Phylogeny of haplolepideous mosses —
challenges and perspectives

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The haplolepideous mosses (Dicranidae) form the second largest group of mosses and are morphologically and ecologically highly diverse. This review summarizes the current state and addresses the most urgent remaining problems in unravelling systematic relationships in the haplolepideous mosses. The main results of early molecular phylogenetic reconstructions based on few chloroplast markers are compared with recent approaches based on markers from different genomes as well as with a new phylogeny based on a novel combination of non-coding plastid markers (rps4-trnF region and atpB-rbcL spacer). According to the available molecular data, three major groups are provisionally distinguished within Dicranidae. The first group comprises morphologically diverse species from different families (Bryoxiphiaceae, Catascopiaceae, Distichiaceae, Ditrichaceae p.p., Drummondiiaceae, Pottiaceae p.p., Rhabdoweisiaceae p.p., and Scouleriaceae p.p.), which form grades branching off first in the phylogenetic reconstructions. The second group, which appears as a grade or unsupported clade, includes Grimmiales, Leucobryaceae, Archidiaceae, Eustichiaeeae, and Saelania glaucescens (Ditrichaceae). The third group comprises the largest portion of the haplolepideous mosses, namely most families of Dicranales as well as the most speciose Pottiales; the respective clades receive significant statistical support in part of the analyses. The position of Amphidium in between the second and third group remains ambiguous. It is concluded that further phylogenetic analyses based on new combinations of markers are necessary at different taxonomic levels, especially to resolve the backbone of the Dicranidae phylogeny, but also to tackle large and taxonomically complex genera that are severely understudied. Implications of the molecular phylogenetic reconstructions for morphological character evolution are exemplarily discussed for the different types of haplolepideous peristomes. Furthermore, genetic and genomic research using haplolepideous taxa is briefly reviewed.

Keywords: Dicranidae, Haplolepideous mosses, Non-coding plastid markers, Phylogeny, Review

Introduction

With about 4000 species in 232 genera and 30 families (Frey & Stech, 2009), the haplolepideous mosses or haplolepiods (Dicranidae) form the second largest subclass of mosses (Bryophyta). Dicranidae are morphologically and ecologically highly diverse and occur in almost all terrestrial ecosystems. They are characterized by the arthrodontous–haplolepideous (Dicranum-type) peristome, which usually consists of a single row of teeth around the capsule mouth. All molecular phylogenetic reconstructions so far support the monophyly of Dicranidae and a position nested within the arthrodontous–diplolepideous mosses, as sister to the largest subclass of mosses, the diplolepideous-alternate Bryidae (reviewed in Stech & Frey, 2008 and Cox et al., 2010). The molecular data therefore indicate that the haplolepideous peristome evolved from a diplolepideous ancestor.

In the first half of the last decade, a booming period of phylogenetic analyses of the major moss lineages, several analyses were published from which ordinal and family-level relationships within the Dicranidae could be inferred. These studies either explicitly focused on the haplolepideous mosses (Stech, 1999a,b; La Farge et al., 2000, 2002; Tsubota et al., 2003; Hedderson et al., 2004) or included a considerable number of haplolepideous taxa in analyses of a broader range of mosses (Goffinet et al., 2001;
Tsubota et al., 2004). They were all based on one or more of three plastid DNA regions (trnL-F, rps4, and rbcL) that are still among the most commonly used standard markers in moss phylogenetics (cf. Stech & Quandt, 2010). Thereafter only few new phylogenies of mosses or land plants in general were published (e.g. Qiu et al., 2006; Stech & Quandt, 2006; Stech & Frey, 2008), which did not contribute much to resolving higher-level relationships within Dicranidae. However, recent approaches based on novel mitochondrial loci (Wahrmund et al., 2009, 2010) as well as the genus-level phylogeny of mosses by Cox et al. (2010) and phylogenetic inference in Dicranidae by Goffinet et al. (2011), both based on markers from all three genomes (rps4, mitochondrial nad5, and nuclear ribosomal 26S), could mark the beginning of a new period of tackling the remaining problems of Dicranidae phylogeny.

This paper reviews the current state of knowledge on phylogenetic relationships at ordinal to (supra-) generic levels within Dicranidae and discusses future challenges, in particular which strategy should be followed to resolve the remaining ambiguous backbone relationships. The earlier approaches are compared with a novel phylogenetic reconstruction of a 50+ taxon set based on non-coding plastid markers, the rps4-trnT-trnL-trnF region (Hernández-Maqueda et al., 2008b) and atpB-rbcL spacer. Implications of the available molecular data for morphological character evolution are exemplarily discussed for the different types of haplolepideous peristomes. Furthermore, genetic and genomic research using haplolepideous model taxa is briefly reviewed.

Materials and Methods

Plant material and compilation of sequence data

The present taxon sampling comprised 54 species of Dicranidae as well as Timmia austriaca Hedw. (Timmiidae) and Encalypta streptocarpa Hedw. (Encalyptidae) as outgroup representatives. Specimens from Stech (1999a, 2004), Stech et al. (2006) and Stech & Frey (2008) as well as additional herbarium collections from L were used (Appendix). In addition to available trnL-F and atpB-rbcL sequences from the above mentioned studies, rps4-trnL sequences and, as far as possible, missing sequences of trnL-F and atpB-rbcL were newly generated for the present study. In some cases, the dataset was completed with sequences from GenBank (Appendix). Classification of Bryophyta follows Frey & Stech (2009).

DNA extraction, PCR, and sequencing

Distal parts of shoots were thoroughly cleaned with distilled water. Total genomic DNA was extracted using the DNeasy® Plant Kit (Qiagen, Hilden, Germany). The rps4-trnF region was amplified and sequenced in two parts, rps4-trnL and trnL-F. The first part comprised the 3’ end of the rps4 gene, the rps4-trnTUGU and trnTUGU-trnL intragenic spacers, the trnTUGU gene as well as the trnL intron and 5’ end of the trnL intron, while the second part spanned the complete trnL intron, trnL-3’ exon, and trnL-trnF spacer. PCR protocols and primers used were as described in previous studies: rps4-trnL (Hernández-Maqueda et al., 2008b; primers rps4-166F and P67), trnL-F (Hernández-Maqueda et al., 2008b; primer CM by Frey et al., 1999; primer F by Taberlet et al., 1991), and atpB-rbcL (Stech, 2004; primers atpB-1 and rbcL-1 by Chiang et al., 1998). In cases of difficulties with obtaining PCR products, the rps4-trnL part was split into two halves, which were amplified and sequenced separately with primers rps4-166F/A-Rbryo and A-Fbryo/P67, respectively (Hernández-Maqueda et al., 2008b). PCR products were purified using the Wizard® DNA Clean-up kit (Promega, Madison, WI, USA) or by Macrogen Inc. (www.macrogen.com), where the automated sequencing was performed as well. Sequencing primers were those used for PCR. GenBank accession numbers for all sequences used in this study are given in the Appendix. The rps4-trnF region is comprised in one accession per specimen; if earlier accession numbers were already available for parts of the region (e.g. trnL-F), these were updated.

Alignment, sequence analysis, and phylogenetic reconstructions

DNA sequences were manually aligned in PhyDE® v0.995 (Müller et al., 2006). Phylogenetic reconstructions according to the maximum parsimony (MP) optimality criterion were performed using PAUP 4.0b10 (Swofford, 2002). Heuristic searches under parsimony were implemented using random sequence addition with 1000 replicates and tree bisection-reconnection branch swapping. Gaps were either treated as missing data or coded as informative by a simple indel coding (SIC) strategy (Simmons & Ochoterena, 2000) as implemented in SeqState (Müller, 2004a). To search the tree space for islands of more parsimonious trees, parsimony ratchet analyses were performed with PRAP2 (Müller, 2004b) in combination with PAUP, employing the default options (200 iterations, 25% of randomly chosen positions up-weighted to 2) and superimposed 10 random addition cycles. Heuristic bootstrap searches under parsimony were performed with 1000 replicates and 10 random addition cycles per bootstrap pseudo-replicate with the same options in effect.

A maximum likelihood (ML) analysis was also performed with PAUP. Bayesian posterior probabilities (PP) were calculated based on the Metropolis-coupled Markov chain Monte Carlo method, using MrBayes v3.1 (Huelsenbeck & Ronquist, 2001). Prior to these model-based analyses, model testing was
performed in Modeltest 3.7 (Posada & Crandall, 1998) employing MrMRTgui (Nuin, 2005). Both the hierarchical likelihood ratio test and the AIC criterion indicated GTR + I + G as best-fit model. Consequently, the settings Basefreq = (0.4531 0.0722 0.0845), Nst = 6, Rmat = (0.7917 2.9656 0.1421 1.5770 2.9656), Rates = gamma, Shape = 1.3539 and Pinvar = 0.2714 were used for ML analysis and Nst = 6 and Rates = invgamma for the Bayesian analysis. In a second Bayesian analysis, the indels coded by SIC were included, with sequence and indel data treated as separate and unlinked partitions, employing the restriction site model (‘F81’) for the indel matrix. The a priori probabilities supplied were those specified in the default settings of the program. Four runs with four chains (10⁶ generations each) were run simultaneously, with the temperature of the single heated chain set to 0.2. Chains were sampled every 1000 generations and the respective trees were written to a tree file. Fifty percent majority rule consensus trees and PP of clades were calculated by combining the four runs and using the trees sampled after the chains converged. Trace plots generated in Tracer v1.5 (Rambaut & Drummond, 2007) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods) and to infer the ‘burnin’, which approximately ranged between the first 100 000 and 120 000 generations (first 100–120 sampled trees). Consequently, the first 150 trees (15%) were deleted to ensure that only trees of the stationary phase were included.

Results

In the present dataset, length ranges of the sequenced non-coding markers within Dicranidae were 261–331 nucleotides (nt) for rps4-trnT spacer, 252–328 nt for trnT-L spacer, 243–325 nt for trnL intron, 59–78 nt for trnL-F spacer, and 445–567 nt for atpB-rbcL spacer. No length variation was observed in the sequenced coding regions except for one additional nt in the trnL 3’ exon in Catsopticum nigratum (Hedw.) Brid. The combined alignment comprised 3079 positions. Of these, 1176 (38.2%) were variable, and 719 (23.4 or 61.1% of the variable positions) were parsimony-informative. Inclusion of indel characters by SIC yielded another 467 parsimony-informative characters, resulting in 1186 parsimony-informative characters in total.

MP analyses without and with indels included by SIC retained eight or two most parsimonious trees, respectively [without indels: lengths 3325, CI 0.523, RI 0.623, RC 0.326; with SIC: lengths 4924, CI 0.548, RI 0.620, RC 0.452]. PRAP searches recovered trees of the same lengths but did not find shorter trees. In the ML analysis, a single optimal tree was found (ln L = -20030.388180), which is shown in Figure 1, with bootstrap support and PP from the respective MP and Bayesian analysis without indels indicated. One of the two most parsimonious trees including indels is depicted in Figure 2, showing BS and PP values of the respective analyses with SIC. The second most parsimonious tree differs only within the Leucobryaceae, showing Atracylocarpus and Campylopodiella on separate branches, but without significant support.

In all phylogenetic reconstructions, the species Catsopticum nigratum, Hymenoloma crispulum (Hedw.) Ochyra, Ditrichum flexicaule (Schwägr.) Hampe, and Drummundia prorepens (Hedw.) E.Britton were placed sister to the rest of the Dicranidae in the phylogenetic reconstructions, either as grades or as an unsupported clade (Figures 1 and 2). Bryophyllum norvegicum (Br.) Mitt. branched off next (84% BS in the MP SIC analysis, PP 1.00) and was sister to a clade of the remaining species, which was statistically supported only by the Bayesian analyses (PP 1.00). Within the latter, the third clade was resolved with moderate to high support. A close relationship of Grimmales (91% BS, PP 1.00), the second Leucobryaceae as well as Archidium alternatifolium (Hedw.) Mitt. and Eustichia longirostris (Br.) Brid. (PP 1.00), and the third the remaining included taxa of Dicranales and Pottiaceae (PP 1.00). In the analysis with SIC (Figure 2), the first two of these clades formed one unsupported clade, and the third clade of the remaining taxa received BS (89%) in addition to a PP of 1.00. Within this latter clade, all families except Ditrichaceae were monophyletic with significant support. A close relationship of Dicranaceae s.str., Hypodontiaceae, and Calympeaceae was resolved with moderate to high support (BS 78–80%, PP 1.00). Other family relationships remained unsupported in the MP analyses, and for some clades also in the Bayesian analyses, or were contradictory. For example, Amphidium was placed sister to the other taxa based on substitutions only (Figure 1) and in the Bayesian analysis with SIC (tree not shown), but nested inside the clade as sister to Fissidens in the MP SIC analysis (Figure 2), albeit without support.

Discussion

Molecular phylogeny of Dicranidae — the first decade (1999–2009)

From the first molecular phylogenetic reconstructions of Dicranidae (Stech, 1999a,b; De Farge et al., 2000, 2002; Goffinet et al., 2001; Tsubota et al., 2003, 2004; Hedderston et al., 2004), four main results emerged. First, Dicranidae were resolved as monophyletic, including a number of families with either reduced or peculiar double peristomes formerly considered as diplolepideous, whose systematic position had long been debated (Archidiaceae, Amphidiateae, Catsoptiaceae, Drummioniaceae, Ephemeraceae, Erpodiaeae,
Stech et al. Phylogeny of haplolepideous mosses

Figure 1 Single optimal maximum likelihood tree of 54 representatives of haplolepideous mosses (Dicranidae) based on chloroplast DNA sequences (rps4-trnF region, atpB-rbcL spacer). Timmia austriaca (Timmiidae) and Encalypta streptocarpa (Encalyptidae) were used as outgroup representatives. Thick lines indicate bootstrap support (BS) values from a respective maximum parsimony analysis and significant posterior probabilities (PP) from a respective Bayesian analysis: BS >90%/PP >95 (black), BS >70%/PP >95 (dark grey), PP >95 (light grey).
Mitteniaceae, Rhachithecaceae, Splachnobryaceae, and Wardiaceae). Second, some of the more speciose families, such as the Calymperaceae, Fissidentaceae, Grimmiaeae, and Pottiaceae, were monophyletic almost in their traditional circumscription or with certain changes in their generic composition (Indusiella and Jaffueliobryum removed from Grimmiaeae; Hernández-Maqueda et al., 2008b). Other families, in contrast, were resolved as polyphyletic, especially Dicranaceae and Ditrichaceae. Third, the backbone of the phylogeny was rather weakly supported and the ordinal classification of Dicranidae was not recognizable in the phylogeny. Fourth, a morphologically diverse assembly of species from different families, called ‘proto-haplolepideous’ taxa (Hedderon et al., 2004), branched off first in the phylogeny (see enumeration below). Subsequently, a number of systematic rearrangements were made and incorporated in the two main recent synopses of classification of Bryophyta (Frey & Stech, 2009; Goffinet et al., 2009). Hypodontiaceae were segregated from the Pottiaceae as a new family and Oncophoraceae (Rhabdoweisiaceae) separated from the Dicranaceae s.l. Furthermore, Dicnemonaceae were re-included in the Dicranaceae s.str., Cinclidotaceae and Ephemereaceae were included in the Pottiaceae, and Leucobryaceae were expanded by the former subfamilies Campylopoideae and Paraleucobryoideae p.p. of the Dicranaceae, which resulted in a more heterogeneous circumscription of the Leucobryaceae comprising both ‘leucobryoid’ and ‘dicranoid’ genera (cf. Frey & Stech, 2009). Further segregates of Dicranaceae s.l. were placed into the resurrected or newly described families Amphidiaceae, Aongstroemiaceae, and Dicranellaceae (Stech & Frey, 2008), which were incorporated in Frey & Stech (2009), but not in Goffinet et al. (2009).

Of the three largest orders, namely Grimmiales, Dicranales, and Pottiaceae, only Grimmiales (Campylostheliaceae, Grimmiaeae, Ptychomitriaceae, and Selligeraceae) have unequivocally been shown to represent a monophyletic group with molecularly well-resolved relationships (Tsubota et al., 2003; Hernández-Maqueda et al., 2008b). Dicranales were clearly not monophyletic. Despite efforts to resolve relationships within the large family Pottiaceae (see below), circumscription of Pottiaceae remained difficult to assess. Representatives of the monogenic Pleurophascaceae and Serportletellaceae were only included in Shaw et al. (2005), who assessed molecular diversity in mosses based on a large-scale phylogenetic reconstruction of moss genera. Inference on the systematic position of individual taxa, however, was not possible from that article as the taxon names were only given in the appendix and not indicated in the phylegetonic tree. The position of Pleurophascum within the diplolepideous Bryaceae in Goffinet et al. (2001) might be an artefact. The monotypic Mitteniaceae were either included in Pottiaceae (Goffinet et al., 2009) or treated as a separate order Mitteniales (Shaw, 1985; Frey & Stech, 2009; cf. also O’Brien, 2007 and discussion in Stech & Frey, 2008).

Molecular phylogeny of Dicranidae — recent developments and current state

The most recent molecular phylogenetic reconstructions allowing further inferences of relationships within the haplolepideous mosses comprise Cox et al. (2010), Wahrmund et al. (2010), Goffinet et al. (2011), and the present study. The circumscription of Dicranidae has been expanded by including Bryowijkia (Cox et al., 2010), which was already separated as family Bryowijkiaeae within the Hedwigiales by Frey & Stech (2008). One of the most surprising findings of several earlier studies, the existence of a number of morphologically diverse taxa branching off first in the Dicranidae phylogeny, seems to be real, as such a topology is also resolved in Cox et al. (2010), Wahrmund et al. (2010), and the present study (Figures 1 and 2). Although not every study included all respective taxa, they seem to comprise, in summary, Bryoxiphiaceae, Catoscopiaceae, Distichiacaeae, Scouleriaceae (Drummondiaecaeae, Scouleriaceae p.p.: Scouleria aquatica Hook.) as well as Hymenoloma crispulum (Rhabdoweisiaceae), Ditrichium flexicaule (Ditrichiaceae), and Timmiella anomala (Bruch & Schimp.) Limpr. (Pottiaceae, see below). The close relationship between Drunmnondia prorepens and Hymenoloma crispulum (Hedderon et al., 2004; present study), the position of Chrysoblastella chilensis (Mont.) Reimers (Ditrichiaceae) as sister to Distichium and Timmiella (Cox et al., 2010), and the position of Tridontium tasmanicum Hook.f. (Scouleriaceae) in Pottiaceae (Cox et al., 2010; Goffinet et al., 2011) need further study. Hymenoloma crisrulpm was traditionally included in Dicranoweisia, but separated by Ochyra et al. (2003) based on morphological characters, a point of view supported by molecular data. Dicranoweisia s.str. clearly belongs to Rhabdoweisiaceae according to the position of Dicranoweisia cirrata (Hedw.) Lindb. ex Milde close to Rhabdoweisia in phylogenetic reconstructions (La Farge et al., 2002; Hedderon et al., 2004; Tsubota et al., 2004).

The other haplolepideous taxa seem to be divided into two large groups. The first group, which appears as a grade or unsupported clade in the phylogenies, comprises Grimmiales, Archidiaeae, Leucobryaceae (Hedderon et al., 2004; Stech & Frey, 2008; Cox et al., 2010; Wahrmund et al., 2010) as well as the recently described Micromitriaceae (Goffinet et al., 2011), Saelania glaucescens (Hedw.) Broth. of Ditrichiaceae (Cox et al., 2010; Goffinet et al., 2011), and Eustichiacaeae (this study). The second group comprises the largest portion of the haplolepideous mosses, namely
Figure 2. One out of two most parsimonious reconstructions (shown as phylogram) of 54 representatives of haplolepideous mosses (Dicranidae) based on chloroplast DNA sequences (rps4-trnF region, atpB-rbcL spacer), with indel characters coded by simple indel coding (SIC) included. *Timmia australica* (Timmiidae) and *Encalypta streptocarpa* (Encalyptidae) were used as outgroup representatives. Bootstrap support values >70% and significant posterior probabilities >95 from a respective Bayesian analysis with indels included are given at the branches.
most families of Dicranales sensu Frey & Stech (2009) (Aongstroemiacae, Bruchiaceae, Calymperaceae, Dicranaceae, Dicranellaceae, Ditrichaceae p.p., Erpodiaeae, Fissidentaceae, Hypodoniaceae, Oncophoraceae, Rhachithecaceae, and Schistostegaceae) plus Bryowijkiaceae as well as the most speciose Pottiaceae. The respective clade of this group receives significant statistical support in Wahrmund et al. (2010), in the present analysis with indels (Figure 2) and, excluding Amphidium (Amphidiaceae), in Cox et al. (2010) and Goffinet et al. (2011). The position of Amphidium remains ambiguous in the present study as well (Figure 1 versus Figure 2).

Except for Chrysoblastella chilensis, Ditrichum flexicaule, and Saelania glaucescens, representatives of Ditrichaceae analysed so far seem to cluster into two groups in the molecular phylogenetic reconstructions. Ecremidium and Garekea form a well-supported clade with Aongstroemia (Aongstroemiacae) and Cladophascum (Bruchiaceae) (Cox et al., 2010; Goffinet et al., 2011). In contrast, a close relationship is indicated between Astomiopsis, Ceratodon, Ditrichum pallidum (Hedw.) Hampel/D. heterornallum (Hedw.) E.Britton, Pleuridium, Pseudephemurum, and Trichodon (La Farge et al., 2002; Tsubota et al., 2003, 2004; Heddersen et al., 2004; Cox et al., 2010; Goffinet et al., 2011; present study), although statistical support is lacking. This latter group might represent Ditrichaceae s.str. Cheilothela should be close to Ceratodon as well (McDaniel, 2005); its position in the trees of the present study (Figures 1 and 2) needs to be confirmed by further material.

The available phylogenetic reconstructions and the systematic rearrangements inferred from them represent an important step towards a classification of haplolepideous mosses that better reflects phylogenetic relationships. However, further molecular analyses based on increased taxon and marker sampling are necessary to clarify still ambiguous relationships within the Dicranidae at different taxonomic levels. At the highest level, this concerns the preliminary distinction of three major groups versus an ordinal classification. Whether the distinction of formal orders within the Dicranidae will remain useful or should be replaced by informal node-based names to characterize major lineages above the family level, as has been done, e.g. by Bell et al. (2007) for the main pleurocarpous lineages, needs to be discussed based on such extended phylogenies. Besides, circumscriptions and relationships of families such as Aongstroemiacae, Bruchiaceae, Dicranellaceae, Oncophoraceae, and especially Ditrichaceae, remain preliminary and need further study. Although in some organism lineages higher level relationships might not be completely solved with tree-based approaches (e.g. Hallström & Janke, 2010), the recent developments of phylogenetic research in the Dicranidae indicate that considerable progress can still be made based on phylogenetic reconstructions of extended data sets.

**Molecular marker sampling**

A general problem of many phylogenetic analyses in bryophytes has been the focus on only few molecular markers (cf. Stech & Quandt, 2010). Concerning the Dicranidae, all initial higher-level phylogenetic analyses were based on three widely used plastid DNA regions, *trn*F, *rps*4, and *rbc*L. Aside from problems with single-marker analyses (e.g. Slowinski & Page, 1999; Gontcharov et al., 2004; Bell & Hyvönen, 2010), all initial analyses of Dicranidae thus only reflect evolutionary patterns of the plastid genome. Fortunately, recent approaches to improve phylogenetic reconstructions of mosses (or all land plants) have evaluated new markers, especially from the mitochondrial genome (Wahrmund et al., 2009, 2010), and used combined markers from two or even all three different plant genomes (Qi et al., 2006; Quandt et al., 2007; Cox et al., 2010; Wahrmund et al., 2010; Goffinet et al., 2011). Another strategy was followed by Stech & Frey (2008), who evaluated the suitability of combined non-coding plastid markers for phylogeny reconstruction of mosses, which is continued and extended in the present study for the Dicranidae.

Which strategy of marker selection should be followed to resolve the remaining uncertainties in Dicranidae phylogeny? As Stech & Quandt (2010) have recently discussed for bryophytes in general, the trend of using multiple markers and comparing markers from different genomes should be continued. But at the same time new markers must be identified that provide sufficient variability and phylogenetic structure at the respective taxonomic level under study. For example, the coding markers used to infer land plant relationships by Qi et al. (2006), which show slow to moderate evolutionary rates, did not resolve relationships within Dicranidae and are thus not useful to employ with a larger taxon sampling. Non-coding markers, as tested in Stech & Frey (2008) and especially in the present study, are very well suited to resolve and support the different families of Dicranidae, whereas their relationships remain largely unsupported, at least in the MP analyses. Compared to Stech & Frey (2008), the present marker combination provides more phylogenetic information, as the very short *psb*A-*trn*H spacer was replaced by spacers of the *rps*4-*trn*L region, which were already employed successfully at family level in haplolepideous mosses (Stech, 2004 [only *trn*T-L spacer]; Hernández-Maqueda et al., 2008b). One problem of resolving the backbone phylogeny of Dicranidae with non-coding markers might be the
considerable amount of homoplasy, as can be inferred, e.g. from the low RC values of the present most parsimonious reconstructions (cf. results). The amount of homoplasy seems to be even higher in the substitutions than in the indels coded by SIC. Although it might be considered critical to use indel characters at higher taxonomic levels given the high length variability of non-coding DNA regions, these characters are generally congruent with the substitution data, and even provide higher support for some clades, in the present study.

As Stech & Quandt (2010) further discussed, one perhaps has to combine several suboptimal markers to collect the small amount of synapomorphic sites in each of them (thereby also considering indel characters) until well-resolved phylogenetic trees can be produced. To do so, further plastid markers such as group 2 introns (trnV, rpl16, and trnK introns) and fast-evolving genes such as matK or ndhB, should be tested. The most suitable plastid markers should be combined with mitochondrial markers and newly developed single- or low-copy nuclear markers, taking into account potentially different evolutionary patterns between the organelle and nuclear markers.

Examples of single- or low-copy nuclear markers already utilized at lower taxonomic level in Dicranidae are adk and phy2 (McDaniel & Shaw, 2005) as well as gpd (Wall, 2002, 2005). Nuclear introns are essential for evolutionary genetic analyses at this scale for two reasons. First, nuclear introns are often sufficiently variable to distinguish closely related species or populations; and second, multiple, independent loci are critical for distinguishing between incomplete lineage sorting and hybridization (including polyploidy) as explanations for close relationships among species. Because introns diverge much faster than the coding portions of duplicate genes, it is relatively straightforward to design primers to amplify a single paralog of a multi-copy gene family. Nuclear genes are likely to be equally important for deep phylogenetics, particularly for thorny problems like resolving the backbone of the Dicranidae. For this level of analysis, however, the choice of loci is more challenging. Analyses of loci that contain few variable sites, either because they are small genes or because they are under rigid functional constraints, may be misleading because the few sites that can change have already experienced multiple changes across the phylogeny. However, markers that are too freely evolving, such as nrITS, may contain insertions, deletions, micro-inversions, and gene duplications that dramatically increase the complexity of the analysis. A concerted effort to identify a set of markers with appropriate characteristics based on a comparison of available genomic data (see below), using approaches like those outlined in Tekle et al. (2010), provides a way forward. Preliminary analyses (McDaniel, unpublished data) suggest that the phytochrome gene family is a strong candidate, but the rate of diversification at the base of the Dicranidae indicates that additional loci may be required.

**Implications for character evolution**

The available molecular data allow preliminary inferences of the evolution of key morphological characters, as exemplarily discussed for the haplolepideous peristome below. More precise insights into character evolution should be based on cladistic analyses of morphological characters as well as ancestral state reconstructions. These, in turn, need to be based on expanded and better supported phylogenies and will probably also need further morphological-anatomical analyses for homology assessment and character coding. All three major groups of haplolepideous mosses distinguished here comprise taxa with very different morphologies that will pose challenges for the interpretation of character evolution in Dicranidae. For example, the morphological diversity of the first diverging taxa is already considerable, including peristome reductions (in Bryoxiphium, Catoscopium, Drummondi, and Scouleria p.p.) and similar morphologies with other haplolepid mosses, such as the Fissidens-like leaf architecture in Bryoxiphium and the pottiaceous morphology of Timmiella (see below). A striking example of parallel gametophyte reduction is displayed by Ephemerum and Micromitrium of the former Ephemeraceae, which are molecularly unrelated (Goffinet et al., 2011), in addition to the long known differences in chromosome numbers and sporophyte characters between both genera (Bryan & Anderson, 1957).

The early stages of peristome development, up to the point where the amphithecium is differentiated into three layers, namely the outer (OPL), primary (PPL), and inner peristomial layer (IPL), are virtually identical between both genera (Bryan & Anderson, 1957). Thereafter, a unique sequence of cell divisions leading to a PPL:IPL arrangement of 2:3 cells for a two-cell segment of the PPL (one-eighth of the peristome) characterizes the haplolepideous peristome developmentally (Shaw et al., 1989). The formula OPL: PPL:IPL 4:2:3 (or 0:2:3 as the OPL/outer PPL walls have disappeared in the mature peristome), however, is modified in several haplolepideous taxa due to the formation of a second row of teeth or reduction to a 2:2 pattern. Resulting formulas are, e.g. 4:2:2 (–3) in Seligeriaeae, (4):2:2 (–3) in Calymperaceae, (8:4):2: 2(–3) in Hypodontiaeae (Edwards, 1979), and a final pattern of 8:4:2 in Glyphomitrium humilillimum (Estébanez et al., 2006). Double haplolepideous
peristomes mostly result from preperistome formation on the OPL side, which is usually restricted to the base of the teeth, or rarely (Mittenia) by involving the inner periclinal walls of the IPL and adjacent cell walls of the outermost endothelial layer (Shaw, 1985). Morphological variation of the haplolepideous peristome is furthermore considerable with respect to the shape, degree of incision, and ornamentation of inner and outer surfaces of the peristome teeth. Peristome reductions obviously occurred several times independently across the whole Dicranidae.

Nevertheless, haplolepideous peristomes can be grouped into four main types, namely the dicranoid, seligerioid, syrrhopodontoid, and pottioid type (cf. Frey & Stech, 2009). The syrrhopodontoid and pottioid types seem to be synapomorphic for the monophyletic and well-supported Calymperaceae (except K. pottioid types seem to be synapomorphic for the Frey & Stech, 2009). The syrrhopodontoid and cf. seligerioid, syrrhopodontoid, and pottioid type (grouped into four main types, namely the dicranoid, times independently across the whole Dicranidae. Peristome reductions obviously occurred several times independently across the whole Dicranidae.

The expression of the pottioid peristome displayed by Timmiella, with 32 filamentous, spiculose, twisted teeth arising from a basal membrane, seemed to have evolved several times in different genera of Pottiaceae as well as in the molecularly distant Timmiella (molecular dataset by Werner et al., 2004; re-analysed by Zander, 2006). However, Zander (2006) argued that the twisted peristome, similar to other morphological traits of Timmiella, is plesiomorphic and represents an example of homology, i.e. a gene cluster determining the existence of major organs that is highly adaptive and, once evolved, can be silenced and re-activated later in another phylogenetic lineage. In this interpretation, the twisted peristome of Timmiella and (other) Pottiaceae resulted from a ‘deep’ developmental homology (a shared deep ancestor with a twisted peristome), not on independent parallel evolution. Whether the development of the Timmiella peristome is in fact developmentally homologous to the twisted peristomes in (other) Pottiaceae remains to be investigated.

Taxa with seligerioid peristomes occur in Scouleriales, Grimmiales, Rhachitheciaeae, and Oncophoraceae (Glyphomitrium), which belong to different haplolepideous lineages (Cox et al., 2010; Goffinet et al., 2011). Similarly, taxa with dicranoid peristomes are found in several different families such as Ditrichaceae p.p., Leucobryaceae, Dicranaceae s.str., Fissidentaceae, and Oncophoraceae p.p. The seligerioid and dicranoid types could thus have evolved several times independently, or could represent artificial assemblies of different non-related peristome morphologies. The latter hypothesis is supported by the large variation especially of dicranoid peristomes with respect to the degree of incision and ornamentation of inner and outer surfaces of the peristome teeth, and the presence of peristomes putatively reduced from the dicranoid type, especially in Ditrichaceae (cf. Frey & Stech, 2009). Besides, the comparison of mainly seligerioid peristomes by Estébanez et al. (2002) showed that peristome movement in relation to histochemical properties can vary greatly between species of the same family, although certain properties (pectin distribution, stages with maximum quantity of phenolics) seemed to characterize the Grimmiaeae. Further comparative morphological, histochemical, and developmental analyses of selected taxa covering the diversity of dicranoid and seligerioid peristome types are clearly necessary to complement earlier studies (e.g. Edwards, 1979; Shaw et al., 1989) and to infer the systematic relevance of characters in these peristome types.

Genus-level phylogenetics

Although phylogenetic analyses in the beginning of the ‘molecular era’ focussed on higher-level systematic relationships in mosses, most studies published so far tackled systematic and biogeographic relationships between and within genera or single species. Dicranidae comprise about 30% of the total moss species diversity. In the publication record, however, they seem to be underrepresented. Out of a total of 292 molecular systematic studies on mosses, only 65 (22%) deal with haplolepideous taxa (literature compiled in Stech & Quandt, 2010; extended by publications up to the end of 2010). Especially with respect to large genera, Dicranidae seem understudied. The 11 largest haplolepideous genera (100+ species each) are covered by only 18 more detailed molecular phylogenetic publications, six of which deal with Campylopus (Stech, 2004; Stech & Dohrmann, 2004; Stech & Wagner, 2005; Frahm & Stech, 2006; Stech et al., 2007, 2010). Relationships within Grimmiaeae are already quite well studied, with phylogenetic analyses of the largest genera, Grimmia s.l. (Streff, 2006; Hernández-Maqueda et al., 2007, 2008a,b), Schistidium (Ignatova et al., 2009; Milyutina et al., 2010), and Racotritium s.l. (Larrain et al., 2011), providing a basis for assessing taxon circumscriptions and relationships. Other large haplolepideous genera, such as Fissidens...
(c. 440 spp.) or Dicranella/Leptotrichella (c. 220 spp.) remain almost unknown at the molecular level (Werner et al., 2009). However, also quite well-studied genera such as Campylolus remain a challenge due to incongruence between morphological species circumscriptions and molecular data (Stech et al., 2010 and references therein).

Unravelling relationships within Pottiaceae s.str. are particularly difficult because it is the largest moss family, with about 1425 species in 83 genera (Frey & Stech, 2009), and because it includes several large and taxonomically difficult genera (e.g. Barbula, Didymodon, Hyophila, Syntrichia, Tortella, Tortula, Trichostomum, and Weissia). For some of these genera such as Barbula and Hyophila, molecular data are almost unavailable. The few published sequences included in, e.g. Werner et al. (2004) and Köckinger & Kucˇera (2011), seem to indicate that Barbula is polyphyletic, but a combined molecular–morphological analysis is clearly needed. Other genera like Didymodon seem to be monophyletic, but their subgeneric taxonomy based on morphological characters is not supported by nrITS data (Werner et al., 2005a). Especially complex is the circumscription of Tortula. While the available data support the view that a part of the species traditionally included in the genus Pottia are indeed morphologically reduced members of Tortula, also Crossidium, Phascum, Pterygoneurum, and Stegonia are part of a Tortula s.l. clade (Werner et al., 2002), with several well-supported clades being formed by species of both Crossidium and Tortula. Aside from general considerations of how to treat such molecular topologies, further molecular phylogenetic analyses of Tortula and related genera based on additional markers should be performed. In contrast, Syntrichia is molecularly clearly separated from Tortula, although some species like Tortula subulata Hedw. show some similarity with Syntrichia on a morphological basis (Werner et al., 2002, 2003). The subfamily Trichostomoideae is particularly complex at all taxonomic levels. On the one hand, molecular data in many cases contradict traditional generic delimitations, for example between Weissia, Trichostomum, Pottiopsis, Tortella, Plectochaeae, Oxystegus, Chionoloma, and Pseudosymblepharis. On the other hand, the genus Weissia seems to evolve extremely fast morphologically as compared with the degree of molecular variation. Even quickly evolving molecular markers like nrITS show almost identical sequences in samples that some authors separated into different genera, e.g. Weissia [Astonum] levieri (Limpr.) Kindb. and W. controversa Hedw. (Werner et al., 2005b).

In summary, despite molecular efforts to resolve relationships of Pottiaceae at (supra-)generic level (e.g. Werner et al., 2002, 2004, 2005a,b; Grundmann et al., 2006; Zander, 2006), a more complete analysis of Pottiaceae, and especially of the larger genera, based on a comprehensive taxon and marker sampling, is still missing. Besides, several remarkable new species and genera were recently described based on molecular and/or morphological data (Hedderson & Zander, 2007, 2008a,b; Jiménez & Cano, 2007, 2008a,b; Gallego & Cano, 2007, 2009; Erdäg & Kürrschner, 2009; Cano et al., 2010; Jiménez et al., 2010; Köckinger et al., 2010; Akiyama & Goffinet, 2011; Zander & Heddderson, 2011), indicating that the total diversity within Pottiaceae is still insufficiently known.

**Genetics and genomics**

Apart from the most prominent ‘genetic model moss’ *Physcomitrella patens* (Hedw.) Bruch & Schimp. (Funariidae) (reviewed in Beike et al., 2010), research on genetic mechanisms and genomic structure in mosses has so far mostly focussed on haplolepideous taxa, namely Ceratodon purpureus (Hedw.) Brid. and Syntrichia species. *Syntrichia ruralis* (Hedw.) F. Weber & D.Mohr is the second moss species, after *P. patens* (Sugiura et al., 2003), from which the complete chloroplast genome was sequenced (Oliver et al., 2010), and Syntrichia species are well-known as a model for research on sex ratio variation (Bowker et al., 2000), sexual dimorphism (Stark et al., 2001), and desiccation tolerance (e.g. Oliver et al., 2005; Stark et al., 2006). *Ceratodon purpureus* is amenable to mutagenesis and growth under laboratory conditions, and is widely used as a model for the study of developmental responses to light and gravity (Cove et al., 1996; Sack et al., 2001; Thornton et al., 2005; Cove & Quatrano, 2006). Besides, *C. purpureus* is the only eukaryote, other than yeast and *P. patens*, that is known to undergo efficient gene targeting via homologous recombination (Brucker et al., 2005; Trouiller et al., 2007; Mittmann et al., 2009). In work with natural populations, Jules & Shaw (1994) demonstrated that *C. purpureus* can adapt to growth on heavy metal containing soils, and Shaw & Beer (1999) and McDaniel (2005) conducted the most in-depth description of within and among-population quantitative genetic variation in a moss species in *C. purpureus* as well. The study of hybridization has a long history in the haplolepideous mosses, with several studies documenting hybrid sporophyte morphology and spore germination patterns in the families Ditrichaceae, Pottiaceae, Dicranaceae, and Grimmiaaceae (reviewed in Natcheva & Cronberg, 2004). More recently, McDaniel et al. (2007, 2008) used a genetic map to dissect the genetic architecture of spore inviability and abnormal development in the progeny from a cross between a temperate and a tropical population of *C. purpureus*. Increasing the resolution of the phylogeny of...
Dicranidae will enable evolutionary biologists to develop sophisticated tests for hypotheses of character correlations derived from developmental or population studies, as well as provide a critical framework for studying gene family and genome evolution across this group.

Acknowledgements

Additional sequencing was made possible by a SYNTHESYS grant to RHM. JM is supported by Ministry of Science and Technology of Spain grant CGL2009-09530-BOS. Sincere thanks are due to M. C. M. Eurlings (Leiden) for technical assistance.

Taxonomic Additions and Changes: Nil.

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