (Cardot) Broth., *Rhamphidium* Mitt., *Trematodon* Michx.). *Encalypta streptocarpa* (Encalyptidae) and *Timmia austriaca* (Timmiidae) were included as outgroup representatives, based on their positions in previous published reconstructions (Cox et al., 2010; Tsubota et al., 2004).

Voucher information and GenBank accession numbers are listed in Appendix 1, with indications of the newly generated sequences and of the sequences that are missing from the dataset (either due to unsuccessful sequencing from our vouchers or because the sequences were not available for the vouchers from published phylogenetic studies included in our analyses).

In some cases, vouchers of specimens included in earlier phylogenies were requested on loan for morphological study, in particular when these were resolved in incongruent positions, indicating possible misidentification. The most prominent case concerns *Chrysoblastella chilensis*, which was resolved as sister to *Dicranella heteromalla* (specimen *R.D. Seppelt 26697*, HIRO; Inoue & Tsubota, 2014) or as part of the protohaplolepideous mosses, either as a separate lineage (specimen *Buck 39507*, DUKE; Cox et al., 2010) or as sister to *Pseudoditrichum* (Pseudoditrichaceae) in Fedosov et al. (2016a), the latter combining *nad*5 from *Buck 39507* and *rps*4 and *rbc*L from *R.D. Seppelt 26697*.

Procedures for DNA extraction, amplification and sequencing followed Bonfim Santos & Stech (2017a) and Fedosov et al. (2016a, 2016b). Sequences were manually aligned in Geneious®

v8.0.5 (Biomatters Ltd.; https://www.geneious.com), using the alignment from Bonfim Santos & Stech (2017a) as a starting point.

Phylogenetic reconstructions

Phylogenetic reconstructions were performed under maximum likelihood (ML) using RAxML v.8 (Stamatakis, 2014) and Bayesian inference (BI) using MrBayes v.3.2.6 (Ronquist et al., 2012), both on the CIPRES Science Gateway v.3.3 (M. A. Miller et al., 2010). Analyses were run for each marker separately to check for supported incongruence (conflicting topologies with >70% maximum likelihood bootstrap support or >0.95 Bayesian posterior probability, assessed by visual comparison of the respective trees) and for the concatenated alignment of all markers. Gaps were treated as missing data. Evolutionary model testing was performed in PartitionFinder v1.1.1 (Lanfear et al., 2012) for the models that can be implemented in RAxML (GTR) and MrBayes (GTR and several of its nested models), respectively, both with or without a gamma-distributed rate variation among sites (Γ) and/or a proportion of invariable sites (I). According to the Akaike information criterion (AIC), the selected evolutionary models by both tests (one for each RAxML and MrBayes implemented models) were GTR+Γ for the *nad*5 G1 intron and GTR+Γ+I for the *trn*S-*rps*4 spacer/*rps*4 gene and *trn*L gene/*trn*L-*trn*F spacer, which were implemented in the BI analyses. In RAxML a single type of rate heterogeneity pattern (either +Γ, +I or +Γ+I) can be applied for all partitions per analysis; thus, we implemented GTR+Γ in the ML analysis of the concatenated markers. In the concatenated marker analyses, model parameters were independently estimated for each partition. For all maximum likelihood analyses, rapid bootstrapping with the majority-rule criterion automatic halt (autoMRE) was performed. For Bayesian inferences, four runs with four chains (5×10^6 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.4. Chains were sampled every 1000 generations, and the respective trees were written to tree files. After verifying the convergence of runs in Tracer v1.6 (Rambaut et al., 2014), 50 percent majority-rule consensus trees and posterior probabilities of clades were calculated, discarding the burn-in phase (25%).

The Shimodaira-Hasegawa (SH) test (Goldman et al., 2000; Shimodaira & Hasegawa, 1999) was applied to test phylogenetic hypotheses related to the monophyly of *Dicranella*. In test 1, the ML tree (topology as in Figure 14) was compared with selected hypotheses obtained from the literature for the circumscriptions of *Aongstroemia*, *Dicranella*, and related genera. These hypotheses are listed in Table 4 along with the results. The generic placement of the species included in this study according to each hypothesis, as well as the constraint applied to each analysis, are provided in Appendix 5. In test 2 (Table 5), the ML tree was compared with alternative hypotheses of relationships between *Dicranella* representatives that were resolved in unsupported places in the ML tree, namely (1) the sister-group relationship of the *D.*

crispa/*D. subulata* clade and the *D. rufescens* clade, (2) the sister-group relationship of the *D. crispa*/*D. subulata* clade and the *D. staphylina* clade, (3) the sister-group relationship of the *D. staphylina* clade and the *D. rufescens* clade, and (4) all the *Dicranella* clades of uncertain placement (*D. staphylina*, *D. crispa* /*D. subulata*, *D. rufescens*) forming a clade. Constraint trees were used as an input to ML analyses with RAxML. The resulting trees with branch length values and corresponding alignment were loaded into PAUP* v.4.0b10 (Swofford, 2002), where these trees were compared with the respective unconstrained topologies using the SH test with 10,000 bootstrap replicates and the resampling estimated log-likelihood (RELL) method.

Table 4. Results from the SH test of selected hypotheses for the circumscriptions of Aongstroemia, Dicranella*, and related genera.*

Constrained topology	Diff lnL	*P*
Dicranella sensu Frey & Stech (2009)	367.77408	0.0000*
Dicranella sensu Crum (2007)	555.31960	0.0000*
Dicranella sensu Crosby et al. (1999)	378.05984	0.0000*
Dicranella sensu Frey & Stech (2009) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	404.65213	0.0000*
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	592.33283	0.0000*
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987) and exclusion of *Diobelonella palustris* (Dicks.) Ochyra (cf. Stech 1999c, Ochyra et al., 2003)	584.39542	0.0000*
Aongstroemia sensu Crosby et al. (1999)	399.13529	0.0000*
Aongstroemia monophyletic with the exclusion of *A. orientalis* (suggested to be closely related to Ditrichaceae genera; cf. Allen, 1994)	192.13965	0.0000*
Dichodontium and *Diobelonella palustris* forming a clade (*D. palustris* included in *Dichodontium* cf. Stech, 1999c)	2.55658	0.8423

The test was applied to the haplolepideous moss alignment of the concatenated molecular markers (mitochondrial *nad*5 G1 intron, and plastid *trn*S/*rps*4 gene and *trn*L gene-*trn*L-*trn*F spacer). * Statistically worse trees at $P < 0.05$.

Table 5. Results from the SH test of four alternative hypotheses of relationships between the Dicranella *clades of unsupported placement in the maximum likelihood tree presented in this study.*

Constrained topology	Diff lnL	*P**
D. crispa/*D. subulata* sister to *D. rufescens*	2.04326	0.7089
D. crispa/*D. subulata* sister to *D. staphylina*	7.20512	0.4403
D. rufescens sister to *D. staphylina*	6.88519	0.4401
Dicranella staphylina, *D. crispa*, *D. subulata*, *D. rufescens* forming a monophyletic group	7.02972	0.4498

The test was applied to the haplolepideous moss alignment of the concatenated molecular markers mitochondrial *nad*5 G1 intron, and plastid *rps*4 gene and *trn*L-*trn*F spacer.

* This test resulted in no statistically worse trees at $P < 0.05$.

Results

The alignment lengths for the *nad*5 G1 intron, *trn*S-*rps*4 spacer/*rps*4 gene, and *trn*L gene/*trn*L-*trn*F spacer were 967, 750, and 831 bp, respectively.

Figure 14 shows the single optimal maximum likelihood (ML) tree calculated from the concatenated mitochondrial and chloroplast markers, with indication of ML bootstrap support (BS) and posterior probabilities (PP) from Bayesian inference. No supported incongruences for the higher-level relationships discussed here were observed between the combined analysis and analyses run for each DNA region separately (Appendix 6). Some incongruences regarding relationships at the infrafamilial or infrageneric level were detected between the separate analyses (e.g., relationships between specimens of *Dicranella curvipes* and *D. heteromalla*).

Most relationships of the protohaplolepideous lineages, from *Catoscopium* to *Bryoxiphium* Mitt., were statistically supported in the phylogeny (Figure 14). These taxa were separated from a clade comprising *Dicranella staphylina*, the protohaplolepideous *Pseudoditrichum mirabile* Steere & Z. Iwats., and the remaining haplolepideous taxa with 84% BS and PP 1. Backbone relationships within the latter clade were poorly supported, except for the clade comprising *Amphidium* Schimp. and the core haplolepideous moss families, with a support value of PP 0.99.

The type species of *Aongstroemia*, *A. longipes*, and the conserved type of *Dicranella*, *D. heteromalla*, were resolved in separate clades. Five *Dicranella* species were resolved as more closely related to the type of *Aongstroemia* than to the type of *Dicranella*. Of these, *D. campylophylla*, *D. grevilleana*, and *D. schreberiana* formed a well-supported clade (BS 98%, PP

1) with *A. longipes* and two *Hygrodicranum* species (*H. bolivianum* Herzog, *H. herrerai* R.S. Williams). The clade including *A. longipes* and the above mentioned *Dicranella* and *Hygrodicranum* taxa was sister to *Dichodontium* (BS 77%). This larger clade was sister to *Diobelonella palustris* (Dicks.) Ochyra (BS 98%, PP 1). One further *Dicranella* species, *D. cardotii*, for which only *nad*5 G1 intron sequences were obtained, was also resolved within the Aongstroemiaceae (BS 97%, PP 1; Appendix 6). *Dicranella howei* and *D. varia* formed a clade (BS 87%, PP 0.95) that was resolved as sister to the clade formed by the Aongstroemiaceae genera (*Aongstroemia* s.str., *Dichodontium*, *Diobelonella*) plus the above mentioned *Dicranella* and *Hygrodicranum* species (BS 74%).

Dicranella heteromalla and the Asian *D. curvipes* (BS 96%, PP 1) formed a clade (BS 100%, PP 1) that was resolved as sister to *D. cerviculata* (BS 100%, PP 1). These three species formed the *Dicranella* s.str. clade (BS 100%, PP 1). The clade comprising species of the other Dicranellaceae genera (*Campylopodium*, *Leptotrichella*, *Microcampylopus*), as well as *Aongstroemia filiformis*, *Garckea phascoides* Müll.Hall. and *Trichodontium falcatum* (R.Br. bis) Fife (BS 100%, PP 0.95), was resolved as sister to *Dicranella* s.str. (BS 100%, PP 1), together forming the Dicranellaceae clade (BS 100%, PP 1). *Cladophascum gymnomitrioides* (Dixon) Dixon (Bruchiaceae) and *Eccremidium floridanum* H.A. Crum (Ditrichaceae) were sister species (BS 100%, PP 1), and together resolved as sister to the above described Dicranellaceae clade (BS 100%, PP 0.99). A clade formed by the two *Bryowijkia* species (BS 100%, PP 1) was sister to the *Cladophascum*-*Eccremidium*-Dicranellaceae clade (BS 100%, PP 1), and this larger clade including *Bryowijkia* was in turn sister to the specimen of *Chrysoblastella chilensis* labelled MS Cc (BS 74%, PP 1). The specimen of *Ditrichum* sp. labelled *Buck 39507* (as *Chrysoblastella chilensis* in Cox et al., 2010) was resolved as sister to a specimen identified as *Ditrichum* cf. *cylindricarpum* (Müll.Hal.) F. Muell. (BS 100, PP 1) within the protohaplolepideous grade.

A third *Aongstroemia* species, *A. orientalis*, and other three clades with *Dicranella* specimens, namely *D. staphylina*, *D. crispa*/D. *subulata*, and *D. rufescens*, all with significant support, did not belong to either the Aongstroemiaceae or Dicranellaceae. *Aongstroemia orientalis* was resolved as sister to *Astomiopsis amblyocalyx* Müll.Hall. (Ditrichaceae) (BS 100%, PP 1) within a clade including other Ditrichaceae and representatives of the Pottiaceae (BS 85%, PP 1). *Dicranella staphylina* showed affinities with the protohaplolepideous taxa (see above), while *D. crispa*/*D. subulata* and *D. rufescens* were resolved (without support) as the two clades closest to the core haplolepideous clade.

Kiaeria (*Dicranella*) *riparia* was resolved within the Rhabdoweisiaceae Limpr. clade, sister to the clade formed by *Arctoa fulvella* (Dicks.) Bruch & Schimp., *Glyphomitrium daviesii* (Dicks. ex With.) Brid., and *Oncophorus integerrimus* Hedenäs (BS 84%, PP 0.99).

None of the resolved clades corresponds to the genus *Anisothecium* as circumscribed in the consulted literature (cf. Appendix 5). The species of *Leptotrichella* included in our analyses (*L.*

flaccidula (Mitt.) Ochyra) was resolved as separate from any of the clades containing *Dicranella* species.

The SH test 1 rejected all the selected hypotheses of circumscriptions for *Aongstroemia*, *Dicranella* and related genera obtained from the literature (see Appendix 5) except the hypothesis of *Dichodontium flavescens* (Dicks.) Lindb., *D. pellucidum* (Hedw.) Schimp. and *Diobelonella palustris* forming a monophyletic group (Table 4). The SH test 2 did not reject any of the tested alternative hypotheses for the relationships between the three clades of *Dicranella* s.l. representatives of uncertain placement in the ML tree (*D. staphylina*, *D. crispa*/*D. subulata*, *D. rufescens*) (Table 5).

Discussion

Phylogeny of the haplolepideous mosses

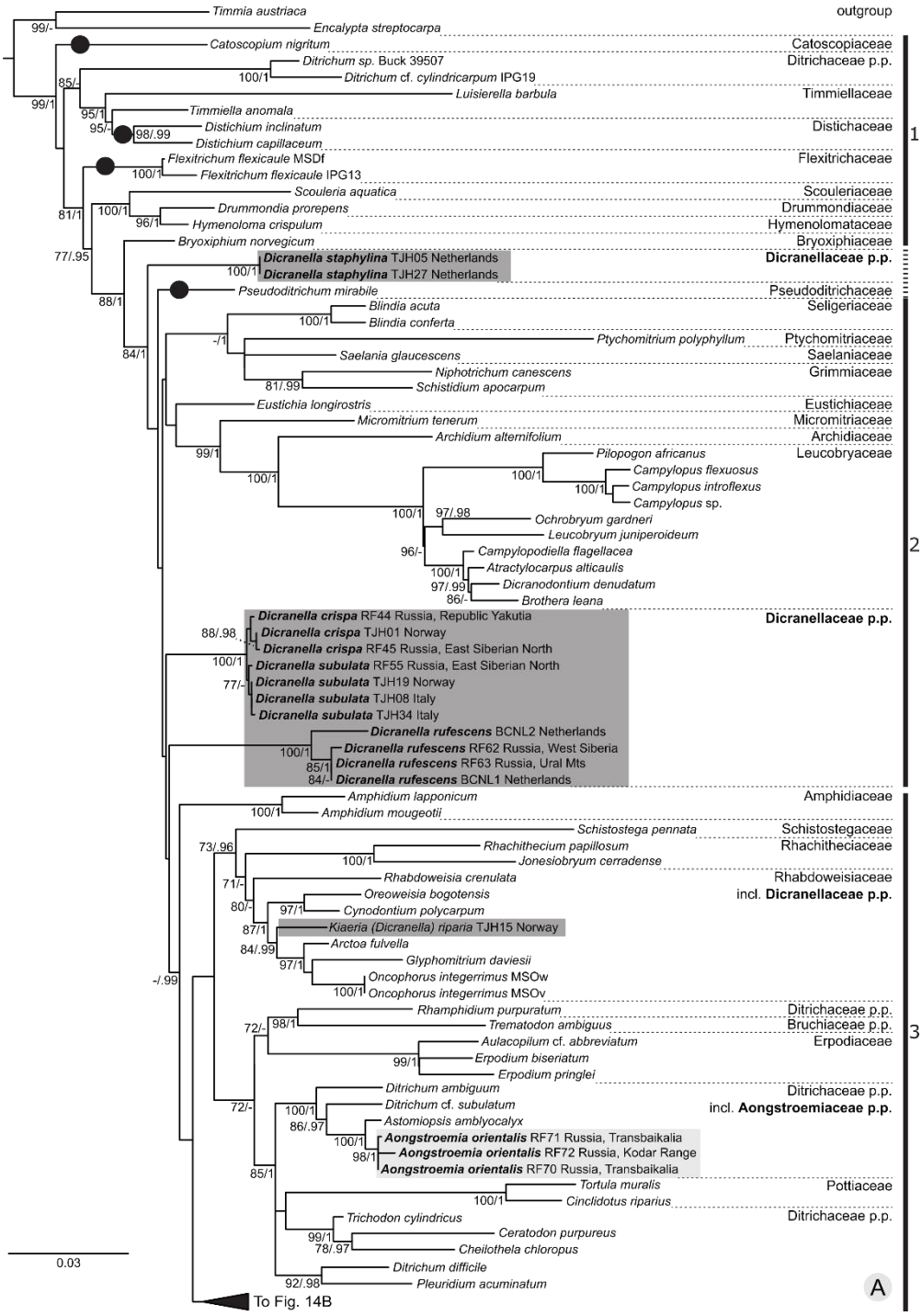
Relationships of the major lineages in Dicranidae (paraphyletic assemblage of 'protohaplolepideous' taxa, an intermediate grade or clade, and a 'core' clade comprising the largest portion of the haplolepideous mosses) are generally concordant in all recent phylogenies (e.g. Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a; Inoue & Tsubota, 2014; Liu et al., 2019; Stech et al., 2012; present study). As was previously shown for Ditrichaceae (Fedosov et al., 2015; Fedosov et al., 2016a), adding species from underrepresented genera (*Aongstroemia*, *Dicranella*, but also *Ditrichum*, see below) sheds new light on the phylogenetic diversity in the haplolepideous mosses. Based on the obtained topologies and morphological evidence, several of the newly discovered lineages should probably be recognised as separate genera and, in some cases, families. However, still only a small percentage of the species diversity of large genera such as *Dicranella*, *Ditrichum*, and *Leptotrichella* have been analysed. More extensive molecular phylogenetic reconstructions may resolve currently unsupported relationships with more confidence and may result in an even higher number of separate lineages that need to be addressed taxonomically. Nevertheless, the present results provide a new, more robust framework on which subsequent studies can build to eventually present a fully revised taxonomy of the Dicranidae.

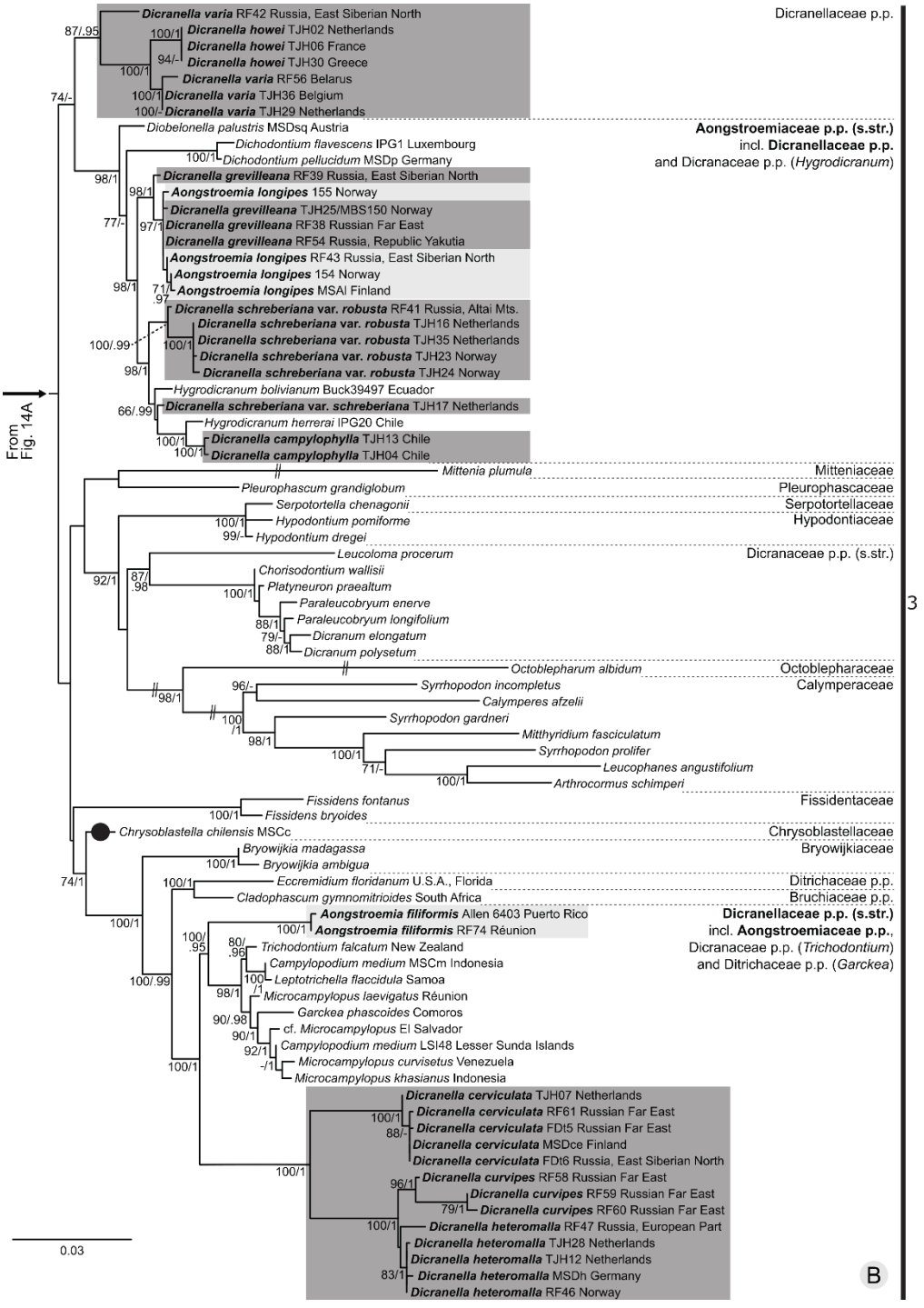
Apart from incomplete taxon sampling, the low and varying support for the backbone in the present and other Dicranidae phylogenies hampers inferences of relationships. Such low resolution was ascribed to a rapid radiation in the evolutionary history in the pleurocarpous Hypnales (M. Fleisch.) W.R. Buck & Vitt (Huttunen et al., 2012; Shaw et al., 2003), which may have occurred in the haplolepideous mosses as well (Cox et al., 2010). Molecular dating indicated that Dicranidae diversified within the last approximately 130 million years (Laenen et al., 2014), which is in accordance with the oldest reliable fossil evidence from the Cretaceous (older fossils that may represent haplolepideous species do exist but their affinities are less

clear; cf. discussion in Savoretti et al., 2018). As part of the first shift in diversification rate in mosses in the Cretaceous (Laenen et al., 2014), the evolution of the main haplolepideous lineages may thus be an example supporting the 'shadow of angiosperm' hypothesis, as a response to an explosive increase in the structural diversity of flowering plants (Laenen et al., 2014; Schmidt et al., 2010). However, low clade support may also result from using too few markers or markers with little variation and/or considerable homoplasy. Most backbone phylogenetic studies of Dicranidae were based on more markers but fewer taxa (e.g. Chang & Graham, 2014; Ignatov et al., 2015), but comparative analyses of possible correlations between (lack of) clade support, taxon sampling, and marker characteristics are still missing.

The present data show that the diversity of the protohaplolepideous grade is still incompletely known. A protohaplolepideous lineage with a ditrichoid morphology (specimens *Ditrichum* sp. *Buck 39507 p.p.* and *D.* cf. *cylindricarpum* IPG19) was discovered, adding to the polyphyly of *Ditrichum*. A detailed morphological and molecular study of this clade will be performed separately. Additionally, our phylogenetic analyses support the classification of *Chrysoblastella chilensis* in its own family, the Chrysoblastellaceae, which is, however, not closely related to the protohaplolepideous Pseudoditrichaceae and should be removed from the Pseudoditrichales, in contrast to the findings of Fedosov et al. (2016a).

Figure 14. Maximum likelihood tree of Dicranidae representatives, with Encalypta streptocarpa *(Encalyptidae) and* Timmia austriaca *(Timmiidae) as outgroup. The phylogenetic reconstruction was based on a concatenated dataset of mitochondrial* nad*5 intron and chloroplast* trn*S*-rps*4 and* trn*L*-trn*F regions, using the GTR+ Γ substitution model. Branch lengths are to scale, except those indicated by "//" (shortened to 50% of their original length). Maximum likelihood bootstrap support values ≥70% and posterior probabilities ≥0.95 from Bayesian inference are shown at the branches. Names of families resolved as para- or polyphyletic based on their latest circumscription are followed by 'p.p.'. Lineages representing the polyphyletic genera* Aongstroemia *and* Dicranella *are highlighted with lighter or darker grey boxes, respectively, and the respective family clades including the type species are indicated by 's.str.'. Vertical lines and numbers on the right indicate the main haplolepideous groups distinguished based on molecular data:* ***1****, protohaplolepideous grade;* ***2****, intermediate grade;* ***3****, core haplolepideous clade. The yet ambiguous transition between 1 and 2 is indicated by a dashed line (see text for details). Black circles indicate lineages with double opposite peristomes (complete in* Pseudoditrichum*, reduced in the other lineages).* →


outgroup
Timmia austriaca
Encalypta streptocarpa
Catoscopium nigritum
Catoscopiaceae
Ditrichum sp. Buck 39507
Ditrichum cf. cylindricarpum IPG19
Ditrichaceae p.p.
Luisierella barbula
Timmiellaceae
Timmiella anomala
Distichium inclinatum
Distichium capillaceum
Distichaceae
Flexitrichum flexicaule MSDf
Flexitrichum flexicaule IPG13
Flexitrichaceae
Scouleria aquatica
Scouleriaceae
Drummondia prorepens
Drummondiaceae
Hymenoloma crispulum
Hymenolomataceae
Bryoxiphium norvegicum
Bryoxiphiaceae
Dicranella staphylina TJH05 Netherlands
Dicranella staphylina TJH27 Netherlands
Dicranellaceae p.p.
Pseudoditrichum mirabile
Pseudoditrichaceae
Blindia acuta
Blindia conferta
Seligeriaceae
Ptychomitrium polyphyllum
Ptychomitriaceae
Saelania glaucescens
Saelaniaceae
Niphotrichum canescens
Schistidium apocarpum
Grimmiaceae
Eustichia longirostris
Eustichiaceae
Micromitrium tenerum
Micromitriaceae
Archidium alternifolium
Archidiaceae
Pilopogon africanus
Campylopus flexuosus
Campylopus introflexus
Campylopus sp.
Ochrobryum gardneri
Leucobryum juniperoideum
Leucobryaceae
Campylopodiella flagellacea
Atractylocarpus alticaulis
Dicranodontium denudatum
Brothera leana
Dicranella crispa RF44 Russia, Republic Yakutia
Dicranella crispa TJH01 Norway
Dicranella crispa RF45 Russia, East Siberian North
Dicranella subulata RF55 Russia, East Siberian North
Dicranella subulata TJH19 Norway
Dicranella subulata TJH08 Italy
Dicranella subulata TJH34 Italy
Dicranella rufescens BCNL2 Netherlands
Dicranella rufescens RF62 Russia, West Siberia
Dicranella rufescens RF63 Russia, Ural Mts
Dicranella rufescens BCNL1 Netherlands
Dicranellaceae p.p.
Amphidium lapponicum
Amphidium mougeotii
Amphidiaceae
Schistostega pennata
Schistostegaceae
Rhachithecium papillosum
Jonesiobryum cerradense
Rhachitheciaceae
Rhabdoweisia crenulata
Oreoweisia bogotensis
Cynodontium polycarpum
Rhabdoweisiaceae
incl. Dicranellaceae p.p.
Kiaeria (Dicranella) riparia TJH15 Norway
Arctoa fulvella
Glyphomitrium daviesii
Oncophorus integerrimus MSOw
Oncophorus integerrimus MSOv
Rhamphidium purpuratum
Ditrichaceae p.p.
Trematodon ambiguus
Bruchiaceae p.p.
Aulacopilum cf. abbreviatum
Erpodium biseriatum
Erpodium pringlei
Erpodiaceae
Ditrichum ambiguum
Ditrichum cf. subulatum
Astomiopsis amblyocalyx
Ditrichaceae p.p.
incl. Aongstroemiaceae p.p.
Aongstroemia orientalis RF71 Russia, Transbaikalia
Aongstroemia orientalis RF72 Russia, Kodar Range
Aongstroemia orientalis RF70 Russia, Transbaikalia
Tortula muralis
Cinclidotus riparius
Pottiaceae
Trichodon cylindricus
Ceratodon purpureus
Cheilothela chloropus
Ditrichaceae p.p.
Ditrichum difficile
Pleuridium acuminatum
0.03
To Fig. 14B
A
1
2
3


Dicranellaceae p.p.
Dicranella varia RF42 Russia, East Siberian North
Dicranella howei TJH02 Netherlands
Dicranella howei TJH06 France
Dicranella howei TJH30 Greece
Dicranella varia RF56 Belarus
Dicranella varia TJH36 Belgium
Dicranella varia TJH29 Netherlands
Aongstroemiaceae p.p. (s.str.)
incl. Dicranellaceae p.p.
and Dicranaceae p.p. (Hygrodicranum)
Diobelonella palustris MSDsq Austria
Dichodontium flavescens IPG1 Luxembourg
Dichodontium pellucidum MSDp Germany
Dicranella grevilleana RF39 Russia, East Siberian North
Aongstroemia longipes 155 Norway
Dicranella grevilleana TJH25/MBS150 Norway
Dicranella grevilleana RF38 Russian Far East
Dicranella grevilleana RF54 Russia, Republic Yakutia
Aongstroemia longipes RF43 Russia, East Siberian North
Aongstroemia longipes 154 Norway
Aongstroemia longipes MSAI Finland
Dicranella schreberiana var. robusta RF41 Russia, Altai Mts.
Dicranella schreberiana var. robusta TJH16 Netherlands
Dicranella schreberiana var. robusta TJH35 Netherlands
Dicranella schreberiana var. robusta TJH23 Norway
Dicranella schreberiana var. robusta TJH24 Norway
Hygrodicranum bolivianum Buck39497 Ecuador
Dicranella schreberiana var. schreberiana TJH17 Netherlands
Hygrodicranum herrerai IPG20 Chile
Dicranella campylophylla TJH13 Chile
Dicranella campylophylla TJH04 Chile
From Fig. 14A
Mittenia plumula
Mitteniaceae
Pleurophascum grandiglobum
Pleurophascaceae
Serpotortella chenagonii
Serpotortellaceae
Hypodontium pomiforme
Hypodontiaceae
Hypodontium dregei
Leucoloma procerum
Dicranaceae p.p. (s.str.)
Chorisodontium wallisii
Platyneuron praealtum
Paraleucobryum enerve
Paraleucobryum longifolium
Dicranum elongatum
Dicranum polysetum
Octoblepharum albidum
Octoblepharaceae
Syrrhopodon incompletus
Calymperaceae
Calymperes afzelii
Syrrhopodon gardneri
Mitthyridium fasciculatum
Syrrhopodon prolifer
Leucophanes angustifolium
Arthrocormus schimperi
Fissidens fontanus
Fissidentaceae
Fissidens bryoides
Chrysoblastella chilensis MSCc
Chrysoblastellaceae
Bryowijkia madagassa
Bryowijkiaceae
Bryowijkia ambigua
Eccremidium floridanum U.S.A., Florida
Ditrichaceae p.p.
Cladophascum gymnomitrioides South Africa
Bruchiaceae p.p.
Aongstroemia filiformis Allen 6403 Puerto Rico
Aongstroemia filiformis RF74 Réunion
Dicranellaceae p.p. (s.str.)
incl. Aongstroemiaceae p.p.,
Dicranaceae p.p. (Trichodontium)
and Ditrichaceae p.p. (Garckea)
Trichodontium falcatum New Zealand
Campylopodium medium MSCm Indonesia
Leptotrichella flaccidula Samoa
Microcampylopus laevigatus Réunion
Garckea phascoides Comoros
cf. Microcampylopus El Salvador
Campylopodium medium LSI48 Lesser Sunda Islands
Microcampylopus curvisetus Venezuela
Microcampylopus khasianus Indonesia
Dicranella cerviculata TJH07 Netherlands
Dicranella cerviculata RF61 Russian Far East
Dicranella cerviculata FDt5 Russian Far East
Dicranella cerviculata MSDce Finland
Dicranella cerviculata FDt6 Russia, East Siberian North
Dicranella curvipes RF58 Russian Far East
Dicranella curvipes RF59 Russian Far East
Dicranella curvipes RF60 Russian Far East
Dicranella heteromalla RF47 Russia, European Part
Dicranella heteromalla TJH28 Netherlands
Dicranella heteromalla TJH12 Netherlands
Dicranella heteromalla MSDh Germany
Dicranella heteromalla RF46 Norway
0.03
3
B

When including all available *Chrysoblastella chilensis* accessions as separate samples in preliminary analyses, they were resolved in very distant positions: *Buck 39507* and our voucher IPG19 formed the protohaplolepideous lineage mentioned above, whereas *R.D. Seppelt 26697* (not included in Figure 14) was resolved closely related to Bryowijkiaceae and Dicranellaceae s.str., sister to our voucher MSCc (herbarium B; published as *Cheilothela chloropus* (Brid.) Lindb. in Stech et al., 2012; Bonfim Santos & Stech, 2017a). None of these vouchers was sister to *Pseudoditrichum mirabile* as resolved in Fedosov et al. (2016a). Morphological identification of the available specimens revealed that voucher MSCc matches the description of *Chrysoblastella chilensis*, IPG19 was identified as *Ditrichum* cf. *cylindricarpum*, and *Buck 39507* turned out to be a mixed voucher of an undetermined *Ditrichum* species (likely the plant sequenced in Cox et al., 2010) and a true plant of *C. chilensis*. The morphological description of Chrysoblastellaceae in Fedosov et al. (2016a), which is in line with other descriptions of the genus *Chrysoblastella* (e.g. Buck, 1981), was based on a third specimen that has not yet been included in phylogenetic analyses (*Ireland & Bellolio 32976*; NY, duplicate MHA).

The present results have important implications for the evolution of the double-opposite peristome, indicating that this peristome type is not confined to the protohaplolepideous mosses, but evolved independently in the core haplolepideous mosses as well, or appeared as a rudimentary plesiomorphic trait. Furthermore, the present study, together with other phylogenies (e.g., Carter et al., 2014; Fedosov et al., 2021; Goffinet et al., 2011), suggests multiple losses of the peristome (capsules gymnostomous or cleistocarpous) during Dicranidae evolution, which occurred in the protohaplolepideous mosses (e.g., *Scouleria* Hook. p.p., *Bryoxiphium*), the intermediate grade or clade (e.g., Micromitriaceae) and the core haplolepideous clade (e.g., *Amphidium*, *Schistostega* D. Mohr, Rhabdoweisiaceae p.p., the *Astomiopsis-Aongstroemia orientalis* clade, Pottiaceae p.p., *Pleurophascum* Lindb., and the *Cladophascum-Eccremidium* clade).

Phylogeny of the Aongstroemiaceae, Dicranellaceae, and their types

The Aongstroemiaceae and the Dicranellaceae were resolved in our analyses as separate families within the core haplolepideous clade. Their present circumscriptions, however, are not in line with the inferred phylogenetic relationships, for two reasons. Firstly, *Aongstroemia* and *Dicranella* are polyphyletic according to the present data, and secondly, species of other genera were resolved within the Aongstroemiaceae and Dicranellaceae clades, as discussed in the following sections. A similar result was obtained before for the morphologically weakly delimited *Ditrichum* and the Ditrichaceae (Fedosov et al., 2015; Fedosov et al., 2016a). Only three out of the 13 species included species of *Dicranella* actually belong in the Dicranellaceae. The other 10 species are either resolved closer to the type of *Aongstroemia*, *A. longipes* (Aongstroemiaceae), or form clades not closely related to any of the currently recognised

families. Likewise, the three sampled *Aongstroemia* species are each resolved in a different core haplolepideous family (i.e., *A. longipes* in the Aongstroemiaceae, *A. filiformis* in the Dicranellaceae, and *A. orientalis* in the Ditrichaceae). The position of *Kiaeria* (*Dicranella*) *riparia* in the Rhabdoweisiaceae is confirmed by the detailed phylogenetic analysis of the latter family in Fedosov et al. (2021).

None of the tested circumscriptions of *Aongstroemia* and *Dicranella* from the literature are supported by our data (SH test: Table 4, Appendix 5). *Anisothecium* (e.g. sensu Crosby et al., 1999) is not supported as a separate genus from *Dicranella*, since the analysed *Dicranella* species placed in *Anisothecium* (e.g. by Crosby et al., 1999; *Dicranella campylophylla*, *D. grevilleana*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. varia*; cf. Appendix 5) are divided into different and not closely related clades as well. Based on the single species included, *Leptotrichella* (sensu Ochyra, 1997) is supported as separate from all *Dicranella* lineages.

Current circumscriptions of *Aongstroemia* and *Dicranella* originated from classifications published in the 19th century and, in fact, are based on plesiomorphic characters (stegocarpous capsules with a well-developed dicranoid peristome) and highly homoplastic traits, which likely originated independently in several lineages of pioneer mosses (small and slender plants, julaceous appearance or, in contrast, linear to subulate leaves), but ignored morphological characters of higher taxonomic value (e.g., presence of an annulus, shape of rhizoid tubers, etc.). Similarly, molecular phylogenetic approaches revealed numerous cases of deep polyphyly of traditionally circumscribed genera in pleurocarpous mosses, for instance *Calliergon* (Sull.) Kindb., *Drepanocladus* (Müll.Hal.) G. Roth, and *Hygrohypnum* Lindb. (Vanderpoorten et al., 2002b, 2002a) as well as *Hypnum* Hedw. (Câmara et al., 2018; Kučera et al., 2019; Schlesak et al., 2018), and these results were immediately followed by corresponding taxonomical solutions.

The re-evaluation of the broad morphological variation in *Dicranella* and *Aongstroemia* based on the current sampling revealed that the different molecular lineages resolved in our analyses possess distinctive (combinations of) morphological characters, as described in the following sections. In particular, the taxonomic significance of the morphology of rhizoid tubers (rhizoid-borne vegetative propagules) for *Dicranella* s.l., first suggested by Risse (1986) but not considered in subsequent studies, was supported by our molecular results. We provide tentative morphological descriptions for the Aongstroemiaceae and *Dicranella* clades based on the sampled specimens and the literature, as a basis for further study and taxonomical consequences. A densely sampled phylogeny coupled with extensive morphological study, as a follow up of the present research, may identify informative morphological characters to circumscribe Aongstroemiaceae and Dicranellaceae.

Revised circumscription of Dicranella *s.str. and the Dicranellaceae*

The clade referred here as *Dicranella* s.str. comprises the conserved type species, *D. heteromalla* (cf. Margadant & Geissler, 1995), *D. cerviculata*, and the Asian *D. curvipes*. Based on these three species, *Dicranella* s.str. would be recognised by the wide (⅓–½ of the leaf width at base) and excurrent costae and yellow setae, combined with stem leaves that are not sheathing but perichaetial leaves with sheathing bases that suddenly contracts into long, narrow subulas, capsules that are inclined to horizontal, asymmetric, curved, and furrowed to sulcate when dry, with annuli poorly differentiated (Nyholm, 1987; Smith, 2004), and the absence of rhizoid tubers (Correns, 1899; Risse, 1986; Whitehouse, 1966). *Dicranella cerviculata* has entire to slightly serrulate leaf apices, weakly delimited costae and strumose capsules, while *D. heteromalla* has distinctly serrulate leaf margins from the apex up to midleaf, strong costae, and capsules not strumose (Nyholm, 1987). *Dicranella curvipes*, distinct from *D. heteromalla* by their cygneous setae, was described as *D. heteromalla* var. *curvipes* Lindb. (Lindberg, 1872) and recently raised to the species level by Ignatov et al. (2006). The present molecular data do not unequivocally separate *D. curvipes* from *D. heteromalla* (Appendix 6), and further study is needed to assess the taxonomic status of *D. curvipes.*

Analyses by Cox et al. (2010) and Fedosov et al. (2016a) had already shown a close relationship of *Aongstroemia filiformis* (= *A. jamaicensis*, cf. Allen, 1994), *Cladophascum* (Bruchiaceae), *Eccremidium floridanum* (Ditrichaceae) and the type of *Garckea*, *G. phascoides* (Ditrichaceae). However, Cox et al. (2010) did not include any *Dicranella* species, whereas in Fedosov et al. (2016a) a specimen of *Dicranella heteromalla* was part of a clade containing the same vouchers of *A. filiformis*, *E. floridanum*, and *G. phascoides*. According to the present results, Dicranellaceae comprise the taxa listed above together with *Trichodontium* and the core genera already included in the family in Frey & Stech (2009) (*Campylopodium*, *Dicranella* s.str., *Leptotrichella*, *Microcampylopus*).

Aongstroemia filiformis differs morphologically from the type of *Aongstroemia*, *A. longipes* (see below), by its larger leaves (4–6 mm vs. 0.5–1 mm) that are abruptly subulate from oblong leaf bases (vs. scale-like to ovate-lanceolate in *A. longipes*), excurrent costae (vs. subpercurrent to percurrent or only rarely excurrent), laminal cells that are short rectangular at the leaf base to linear-vermicular at the apex (vs. elongate, irregularly hexagonal, rhomboid or rectangular, and shorter at the apex), capsules with stomata, and peristomes that are divided above into two or three prongs (vs. divided, perforated or entire) (Allen, 1994; Crum, 1994; Eckel, 2007a).

Based on the phylogenetic relationships resolved here, the Dicranellaceae would include plants with three different sporophytic morphologies, with either (1) long setae and emergent to exserted, peristomate capsules (in the initially included genera, plus *A. filiformis* and *Trichodontium*); (2) short setae and immersed, peristomate capsules (in *Garckea*); and (3) short setae and eperistomate capsules (immersed in *Cladophascum*, most commonly laterally

emergent and pendulous but sometimes erect and immersed in *Eccremidium*) (Buck, 2007; Crum, 1994; Sim, 1926). The family thus would include at least two lineages with independent sporophyte reduction (*Garckea* and *Cladophascum*/*Eccremidium*), and the present results add to the understanding of the relationships of these lineages as inferred in previous studies (Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a). Gametophytically, all Dicranellaceae taxa share long lanceolate leaves (in *Cladophascum*, at least the perichaetial leaves) with a strong costa (Frey & Stech, 2009), which are not very distinctive among the haplolepideous mosses. Dicranellaceae are markedly morphologically distinct from their well-supported sistergroup *Bryowijkia* (Bryowijkiaceae), which has cladocarpous, profusely branched plants, plicate leaves with differentiation between stem and branch leaves, and microstomous capsules (Frey & Stech, 2008; Vitt & Buck, 1984).

Revised circumscription of Aongstroemia *s.str. and the Aongstroemiaceae*

Based on the present results, the Aongstroemiaceae clade comprises *Aongstroemia longipes*, *Dichodontium*, and *Diobelonella* (as in Frey & Stech, 2009) as well as five species presently placed in *Dicranella* (three of which have been previously combined under *Aongstroemia* by Carl Müller (1849): *D. campylophylla*, *D. grevilleana*, and *D. varia*) and two *Hygrodicranum* species. Within this clade, there was less molecular support for the sister-group relationship of the *Dicranella* lineage composed by *D. howei* and *D. varia* to the Aongstroemiaceae s.str. clade. *Dicranella howei* and *D. varia* have in common with the Aongstroemiaceae s.str. clade peristome teeth that are vertically pitted-striolate at base. *Dicranella howei* and *D. varia* further resemble part of the taxa in the Aongstroemiaceae s.str. in their inclined, ovoid, asymmetric, gibbous capsules that remain smooth when dry. On the contrary, they differ from *Dichodontium* by the presence of rhizoid tubers, from *Aongstroemia* and *Diobelonella* by the irregular instead of spherical shape of the tubers (although *Aongstroemia* does not always present tubers), and further from *Aongstroemia* by having undifferentiated stem and perichaetial leaves without sheathing bases (Crum, 2007; Eckel, 2007a; Renauld & Cardot, 1893; Smith, 2004; Whitehouse, 1966). With or without the inclusion of *D. howei* and *D. varia* as part of the Aongstroemiaceae, the family remains morphologically heterogeneous and without distinctive characters that separate it from other haplolepideous moss families.

The current characterization of *Aongstroemia* based on the possession of julaceous gametophytes (see Eckel, 2007) does not hold, since the three included *Aongstroemia* species belong to different families, whereas the *Dicranella* and *Hygrodicranum* species that were resolved as closely related to *A. longipes* do not have julaceous gametophytes. The re-circumscribed *Aongstroemia* would be recognised by stem leaves with a broad sheathing base that is abruptly contracted to a short- to long-pointed, spreading to squarrose leaf apex (the latter also present in well-developed *A. longipes* plants, according to Drugova, 2010),

rectangular lamina cells, spherical rhizoid tubers (if present) without protruding cells, capsules erect to inclined, symmetric to asymmetric, oval/obloid, straight to curved and sometimes slightly strumose, on a straight, erect, red to darkened seta (Smith, 2004; Whitehouse, 1966). The leaves of *A. longipes* may have originated from a reduction of the apex, eliminating the subulate awn and thus resulting in the ovate-lanceolate leaf shape and julaceous habit. A broad range of lengths of the leaf awn is not unusual among haplolepideous mosses, for instance, in some species of the genus *Ditrichum* (see the complex *D. lineare* (Sw.) Lindb./*D. plumbicola* Crundw.; Atherton et al., 2010; Frahm et al., 2008). The close relationship of *Aongstroemia longipes* and *Dicranella grevilleana*, which cannot be separated with the present molecular markers, may indicate that the gametophyte morphology of *A. longipes* represents a unique derived state within the clade. Capsule morphology markedly differs between *A. longipes* (capsules ovoid, symmetric, erect, smooth) and *D. grevilleana* (capsules curved, asymmetric, inclined, furrowed when dry), although in their sister clade morphological transitions exist within single species: In both *D. campylophylla* and *D. schreberiana* capsule shape ranges between that of *A. longipes* and *D. grevilleana* (Ochyra et al., 2008; Smith, 2004). Nevertheless, *A. longipes* and *D. grevilleana* should be maintained as separate species unless evidence to the contrary arises from further phylogenetic analyses.

Further problems of species delimitation to be addressed in subsequent studies concern *Dicranella varia*/*D. howei* and *D. schreberiana*. The former two species were regarded as conspecific by some authors (e.g., Crum, 2007) but not yet formally synonymised (cf. Tropicos, 2020). The results of our phylogenetic analyses support the monophyly of *D. howei* but not of *D. varia*. The split of *D. varia* into a clade of European samples sister to *D. howei*, and a single specimen from Siberia, together with differences between the type specimen of *D. howei* from California and Mediterranean material (Crundwell & Nyholm, 1977), and the occurrence of intermediate forms between the two species, support the need of further study. The same holds for the two varieties of *D. schreberiana* included in this study, which were resolved in separate positions, with *D. schreberiana* var. *schreberiana* resolved as more closely related to *Hygrodicanum* and *D. campylophylla* than to *D. schreberiana* var. *robusta* (Schimp. ex Braithw.) H.A. Crum & L.E. Anderson.

The clade formed by *D. campylophylla*, *D. cardotii*, *D. schreberiana*, *Hygrodicranum bolivianum* and *H. herrerai* (Figure 14, Appendix 6) is the only one in our analysis to include former *Dicranella* species with mamillose or papillose lamina cells. Moreover, this clade includes species with a bistratose lamina, also found in the *D. howei*/*D. varia* clade, and in the genus *Dichodontium* (Smith, 2004). A regularly to irregularly bistratose lamina (homogeneously two-layered or with an interrupted, irregular second layer) is the main diagnostic character of *Hygrodicranum*, which comprises three aquatic species (Cook et al., 1974) never collected with sporophytes, but is found in some *Dicranella* species (as *D. campylophylla* and *D. cardotii*) as well. Earlier molecular analyses of aquatic, especially rheophytic, pleurocarpous mosses have

already shown that the character of bi- to multistratose laminae was taxonomically overrated (e.g., Spitale & Petraglia, 2010; Stech & Frahm, 2000). A similar example from the haplolepideous mosses is *Fissidens grandifrons* Brid., which was earlier classified in its own genus *Pachyfissidens* (Müll.Hal.) Limpr. (Limpricht, 1887: 454). The present data indicate that *Hygrodicranum* does not deserve recognition as a separate genus, but the type, *H. falklandicum* Cardot, is yet to be included in phylogenetic analyses.

The separation of *Diobelonella* from *Dichodontium* (Ochyra et al., 2003; as opposed to Stech, 1999c) is supported by our molecular results and morphology. While *Dichodontium* has short, thick-walled, coarsely-papillose or mamillose distal lamina cells, irregularly dentate upper leaf margins, and strong costae with two stereid bands, *Diobelonella* has prosenchymatous, thin-walled, entirely smooth distal lamina cells, entire to crenulate leaf margins, and weak costae with a single stereid band (Ochyra et al., 2003). Furthermore, *Diobelonella palustris* has spherical rhizoid tubers similar to those of *Dicranella campylophylla*, *D. grevilleana*, and *D. schreberiana*, although the tubers of the latter three species do not have protruding cells (Ochyra et al., 2003; Risse, 1986; Whitehouse, 1966). *Dichodontium* is not reported to have typical rhizoid tubers but bears ellipsoid or clavate multicellular gemmae on filamentous branches on the leaf axils (Eckel, 2007b; Smith, 2004). On the other hand, the results of the SH test (Table 4, Appendix 5) do not reject the hypothesis of *Dichodontium* and *Diobelonella palustris* forming a single clade.

Dicranella *and* Aongstroemia *segregates outside Dicranellaceae and Aongstroemiaceae*

Regarding species so far still considered in *Dicranella*, the precise placement of three remaining supported *Dicranella* clades (*D. staphylina*, *D. crispa*/*D. subulata* and *D. rufescens*), intermediate between the protohaplolepideous and the main haplolepideous clade, is still unclear. The SH test rejected the hypothesis of the monophyly of a clade formed by all *Dicranella* species included in this study but did not exclude the hypothesis that these three *Dicranella* clades form a monophyletic group or that any two of the three clades are sister groups (Table 5). Nevertheless, considering that these clades can each be recognised by a combination of morphological features, but have little in common, they might be considered as different genera and families.

Dicranella staphylina is a very small species known from cultivated fields across North America and Europe. The epithet is based on the characteristic rhizoid tubers shaped like bunches of grapes (from the Greek *staphyle*), which are regularly found (Miguel Velasco, 1986; Whitehouse, 1969, 2001). In addition to the tubers, plants of *D. staphylina* can be recognised by bright green color, stems ramified only at base, and stem leaves not sheathing, often with a recurved margin at the base (Nyholm, 1987; Whitehouse, 1969). Its sporophytes are little known, which is a common phenomenon among tuber-bearing moss species (Whitehouse,

1966). In fact, the only report of this life phase corresponded to 10 immature sporophytes and lacked information about some features as annulus and basal membrane (Arts, 1985). Characters that could be inferred from the perichaetial leaves (differentiated, with a sheathing base and abruptly contracted into the spreading apex) and the immature sporophytes (seta yellow to orange, capsules erect, symmetrical, smooth, with few stomata, and peristome teeth bifid to the middle; Arts, 1985), are little informative for the relationships with other haplolepideous mosses and yet to be confirmed based on mature sporophytes. Nevertheless, the phylogenetic position of *D. staphylina* (represented here by Dutch specimens), branching off early in the haplolepideous moss tree, although without support (Figure 14), indicates that it was assigned to *Dicranella* based on rather superficial gametophytic similarities.

The clade composed of *Dicranella crispa* and *D. subulata* can be recognised by having an oblong leaf base gradually narrowed into a long subulate apex (abruptly so in *D. subulata* perichaetial leaves), percurrent to excurrent costa filling most of the subula, and capsules +/- erect, +/- symmetric, striate to furrowed when dry, with well-differentiated annulus formed by 2–3 rows of widened cells (Crum, 2007; Nyholm, 1987). It is morphologically close to the *Dicranella schreberiana* clade, but the latter differs in the very broad, abruptly narrowed ('quadrate') and tightly clasping sheathing base in stem leaves, the most frequently inclined to horizontal and slightly asymmetric to gibbous capsules (Nyholm, 1987; Smith, 2004), spherical rhizoid tubers (Whitehouse, 1966) and not or poorly differentiated annulus. Tubers in *D. subulata* resemble the other basal lineage corresponding to *D. rufescens* and are considered structurally homologous to rhizoids (different from, e.g., those of *D. campylophylla*, considered to develop from a tuber initial cell; cf. Risse, 1986). *Dicranella crispa* and *D. subulata* are molecularly (Figure 14) and morphologically distinct from each other. *Dicranella crispa* has a squarrose leaf apex from the sheathing base and an erect capsule, while *D. subulata* has leaves +/- erect spreading or secund (only perichaetial leaves with sheathing base) and capsules sometimes slightly inclined and asymmetric (Nyholm, 1987; Smith, 2004).

Dicranella rufescens, which has also been combined into the genera *Anisothecium*, *Aongstroemia*, and *Dicranum*, differs from all other *Dicranella* lineages included in this study by two characters: its peristome with a high basal membrane, contrasting to the short basal membranes up to three cells high in the other lineages, and the red color of its stems (Hallingbäck et al., 2006). *Dicranella rufescens* is morphologically close to *D. humilis* R. Ruthe. The latter species shares with *D. rufescens* the red coloration of the stem but differs by inclined, slightly curved and asymmetric capsules (upright, straight, symmetric in *D. rufescens*) (Hallingbäck et al., 2006). Additionally, Kučera (2004) describes that *D.* rufescens has exothecial walls that are always equally thickened, while *D. humilis* has sometimes weaker transverse walls, even though this character does not seem to be a stable distinguishing trait. Among our specimens originally labelled as *D. rufescens,* BCNL1 was sterile, and BCNL2 had the typical capsules of *D. rufescens*. RF63, however, was initially identified as *D. humilis*, based

on its slightly inclined capsules and slightly different thickness of the longitudinal and transverse exothecial cell walls.

Based on the absence of peristome teeth, Allen (1994) considered some species of *Aongstroemia* to resemble the Ditrichaceae genera *Astomiopsis* and *Bryomanginia*. As predicted based on morphology, *Aongstroemia orientalis* was found to be closely related to *Astomiopsis amblyocalyx* (Ditrichaceae) and should probably be transferred to that genus. *Aongstroemia orientalis* and also *A. julacea* (Hook.) Mitt. (the latter not yet included in molecular phylogenetic analyses) differ from *Aongstroemia* as defined here (see above) not only by eperistomate capsules but also in having gemmae in the leaf axils (Allen, 1994; Drugova, 2010). The other three currently accepted *Aongstroemia* species not yet included in molecular phylogenetic studies, *A. appressa* Hampe ex Müll.Hal., *A. gayana* (Mont.) Müll.Hal., and *A. subcompressa* Hampe ex Müll Hal. are little known, and their affinities are unclear.