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Molecular evolution and diversification of the moss family Daltoniaceae (Hookeriales, Bryophyta) with emphasis on the unravelling of the phylogeny of *Distichophyllum* and its allies

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Phylogenetic relationships in Daltoniaceae (~200 species in 14 genera) are inferred from nucleotide sequences from five genes, representing all genomic compartments, using parsimony, likelihood and Bayesian methods. Alternative classifications for Daltoniaceae have favoured traits from either sporophytes or gametophytes; phylogenetic transitions in gametophytic leaf limbidia and sporophytic exostome ornamentation were evaluated using ancestral state reconstruction to assess the levels of conflict between these generations. Elimbate leaves and the cross-striate exostome are reconstructed as plesiomorphic states. Limbate leaves and papillose exostomes evolved at least two and six times, respectively, without reversals. The evolution of leaf limbidia is relatively conserved, but exostome ornamentation is highly homoplasious, indicating that superficial similarity in peristomes gives unreliable approximations of phylogenetic relatedness. Our phylogenetic analyses show that *Achrophyllum* and *Calyptrochaeta* are reciprocally monophyletic. Within core Daltoniaceae, relationships among taxa with elimbate leaves are generally well understood. However, taxa with limbate leaves form a monophyletic group, but resolved subclades correspond to biogeographical entities, rather than to traditional concepts of genera. *Daltonia* (~21 species), *Distichophyllum* (~100 species) and *Leskeodon* (~20 species) are polyphyletic. Seven nomenclatural changes are proposed here. As the current taxonomy of Daltoniaceae lacks phylogenetic consistency, critical generic revisions are needed. *Botanical Journal of the Linnean Society*, 2012, **170**, 157–175.

ADDITIONAL KEYWORDS: *Achrophyllum* – ancestral states – *Calyptrochaeta* – *Daltonia* – exostome ornamentation – *Leskeodon* – limbidium.

INTRODUCTION

Insights into phylogenetic relationships enable a better understanding of morphology and morphological evolution. Because all land plants are characterized by an alternation of sporophytic and gametophytic generations, a phylogenetic context can help to clarify how evolutionary change has occurred in the two generations, in concert and independently. In mosses (Bryophyta), conflicting classifications with an emphasis on either sporophytes or gametophytes are common because both generations are relatively well developed and provide characters that may be useful for phylogenetic inference and classification.

Daltoniaceae (Hookeriales: Bryophyta) exemplifies the systematic challenge that may result from focusing taxonomic inferences on traits from just one generation (i.e. gametophytes vs. sporophytes). Daltoniaceae comprises a prominent group of tropical and south-temperate mosses that prefer humid forest habitats.

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Members of the family can be found in a wide range of habitats from terrestrial to epiphytic and occasionally epiphyllous (Fleischer, 1908; Miller, 1982; Buck, 1998). A few taxa are facultatively aquatic, being entirely submerged under water (de Winton & Beever, 2004), and there are reports of an epizoic species of Daltoniaceae living on the backs of weevils (Gressitt, Samuelson & Vitt, 1968; Gradstein, Vitt & Anderson, 1984). The family (~200 species in 14 genera) is characterized by: (1) plants sparingly branched and usually complanate foliate; (2) foliate shoots not well differentiated into primary and secondary axes; (3) leaves with one midrib or costa (unicostate), rarely none (ecostate only in *Distichophyllidium* M.Fleisch.); (4) marginal laminal cells mostly differentiated as a distinct border of elongate cells (limbate); (5) median laminal cells ± isodiametric; and (6) calyptra mitrate. About half the genera include only one or two species each (*Adelothecium* Mitt., *Beeveria* Fife, *Benitotania* H.Akiyama, T.Yamag. & Suleiman, *Bryobrothera* Thér., *Crosbya* Vitt., *Ephemeropsis* K.I.Goebel, *Metadistochophyllum* Nog. & Z.Iwats. and *Leskeodontopsis* Zanten), whereas the largest genus, *Distichophyllum* Dozy & Molk., contains about half the species in the family.

The circumscription of Daltoniaceae and the relationships among genera are not entirely settled. In particular, the inclusion of *Calyptrochaeta* Desv. in the family still awaits confirmation. The relationships between *Achrophyllum* Vitt & Crosby, *Calyptrochaeta* and the rest of Daltoniaceae are uncertain. Furthermore, infrageneric relationships within *Achrophyllum* have never been assessed. A first approach exists for *Calyptrochaeta* (Pokorny, Oliván & Shaw, 2011). Within the well-supported core Daltoniaceae (i.e. Daltoniaceae *s.l.*, excluding *Calyptrochaeta* and *Achrophyllum*), relationships among the genera, especially with regard to the species-rich *Distichophyllum*, are still in question. An understanding of morphological evolution in these mosses requires a better understanding of phylogenetic relationships (Buck, Cox & Shaw, 2005).

History of daltoniaceae

The traditional widely adopted and broadly defined Hookeriaceae, which includes Daltoniaceae, was first proposed by Fleischer (1908). According to Fleischer, Daltonieae [with *Daltonia* and *Crosbya* (as *Bellia* Broth.)] and Distichophylleae [with *Achrophyllum* (as *Pterygophyllum* Broth.), *Adelothecium*, *Calyptrochaeta* (as *Eriopus* (Brid.) Brid.), *Distichophyllidium* M.Fleisch., *Distichophyllum* and *Leskeodon* Broth.] were recognized as two separate, but closely related, tribes with unicostate leaves. In general, Fleischer classified groups below the ordinal level primarily on gametophytic similarities and above that on sporophytic characters. Brotherus (1925) followed Fleischer's classification, but recognized the tribes as subfamilies in Hookeriaceae.

Subsequent systematic views differed in their emphasis on sporophytic vs. gametophytic traits for inferring relationships in the group. Crosby (1974) proposed a novel classification in which genera were grouped according to two basic peristome types (teeth surrounding the mouth of the sporophytic capsule or sporangium), regardless of their gametophytic similarities. The so-called hookeriaceous peristome has the exostome teeth (outer peristome) horizontally cross-striolate on the outer surfaces near their bases (Fig. 1C), endostomes (inner peristome) with high basal membranes and finely papillose segments and cilia absent to rudimentary. The daltoniaceous peristome, in contrast, has exostome teeth papillose throughout (Fig. 1D), endostomes with low or absent basal membranes and low papillose segments and cilia absent (Crosby, 1974). The genera *Leskeodon* and *Crosbya* were initially segregated from *Distichophyllum* and *Daltonia*, respectively, because of their dissimilar peristome (Brotherus, 1907). In Crosby's classification, *Daltonia*, *Distichophyllidium*, *Leskeodon*, *Leskeodontopsis* and other ecostate and bicostate genera that share daltoniaceous peristomes were classified into one family, Daltoniaceae, whereas genera characterized by hookeriaceous peristomes were classified into Hookeriaceae. As a result, both families were highly heterogeneous in gametophytic structure.

Buck (1987, 1988) took the opposite approach, emphasizing gametophytic rather than sporophytic features, and re-circumscribed Daltoniaceae to include the neotenic *Ephemeropsis* (gametophytes consisting of little more than protonemata, and a hookeriaceous peristome). As a consequence, the families distinguished in Hookeriales were heterogeneous with regard to hookeriaceous and daltoniaceous peristome morphologies. Hedenäs (1996) conducted a cladistic study on Hookeriales based on 75 morphological characters, about one-third of which came from the sporophyte and 14 came from the peristome. Not surprisingly, genera with similar peristomes were not grouped together, but few relationships received strong bootstrap support (BS). Buck *et al*. (2005) resolved the relationships within Hookeriales using nucleotide sequences from four genes. In general, this analysis supported the view that peristome characters can be homoplasious and should not, a priori, be relied upon to infer relationships. They found that Adelotheciaceae is phylogenetically nested in Daltoniaceae, but relationships among *Achrophyllum*, *Calyptrochaeta* and the rest of Daltoniaceae were ambiguous. Although only two *Distichophyllum* spp. were sampled, the genus appeared to be non-monophyletic.

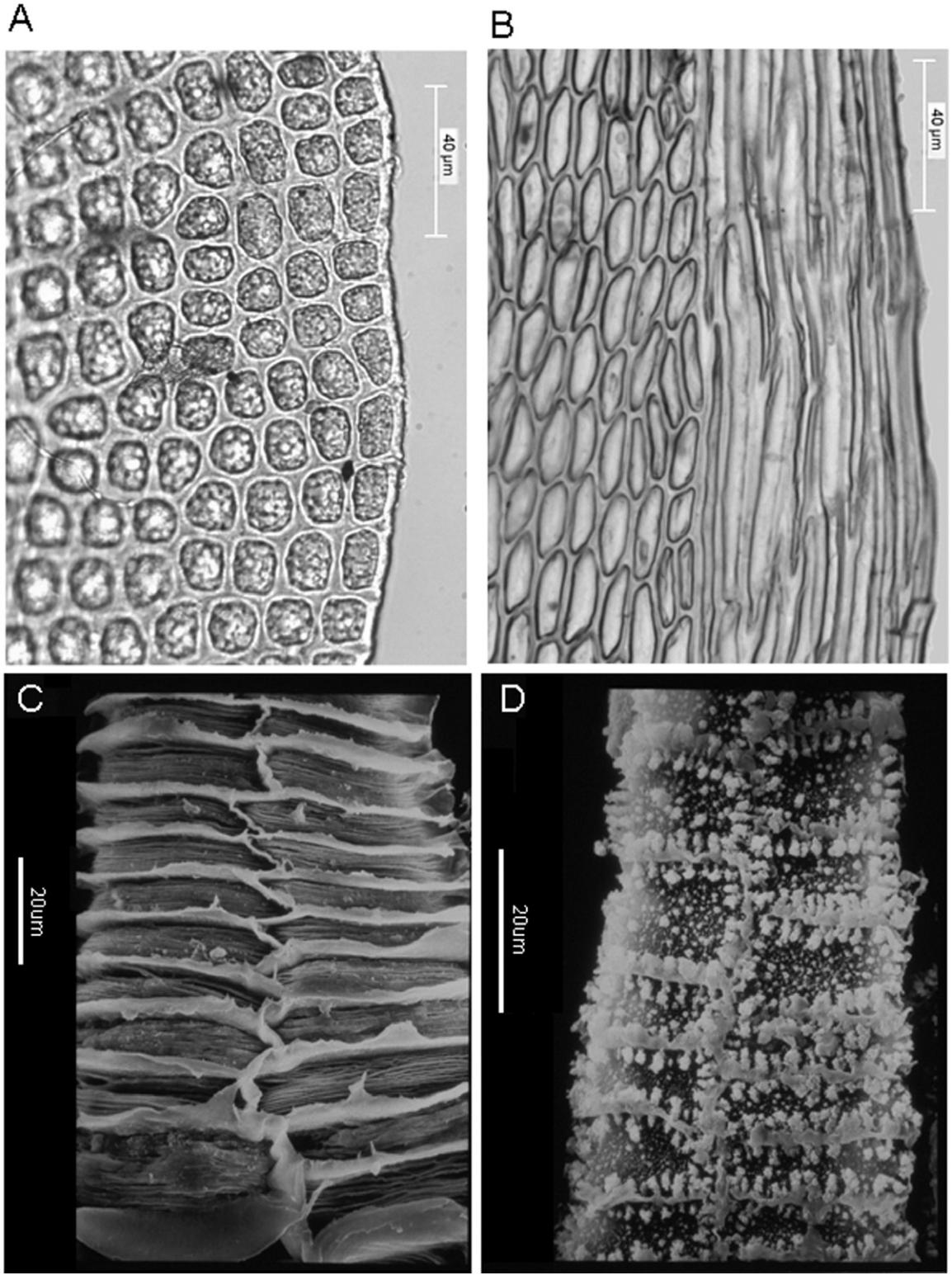

Figure 1. Characters for which character evolution was reconstructed using BayesTraits. A, Undifferentiated marginal laminal cells in mid-leaf in *Benitotania elimbata* (*Suleiman 1901*). B, Differentiated marginal laminal cells in mid-leaf in *Daltonia marginata* (*Juri 10*). C, Horizontally cross-striate ornamentation on the lower outside of the exostome in *Hookeria acutifolia* (*Robbinson & Sharp 1955*). D, Papillose ornamentation on the lower outside of the exostome in *Daltonia angustifolia* (*Brass 20740*).

Evolution of taxonomically relevant morphological characters in Daltoniaceae

Two characters have been considered to be especially important for generic separation in this group, i.e. gametophytic leaf limbidia (elimbate vs. limbate, see Fig. 1A,B) and sporophytic exostome ornamentation (cross-striate vs. papillose, see Fig. 1C,D). Gametophytic leaf limbidia, differentiated leaf borders consisting of narrower elongated, thick-walled cells (Fig. 1B), have long been associated with Hookeriales (Hedenäs, 1999). The character is commonly used to separate the genera of Hookeriaceae *s.l.* (e.g. Brotherus, 1907, 1925), although the functional importance of limbidia is obscure.

There is now general agreement that sporophytic features are not as stable, reliable or informative for inferring phylogenetic relationships as formerly supposed (e.g. Hedenäs, 2001, 2002; Vanderpoorten *et al*., 2002; Huttunen *et al*., 2004; Olsson *et al*., 2009a; Quandt *et al*., 2009; Liu, Budke & Goffinet, 2012; Pokorny *et al*., 2012). Nonetheless, Buck (1991, 2007) considered that peristome features could still be valuable for distinguishing genera in some families. In Hookeriales, ancestral state reconstructions using phylogenetic trees inferred from molecular data indicate that exostome ornamentation is homoplasious (Pokorny *et al*., 2012). This result corroborates earlier suggestions by Buck (1987), Whittemore & Allen (1989) and Tan & Robinson (1990).

In Daltoniaceae, there are at least two generic pairs that have similar gametophytic traits, but different peristome structure and ornamentation (*Crosbya* – hookeriaceous vs. *Daltonia* – daltoniaceous; *Distichophyllum* – hookeriaceous vs. *Leskeodon* – daltoniaceous). The resolution of phylogenetic relationships in the family will facilitate an understanding of the patterns of evolution in both leaf limbidia and peristome structure, especially exostome ornamentation (Fig. 1). Consequently, their value in generic delimitation can be assessed.

This study was conducted with the following aims: (1) to assess the evolution of two taxonomically important morphological characters (leaf limbidia and exostome ornamentation); (2) to resolve relationships among the genera of Daltoniaceae; (3) to infer infrageneric relationships in *Calyptrochaeta* and *Achrophyllum*; and (4) to assess interspecific relationships in the large and widespread (and apparently polyphyletic) genus *Distichophyllum*.

MATERIAL AND METHODS

Plant names used for taxa in this study follow the Tropicos database (http://www.tropicos.org), except when otherwise indicated. Authorities of species and variety names are indicated in Supporting Information Table S1. Each voucher is annotated with the taxon name followed by a two-letter country code in parentheses according to the 'ISO 3166-1 alpha-2' (http://www.iso.org/iso/english_country_names_and_code_elements). In some cases, a single letter suffix was added to indicate collection from different regions within a large country (see Table S1).

Taxon sampling and molecular protocols

One hundred and twenty-six accessions were sampled for DNA, including 18 exemplars from the other seven hookerialean families *sensu* Buck *et al*. (2005) as outgroups. The ingroup consisted of 94 species from 12 of the 14 genera in Daltoniaceae *s.l.* As phylogenetic relationships in *Daltonia* have recently been assessed (Yu *et al*., 2010), only a selection of representatives of *Daltonia* to cover the genetic diversity of the genus was included in this study (10 of 21). However, as many species as possible were sampled from other larger genera, such as *Achrophyllum* (six of eight), *Calyptrochaeta* (11 of 29) and *Leskeodon* (seven of 20). Sampling of *Distichophyllum* (37 species and eight varieties) represented about one-third of the ~100 accepted species (Crosby *et al*., 1999). In *Distichophyllum*, the sampling included two or more accessions for species that showed large morphological variability and/or formed species complexes that were difficult to resolve morphologically.

Nucleotide sequences (593 of 630 available) were obtained from five regions representing all three genomes, including 320 newly generated nucleotide sequences for this study (Table S1). Sequenced regions comprised: (1) the plastid *trnS-rps4* region [i.e. *rps4* plus the *trnS–rps4* intergenic spacer (IGS), hereafter *rps4*]; (2) the plastid *trnL–F* region, including the *trnL*$_{UAA}$ group I intron and the *trnL–F* IGS (hereafter *trnLF*); (3) the mitochondrial *nad*5 group I intron (hereafter *nad5*); (4) the nuclear ribosomal ITS1–5.8S–ITS2 (hereafter ITS) region; and (5) partial nuclear large ribosomal RNA subunit (hereafter 26S). Voucher information and GenBank accession numbers are summarized in Table S1. All 73 new vouchers used in this study were identified or re-confirmed by the first author, as many species have not been critically evaluated in a taxonomic revision.

Total genomic DNA extractions were performed from dried herbarium vouchers using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1990), as in Shaw (2000). Amplifications of the selected DNA regions were carried out following standard protocols and primers, as outlined in Olsson *et al*. (2009b) and Shaw, Cox & Boles (2003).

Table 1. List of hotspots (ambiguous alignments) and inversions with their corresponding positions in the final concatenated data matrix (Supporting Information)

Nr.	Position	Gene	Nr.	Position	Gene
Hs1	957–960	*trnL-F*	Hs8	2526 2530	*nad*5
Iv1	961–965	*trnL-F*	Hs9	2993–3042	*nad*5
Hs2	970–973	*trnL-F*	Hs10	3109–3111	ITS
Iv2	1131–1137	*trnL-F*	Hs11	3131–3135	ITS
Hs3	1212–1215	*trnL-F*	Hs12	3432–3437	ITS
Hs4	1281–1289	*trnL-F*	Hs13	3601–3603	ITS
Hs5	1339–1347	*trnL-F*	Hs14	3763–3766	ITS
Hs6	1553–1558	*trnL-F*	Hs15	4192–197	ITS
Iv3	1604–1610	*trnL-F*	Hs16	4280–4290	ITS
Hs7	1650–1655	*trnL-F*	Hs17	5016–5018	26S
			Hs18	5042–5043	26S

Genomic region sequences in the concatenated data matrix were arranged in the order: *rps4*, 1–837; *trnL-F*, 838–1695; *nad5*, 1696–3042; ITS, 3043–4501; 26S, 4502–5525.
Hs, hotspot; Iv, inversion.

DNA sequence editing and alignment

Forward and reverse sequences were assembled and edited for inaccuracy using either PhyDE 0.995 (http://www.phyde.de) or Sequencher v4.1 (Gene Codes Corp.). Consensus sequences were aligned manually in PhyDE 0.995 applying the guidelines outlined in Kelchner (2000), Borsch *et al*. (2003), Quandt & Stech (2005) and Morrison (2006). Simple sequence repeats were positionally isolated on the basis of strict motif recognition, as advocated by Kelchner (2000) and Quandt & Stech (2005). Regions of ambiguous alignment (hotspots) in the data matrix were defined as outlined in Olsson *et al*. (2009b) and excluded from phylogenetic analyses (Table 1). Hairpin-associated inversions, which were visually identified, were positioned separately in the alignment (see Table 1). Instead of coding for the presence or absence of inversions for the phylogenetic analyses, they were reversed and complemented in a second alignment file to retrieve the information within the detected inversion (cf. Quandt, Müller & Huttunen, 2003; Borsch & Quandt, 2009). Both alignments can be found as online supporting information (Data files S1 & S2) and are also deposited in TreeBASE (http://www.treebase.org) under http://purl.org/phylo/treebase/phylows/study/TB2:S11127.

DNA sequence data analyses

Analyses using maximum parsimony (MP), maximum likelihood (ML) and Bayesian Inference (BI) were performed with or without additional information from the simple indel coding (abbreviated as sic) approach of Simmons & Ochoterena (2000). Preliminary analyses on the concatenated nuclear and organellar datasets were first carried out to check for conflicts [i.e. compare nodes with at least 70% BS or 0.95 posterior probability (PP); see method in Mason-Gamer & Kellogg, 1996) before final analyses on the total combined data matrix. The concatenated combined data matrix was analysed without indel coding, with indel coding in the organellar dataset only and with indel coding for the complete dataset (written as subscript w/o, sic-org and sic, respectively; see Table S2 for detailed definition).

The computer program SeqState (Müller, 2005) was used to generate a ready-to-use NEXUS file containing the sequence alignment with an automatically generated binary indel matrix appended. Command files for the parsimony ratchet (Nixon, 1999) and likelihood ratchet (Morrison, 2007) under the GTR + Γ + I model were both generated using the program PRAP2 (Müller, 2007), applying the default configuration, and executed in PAUP 4.0b10 (Swofford, 2002). Heuristic bootstrap searches under MP were performed with 10 000 replicates (Hillis & Bull, 1993; cf. Müller, 2005) in PAUP 4.0b10, whereas, under ML, 400 replicates (cf. Pattengale *et al*., 2009) were performed in GARLI 0.96b8 (Zwickl, 2006) with default settings.

Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), applying the GTR + Γ + I substitution model and the restriction site model (an F81-like model; MrBayes manual) for the sequence data and the binary indel partitions, respectively. To allow for heterogeneous DNA substitution patterns, the dataset was divided into four sequence data partitions including partition 1 [plastid (*rps4 + trnLF*)], 2 [mitochondrial (*nad5*)], 3 [nuclear (ITS + 26S)] and 4

[coded indel] scores. Model parameters for each partition were optimized independently. We performed an analysis with partitioning of the dataset, but without including the indels, to evaluate the effect of indel inclusion. Another analysis of the dataset without sequence-based partitioning (and without indel coding) was carried out to evaluate the effects of data partitioning. The a priori probabilities supplied were those specified in the default settings of the program. PP distributions of trees were estimated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. Eight runs with four chains of 6×10^6 generations each were run simultaneously. Chains were sampled every 1000 generations with trees written to a tree file. The program Tracer v1.5 (Rambaut & Drummond, 2009) was used to evaluate the burn-in and to examine the log likelihoods, ensuring that all parameters converged to a stationary phase with sufficient effective sample size (ESS). Calculations of the consensus tree and PP of the clades were performed based on the trees sampled after the chains converged at generation 1 000 000 for the dataset with partitioning (with or without simple indel coding) and 600 000 without partitioning.

Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph 2.0.45-197 beta (Stöver & Müller, 2010).

Morphological data and ancestral state reconstruction

As leaf limbidia (character C1: elimbate, 0; limbate, 1) and exostome ornamentation (character C2: cross-striate, 0; papillose, 1) are key characters used to distinguish genera in Daltoniaceae (Fig. 1), their evolution was reconstructed. Character states were scored from the voucher specimens, supplemented by published descriptions where necessary. Literature-based information was necessary, especially for exostome structure, as sporophytes at the correct developmental stage are uncommon and molecular vouchers often lacked them.

Ancestral states were evaluated analytically using an MCMC approach implemented in the BayesMultiState module in the BayesTraits v1.0 package (Pagel, Meade & Barker, 2004; Pagel & Meade, 2006). The MCMC method has the advantage of accounting for uncertainty in both phylogenetic topology and character mapping (Pagel *et al*., 2004; Ronquist, 2004). A total of 1000 BI trees (i.e. 125 random trees after burn-in from each of the eight BI_{hom} runs (i.e. BI with a single homogeneous model, see Table S2) were used for character evolution analyses. To enable bifurcating branching at the root node, a prerequisite for BayesTraits to execute, Hypopterygiaceae (the most distant outgroup) was pruned from these 1000 rooted trees with PAUP 4.0b10 (Swofford, 2002) prior to the analyses. A reversible-jump MCMC approached was utilized, as advocated by Pagel & Meade (2006), because it can simultaneously test the five models of character state transformation available in the program and reconstruct ancestral states. A reversible-jump hyperprior with a uniform distribution on the values of 0–30 was used to seed the mean of the prior exponential distribution of the rates of state transition.

Ancestral states were only reconstructed for deeper nodes of the backbone and other selected nodes of interest. Analyses were carried out using the 'addMRCA' (most recent common ancestor) command so that the reconstruction will consider the node in any sampled tree that minimally contains all the specified taxa (i.e. the node might include a number of other taxa). Rate deviation (rd) was adjusted (rd = 70 for C1, rd = 60 for C2) to yield an acceptance rate of about 15–40%, as recommended in the program manual. The analyses were performed for 100×10^6 iterations with a sampling frequency of 5000. Tracer v1.5 was used to ascertain that the chains in both analyses had reached convergence after the default burn-in (50 000 iterations). Ancestral states (i.e. state 0 and 1) for each reconstructed node were evaluated by taking the arithmetic means of the sampled PPs for each character state. Mean PPs, shown as proportions on piecharts plotted on a cladogram, were drawn with TreeGraph 2.0.45-197 beta.

RESULTS

Alignment and sequence analyses

Ninety-four per cent of the sequences were successfully obtained: all *rps4* and *trnL-F*, 87% *nad5*, 95% ITS and 88% 26S accessions (Table S1), with unaligned amplicon lengths of 623–729, 406–479, 920–1149, 655–797 and 985–999 nucleotide bases, respectively. Excluding the hotspots, the concatenated and aligned data matrix consists of 5365 characters in total: 1634 plastid, 1291 mitochondrial and 2440 nuclear positions. In total, 18 hotspots were identified, occurring mostly in *trnL-F* and ITS (see Table 1).

A total of 960 indels was coded, two-thirds belonging to ITS sequences. Simple sequence repeats contributed to most of the length variation in *trnL-F*. Within *rps4*, the *rps4-trnS* IGS accounted for most of the sequence length variability. However, a 90-base nucleotide repeat within *rps4*, characterizing *Ephemeropsis trentepohlioides* (Renner) Sainsbury, is noteworthy. Length mutations in the *nad5* and 26S sequences were limited. The coded indels also increased the number of potentially parsimony informative (PI) characters from 1120 to 1630

(Table S2). The PI characters of the nuclear ribosomal genome nearly doubled with the inclusion of coded indels as characters (from 483 to 870). Among the five markers, ITS contributed the highest number of PI characters (380) in the combined data matrix, similar in number to the combined plastid genome sequences (i.e. *rps4* + *trnL-F*).

Phylogenetic analyses

Phylogenetic trees were rooted with Hypopterygiaceae (*Cyathophorum* P.Beauv., *Hypopterygium* Brid. and *Lopidium* Hook.f. & Wilson) based on the results of Buck *et al*. (2005). Both MP and BI analyses of the combined nuclear (ITS + 26S) and organellar (*nad5* + *rps4* + *trnL-F*) datasets revealed no significant conflicts (i.e. nodes with at least 70% BS or 0.95 PP; Supporting Information Figs S1 and S2). The two datasets were hence combined for the final analyses.

Clade support was assessed by BS for parsimony (three datasets: $BS_{w/o}$, BS_{sic}, $BS_{sic\text{-}org}$, see Table S2 for abbreviations of the subscripts) and likelihood (BS_{ML}) analyses, and by PP for BIs (four datasets: PP_{hom}, $PP_{w/o}$, PP_{sic}, $PP_{sic\text{-}org}$, see Table S2 for abbreviations of the subscripts). Support values were considered to be 'adequate' when BS ≥ 70% (Hillis & Bull, 1993) (100%, 'maximum support'; ≥ 80%, 'well supported'; 80–70%, 'moderately supported'; < 70%, 'poorly supported') or when PP ≥ 0.95 (1.00, 'maximum support'; ≥ 0.99, 'well supported'; 0.95–0.99, 'moderately supported'; < 0.95, 'poorly supported').

All MP analyses of the concatenated datasets with various simple indel coding schemes gave almost identical results. However, MP analyses of the datasets with simple indel coding (i.e. MP_{sic} and $MP_{sic\text{-}org}$) generally provided stronger BS than analyses without coded indels ($MP_{w/o}$) (Fig. S1).

Results from BIs of all four datasets (i.e. BI_{hom}, $BI_{w/o}$, $BI_{sic,}$ $BI_{sic\text{-}org}$) showed no conflict with the MP consensus trees and had higher resolution with generally higher support. The tree topologies from the three datasets with sequence partitioning were almost identical, except for a few distal branches with poor PP values (see Fig. S2). For all analyses of the partitioned datasets, individual runs did not have sufficient ESS (<200) for the 'Tree Length' (TL{all}) parameter. Nevertheless, BI_{hom} gave the best overall scores for each parameter among the four BI analyses (Table S2).

ML analyses were carried out only on the dataset without indel coding. Likelihood ratchet analysis resulted in 12 best trees, with two main topologies that differed in the placement of the *Calyptrochaeta* clade. One-third of the ML trees ($\ln L = -36831.66$) resolved *Calyptrochaeta* as the sister group of the rest of Daltoniaceae, and this relationship was also supported maximally in $BI_{w/o}$. The remaining nine trees ($\ln L = -36831.15$) had *Calyptrochaeta* forming a sister-group relationship with the Schimperobryaceae–Hookeriaceae–Leucomiaceae–Pilotrichaceae clade. One of the ML trees with the latter topology is shown in Figure 2A and B, with support values BS_{sic}, BS_{ML}, PP_{sic} and $PP_{w/o}$. Alternative topologies relating to the placement of *Calyptrochaeta* are shown by dotted lines. The same tree is also presented as a phylogram to illustrate the branch lengths within and among clades (Fig. S3).

The precise phylogenetic position of *Calyptrochaeta* remains uncertain (Fig. 2A). Similarly, the placement of *Achrophyllum*, emerging as the sister group of the remaining Daltoniaceae (i.e. core Daltoniaceae), does not have adequate support in all analyses, except BI_{sic} and $BI_{sic\text{-}org}$.

Monophyly of both *Calyptrochaeta* and *Achrophyllum* is maximally supported. In *Calyptrochaeta*, the position of *C. asplenioides* (Brid.) Crosby is ambiguous and different sister-group relationships are resolved under ML and BI (Fig. 2A). With MP (Fig. S1A), *C. asplenioides*, *C. cristata* (Hedw.) Desv. and the other nine *Calyptrochaeta* spp. form a trichotomy. The species-rich clade sits on a long branch that bifurcates into an Australasian–Patagonian subclade and an Asian subclade (Fig. S3). The relationships of the two Australasian species *C. brownii* (Dixon) J.K.Barlett and *C. otwayensis* Streimann are unresolved in all analyses. All six Asian species sampled form a monophyletic group, although support values are inadequate ($BS_{ML} = 67$, $PP_{hom} = 0.85$). In *Achrophyllum*, *A. haesselianum* (Matteri) Matteri is the sister group of all other species in the genus. The type of the genus, *A. quadrifarium* (Sm.) Vitt & Crosby, forms a sister-group relationship with the remaining four species, which are largely unresolved (Fig. 2A).

Within the maximally supported core Daltoniaceae, *Beeveria distichophylloides* (Broth & Dixon) Fife and *Distichophyllum microcarpon* (Hedw.) Mitt. form the sister group of the remaining family. These two species, both on long branches (Fig. S3), are moderately supported as sister groups by the dataset with indels coded (Fig. 2A). Support values are lower without indels coded. Remaining exemplars of core Daltoniaceae comprise two sister clades of unequal size, the smaller including *Ephemeropsis*, *Bryobrothera*, *Adelothecium* and *Benitotania*. *Adelothecium* and *Benitotania* form the sister group of *Bryobrothera* and these three monotypic genera constitute, in turn, the sister group of *Ephemeropsis*.

The well-supported and larger clade consists of various assemblages of different species in *Daltonia*, *Distichophyllum*, *Leskeodon* and a few other small genera. Within this clade, a group of Neotropical

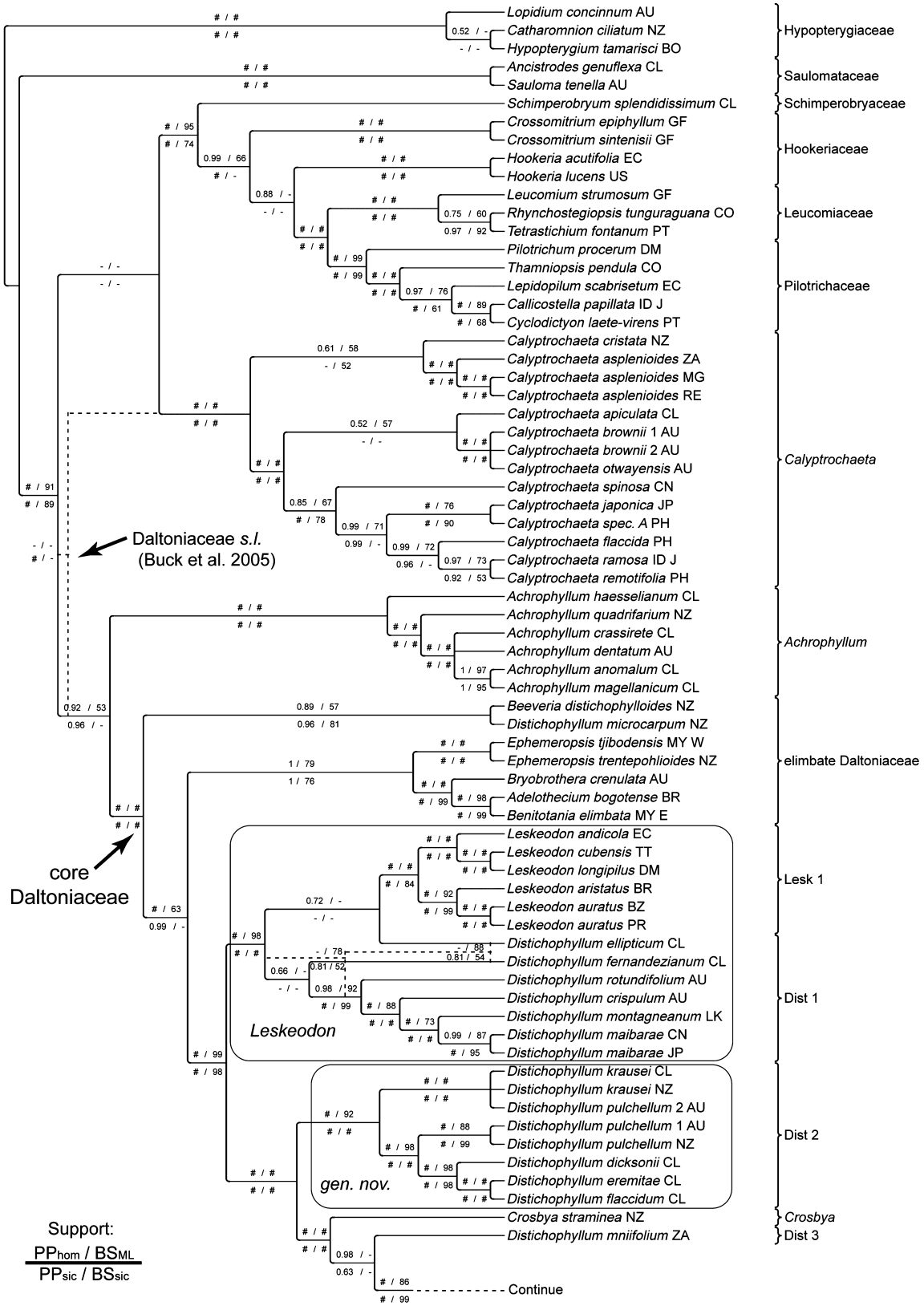

Figure 2. See caption on next page.

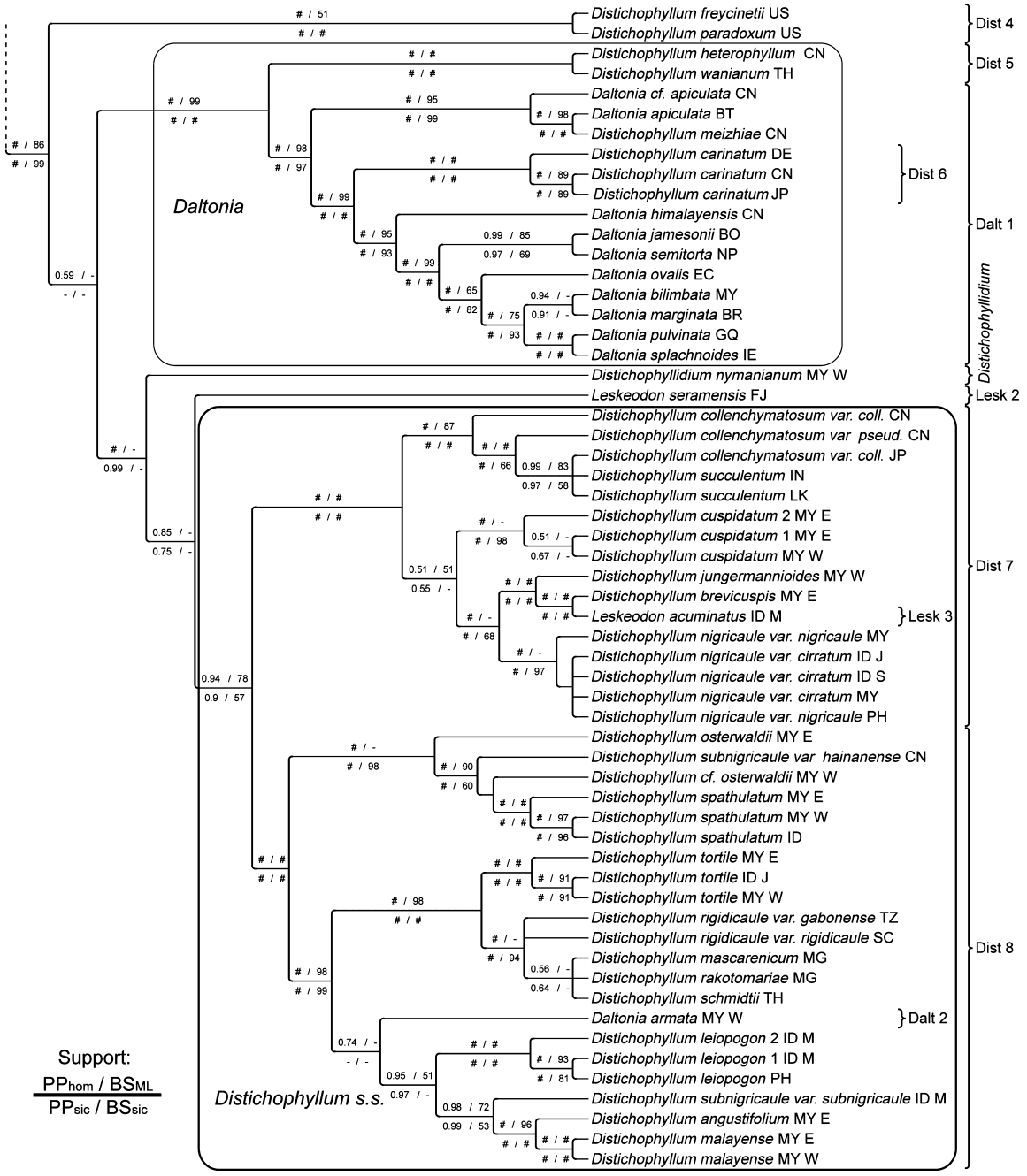

Figure 2. Cladogram of one of 12 maximum likelihood trees found using a ratchet approach (see text). Support values above the branches are posterior probabilities based on a nonpartitioned model (PP_{hom}), followed by likelihood bootstrap support values (BS_{ML}); both excluded indels in the analysis. Values below the branches are posterior probabilities based on a partitioned model (PP_{sic}), followed by parsimony boostrap support values (BS_{sic}), both with indels coded in the matrix. Alternative topologies of parts of branches are shown as dotted lines. '#' denotes maximum support (BS = 100%, PP = 1.00); '-' denotes conflicting or unresolved topology of the corresponding analysis; Dalt 1–2, Dist 1–8 and Lesk 1–3 are abbreviations for *Daltonia* clade 1–2, *Distichophyllum* clade 1–8 and *Leskeodon* clade 1–3, respectively.

Leskeodon (Lesk1), together with six *Distichophyllum* spp. from South America, Australasia and Asia (Dist1), is sister group to the rest of the sampled taxa. The Lesk1–Dist1 clade is on a long branch (Fig. S3) and receives maximum support in nearly all analyses (Fig. 2A). The placement of *Distichophyllum ellipticum* Herzog within this clade is uncertain, with some analyses favouring a topology of *D. ellipticum* forming

a sister-group relationship with *D. fernandezianum* Broth., which is in turn sister group to the rest of Dist1 (Fig. 2A, shown as dotted lines).

Next, a well-supported group of *Distichophyllum* spp. confined to southern Australasia and Patagonia (Dist2) is sister to the rest of the taxa (bottom of Fig. 2A plus 2B). *Crosbya straminea* (Mitt. ex Beckett) Vitt and *Distichophyllum mniifolium* (Hornsch.) Sim (clade Dist3), New Zealand and South African endemics, respectively, are the next two successive splits, forming a grade. Following these, there are three successive clades that form three short branches lacking support (Figs 2B and S3). These include a small clade of Hawaiian endemics, *Distichophyllum freycineti* (Schwägr.) Mitt. and *D. paradoxum* (Mont.) Mitt. (Dist4), a clade consisting largely of *Daltonia* spp. plus a few morphologically atypical (see discussion for details) *Distichophyllum* spp. (Dist5 + Dalt1), and a large clade of exclusively Old-World-Pacific taxa. Most internal nodes within the well-supported Dist5–Dalt1 clade receive maximum PP and at least 85% support from BS_{ML} and $BS_{w/o}$.

The Old-World-Pacific clade is well supported in Bayesian analysis, but lacks BS support in the MP and ML analyses. Within this clade, *Distichophyllidium nymanianum* M.Fleisch., *Leskeodon seramensis* H.Akiyama (Lesk2) and the rest of the taxa (Dist7 + Dist8) form another three short branches with almost zero branch lengths (Fig. S3). Nevertheless, topologies in almost all analyses (except MP_{sic}) indicate that at least Lesk2 is sister group to the Dist7–Dist8 clade (Fig. 2B). The latter clade is poorly supported (except $BS_{ML} = 78$), but its two main subclades (Dist7 and Dist8) are maximally supported.

The Dist7 clade (within which Lesk3 is nested) consists of epiphytes and two species complexes surrounding *Distichophyllum nigricaule* Mitt. ex Bosch. & Sande Lac. and *D. collenchymatosum* Cardot. Both species complexes receive good support, but it is unclear which is the sister group of *D. cuspidatum* (Dozy & Molk.) Dozy & Molk. (Fig. 2B). This group is the sister group of Dist8, within which Dalt2 is nested. Most of the internodes within the Dist8 clade are well supported, although the position of *Daltonia armata* E.B.Bartram is ambiguous. Species with multiple accessions from different islands, such as *Distichophyllum leiopogon* Dixon (better known as *D. cucullatum* E.B.Bartram, see Ho, Tan & Nathi, 2010), *D. spathulatum* Broth. and *D. tortile* Dozy & Molk. ex Bosch & Sande Lac., are resolved as monophyletic with maximum support. The sister group of *D. tortile* is a well-supported clade consisting of *D. schmidtii* Broth. plus all species sampled from southeast Africa and adjacent islands in the western Indian Ocean. One of the 12 ML trees has *D. schmidtii* resolved as sister group of *D. mascarenicum* Besch. and *D. rakotomariae* Crosby (not shown), which also has some poor support from all BI analyses (Fig. S2B).

Ancestral state reconstruction

Reconstructed evolutionary transitions in leaf limbidia (C1) and exostome ornamentation (C2) are plotted on the tree shown in Figure 3. The reversible jump MCMC chains visited the one-rate '0Z' model (i.e. $q_{01} > 0$, $q_{10} = 0$, where q_{01} is the transition rate from state 0 to 1 and vice versa) ~96% and 68% of all post-burnin iterations for characters C1 and C2, and, for the '00' model (i.e. $q_{01} = q_{10}$), ~4% and 31%, respectively (visited other models occasionally). No state reversals were detected for either character. Reconstruction of leaf limbidia indicates that the ancestral state at the root node is elimbate (mean PP of 0.9999) and that leaf limbidia evolved twice (Fig. 3, circles above the branches). A transition from elimbate to limbate leaves occurs at the root of the *Calyptrochaeta* clade and the clade corresponding to the *Daltonia–Distichophyllum–Leskeodon* complex (Lesk1, Dist1 in Fig. 2A).

For exostome ornamentation, the ancestral state at the root is reconstructed as striate (PP = 0.9961). At least six independent transitions to papillose from striate exostome teeth were detected (Fig. 3, circles below branches). These transitions correspond to the Neotropical *Leskeodon* (Lesk1), the main *Daltonia* clade (i.e. Dalt1 + Dist5) and four scattered individual species (*Distichophyllidium nymanianum*, *Leskeodon seramensis*, *L. acuminatus* and *Daltonia armata*). The last two taxa clearly represent cases of papillose exostomes arising within clades with striate exostome. Although character reconstructions of the ancestral nodes immediately before *Distichophyllidium nymanianum* and *L. seramensis* have a higher mean PP of being cross-striate (0.7887 and 0.9138, respectively) than papillose, the precise position of these two taxa is uncertain (Fig. 2B). This may play a major role in obscuring a definitive tally for the number of times papillose exostome teeth arose.

DISCUSSION

Assessing the evolution of two taxonomically important morphological characters

Ancestral state reconstructions show that the elimbate leaves and cross-striate exostome are plesiomorphic states in Daltoniaceae *s.l.* Limbate leaves evolved twice and represent a synapomorphy for *Calyptrochaeta* and for *Distichophyllum* plus its allied genera. The majority of species in core Daltoniaceae have limbate leaves (without known

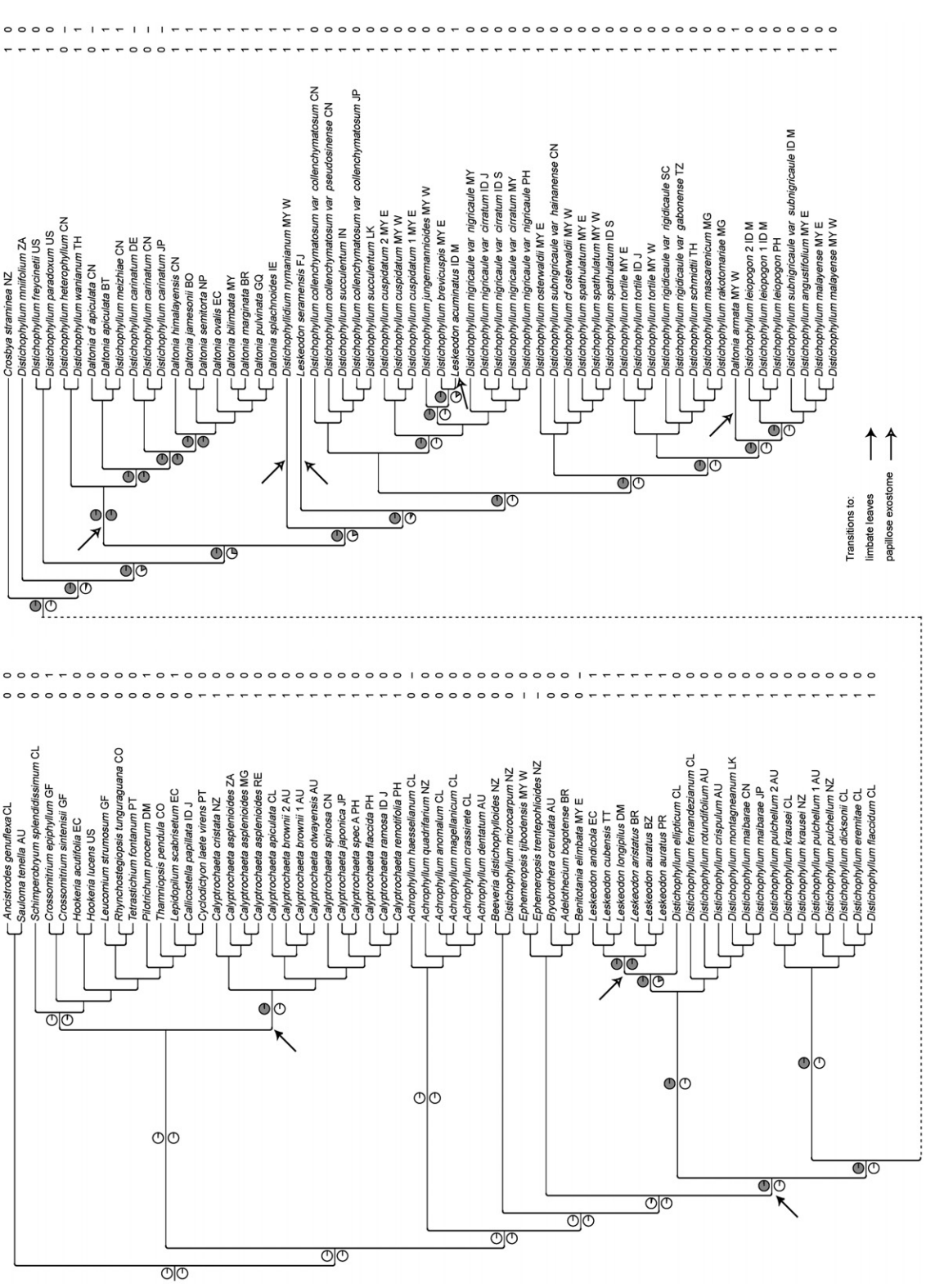

Figure 3. Majority consensus tree of 1000 resampled trees used for character evolution analysis in BayesTraits (see text). Circles plotted beside the nodes represent the proportions of the mean posterior probability (PP) of each state in Character C1 (above branches) and C2 (below branches). Numbers following the sample names correspond to character codings: C1 (leaf limbidia): elimbate (white in circle plot, coded as 0), limbate (grey, coded as 1); C2 (exostome ornamentation): cross-striate (white in circle plot, coded as 0), papillose (grey, coded as 1). Unknown or inapplicable states are denoted by '–'.

functionality) and are all resolved in one clade, corresponding to the traditional *Daltonia*, *Distichophyllidium*, *Distichophyllum*, *Crosbya* and *Leskeodon*. All species in these five genera (except *Distichophyllum microcarpon*, discussed below), including those not sampled here, possess at least some traces of a limbidium. Thus, states of this character correspond well with phylogenetic relationships. The only exception, *Calyptrochaeta*, has limbate leaves, but with short and unequally forked costae near the base. Costae in other genera in the family are single, except for *Distichophyllidium*, which is ecostate, and *Achrophyllum*, which has elimbate leaves with a single costa that is forked above the base.

Papillose exostome teeth, characteristic of daltoniaceous peristomes, evolved independently from striate teeth multiple times in Daltoniaceae. Species with papillose exostomes were traditionally placed in the largely epiphytic *Daltonia* or *Leskeodon*. Although papillose exostomes are consistent in clades Lesk1 and Dalt1, some species in the traditional *Daltonia* and *Leskeodon* are intermixed with species of *Distichophyllum*, a genus traditionally circumscribed as having striate exostomes. *Daltonia armata* was described in *Daltonia* based mainly on leaf morphology without description of the peristome (Bartram, 1944). Its papillose exostome is confirmed here from the type specimens (*A. Lynn Zwickey 638* FH!, studied by the first author). *Leskeodon acuminatus* was first described as a *Distichophyllum*, but was transferred to *Leskeodon* because of its peristome type (Fleischer, 1908). *Daltonia armata* and *Leskeodon acuminatus* are nested deeply within a clade of taxa with striate peristomes. Traditional segregation of *Leskeodon* from *Distichophyllum* merely by its daltoniaceous peristome (papillose exostome) is rejected here. For the other generic pair, the separation of the hookeriaceous *Crosbya*, with only two accepted close species, from *Daltonia* is supported. Thus, contrary to Buck's (1991, 2007) suggestion, exostome ornamentation is not always a reliable character for distinguishing genera in Daltoniaceae.

Whittemore & Allen (1989) observed that the two types of perisome exhibit different and opposite hydroscopic movements in response to changing humidity. Shifts to a daltoniaceous peristome could be associated with the change to an epiphytic lifestyle during evolution. Our observations may have implications for generic delimitation in the large family Pilotrichaceae, in which genera are similarly distinguished by peristome types regardless of gametophytic similarities (e.g. *Lepidopilum* vs. *Lepidopilidium*, *Stenodictyon* vs. *Stenodesmus*) (Buck, 1998). Although molecular data are available for some of these taxa, a study focus in Pilotrichaceae, with better sampling, is necessary to ascertain our hypothesis (see also Pokorny *et al*., 2012).

Basic peristome types that differ in development correspond to deep lineages in the mosses (Shaw, Anderson & Mishler, 1987, 1989a, b; Shaw & Anderson, 1988; Goffinet *et al*., 1999; Shaw, Szövényi & Shaw, 2011). The differences, however, relate to patterns of cell division in the apical region of young sporophytes, long before the teeth develop, whereas the sorts of differences that have historically been used to distinguish genera and families in Hookeriales and other groups of pleurocarpous mosses pertain to superficial ornamentation of the teeth. Differences in ornamentation develop just before maturation and the release of spores. Our results add to a growing body of evidence that peristomal differences that occur late in development are phylogenetically mutable (e.g. Hedenäs, 2001, 2002; Vanderpoorten *et al*., 2002; Huttunen *et al*., 2004; Olsson *et al*., 2009a; Quandt *et al*., 2009; Liu *et al*., 2012; Pokorny *et al*., 2012). Nevertheless, the systematic significance of peristome variation needs to be assessed on a group-by-group basis, ideally using independent evidence to resolve phylogenetic relationships.

Resolving relationships among the genera in Daltoniaceae

The topology of the Hookeriales backbone closely resembles results from Buck *et al*. (2005). Buck *et al*. (2005) were unable to unambiguously resolve the relationships of *Calyptrochaeta* and *Achrophyllum* relative to Hookeriaceae and Daltoniaceae. Despite the increased taxon sampling in our study, resolution of their relationships remains inconclusive. Nevertheless, both genera are resolved as monophyletic with maximum support. Other approaches to resolving their relationships to other genera could be to increase genomic sampling with a reduced taxon dataset or to try other analysis methods, such as network-based techniques.

The topology of the elimbate taxa of core Daltoniaceae differs slightly from that reconstructed by Buck *et al*. (2005) and Gradstein & Wilson (2008). In their studies, *Beeveria* and *Ephemeropsis* formed a clade (PP < 0.95, BS = 52) that is the sister group to the rest of core Daltoniaceae. In our study, *Beeveria* plus *Distichophyllum microcarpon* (not sampled in their studies) form a sister-group relationship to the rest of core Daltoniaceae, and *Ephemeropsis* is the sister group of the remaining elimbate taxa (Fig. 2A). Leaves of *D. microcarpon*, unlike any other species of *Distichophyllum*, have no trace of differentiated leaf border or limbidium, and hence the taxon is unlikely to belong to *Distichophyllum* or any of its allied genera. *Distichophyllum microcarpon* could be

transferred to *Beeveria* to reflect its closer affinity to that genus than to other limbate groups, although the relationship is not adequately supported in all analyses here. Allan Fife (unpubl. data) intends to create a new genus to accommodate this taxon based on morphology; our findings would lend additional support to this taxonomic change.

The monophyly of, and relationships among, *Adelothecium*, *Benitotania* and *Bryobrothera* have been assessed without disagreement (Akiyama *et al*., 2003; Buck *et al*., 2005; Gradstein & Wilson, 2008) and are re-confirmed here. However, the sister-group relationship of this clade with the genus *Ephemeropsis*, found here, does not agree with the findings of Buck *et al*. (2005) or Gradstein & Wilson (2008). The relationships of *Ephemeropsis trentepohlioides* and *Beeveria distichophylloides* in their studies could be attributed to long-branch attraction. Our results indicate an adequately supported clade consisting of *Adelothecium*, *Benitotania*, *Bryobrothera* and *Ephemeropsis*, which are all epiphytic/epiphyllous taxa.

The sister clade of *Adelothecium*, *Benitotania*, *Bryobrothera* and *Ephemeropsis*, which consists of taxa with limbate leaves, is well supported as monophyletic in our study. Nevertheless, phylogenetic relationships within this clade contradict classical concepts of *Daltonia*, *Distichophyllum* and *Leskeodon*. Specifically, the small genera *Crosbya* and *Distichophyllidium* are positioned within these genera. Adequately supported (i.e. BS $\geq$ 70%, PP $\geq$ 0.95) clades show various combinations of species currently classified in different genera. The phylogenetic evaluation of the limbate taxa is discussed below with regard to the reorganization of *Distichophyllum*.

Infrageneric relationships within *Calyptrochaeta* and *Achrophyllum*

In terms of morphology, the type species *C. cristata* deviates from the most common appearance of the species in the genus, with laminal cells evenly thin-walled and leaf marginal teeth consisting of a variable number of cells. Typical *Calyptrochaeta* spp. often have thick-walled laminal cells, at least at the cell corners, and the majority of the leaf marginal teeth consist of parts of two adjacent border cells. These morphological differences seem to support the topology in BI (Fig. S2A), in which *C. cristata* forms a sister-group relationship to the rest of the sampled species. The Australasian species, *C. brownii* and *C. otwayensis*, have almost identical sequences, which may explain the unresolved topology. In our opinion, the morphological variation between the two species is sufficiently low to be considered conspecific.

This is the first attempt to evaluate species relationships in the genus *Achrophyllum* using molecular data. Within *Achrophyllum*, *A. haesselianum* and *A. quadrifarium* are morphologically distinct, as the plants have pale green coloration and scarcely toothed to subentire leaf margins (Sainsbury, 1955; Matteri, 1972). The four species reconstructed as the sister group of *A. haesselianum* are dark green plants with erose-dentate leaf margins. Species in the latter group are difficult to distinguish morphologically and largely unresolved in the phylogenetic tree. Matteri (1972) and Robinson (1975) had different concepts for taxa in this species complex, evident from contrasting morphological features used in their identification keys. Moreover, features used by both authors for species identification, such as size of marginal teeth, length of costa, laminal cell size, degree of wall thickening at cell corners etc., are quite variable. Notably, Robinson (1975) proposed synonymy of *A. crassirete* (Matteri) Matteri and *A. magellanicum* (Besch.) Matteri under *A. anomalum* (Schwägr.) H.Rob. and *A. dentatum* Hook.f. & Wilson) Vitt & Crosby, respectively.

Reorganization of *Distichophyllum* and its allies

This study has confirmed the heterogeneity of *Distichophyllum* and shows the complexity of its relationships to *Crosbya*, *Daltonia*, *Distichophyllidium* and *Leskeodon*. Considering the difficulty of identifying morphological synapomorphies for internal clades, one option is to consider the entire clade as a single genus. This would require the generic names *Distichophyllum* and *Leskeodon*, together with a few others, to be synonymized with the oldest name, *Daltonia*. This approach would require numerous new combinations and would tend to disrupt nomenclatural stability. We propose that it is a more reasonable solution to reorganize and adjust the traditional concepts of the genera, which will require additional morphological study.

Resolved clades within *Distichophyllum* and its allies correspond more to biogeographical entities than to traditional concepts of genera. For instance, Lesk1 consists of species limited to the Neotropics, species in the Dist2 clade appear to have a so-called '*Nothofagus*-type' distribution (Seki, 1973), the two Hawaiian endemics are closely related (Dist4) and all species in Dist7 and Dist8 are restricted to the Old World. The only exception is found in the Dist1 clade, where Asian species are nested within groups of southern South American and Australasian species. Our phylogenetic analyses also show that *Daltonia* species occurring almost exclusively in the Himalayan region belong to species-poor clades that constitute a paraphyletic

assemblage, whereas species with a transcontinental distribution form a monophyletic group, thus agreeing with Yu *et al*. (2010).

Although the genus *Leskeodon* is phylogenetically heterogeneous, excluding the Old World species would make the remaining members monophyletic and exclusively Neotropical (Lesk1 in Fig. 2A). However, some species currently in *Distichophyllum* (Dist1) are the sister group of Lesk1. These two clades are separated by short branches, but together are subtended by a longer branch (Fig. S3). Morphologically, all species in this Lesk1–Dist1 clade have small isodiametric laminal cells that are more or less homogeneous in size, except along the costa near the base. Moreover, plants of *D. maibarae* Besch. and *D. montagneanum* from Asia have remarkably similar leaf morphology to those of *L. andicola* from the New World. Although W. R. Buck (pers. comm., February 2010) believes that members of the two clades are sufficiently distinct to be different genera, we consider it better to treat both Dist1 and Lesk1 as a single genus. As an exemplar of *L. auratus* (type species of *Leskeodon*; Welch, 1966) belongs in the Lesk1–Dist1 clade, we thus refer to the Lesk1–Dist1 clade as *Leskeodon*. However, the formal transfers of the names *D. crispulum*, *D. ellipticum*, *D. fernandezianum*, *D. montagneanum* and *D. rotundifolium* to *Leskeodon* are postponed because the types of these names have not been examined.

Within this clade, *D. maibarae* and *D. montagneanum* are distinguished exclusively by the presence or absence of some long erect hairs on the calyptrae (Mohamed & Robinson, 1991). The taxonomic value of this character has been questioned (Ho *et al*., 2010). The sampled Chinese accession of this species complex has naked calyptrae and should be named *D. montagneanum*, a new country record. However, the tree topology suggests that this plant is closer to the Japanese plants, where only *D. maibarae*, with hairy calyptrae, is known. The two species are gametophytically inseparable and polymorphic in terms of size and colour. No molecular or morphological evidence currently supports the separation of the two species, but a more detailed study with populations sampled throughout the geographical range of occurrence may resolve this taxonomic issue.

The position of the New Zealand endemic genus *Crosbya* is almost identical to that in earlier published results (Buck *et al*., 2005). Gametophytes of *Crosbya* closely resemble those of *Daltonia*, except for the excurrent costa. Unlike *Daltonia* spp., the two species of *Crosbya* are both dioecious and have a hookeriaceous peristome (Vitt, 1977). Gametophytic similarity of these two genera may reflect convergence, as both are mostly epiphytes (Vitt, 1977). However, *Crosbya* spp. appear to be limited to tree trunks and branches, sometimes on boulders, whereas *Daltonia* spp. most commonly grow on twigs and leaves.

All *Daltonia* spp., except *D. armata*, fall within the well-supported Dist5–Dalt1 clade. A few peculiar Asian (Himalayan) *Distichophyllum* spp. with more or less carinate leaves also belong to this clade. *Daltonia* cf. *apiculata*, *Distichophyllum heterophyllum*, *D. meizhiae* B.C.Tan & P.J.Lin and *D. wanianum* B.C.Tan & P.J.Lin produce gemmae on the dorsal side of the leaf costa (Ho *et al*., 2010). These *Distichophyllum* spp. also have ± rectangular basal laminal cells, which is a typical trait of some *Daltonia* spp. (Yu *et al*., 2010). Hence, the nomenclatural transfer of these species of *Distichophyllum* into *Daltonia* can be justified and proposed in this article.

The limited sampling of the International Union for the Conservation of Nature (IUCN) red-listed *Distichophyllum carinatum* reveals that the Asian exemplars are the sister group of the European (German) exemplar, but this should not be interpreted as meaning that the species originated in Europe (Fig. 2B). The two available sequences from the Japanese voucher are identical to those of the Chinese exemplar. Comparing all gene sequences from German and Chinese vouchers (Table S1), only five nucleotide differences are detected in ~4500 nucleotides. It is most likely that the species originated in continental Asia (see discussion in Ho *et al*., 2010) where the majority of the closely related *Daltonia* spp. occur.

The small genus *Distichophyllidium* is represented in the present study by only the type species. In the absence of the other four species in the genus, its monophyly and generic relationships cannot be determined. Buck *et al*. (2005), with the sampling of only two species each of *Distichophyllum* and *Daltonia*, showed a well-supported sister-group relationship between *Distichophyllidium* and *Daltonia*, a topology not resolved in our study with better sampling.

The Dist7–Dist8 clade is considered to be the core of *Distichophyllum*, because an exemplar of the type species, *D. spathulatum* (Buck *et al*., 2005), is included here. The core *Distichophyllum* is a poorly supported clade consisting of two well-supported sister clades. We provisionally recognize the whole clade as *Distichophyllum* in the strict sense. At best, the two subclades Dist7 and Dist8 should be recognized at the infrageneric level in *Distichophyllum*, as there appears to be no morphological characters distinguishing them.

Within Dist7, the nesting of *Distichophyllum succulentum* within *D. collenchymatosum* corroborates the suggestion in Ho *et al*. (2010) that the two names might be conspecific. However, without examination of type specimens, particularly those of the little known *D. succulentum*, it is better to postpone the synonymization of these names.

Two clades of epiphytic species occur within Dist7. The first consists only of *Distichophyllum cuspidatum*. In the other epiphytic clade, *D. jungermannioides* (Müll.Hal.) Bosch & Sande Lac., a species commonly found at the base of trees and shrubs, is the sister group of two other true epiphytes including *Leskeodon acuminatus*. Consequently, revival of the original basionym *Distichophyllum acuminatum* Bosch & Sande Lac. is proposed here for the latter species.

All exemplars of the *D. nigricaule* complex, including plants that vary in size and degree of laminal cell size differentiation, are grouped together, but without resolution. The morphological variability within this species appears to be unrelated to genealogy. Thus, synonymy of the two varietal names, as proposed previously by some authors (e.g. Bartram, 1939; Gangulee, 1977), is supported here.

Yu *et al*. (2010) speculated that *Daltonia armata* (not sampled in that study) may belong to the paraphyletic assemblage of *Daltonia* with limited geographical distribution, and not within the monophyletic transcontinentally distributed clade of *Daltonia*. Current analyses support the nesting of this species (Dalt2) within the Dist8 clade of the core *Distichophyllum*. The removal of this species from *Daltonia* has been suggested (Ho *et al*., 2010), but its placement was uncertain. The current phylogenetic assessment justifies its transfer into *Distichophyllum s.s*. The long branch length leading to this species (Fig. S3) indicates that rapid evolution could explain its highly modified morphology, but this suggestion remains to be tested.

Taxonomic assessment of other species is not attempted in this study because additional morphological evaluation is required. These species belong to clades that either show unresolved relationships or have no known morphological synapomorphies. Hence, the names are tentatively retained in their currently accepted genera. These include the clades Dist2, Dist3 (with only *Distichophyllum mniifolium*), Dist4, (Hawaiian endemics) and Lesk2 (with only *Leskeodon seramensis*).

Distichophyllum subnigricaule is heterogeneous, with its two established varieties appearing in different clades. Morphological similarities of the two varieties may be convergent. Thus, it is best to raise *D. subnigricaule* var. *hainanense* to species level and to treat the two taxa as separate species. For other species that are demonstrably nonmonophyletic, including *Daltonia apiculata*, *Distichophyllum osterwaldii* and *D. pulchellum*, the status of possible new taxa cannot be decisively assessed without further taxonomic study.

Several relationships within the family could not be evaluated because sequences of some critical taxa were not obtained. Future phylogenetic studies need to include: (1) *Metadistichophyllum rhizophorum* (M.Fleisch.) Nog. & Z.Iwats., which is a monotypic South-East Asian genus sometimes considered to be synonymous with *Distichophyllum* (Crosby, 1974; Akiyama, 1990); (2) *Leskeodontopsis pustulata* Zanten, a monotypic genus of rare occurrence in New Guinea; (3) *Distichophyllum flavescens* (Mitt.) Paris, the type species of the genus *Discophyllum* Mitt. from the Pacific Islands; (4) *Distichophyllum noguchianum* B.C.Tan, the type species of section *Platyovatophyllum* B.C.Tan known only from the Philippines; (5) *Leskeodon palmarum* (Mitt.) Broth., morphologically distinctive (Buck, 1998) and the only species in *Leskeodon* section *Longiseti* Broth. from the Neotropics. In addition, species, such as *Achrophyllum javense* (J. Froehl.) Z.Iwats. from South-East Asia, *Calyptrochaeta setigera* (Mitt.) W.R.Buck endemic to Brazil, *Distichophyllidium jungermanniaceum* M.Fleisch., also from South-East Asia, and *Distichophyllum santosii* E.B.Bartram from Borneo and the Philippines, could be important for the clarification of infrageneric relationships.

CONCLUSION

Being lost in a sea of similar gametophytic characters among genera and under the influence of Philibert's principles of peristome conservatism, it is no surprise that workers in the past used easily observed differences in exostome ornamentation as key characters to delimit genera and families. The heterogeneity of papillose exostomes in the limbate Daltoniaceae means that traditional concepts of several genera require taxonomic recircumscription to reflect new insights about phylogenetic relationships. However, finding a set of 'good' morphological features to delimit the newly recognized clades is still challenging. Our study has revealed new information about relationships among genera within the Daltoniaceae. However, precise relationships of certain species, genera and clades still remain obscure. Traditional genera in the limbate Daltoniaceae are, in many cases, not supported by our molecular data and suggest convergent evolution. The abundance of homoplasy in morphological traits has hampered an accurate circumscription of genera to reflect natural groupings. Critical generic revisions of *Daltonia*, *Distichophyllum* and *Leskeodon* are essential for the construction of a new taxonomic system and the identification of morphological synapomorphies for resolved clades, if such synapomorphies exist.

Proposed new nomenclatural combinations and new synonymies

Daltonia carinata (Dixon & W.E.Nicholson) B.C.Ho & L.Pokorny, ***comb. nov.*** – Basionym: *Distichophyl-*

lum carinatum Dixon & W.E.Nicholson in Dixon *Rev. Bryol.* 36: 24. f. 1–7. 1909. – **Type:** Austria, Salzburg, [Salzkammergut], St. Wolfgang See, Zinkenbach, alt. 700 m; creeping on other mosses upon dripping rocks in ravine. *H.N. Dixon & W.E. Nicholson s.n.*, 3.viii.1908 (holotype: BM!; isotypes DUKE!, E!, H!, NY!, MO n.v., S n.v.).

Daltonia heterophylla (Wilson ex Mitt.) B.C.Ho & L.Pokorny, ***comb. nov***. – Basionym: *Mniadelphus heterophyllus* Wilson ex Mitt. *J. Proc. Linn. Soc., Bot.* Suppl. 2: 144. 1859. – *Distichophyllum heterophyllum* (Wilson ex Mitt.) Paris, *Index Bryol*. 389. 1896. – **Type**: [Nepal,] Nangli ['Sikkim'], 10 000 ft; *J.D. Hooker '690'* (holotype: NY-Mitten!; isotype: L!).

The protologue of the name in Mitten (1859) states: 'In Himalayae orient. Reg. temp., Sikkim, *J.D. Hooker no. 690*'. The holotype in NY-Mitten is labeled: '690 (in pencil), Nangkli, 10 000', and it has been confirmed by D. G. Long (Royal Botanic Garden Edinburgh, pers. comm. January 2010) that the type locality of this name 'Nangkli' should be in Nepal, but has been erroneously regarded as Sikkim by Mitten. A supposed isotype in BM bears the label '*690 Herb Ind. Or. Hook. fil. & Thomson* (no. 458 in pencil). *Hab. Tonglo, Reg. temp. Sikkim Himalaya, alt 10 000 ft. Coll. J.D.H.*'. However, on close examination of this BM specimen, it turns out to be a different species, identifiable to *Distichophyllum succulentum* (Mitt.) Broth. It is known that the 'Herb. Ind. Or. Hook. Fil. & Thomson' numbers were issued by the herbarium K for distribution of presumed identical species, not duplicates of the same collection (*fide* D.G. Long); thus, the BM specimen is considered here as not a type.

Although only the above two specimens of this seemingly rare species have been reported in the literature (Gangulee, 1977), one other historical specimen of this species has been located in L (Herb. v.d. Sande Lacoste) labelled: '*Mniadelphus heterophyllus* M., *Himalaya orient. Hb Mitten*'. As with the holotype in NY, this specimen also has a few stems of *Cyathophorum hookerianum* (Griff.) Mitt. mixed in, suggesting that they originated from the same collection. Mitten exchanged specimens with Dutch bryologists (see Touw, 2007), and thus the specimen at L is interpreted here as an isotype.

Daltonia meizhiae (B.C.Tan & P.J.Lin) B.C.Ho & L.Pokorny, ***comb. nov***. – Basionym: *Distichophyllum meizhiae* B.C.Tan & P.J.Lin *Trop. Bryol.* 10: 55. f. 2, 8–12. 1995, '*meizhii*'. – **Type:** China. Yunnan Province, Gongshan-xian (county), Du-long-jiang Commune, on boulder by the Ching-lang-tang river bank, about 1300 m elev. *Mei-zhi Wang 10040*, viii.1982 (holotype: PE!).

Daltonia waniana (B.C.Tan & P.J.Lin) B.C.Ho & L.Pokorny, ***comb. nov***. – Basionym: *Distichophyllum wanianum* B.C.Tan & P.J.Lin, *Trop. Bryol*. 10: 57. f. 1, 13–18. 1995. – **Type**: China. Yunnan Province, Luchun, on branches. *M. Zhang 550* (holotype: IBSC n.v.; isotypes: KUN n.v., FH!).

Distichophyllum armatum (E.B.Bartram) B.C.Ho & L.Pokorny, ***comb. nov***. – Basionym: *Daltonia armata* E.B.Bartram, *Farlowia* 1: 508, f. 21–24. 1944. – **Type:** Philippines, Mindanao, Lanao Prov., vicinity of Dansalan [= Marawi], Sacred Mountain, alt. 700–800 m, on culm of climbing bamboo, *A. Lynn Zwickey 638*, 3.xi.1938. (holotype: FH!; isotype: FH! MICH n.v.).

Distichophyllum hainanense (P.J.Lin & B.C.Tan) B.C.Ho & L.Pokorny, ***stat. nov***. – Basionym: *Distichophyllum subnigricaule* var. *hainanense* P.J.Lin & B.C.Tan *Harvard Pap. Bot.* 7: 43. f. 33: E–I. 1995. – **Type:** China. 'Hainan, Mt. Diao-luo, on root of tree, c. 1050 m.' *P.-J. Lin et al. 945A*, iii.1990 (holotype: IBSC n.v.; isotype: FH!).

Leskeodon maibarae (Besch.) B.C.Ho & L.Pokorny, ***comb. nov***. – Basionym: *Distichophyllum maibarae* Besch., *J. Bot. (Morot)* 13: 40. 1899 – **Type**: Japon, Nippon central [Honshu], Maibara, associé au *Symphyogyna sublobata*, *Faurie 11130*, 7.xi.1893 (holotype: BM!; isotypes: FH, H-Br!).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Strict consensus cladogram obtained from parsimony ratchet analysis of the concatenated dataset without indels coded, with bootstrap support values noted beside the branches. Values above the branches are analyses that exclude indels ($BS_{w/o}$); those below the branches are with indels coded for the entire sequence data (BS_{sic}) or coded for only the organellar sequence data ($BS_{sic\text{-}org}$).

Figure S2. Majority consensus cladogram for the Bayesian analysis of the concatenated dataset in which indels were coded ($BI_{w/o}$), with posterior probabilities (PPs) obtained from various modified dataset and model implementation. Support values above the branches are for analyses with indels excluded and use a homogeneous DNA substitution model for the matrix (PP_{hom}), followed by values for an analysis using partitioned models ($PP_{w/o}$). Values below the branches include indels and consider the entire sequence data (PP_{sic}) or only the organellar sequence data ($PP_{sic\text{-}org}$); in both cases, the matrix was partitioned into different regions.

Figure S3. Phylogram of one of the 12 trees from a maximum likelihood analysis (this is the same tree as in Fig. 2, but shows branch lengths).

Data file S1. Dalt_final_alignm.nex. Final alignment of the concatenated five-gene data matrix in the order *rps*4, *trn*LF, *nad*5, ITS and 26S. See Table 1 for the nucleotide positions of the genes, hotspots (ambiguous alignments) and inversions.

Data file S2. Dalt_comb_hx_ir_ML_trees.nex. Alignment of the five-gene data matrix (without indel coding) used in various phylogenetic analyses. The ambiguous aligned segments (hotspots) have been trimmed. Detected inversions have been reversed and complimented. Twelve resulting maximum likelihood trees were embedded.

Table S1. Species sampled, voucher information and GenBank accessions for *rps4*, *trnLF*, *nad5*, ITS and 26S of 126 samples (593 of 630 available). An asterisk (*) indicates the type species of a genus. Country codes follow those of ISO 3166-1 alpha-2; additional regional abbreviations: ID-C, Celebes; ID-J, Java; ID-M, Moluccas; ID-S, Sumatra; MY-E, East Malaysia (Sarawak and Sabah); MY-W, West Malaysia (Peninsula). Sequences generated for this study are written in boldface, those not available are denoted by 'n.a.'. Herbarium acronyms follow those of the *Index Herbariorum*.

Table S2. Comparsion of likelihood scores and effective sample sizes (ESS) for Bayesian inference under the GTR + Γ + I substitution model and the restriction site model on various modified datasets.

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