Phylogeny of the Polytrichales (Bryophyta) based on simultaneous analysis of molecular and morphological data

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Abstract

Phylogenetic analyses of Polytrichales were conducted using morphology and sequence data from the chloroplast genes *rbcL* and *rps4* plus the *trnL-F* gene region, part of the mitochondrial *nad5* and the nuclear-encoded 18S rDNA. Our analyses included 46 species representing all genera of Polytrichales. Phylogenetic trees were constructed with simultaneous parsimony analyses of all sequences plus morphology and separate combinations of sequence data only. Results lend support for recognition of Polytrichales as a monophyletic entity. *Oedipodium griffithianum* appears as a sister taxon to Polytrichales or as a sister taxon of all mosses excluding Sphagnales and Andreaeles. Within Polytrichales, *Alophosia* and *Atrichopsis*, species without the adaxial lamellae (in *Atrichopsis* present but poorly developed on male gametophyte) otherwise typical of the group are sister to the remaining species followed by a clade including *Bartramiopsis* and *Lyellia*, species with adaxial lamellae covering only the central portion of the leaves. Six taxa with an exclusively Southern Hemisphere distribution form a grade between the basal lineages and a clade including genera that are mostly confined to the Northern Hemisphere.
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1. Introduction

The green plants are one of the major clades of eukaryotes (e.g. Lipscomb et al., 1998). Their morphological and chemical diversity and ecological dominance in almost all habitats make them the most important group of organisms in terrestrial ecosystems. Reconstruction of their phylogenetic relationships is important for understanding some of the most significant evolutionary events, such as the original conquest of dry land habitats. Bryophytes are small plants with a haplo-diplontic life cycle, and they probably were among the first plants to gain a hold in terrestrial environments. Three groups can be clearly distinguished: Marchantiophyta, Anthocerophyta, and Bryophyta (Newton et al., 2000). Traditionally, bryophytes have been recognized as a monophyletic entity but relationships among the three major lineages are still in dispute (Hedderson et al., 1998; Lewis et al., 1997; Nickrent et al., 2000; Renzaglia et al., 2000). While the reason for this may be inadequate sampling, it might well be that we will never resolve this part of organismal history. It would not be surprising that dispersal to practically empty "dry" land habitats caused such an explosive evolutionary diversification that branch lengths of the resulting "tree" are simply too short to recover after several hundred million years and presumably rampant extinction. However, there is no doubt that each of the three major lineages of bryophytes is monophyletic. Bryophyta, the mosses, is the largest of all bryophyte groups. The number of species is estimated at 7000–8000 (Crosby, 1999), and mosses can be found in virtually all terrestrial and in many fresh water habitats.

They are also extremely important, even dominant, ecologically in habitats such as mires and forests of the boreal zone as well as humid cloud forests of the tropical and subtropical mountains.

Within Bryophyta four major lineages can be distinguished: Sphagnopsida, Andreaeaopsida, Polytrichopsida, and Bryopsida (Buck and Goffinet, 2000). In addition, there are genera like *Andreaeobryum* Murray, *Takakia* S. Hatt. and Inoue, *Buxbaumia* Hedw., *Oedipodium* Schwägr., *Tetraphis* Hedw. and *Tetrodontium* Schwägr. whose affinity to the four major groups is still unclear. Buck and Goffinet (2000) include all but the first two genera within Polytrichopsida in order Tetraphidales but it is still equivocal whether they belong here together with Polytrichales or in Bryopsida. Bryopsida is by far the largest group of mosses with well over 90% of all species. Polytrichopsida, whether it is interpreted to include only Polytrichales or also Tetraphidales, is a much smaller group but still the second largest of the major groups both in species number and ecological variability.

Polytrichales are typically pioneer plants of open, sometimes even dry, habitats. Despite the small number of species, the order exhibits great diversity from miniature plants with reduced leaves such as *Pogonatum pensilvanicum* (Hedw.) P. Beauv. of eastern North America to giants of Australasia and New Zealand like *Dawsonia superba* Grev. with the best-developed gametophyte of all land plants. The most typical features of the polytrichalean gametophyte are the closely spaced adaxial lamellae on the leaves, forming a pseudoparenchyma, and differentiation of leaves into a distinct blade and sheathing base. The calyptra is typically hairy in many common species of the Northern Hemisphere, enveloping the developing capsules of the sporophyte generation. This has given the whole group its name, although most genera have a practically naked calyptra. Capsules of the Polytrichales normally have a well-developed peristome with at least 16 teeth formed of whole cells. The epiphragm covering the mouth of the capsule is a unique character that distinguishes Polytrichales from all other groups of mosses. Size and shape of the urn vary greatly among genera (Schofield, 1985; Smith, 1971).

Nineteen genera are currently accepted in Polytrichales, comprising approximately 200 species (Hyvönen et al., 1998). *Eopolytrichum antiquum* Konopka et al. (1997), the sole species of *Eopolytrichum*, is known only from late Cretaceous fossils. Many of the remaining genera are monotypic, and all the others, with the exception of *Pogonatum* (ca. 50 spp.) and *Polytrichum*, (ca. 30 spp.) are fairly small.

Some species, like *Polytrichum juniperinum* Hedw., have almost cosmopolitan distributions, while there are also narrow endemics, some possibly even threatened by extinction. Ecologically, Polytrichales range from xerophytes like *P. piliferum* Hedw. to species of

peaty, wet, and to some extent flooded habitats like *P. commune* Hedw. Although their structure appears obviously adapted to dry environments, Polytrichales are largely absent from extremely arid regions, and the group exhibits greatest diversity in areas with humid or moist subtropical and tropical climates.

Phylogenetic relationships of Polytrichales are particularly relevant to considerations of the evolutionary history of mosses since the group is probably among the first of the lineages that diverged from the common ancestor of all mosses (Mishler and Churchill, 1984). Earlier cladistic analyses of the group have been done by Hyvönen (1989), Forrest (1995), and Hyvönen et al. (1998). The first two were based solely on morphology, and the latter on sequences of three genes (the chloroplast-encoded *rbcL* and *rps4* loci and the nuclear-encoded 18S rRNA gene) plus morphology from 22 species. However, sequences were not obtained from all three genes for all species, and large genera like *Pogonatum* were represented by only a few species. In addition, only morphological characters were available for some of the species. The aim of the current study was to enlarge our matrix significantly with respect to both taxa and characters in order to develop a more robust hypothesis of Polytrichalean phylogeny.

2. Material and methods

2.1. Plant material and data sets

Our morphological matrix includes 43 characters, and the treatment mostly follows Hyvönen et al. (1998). This data set is based on extensive study of specimens from several herbaria and in most cases also included the DNA voucher specimens. The data matrix and list of characters can be found in Appendices A and B.

Our analyses include 46 species, representing all known genera of Polytrichales. With few exceptions, we were able to obtain sequence data for all five loci. Of course, the fossil *Eopolytrichum* is represented only by morphology. *Andreaea rupestris* Hedw., *Buxbaumia aphylla* Hedw., *B. piperi* Best, *Diphyscium foliosum* (Hedw.) Mohr, *Funaria hygrometrica* Hedw., *Oedipodium griffithianum* (Dicks.) Schwaegr., *Sphagnum palustre* L., *Tetraphis pellucida* Hedw., *T. geniculata* Girg. ex Milde, and *Timmia sibirica* Lindb. et Arn. were used as outgroup taxa based on previous higher-level analyses (Cox and Hedderson, 1999; Hedderson et al., 1996, 1998). Our data included sequences for the nuclear-encoded 18S rRNA gene, the chloroplast-encoded *rbcL*, *rps4*, and *trnL-F* regions, and a stretch of ca. 700 bp from the 3′ terminus of the mitochondrial *nad5* gene. We tried to get all sequences from the same voucher specimen but this was not always possible, and matrices were supplemented with available sequences from GenBank. We used composite taxa, i.e.,

combining data of two species, only in two cases: *nad*5 sequences were not available for *Sphagnum palustre* and *Timmia sibirica*, and we used sequences of *S. fallax* H. Klinggr. and *T. bavarica* Hessl., respectively, to supplement our matrix. See Table 1 for details of the vouchers and available sequences.

2.2. DNA extraction, amplification, and sequencing

Total DNA was extracted from fresh, herbarium (oldest specimen 25 years), or silica-dried specimens. Extractions were made using the two different methods given in Hyvönen et al. (1998) and the Dneasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Template DNA suitable for cycle sequencing was prepared via PCR. Amplification was done using either the DynaZyme DNA Polymerase Kit (Finnzymes Oy) and Personal Minicycler (MJ Research) or the *Taq* DNA Polymerase Kit (Promega) and DNA Thermal Cycler 480 (Perkin–Elmer) or AmpliTaq Gold DNA polymerase (Perkin–Elmer) and Gene Amp PCR system 9700 (Perkin–Elmer). For *nad5* and 18S rDNA we used a program comprising a 95 °C initial denaturation step (12 min) followed by 35 cycles of 95 °C denaturation (30 s), 52 °C annealing (1 min), and 72 °C extension (3 min) with a final annealing step at 72 °C for 7 min. The reaction volume was 50 µl. The program used for *rbcL* and *rps4* included a 97 °C initial denaturation step (12 min) followed by 35 cycles of 97 °C denaturation (30 s), 55 °C annealing (1 min), and 72 °C extension (1 min 30 s) with a final annealing step of 72 °C for 7 min. All reactions were done in 50 µl volumes. See also Hyvönen et al. (1998) for two alternative PCR programs. A negative control, including all reaction components except the target DNA, was also used. The PCR products were inspected on agarose gels and product sizes were determined from a DNA size-standard ladder of 50–20,000 bp (Bio-Rad Laboratories).

PCR products were purified with the PCR Purification Kit (QIAquick) according to the manufacturer's instructions. Cycle sequencing reactions were prepared using the DNA Sequence Kit (ABI, Perkin–Elmer), with either Dye Terminator or dRhodamine Terminator Cycle Sequencing reactions or the Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequences were visualized using ABI 373 or 377 automated sequencers. The PCR primers used for 18S rDNA were NS1 and B, and the internal sequencing primers were ERC, G, H, KRC, and Q (Hedderson et al., 1998). Primers rps5 and trnas were used for both PCR amplification and sequencing of *rps4* (Cox and Hedderson, 1999). For *rbcL*, primers M28 (NM34 of Newton et al., 2000) and M1390r were used for PCR and, along with internal primers M740r and M1010r, for sequencing. For some species we were unable to amplify *rbc*L in one piece and therefore had to use an additional primer (M636) for PCRs. For the trnL-F gene region we used primers C and F both for

the PCR and sequencing reactions (see Cox et al., 2000 for details of these oligonucleotide primers). For *nad*5, K- and L-primers were used for PCR and only L-primer for sequencing (Steinhauser et al., 1999).

The individual sequencing products from different primer reactions were aligned as a composite strand using either programs of the Lasergene package (DNASTAR) or manually with a text-editor using a color-coded font BKGCuclc (M. Sogin, Marine Biological Laboratory, Woods Hole, MA). Portions of the completed sequence for each gene are based on reads in only one direction, and the extent of single-read sequence varies among taxa. Discrepancies between reads were solved manually by inspection of the original electropherograms. In doubtful cases IUPAC ambiguity codes were assigned.

2.3. Data analysis

Sequence alignment was performed initially with the program CLUSTAL X (Jeanmougin et al., 1998), and sequences were adjusted manually using a color-coded font BKGCuclc. The chloroplast genes *rps4* and *rbcL* and the mitochondrial *nad5* did not pose alignment problems. Similarly, the nuclear 18S rRNA-coding gene was not particularly length-variable over the range of taxa in this study and thus was easily aligned; only positions 194–199 and 1396–1399 were ambiguous, and these 10 nucleotides were removed from the final matrix. However, whether these 10 nt were included or not did not alter the resulting topologies. Some of the sequences generated were of too poor quality to be used so we do not have complete sequences for every taxon included in the analyses. All sequences are deposited in GenBank as indicated in Table 1, and the complete matrix has been submitted to TreeBase (http://www.treebase.org). Our final matrix included 4794 characters, and of these, 988 (21%) were parsimony-informative. Parsimony-informative characters were distributed among data sets as indicated in Table 2.

Difficulties were experienced with aligning the non-coding region at the 3′ end of the *rps4* sequences, so these positions were excluded from the analyses. Besides this, the *trnL-F* gene region shows considerable length variation in non-coding regions, and therefore alignment was problematic. There are basically two ways to treat ambiguous alignment of sequences. It has been proposed that all such sequences should be excluded from the analyses, which equals ignoring part of the data. The logic behind this is, however, that there genuinely are sequences for which we cannot find optimal alignment. If we adopt this approach for the current material, we have to ignore most of the *trnL-F* sequences as well as the intergenic spacer at the 3′ end of the *rps4* gene, which is typically long for Polytrichales and some other mosses but practically lacking, for example, in Bryales (Goffinet et al., 2001). We made preliminary alignments for the *trnL-F* matrix with Clustal X (Jeanmougin et al., 1998).

Table 1
Voucher numbers for taxa sampled in analysis, followed by GenBank Accession numbers for DNA sequences

Taxon	Collection reference		Accession No.				
			18S	rbcL	rps4	trnL	nad5
Alophosia azorica	Azores	*Rumsey 18.3.1997* (RNG)	AY126951	AF208408	AY137679	AF544997	AY137713
Atrichopsis compressa	Chile. Reg. de Magallanes	*Smith B1407b* (AAS)	AF548459 [a]	AY118233	—	AF544998	—
Atrichum androgynum	Brazil. São Paulo	*Hyvönen 6387*(H)	AY126952	AY118234	—	AF544999	AY137714
A. angustatum	USA Lousiana	*Hedderson 10393* (RNG)	U18492	AF231061	AF208417	AF545000	AY137715
A. oerstaedianum	Mexico. Veracruz	*Hyvönen 6504* (H)	AY126953	AY118235	AY137680	AF545001	AY137716
A. undulatum [b]	Finland. Uusimaa	*Hyvönen 6170* (H)	X85093	AY118236	AY137681	AF545002	AY137717
Bartramiopsis lescurii	Canada. British Columbia	*Hedderson 10044* (RNG)	AY126954	AF208409	AF208418	AF545003	AY137718
Dawsonia papuana	Papua New Guinea	*Baker 662* (RNG)	AF228669	AF208410	AF208419	AF246704	AY150372
D. polytrichoides	Australia. Queensland	*Schulman 125* (H)	AY126956	AY118238	AY137683	AF545005	AY137720
D. superba	New Zealand. N Island	*Stenroos 4677* (H)	AY126955	AY118237	AY137682	AF545004	AY137719
Dendroligotrichum dendroides	New Zealand. N Island	*Hyvönen 6083* (H)	AF208402	AAF208411	AF208420	AF545006	AY137721
D. squamosum	Argentina. Tierra del Fuego	*Hyvönen 2557* (H)	AY126957	AY118239	AY137684	AF545007	AY137722
Hebantia rigida	Chile. Region de los Lagos	*Kelt 26.5.1986* (H)	AY126958	AY118240	AY137685	AF545008	AY137723
Itatiella ulei	Brazil, Sao Paulo	*Ahti 51824*(H)	AY126959	AF208412	AF208421	AF545009	AY137724
Lyellia aspera	Canada. Ellesmere Island	*Hedderson 6825* (RNG)	AF208403	AF208413	AF208422	AF545010	AY137725
Meiotrichum lyallii	USA Colorado	*Weber WWB36612* (H)	AY126960	AY118241	AF208423	AF545011	AY137726
Notoligotrichum australe	New Zealand, S Island	*Hyvönen 6069* (H)	AF208404	AF208414	AY137686	AF545012	AY137727
Oligotrichum austro-aligerum	Argentina. Rio Negro	*Hyvönen 5625* (H)	AY126961	AY118242	AY137687	AF545013	AY137728
O. hercynicum	Finland. Uusimaa	*Enroth 25.7.1998* (H)	AY126962	AY118243	AY137688	AF545014	AY137729
O. parallelum	Canada. British Columbia	*Hedderson 10043* (RNG)	AY126963	AF208415	AF208424	AF545015	AY137730
Pogonatum aloides	Sweden. Skåne	*Hyvönen 6486* (H)	AY126964	AY118244	AY137689	AF545016	AY137731
P. campylocarpum	Brazil. São Paulo	*Hyvönen 6392* (H)	AY126965	AY118245	AY137690	AF545017	AY137732
P. cirratum	Taiwan. Taichung	*Hyvönen 4008* (H)	AY126966	AY118246	AY137691	AF545018	AY137733
P. contortum	Canada. British Columbia	*Hedderson 5803* (H)	AY126967	AY118247	AF208425	AF545019	AY137734
P. dentatum	Finland. Uusimaa	*Hyvönen 6169* (H)	AY126968	AY118248	AY137692	AF545020	AY137735
P. japonicum	Japan. Honshu	*Nishimura 10601*(H)	AY126969	AY118249	AY137693	AF545021	AY137736
P. microstomum	Taiwan. Taichung	*Hyvönen 4087*(H)	AY126970	AY118250	AY137694	AF545022	AY137737
P. neesii	Taiwan. Taichung	*Hyvönen 4021* (H)	AY126971	AY118251	AY137695	AF545023	AY137738
P. nipponicum	Japan. Honshu	*Hayashi 7038* (H)	AY126972	AY118252	AY137696	AF545024	AY137739
P. pensilvanicum	Brazil. São Paulo	*Hyvönen 6393*(H)	AY126973	AY118253	AY137697	AF545025	AY137740
P. spinulosum	Japan. Honshu	*Chishiki 1862* (H)	AY126974	AY118254	AY137698	AF545026	AY137741
P. subulatum	Australia. Queensland	*Hyvönen 6025*(H)	AY126975	AY118255	AY137699	AF545027	AY137742
P. urnigerum	Finland. Uusimaa	*Hyvönen 6173* (H)	AF208406	AY118256	AF208426	AF545028	AY137743
Polytrichadelphus magellanicus	Chile. Region de Los Lagos	*Hyvönen 5865* (H)	AF208407	AY118257	AF208427	AF545029	AY137744
P. pseudopolytrichum	Brazil. Minas Gerais	*Hyvönen 6276* (H)	AY126976	AF261074	AY137700	AF545030	AY137745
Polytrichastrum alpinum	Finland. Etelä-Häme	*Hyvönen 6204* (H)	AY126977	AY118258	AY137701	AF545031	AY137746
P. formosum [b]	Finland. Uusimaa	*Hyvönen 6197*(H)	X80982	AY118259	AY137702	AF545032	AY137747
P. longisetum	Finland, Varsinais-Suomi	*Hyvönen 6506* (H)	AY126978	AY118260	AY137703	AF545033	AY137748
Polytrichum brachymitrium	Brazil. Minas Gerais	*Hyvönen 6230*(H)	AY126979	AY118261	AY137704	AF545034	AY137749
P. commune [b,c]	Finland. Uusimaa	*Hyvönen 6168* (H)	U18518	U87087	AF208428	AF545035	—
P. juniperinum	Finland. Uusimaa	*Hyvönen 6193* (H)	AY126980	AY118262	AY137705	AF545036	AY137751
P. piliferum	Finland. Uusimaa	*Hyvönen 6205* (H)	AY126981	AY118263	AY137706	AF545037	AY137752
P. subpilosum	Malawi. Mulanji	*Wigginton M1397a* (H)	AY126982	AY118264	AY137707	AF545038	AY137753
Psilopilum laevigatum	Canada. Ellesmere Island	*Hedderson 5938* (RNG)	AY126983	AF208416	AF208429	AF545039	AY137754
Steereobryon subulirostrum	Mexico. Veracruz	*Hedderson 12898* (H)	AY126984	AY118265	AY137708	AF545040	AY137755

Taxon	Origin	Collection	18S	rbcL	rps4	trnL-F	nad5
Outgroup taxa							
Andreaea rupestris[c,d]	Canada. New Foundland	Hedderson 10439 (RNG)	*U18490*	*L13473*	*AJ617675*	—	*AJ001227*
A. rothii	USA North Carolina	Anderson 24716(DUKE)	Y17603	AY118230	AY137677	*AF231905*	AY137709
Buxbaumia aphylla[b]	Canada. Nova Scotia	Belland 16889 (DUKE)	AY126949	AY118231	AY137678	*AF231909*	AY137710
B. piperi	Canada. British Columbia	Schofield 81571 (DUKE)	AF230415	AF232692	AF223034	AF544994	AY137711
Diphyscium foliosum	USA North Carolina	Goffinet 4492 (DUKE)	X80212	AF005513	AF023776	AF544995	*Z98959*
Funaria hygrometrica[b,c,d]	Great Britain. England	Cox 148(RNG)	AF228668	AF246289	AF306968	AF023716	AF548458
Oedipodium griffithianum[b]	USA Alaska	Schofield 98670 (DUKE)	—	—	—	AF246290	AJ001225
Sphagnum fallax							
S. palustre[b,d]	USA Hawaii	Hotchkill 98-70	Y11370	AF231887	*AF231892*	—	—
Tetraphis geniculata	Canada. British Columbia	Schofield 103022 (DUKE)	AY126950	AY118232	AF306955	*AF231902*	AY137712
T. pellucida[b]	USA North Carolina	Pittillo 9764(DUKE)	U18527	U87091	*AF231896*	AF544996	AJ224855
Timmia sibirica[d]	Canada. Ellesmere Island	Hedderson 6341(RNG)	AF023678	AF275166	AF023775	*AF231908*	—
T. bavarica			—	—	—	*AF023715*	*Z98963*

Accession numbers in *italics* indicate sequences downloaded from GenBank. See text for further details.

[a] 18S sequences submitted in two pieces, the other No. is AF548460.
[b] 18S sequences not from the same specimen.
[c] rbcL sequences not from the same specimen.
[d] nad5 sequences not from the same specimen.

Table 2
Size of the each matrix and percentage of parsimony informative characters plus values for consistency (CI) and retention indeces (RI) as measured for the two equally parsimonious trees found in the analysis of the whole combined matrix

Matrix	Characters	Informative	(%)	CI	RI
18S	1834	173	9	0.54	0.62
rbcL	1367	404	30	0.40	0.44
rps4	600	208	35	0.53	0.57
trnL-F	261	66	25	0.50	0.67
nad5	689	99	14	0.62	0.66
Morphology	43	39	91	0.34	0.64

This resulted in a matrix of 948 characters. However, none of the individual sequences had this length, and alignment gaps of various length were inserted in all. In order to retain only unambiguously aligned stretches of sequences, we removed nucleotides in positions 1–73, 148–151, 175–202, 240–499, and 627–948 from the matrix. This resulted in a reduced matrix of only 260 nucleotides that were unambiguously aligned, corresponding to parts of the *trnL* intron and the *trnL* 3′ exon in the *trnL-F* region. In addition to this, we were able to align sequences of *Buxbaumia piperi* and *Tetraphis geniculata* only by their 3′ end and therefore had to remove the first 499 bp of these sequences. Unfortunately, we were not able to use the same approach for the 3′ terminus AT-rich indel of *rps4* because even partially unambiguous alignment was not obtained for these sequences.

Parsimony analyses were performed using the program NONA (Goloboff, 1994) in conjunction with a Winclada shell (Nixon, 1999) with the following settings: hold * (holding all trees that memory allows, in current settings with Winclada this is 10,000), mult*100 (search replicated 100 times), hold/2 (keeping 2 starting trees for each replication), and using multiple tree-bisection reconnection algorithm (mult*max*). We also performed more extensive analysis of the whole material (hold*, mult*1000, hold/10) but obtained exactly the same result as with the smaller search. In all sequences, gaps were treated as missing data. In the analyses all characters were weighted equally, with no distinction between transitions and transversions, and morphological characters of the sporophyte and gametophyte generation were treated equally as well. All morphological characters were treated as unordered. Traditionally, more "weight" has been given to sporophytic characters in studies of moss phylogeny. However, we preferred to avoid additional assumptions which a priori character weighting necessitates (Kluge, 1997). The following analyses were performed:

1. Simultaneous analyses (Nixon and Carpenter, 1996) including all five sequence matrices plus morphology based on
   (A) inclusion of all taxa,
   (B) analysis leaving out the fossil taxon *Eopolytrichum antiquum* with a high proportion of missing entries.

2. Analyses based only on sequence data
   (A) from all five available matrices,
   (B) leaving out nuclear 18S rDNA sequence.

Different methods and indices have been proposed to study "reliability" or "strength" of different phylogenetic hypotheses included in each cladogram. The aim of calculating support values is to estimate how well our hypotheses will hold up when more, possibly conflicting, data is obtained. We calculated jackknife values (Farris et al., 1996) for our material using the parsimony jackknifer xac (Farris, 1997a) which includes branch-swapping. The search was performed with 10,000 replicates.

Some authors (e.g., Kluge, 1997) have challenged the use of these metrics altogether, arguing convincingly that comparisons of the equally parsimonious tree(s) with suboptimal topologies or utilizing only part of the available evidence within the cladistic framework is not warranted. Real tests of the current hypotheses will be provided only by further data, i.e., the next added character or taxon.

The congruence between different data sets was tested by performing the incongruence length test (ILD) of Mickevich and Farris (1981) with the program xarn (Farris, 1997a,b). This was performed with 1000 repetitions and three rounds of branch-swapping.

3. Results

Our simultaneous parsimony analysis of the total matrix with all of the 56 taxa included yielded two equally parsimonious trees of 4419 steps, with a consistency index (CI, Kluge and Farris, 1969) of 0.47 and retention index (RI, Farris, 1989) of 0.54. One of these trees is shown in Fig. 1. The only difference between the two trees is in the position of *Pogonatum contortum*, which is either sister to *P. cirratum* or sister to the rest of the same clade. Our material includes a large proportion of missing entries for the fossil *Eopolytrichum antiquum* but this did not seem to have an effect on results. This fossil species was unambiguously nested within the *Polytrichum* clade in all trees, and excluding it from the analysis did not change the general topology.

Excluding morphological characters resulted only in a single, but very surprising, change: *Atrichum angustatum* was nested within *Polytrichum* as the sister of *P. commune*! Both *Polytrichum* and *Atrichum* are very well-defined, distinct genera that can be distinguished unambiguously by their morphology. What is the reason for this spurious grouping? Long-branch attraction has in many cases been given as an explanation when novel and unexpected groupings are encountered (e.g., Buck et al., 2000). However, as pointed out by Siddall and Whiting (1999) the presence of long-branch attraction as an artifact is possible only when two branches are attracted to each other. It should be evident if the topol-

ogy is altered by removing one of the taxa from the analysis. When *P. commune* was removed, *A. angustatum* was back with other species of *Atrichum*. Branches leading to these two problematic species are the longest within *Atrichum* and *Polytrichum*, respectively. However, there are also longer branches on the overall tree. But when we examine changes in 18S sequences, it is evident that branches leading to *A. angustatum* and *P. commune* are exceptionally long (53 and 48 changes, respectively). There are two other branches that come close, one leading to *Atrichopsis compressa* (46 changes) and the one leading to the clade of *Pogonatum neesii* plus *P. subulatum* (47). Reduction of the data set still further by including only plastid sequences in the analysis gave eight equally parsimonious trees (not shown) without an *A. angustatum–P. commune* grouping. It seems that this unexpected grouping was due to the exceptional 18S sequences downloaded from GenBank. Both were deposited by one of us (TAH) as part of an early study of land plant relationships. They were generated from RNA templates using reverse transcriptase, an enzyme with a high known error rate. Resequencing of 18S rDNA for these two taxa seems to be warranted.

The ILD test performed with xarn (Farris, 1997a) revealed all data sets to be highly incongruent with each other. The low α-values (0.001) indicating highly incongruent data sets were obtained for all comparisons between different data sets irrespective of whether they represent the same or different organellar genome or morphology. The only exceptions were comparisons between partial sequences of the mitochondrial *nad5* and the chloroplast *trnL-F* region (0.057) and between the nuclear-encoded 18S and *nad5* (0.002).

4. Discussion

4.1. Sequence analyses

Our data included nuclear, chloroplast, and mitochondrial DNA sequences from coding as well as noncoding regions. The utility of these regions in resolving lineages of different age probably varies a great deal. In the present context this is a clear advantage because in Polytrichales we likely have a group of great antiquity but possibly also with lineages that have undergone speciation quite recently. Nuclear-encoded ribosomal 18S rDNA sequences have been used extensively to address "deep" (Mishler, 2000) reconstruction problems in many groups of organisms from family to kingdom level. It shows the lowest percentage of informative characters (Table 2) in our material, but remains more or less at the same level (8%) even if we compare Polytrichales only, and provides information even within the most apical clade consisting essentially of *Psilopilum*, *Polytrichum*, and *Pogonatum* (5%). Leaving financial
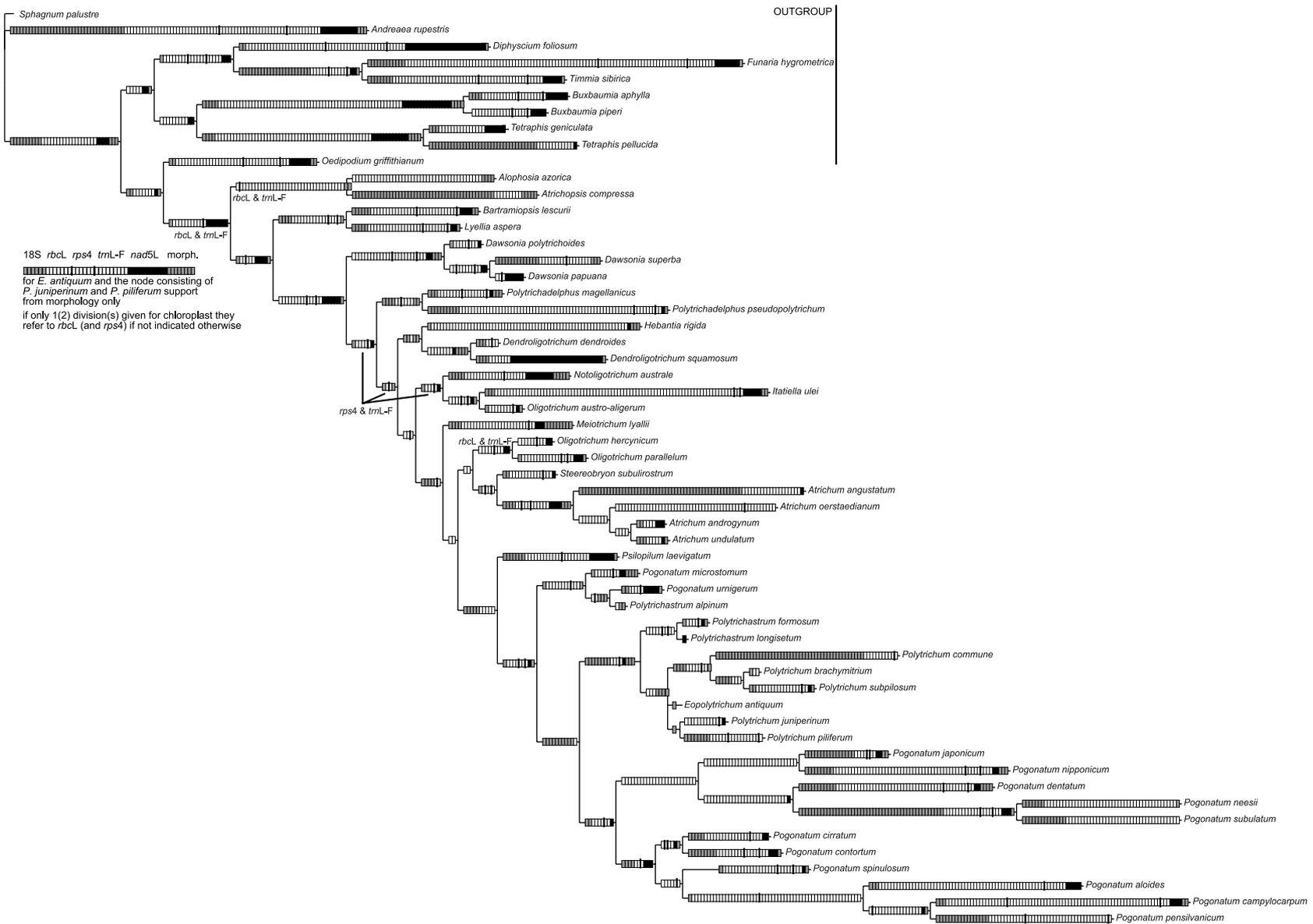
Fig. 1. The other one of the two equally parsimonious trees based on simultaneous analysis of the total matrix composed of six datasets. The length of the trees are 4419 steps, with a consistency index (CI) of 0.47 and retention index (RI) of 0.54. Supporting characters given for each node are shown as divided between different sources of data.

matters aside (18S rDNA as compared to other gene regions in this analysis is expensive to sequence because of its length), it seems that sequencing of even fairly "conservative" gene regions is valuable and can provide information also at "lower" taxonomic levels.

It has been argued that substitutions at certain sites (such as third codon positions in protein coding genes) become randomized (saturated) and will be phylogenetically uninformative (Swofford et al., 1996) but recent findings (e.g. Yang, 1998) show that these concerns are exaggerated. As pointed out by Chase and Albert (1998) and Källersjö et al. (1999), based on examples from large *rbcL* data sets, there is no direct connection between homoplasy and "value" of characters in phylogeny reconstruction; leaving out or down-weighting characters according to their homoplasy would be a mistake. Also in our own *rbcL* matrix the percentage of informative characters was highest in third positions (66%) but they also showed much homoplasy, with lower CI (0.38) than first (0.40) and second (0.56) positions of the gene. Even assuming (based on a model of molecular evolution) that there are undetected multiple substitutions, one is still dealing with many, possibly conflicting characters. It is extremely unlikely that these "saturated" sites would covary to such an extent that they would obscure the phylogenetic signal in the material. Certainly this is far less of a problem when an analysis, such as ours, is based on multiple genes and other sources of data.

When we use simultaneous analysis of all available data, it might still be valuable to explore the data with separate analyses. For example, when we left out nuclear-encoded 18S rDNA sequences the resulting topology included some curious groupings. A lineage including *Pogonatum microstomum*, *P. urnigerum*, and *Polytrichastrum alpinum* is now placed within *Pogonatum*, albeit as a basal branch. In plants plastid genomes are predominantly inherited from the maternal lineage and therefore one can suspect that this conflict in results is possibly due to an ancient hybridization between two genera in Polytrichales. To answer this question other types of data and analyses are needed and even then getting unambiguous answers might be difficult because of missing data due to extinction (Derda and Wyatt, 1999). Other differences from the results obtained with simultaneous analysis include separation of *P. aloides*, *P. campylocarpum*, and *P. pensilvanicum* from other species of the genus forming a clade together with *Alophosia azorica* and *Atrichopsis compressa*. The taxa involved in this unexpected and novel grouping all show exceptionally long-branch lengths for the chloroplast data. Are these novel groupings examples of long-branch attraction? If yes, combining data from different sources might have another benefit for the analyses by leveling out length differences between branches and removing problems associated with exceptionally long branches. When we inspect characters supporting each branch (Fig. 1) one can immediately see that in our material contribution to the total length of branches from different gene regions is not uniform but varies a great deal throughout the tree.

As soon as multiple sequence data sets became available for phylogenetic analyses concerns were raised over their congruence, i.e., if they could (or should) be combined or analyzed separately (e.g. Miyamoto and Fitch, 1995 versus Kluge, 1989; Eernisse and Kluge, 1993). The simplest way to test this is to compare resulting topologies (taxonomic congruence). This does not, however, take into account the strength of support for individual hypotheses included in the topologies that are being compared. Farris et al. (1994) devised an elegant way to explore this with the incongruence length (ILD) test of Mickevich and Farris (1981). While we acknowledge the power of the test, we agree with Siddall (1997) who argued that incongruence per se does not warrant ignoring part of the available data. He also showed with a simple example that incongruence can be caused by a very small number (actually only one!) of characters that are in conflict with other sources of data. Leaving out part of the data would be warranted only if we knew a priori which part of our data is unreliable. This is, however, something we cannot know. Which part of the data should we trust? For example, in our material clear incongruence was observed among practically all partitions. Should we perform independent analyses and pool the results to find out their taxonomic congruence? In our opinion this is not a viable alternative. Homoplasy is encountered in all data sets, and we agree with Wheeler et al. (1993) that congruence between characters from different data sets provides us with the best test to sort out homology from homoplasy and level out noise in our data sets.

When we examine jackknife support values and length of branches it is noteworthy that internal nodes within Polytrichales have either very low values (or they are lacking altogether) and these branches are extremely short as compared with more terminal branches (Figs. 1 and 2). There might be real biological reasons for these short branches. For example, in the present case short branches are characteristic for the part of tree where genera that represent Gondwanan and Northern Hemisphere elements, respectively, branch off. It is possible that short branches indicate dispersal to new "empty" areas and habitats and subsequent rapid diversification.

4.2. Classification, morphology, and biogeography

Our analysis provides support for the monophyly of Polytrichales with *Oedipodium griffithianum*, formerly included in the Bryales, as a sister taxon. The position of *Oedipodium* as a member of Polytrichopsida is, however, ambiguous. In the analysis based only on plastid sequences *O. griffithianum* is in a still more basal position within mosses, being placed between *Andreaea* and a clade leading to Bryopsida (including Tetraphidales

sensu Buck and Goffinet, 2000) and Polytrichales. Similar results were also obtained by Newton et al. (2000) in their analysis of phylogenetic relationships among major moss lineages, and our jackknife tree (Fig. 2) shows the same position. However, in analyses performed by Newton et al. (2000) Polytrichales were represented by only two species whereas in our own analysis we included only few species of Bryales. In order to test whether our results were due to unequal sampling of Polytrichales versus Bryales we expanded our matrix with 10 more

outgroup taxa. Sequences were downloaded from Gen-Bank and many of these additional outgroup taxa were composites as indicated in Table 3 and morphology was not scored for these additional taxa. In this analysis *Oedipodium* still remained as sister-taxon of Polytrichales. However, we should aim for still wider sampling, including morphology, to resolve phylogeny of the most basal lineages of mosses. With the currently available programs with extremely powerful algorithms and access to parallel computing we should aim to expand data sets, not reduce them. Due to extinct lineages, our sampling will always be only representative rather than complete and there is no reason to reduce it still further. In order to obtain reliable hypotheses of the deeper nodes within mosses, such genera as *Diphyscium*, *Buxbaumia*, and *Tetraphis* with their allies are in a pivotal position. More detailed sampling of them is needed and such studies have already been undertaken by Magombo (2003).

Within Polytrichales our data give clear and strong support for some traditionally distinguished genera (e.g., *Atrichum*, *Dawsonia*) while the largest genera, *Pogonatum* and *Polytrichum*, appear as paraphyletic or are distinguished as clades that also include other species, respectively. As mentioned above, most of the internal branches are weakly supported. However, the tree presented is still our best hypothesis given the data we currently have.

Our results are to a large extent compatible with those obtained in a preliminary analysis based on restricted sampling of both taxa and characters (Hyvönen et al., 1998). The traditional division of the order into two families, Polytrichaceae and Dawsoniaceae (e.g. Brotherus, 1925; Crum, 2001), is still strongly contradicted. *Dawsonia* is monophyletic but firmly included within Polytrichaceae as noted by Smith (1971). *Alophosia azorica* appears to be sister to all the other Polytrichales but now joined by another taxon lacking the typical adaxial lamellae—*Atrichopsis compressa*. In this species lamellae are actually present, but poorly developed and found only on the male gametophyte. When we recoded this character for *A. compressa* as "lamellae present" it did not change the topology, but only added one more step. The latter species was represented in our earlier analysis only by morphology. Both of these species are narrow endemics with very restricted distribution. *Alophosia azorica* is found only in Macaronesia (Azores and Madeira) while *Atrichopsis compressa* has been found only in the extremely oceanic areas of the western and southern coasts of southernmost South America.

The species of *Bartramiopsis*, *Lyellia*, and *Dawsonia* and the rest of Polytrichales show a grade of more elaborate development of photosynthetic adaxial lamellae from the species without them (*Alophosia azorica* and *Atrichopsis compressa*), through species with lamellae only in the central part of the leaf (*Bartramiopsis* and *Lyellia*) to species with numerous high lamellae with specialized enlarged apical cells with ornamented outer
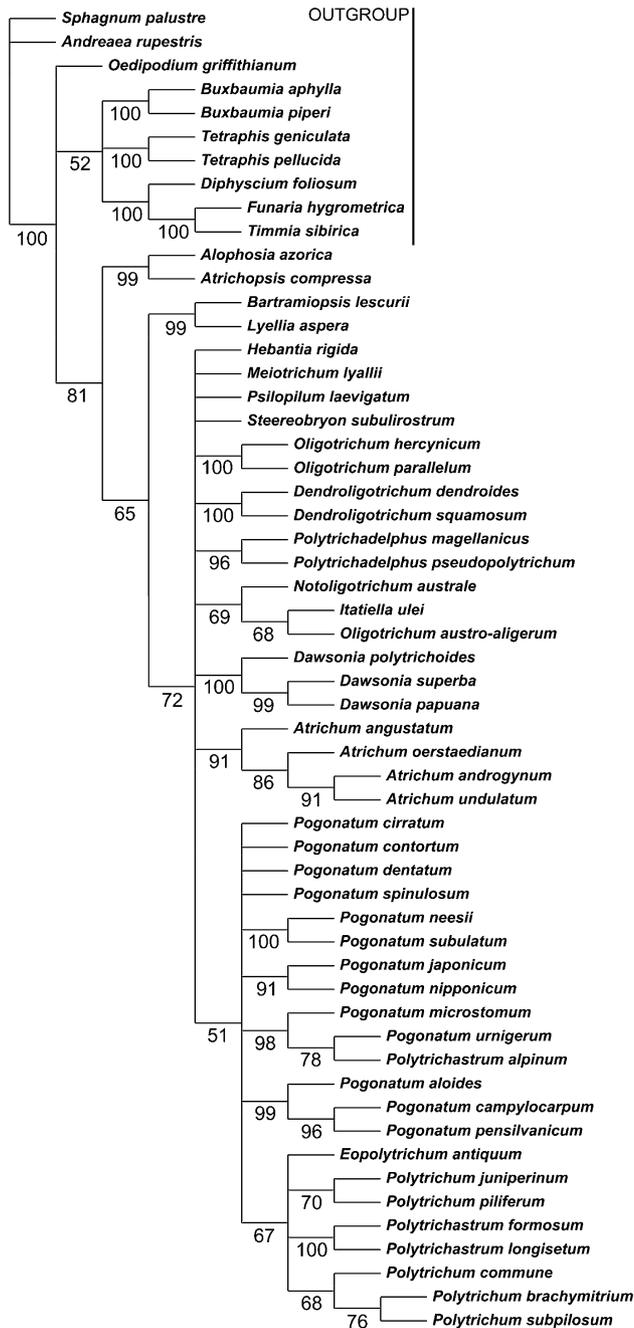

Fig. 2. The tree obtained from the jackknife analysis with the program xac based on the total matrix composed of six datasets. Jackknife support values are given below the nodes.

Table 3
Outgroup taxa sampled in the extended analysis, all sequences downloaded from the GenBank

Taxon	Accession No.				
	18S	*rbc*L	*rps*4	*trn*L-F	*nad*5
Dicranum scoparium	X89874	AF231067	AF234158	AF234159	Z98956
Encalypta rhaptocarpa	AF023680	AJ275167	AF023777	AF023717	—
E. streptocarpa	—	—	—	—	AJ291556
Fontinalis antipyretica	AF023714	AJ275183	AF023817	AF023771	AJ291570
Hedwigia ciliata	AJ275010	AF231073	AJ251309	AF233587	Z98966
Hookeria acutifolia	—	AF158170	—	—	—
H. lucens	AJ243168	—	AJ251316	AF215906	Z98969
Mnium hornum	X80985	AF226820	AF023796	AF023767	AJ291567
Rhodobryum giganteum	AF023699	AJ275176	AF023789	AF023737	Z98964
Takakia lepidozioides	AJ269686	AF244565	AF306950	—	AJ291553
Takakia sp.	—	—	—	AF231904	—
Theriotia lorifolia	AF223007	AF232698	AF223036	AF229893	—
Tortula ruralis	AF023682	AJ275169	AF023831	AF023722	AJ291562

See text for further details.

walls. There are, however, also species of *Atrichum* and *Pogonatum* that lack lamellae. Our results lend support to the view that the absence of these structures in some species of these two genera is due to reduction. Similar variation can be also be seen in the leaf form. *Alophosia azorica* shows the differentiation of leaf parts that is typical for Polytrichales: a distinctly widened leaf-base (sheath) with a long and narrow apical blade. It seems that in most cases undifferentiated leaves are due to reduction but whether this applies also to *Atrichopsis compressa* and *Bartramiopsis lescurii* is not clear. It is equally parsimonious to assume that the common ancestor of all Polytrichales already had a differentiated sheath as it is to suppose that differentiation has taken place independently in *Alophosia*, *Lyellia*, and in the common ancestor of all other Polytrichales.

Leaf margins in Polytrichales vary from entire to distinctly toothed. Taxa with sharp, unicellular teeth on the blade margins do not form a monophyletic group and therefore one has to assume that this kind of teeth evolved independently at least three times. Polytrichales also show variation in the thickness of leaf margins, and this seems to be a highly homoplasious character as well.

The hairy calyptra, a structure typical of many Polytrichales, is present in three different groups. The calyptra of *Alophosia* has both uni- and multiseriate hairs, while those with exclusively multiseriate (*Dawsonia*) or uniseriate hairs (*Polytrichum* and *Pogonatum*) seem to have evolved independently. Whether peristomes of all mosses are homologous is also open to debate according to our results. If *Oedipodium* is a sister taxon to Polytrichales then it is more parsimonious to assume that the peristomes have evolved independently in Bryopsida, *Atrichopsis* and the rest of the Polytrichales. However, if *Oedipodium* is in the more basal position as a sister taxon to all mosses excluding Sphagnopsida and Andreaeaopsida then it is equally parsimonious to

assume that lack of peristome in *Alophosia*, *Bartramiopsis*, and *Lyellia* is due to reduction.

At the moment it seems that none of the morphological characters is a very good indicator of phylogeny, since all of them show considerable homoplasy. However, the level of homoplasy observed does not differ significantly from the values obtained for sequence data. The combined simultaneous analysis provided results that would have been unexpected based solely on morphology as observed in our earlier analysis (Hyvönen et al., 1998). Plants such as *Polytrichadelphus*, *Dawsonia*, and *Polytrichum* with large, well-developed gametophytes and leaves with differentiated hinge-tissue, numerous adaxial lamellae and specialized marginal cells appear to be quite unrelated to each other. These elaborate gametophyte structures seem to have evolved independently in these lineages.

When we examine the current geographical distributions of the taxa, an interesting pattern is quite obvious (Fig. 3). The most basal clades include species that are today geographically widely separated from each other. *Alophosia* is a Macaronesian endemic, *Atrichopsis* is restricted to southernmost South America, *Bartramiopsis* is confined to equally oceanic climates around the northern Pacific coastline and *Lyellia* has species in the high arctic and in the Himalayas. However, conclusions of whether the common ancestor of all Polytrichales was found in the north or in the south should not be made based on the pattern illustrated in Fig. 3. As mentioned above, our sampling of the basal moss lineages is still so unbalanced that such conclusions would be unwarranted. While *Atrichopsis* obviously represents an archaic element in the Southern Hemisphere, most of the genera with contemporary Southern Hemisphere distributions seem to have originated later. All these genera form a grade within Polytrichales, and it is parsimonious to assume that widespread and common Northern Hemisphere genera such as *Atrichum*, *Polytrichum*, and *Pogonatum* represent younger elements of the Northern Hemisphere
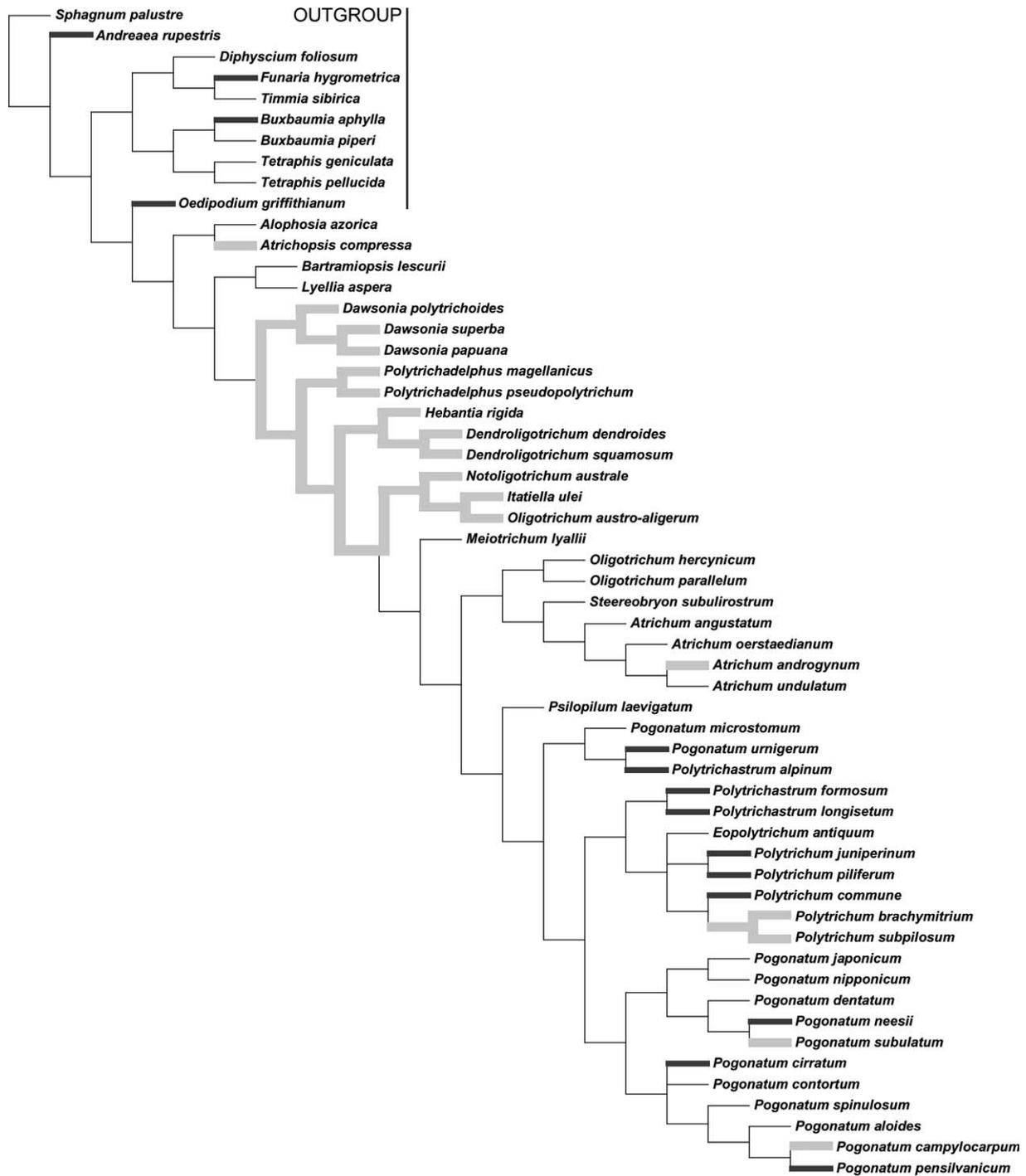
Fig. 3. The distribution of the Polytrichales included in the analysis mapped on the strict consensus tree of the two trees obtained from the analysis based on simultaneous analysis of the total matrix composed of six datasets. Species found exclusively in the southern Hemisphere are marked with thick light gray bars and those that are present in both Hemispheres with dark gray bars. Unmarked species are confined to the northern Hemisphere.

Polytrichales and originated from their common ancestor with *Meiotrichum* and some other smaller northern genera. This corresponds to a large extent with the biogeographic scenario presented by Smith (1972) although at least with the current sampling it seems that *Atrichum*, *Polytrichum*, and *Pogonatum* did not originate in the south but instead spread there later.

If we follow recommendations and conventions by Wiley (1981) our results would necessitate numerous changes in nomenclature. Four of these would require only adoption of the older names of the taxa, which in some cases, have been continuously widely used. However, at this point we decline to make any formal changes because data are accumulating at an ever-increasing pace

and we are sure that our results will be challenged shortly with wider sampling of characters and taxa. If short internal branches that are typical for our results at this point still persist after analyses of much larger data matrices, it will be time to reconsider the existing nomenclature in order to make it compatible with phylogenetic relationships and as informative as possible.

Appendix A

Different character states are coded with (0), (1), (2), and (3). These codes do not, however, designate a priori which of the states is plesiomorphic or apomorphic. Unless otherwise stated treatment of these characters follow Hyvönen et al. (1998) and this paper should be consulted for more detailed discussion of the characters and their states.

 1. Branching: not or sparingly branched (0); dendroid (1); branches in fascicles (2).
 2. Sheath type: sheath differentiated, broad (0); sheath not differentiated (1).
 3. Hyaline sheath margin: present (0); absent (1).
 4. Sheath/leaf base margin: entire (0); ciliate (1); serrate (2).
 5. Hinge tissue: present (0); absent (1).
 6. Leaf (blade) margin: serrate (0); toothed (1); entire (2).
 7. Leaf border: absent (0); *Atrichum*-type (1).
 8. Thickness of leaf margin: unistratose (0); two- or more stratose (1).
 9. Adaxial lamellae: present (0); absent (1).
10. Extent of adaxial lamellae: numerous, occupying full width of lamina (0); restricted to median strip (1). Taxa lacking lamellae were scored as "−" (inapplicable) for this and following three characters.
11. Thickness of lamella-free lamina: unistratose (0); bistratose (1).
12. Lamella marginal cells (LMCs, apical cells of lamellae): single (0); geminate (1).
13. Size of LMC lumen: comparable to lower cells of lamellae (0); elongated as seen in side view (1); higher than lower cells (1).
14. Form of LMCs as seen in cross-section: rounded (undifferentiated) (0); ovoid to bottle-shaped (1); flattened (2); retuse (3).
15. LMC cell-walls: undifferentiated (0); incrassate (1); only outer wall incrassate (2); outer wall notched (3).
16. Lamella cuticle: smooth (0); papillose (1).
17. Paraphyses: present (0); absent (1).
18. Calyptra: present (0); absent (1).
19. Calyptra hair: uniseriate (0); multiseriate (1); sparse or none (2).
20. Pseudopodium: absent (0); present (1).
21. Seta: present (0); absent (1).
22. Seta surface: smooth (0); papillose (1).

The capsule form was treated with two characters (capsule cross-section and angles) by Hyvönen et al. (1998). We now think that it is better to distinguish capsule cross-sectional symmetry from the capsule angles and therefore include here three distinct characters: symmetry, angles, and angle form.

23. Capsule cross sectional symmetry: symmetrical (0); dorsiventral (1); bilaterally compressed (2).
24. Angles of capsule: none (0); two (1); 4–6 (2); numerous (6–8) or practically absent (3).
25. Capsule angle form: blunt (0); sharp, knife-edged (1); ribbed (2).
26. Capsule dehiscence: longitudinal slits (0); operculum (1).
27. Exothecium: smooth (0); mamillose (1); papillose (2).
28. Exothecial pitting: none (0); thin-spots (1); pitted (2).
29. Apophysis: tapering (0); contracted (1); discoid (2).
30. Stomata: present (0); absent (1).
31. Stomata type: superficial (0); cryptopore (1).
32. Stomata extent: restricted to base (0); dispersed (1).
33. Peristome: present (0); absent (1).
34. Peristome type: polytrichoid (0); dawsonioid (1); tetraphid (2); arthrodont (3).
35. Tooth structure: simple (0); compound, sinus broad (1); compound, sinus narrow (2).
36. Tooth number: 32 (0); 64 (1); 4 (2); 16 (3).
37. Peristome pigmentation: pale (0); intensively colored (1).
38. Epiphragm type: discoid (0); absent (1); stopper (2); cylindric (rod) (3).
39. Capsule rim disc: narrow (0); broad (disc) (1).

40. Spore sac: overarching columella (0); cylindrical (1).
41. Spore origin: from endothecium (0); from exothecium (1).

42. Spore surface: papillose (0); echinulate (1); *Bartramiopsis*-type (2); *Oedipodium*-type (3).
43. Brood-bodies: absent (0); present (1).

Appendix B

Different character states are coded with (0), (1), (2), and (3). Polymorphism (0,1) marked with M, and (0,2) with R. (?) denote unknown information and (–) inapplicable characters.

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                                    0000000001111111111222222222233333333334444
                                    1234567890123456789012345678901234567890123

Sphagnum palustre                   211012001-0-----01-11-00-10000011----100100
Andreaea rupestris                  011012001-0-----10211-00-00001-01----1-0000
Funaria hygrometrica                011010001-0-----00200000-100001003-30101000
Timmia sibirica                     001010001-0-----00200000-100000003-30101000
Buxbaumia aphylla                   0--212001-0-----10200111110000l003-30101000
B. piperi                           0--212001-0-----102001111100001003-30101000
Diphyscium foliosum                 011012001-0-----00200100100000003-30101000
Oedipodium griffithianum            011212001-0-----?0200000-10000001----201031
Tetraphis geniculata                011012001-0-----00200100-10001--02-20?01001
T. pellucida                        011012001-0-----00200000-10001--02-20301001
Alophosia azorica                   001010011-1----100M000111110000001----211011
Atrichopsis compressa               011010001-1----100200020010000l000000001000
Atrichum androgynum                 011011001010000000200000-10001--00000001000
A. angustatum                       0110111M010000000000200000-10001--00000001000
A. oerstaedianum                    011011100100000000200000-10001--00000001000
A. undulatum                        011011110100000000200000-10001--00000001000
Bartramiopsis lescurii              011210010110000000200000-10000001----201020
Dawsonia papuana                    001001000000000001000111100000001--0301010
D. polytrichoides                   001001000000000001000111100000001--0301010
D. superba                          001001000001010001001101100000001--0301010
Dendroligotrichum dendroides        10101M01001000000200000-100000100011001000
D. squamosum                        10101M01001100000200000-100000100011001000
Eopolytrichum antiquum              ?0?00000000001000???0000-112200000---201?10
Hebantia rigida                     01101M00010000000000200000-100100100001001000
Itatiella ulei                      011010000000000000200000-10?01--1----001000
Lyellia aspera                      00101001011M000000200011110000001----211010
Meiotrichum lyallii                 000001000000021002000021101100000010001000
Notoligotrichum australe            000012000000021002000200l0000l000000001000
Oligotrichum austro-aligerum        011012000100000?0200000-10000000000M001000
O. hercynicum                       011010000100000000200000-100000000200001000
O. parallelum                       011010000100000000200000-100100000100001000
Pogonatum aloides                   001010000000000000000-12001--00201001000
P. campylocarpum                    01101000000M21000000003212001--00201001000
P. cirratum                         001M100M000M000000003212001--00201001000
P. contortum                        011010000000000000003212001--00201001000
P. dentatum                         001000000000021100000-12001--00201001000
P. japonicum                        00101M010001000100000000-12001--00201001000
P. microphyllum                     001010000012120000003212001--00201001000
P. neesii                           00101000000030000003212001--00201001000
P. nipponicum                       00101000000M212000000000-12001--00201001000
P. pensilvanicum                    0?1010000000000000003212001--00201001000
P. spinulosum                       0?101---1-0-----00000000-12001--00201001000
P. subulatum                        0M1010000000000000003212001--00201001000
P. urnigerum                        000000000000001100000000-12001--00201001000
Polytrichadelphus magellanicus      0000000000002130002000111100000000010001000
P. pseudopolytrichum                001000000030002000111100001000100010?0
Polytrichastrum alpinum             000001000000210000000R01001010000M0001000
P. formosum                         00000100000000000000020100100000010001000
P. longisetum                       000001000000000000000201000000000M10001000
Polytrichum brachymitrium           000001000000300?000002111220000010001010?0
P. commune                          0000010000030000000002111220000010001010
P. juniperinum                      000002000000130000000002111220000010001010?0
P. piliferum                        00000200000013000000002111220000010001010?0
P. subpilosum                       000001000000330000000002111220000010001010?0
Psilopilum laevigatum               011012000100000000200000-100000000000001000
Steereobryon subulirostrum          011010000100000000200000-100000000000001000
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References

Brotherus, V.F., 1925. Musci (Laubmoose) 2. Hälfte. In: Engler, A. (Ed.), Die natürlichen Pflanzenfamilien. 2. Aufl. 11. Engelmann. Leipzig.

Buck, W.R., Goffinet, B., 2000. Morphology and classification of mosses. In: Shaw, A.J., Goffinet, B. (Eds.), Bryophyte Biology. Cambridge University Press, Cambridge, pp. 71–123.

Buck, W.R., Goffinet, B., Shaw, A.J., 2000. Novel relationships in pleurocarpous mosses as revealed by cpDNA sequences. Bryologist 103, 774–789.

Chase, M.W., Albert, V.A., 1998. A perspective on the contribution of plastid rbcL DNA sequences to angiosperm phylogenetics. In: Soltis, D.E., Soltis, P.E., Doyle, J.J. (Eds.), Molecular Systematics of Plants II. Kluwer Academic Publishers, Boston, MA, pp. 488–507.

Cox, C., Hedderson, T.J., 1999. Phylogenetic relationships among the ciliate arthrodontous mosses: evidence from nuclear and chloroplast gene sequences. Plant Syst. Evol. 215, 119–139.

Cox, C.J., Goffinet, B., Newton, A.E., Shaw, A.J., Hedderson, T.A.J., 2000. Phylogenetic relationships among the diplolepideous-alternate mosses (Bryidae) inferred frrom nuclear and chloroplast DNA sequences. Bryologist 103, 224–241.

Crosby, M.R., 1999. An overview of the species of mosses of the world. Lecture in the XVIth International Botanical Congress, St. Louis, MO.

Crum, H., 2001. Structural diversity of bryophytes. The University of Michigan Herbarium, Ann Arbor, MI.

Derda, G.S., Wyatt, R., 1999. Isozyme evidence regarding the origins of three allopolyploid species of Polytrichastrum (Polytrichaceae, Bryophyta). Plant Syst. Evol. 220, 37–53.

Eernisse, D.J., Kluge, A.G., 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Mol. Biol. Evol. 10, 1170–1195.

Farris, J.S., 1989. The retention index and rescaled consistency index. Cladistics 5, 417–419.

Farris, J.S., 1997a. xac. Computer program and documentation distributed by the author.

Farris, J.S., 1997b. xarn. Computer program and documentation distributed by the author.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–320.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12, 99–124.

Forrest, L.L., 1995. A phylogenetic analysis of Polytrichaceae (Musci). M.Sc. thesis, Department of Botany, University of Reading.

Goffinet, B., Cox, C.J., Shaw, A.J., Hedderson, T.A.J., 2001. The Bryophyta (Mosses): systematic and evolutionary inferences from an rps4 gene (cpDNA) phylogeny. Ann. Bot. 87, 191–208.

Goloboff, P.A., 1994. NONA: A tree searching program. Program and documentation, Available from ftp.unt.edu.ar/pub/parsimony.

Hedderson, T.A., Chapman, R.L., Rootes, W.L., 1996. Phylogenetic relationships of bryophytes inferred fron small-subunit rRNA gene sequences. Plant Syst. Evol. 200, 213–224.

Hedderson, T.A., Chapman, R.L., Cox, C.J., 1998. The origins and diversification of land plants: new evidence from molecules. In: Ashton, N.W., Bates, J.W., Duckett, J.G. (Eds.), Bryology for the Twenty-first Century. Maney, Leeds, pp. 65–77.

Hyvönen, J., 1989. A synopsis of genus Pogonatum (Polytrichaceae, Musci). Acta Bot. Fennica 138, 1–87.

Hyvönen, J., Hedderson, T.A., Smith Merril, G.L., Gibbings, J.G., Koskinen, S., 1998. On phylogeny of the Polytrichales. Bryologist 101, 489–504.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., Gibson, T.J., 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci 23, 403–405.

Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). Syst. Zool. 38, 7–25.

Kluge, A.G., 1997. Testability and the refutation and corroboration of cladistic hypotheses. Cladistics 13, 81–96.

Kluge, A.G., Farris, J.S., 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18, 1–32.

Konopka, A.S., Herendeen, P.S., Smith Merrill, G.L., Crane, P.R., 1997. Sporophytes and gametophytes of Polytrichaceae from the Campanian (Late Cretaceous) of Georgia, USA. Int. J. Plant Sci. 158, 489–499.

Källersjö, M., Albert, V.A., Farris, J.S., 1999. Homoplasy increases phylogenetic structure. Cladistics 15, 91–94.

Lewis, L.A., Mishler, B.D., Vilgalys, R., 1997. Phylogenetic relationships of the liverworts (Hepaticae), a basal embryophyte lineage, inferred from nucleotide sequence data of the chloroplast gene rbcL. Mol. Phylogenet. Evol. 7, 377–393.

Lipscomb, D.L., Farris, J.S., Källersjö, M., Tehler, A., 1998. Support, ribosomal sequences and the phylogeny of the eukaryotes. Cladistics 14, 303–338.

Magombo, Z., 2003. Inferences on evolution and phylogeny of peristomate mosses based on chloroplast genes. Syst. Bot. 28, 24–38.

Mickevich, M.F., Farris, J.S., 1981. The implications of congruence in Menidia. Syst. Zool. 30, 351–369.

Mishler, B.D., 2000. Deep phylogenetic relationships among "plants" and their implications for classification. Taxon 49, 661–683.

Mishler, B.D., Churchill, S.P., 1984. A cladistic approach to the phylogeny of the "bryophytes". Brittonia 36, 406–424.

Miyamoto, M.M., Fitch, W.M., 1995. Testing species phylogenies and phylogenetic methods with congruence. Syst. Biol. 44, 64–76.

Newton, A., Cox, C., Duckett, J., Wheeler, J.A., Goffinet, B., Hedderson, T.A.J., Mishler, B.D., 2000. Evolution of the major moss lineages: phyologenetic analyses based on multiple gene sequences and morphology. Bryologist 103, 187–211.

Nickrent, D.L., Parkinson, C.L., Palmer, J.D., Duff, R.J., 2000. Multigene phylogeny of land plants: hornworts are basal and mosses are sister to liverworts. Mol. Biol. Evol. 17, 1885–1895.

Nixon, K.C., 1999. Winclada (beta) ver. 0.9. Published by the author, Ithaca, NY. [Available from http://www.cladistics.com].

Nixon, K.C., Carpenter, J.M., 1996. On simultaneous analysis. Cladistics 12, 221–242.

Renzaglia, K.S., Duff, R.J., Nickrent, D.N., Garbary, D.J., 2000. Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. Philos. T. R. Soc. B 355, 769–793.

Schofield, W.B., 1985. Introduction to Bryology. Macmillan, New York.

Siddall, M.E., 1997. Prior agreement: arbitration or arbitrary? Syst. Biol. 46, 765–769.

Siddall, M.E., Whiting, M.F., 1999. Long-branch abstractions. Cladistics 15, 9–24.

Smith, G.L., 1971. A conspectus of the genera of Polytrichaceae. Mem. N. Y. Bot. Gard. 21, 1–83.

Smith, G.L., 1972. Continental drift and the distribution of Polytrichaceae. J. Hattori Bot. Lab. 35, 41–49.

Steinhauser, S., Beckert, S., Capesius, I., Malek, O., Knoop, V., 1999. Plant mitochondrial RNA editing—extreme in hornworts and dividing the liverworts? J. Mol. Evol. 48, 303–312.

Swofford, D.L., Olsen, G.L., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Morowitz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer, Sunderland. MA, pp. 407–514.

Wheeler, W.C., Cartwright, P., Hayashi, C.Y., 1993. Arthropod phylogeny: a combined approach. Cladistics 9, 1–39.

Wiley, E.O., 1981. Phylogenetics: the theory and practice of phylogenetic systematics. Wiley, New York.

Yang, Z., 1998. On the best evolutionary rate for phylogenetic analysis. Syst. Biol. 47, 125–133.