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On Phylogeny of the Polytrichales[1]

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Abstract. *Phylogenetic analyses on Polytrichales were conducted using morphological characters as well as sequence data from the chloroplast genes* rbc*L and rps4 and the nuclear-encoded 18S rRNA gene. Our analyses included 22 species representing all genera of Polytrichales, plus eight outgroup species. Sequence data were obtained from 25, 22, and 19 taxa for 18S,* rbc*L and rps4 genes, respectively. Phylogenetic trees were constructed with parsimony analyses. Results lend support for recognition of Polytrichales as a distinct, monophyletic entity. After successively approximated weighting,* Oedipodium griffithianum *appears as the sister-group to Polytrichales. Within Polytrichales,* Alophosia, Bartramiopsis, *and* Lyellia *have the most basal placement outside a clade including all other genera.* Atrichopsis, Dendroligotrichum, Itatiella, Meiotrichum, *and* Notoligotrichum *are distinguished as a resolved monophyletic group while other genera are left as an unresolved entity. Resolution between these genera is achieved by successive weighting of data. After this,* Dawsonia *is resolved in the basal position within the clade and* Polytrichastrum *appears as a sister-taxon to* Eopolytrichum *and* Polytrichum.

Polytrichales are typically pioneer plants of open, sometimes even dry, habitats. Despite comprising only a small number of species, the order exhibits great diversity of shapes and sizes, from miniature plants such as *Pogonatum piliferum* (Dozy & Molk.) Touw of SE Asia and *P. pensilvanicum* (Hedw.) P. Beauv. of E North America to giants of Papua New Guinea like *Dawsonia gigantea* Geh. with stems reaching up to 80 cm. The most typical features of Polytrichales are the adaxial leaf lamellae and differentiation of leaves into a distinct blade and sheathing base. The hairy calyptra has given the group its name, although most genera have practically naked calyptrae. Sporophytes of Polytrichales normally have a well-developed peristome with at least 16 teeth that consist of whole cells. The epiphragm that covers the mouth of the capsule is a unique character that distinguishes most of the genera from all other groups of mosses. Size and shape of the urn vary greatly among genera (Schofield 1985; Smith 1971).

The number of currently accepted genera in the Polytrichales is 19, and the approximate number of species in each genus is given in Table 1. One genus, along with its sole species *Eopolytrichum antiquum* Konopka, Herendeen, Merrill & Crane, is known only from beautifully preserved late Cretaceous fossils that reveal its structures in fine detail (Konopka et al. 1997). Many of the remaining genera are monotypic and all the others, with the exception of *Pogonatum* and *Polytrichum,* are fairly small. Schofield (1985) gives a conservative estimate of about 370 for the number of species in Polytrichales, but on the basis of recent critical revisions (e.g., Hyvönen 1989) it is realistic to assume that the number is actually closer to, or even less than, 200.

Some species, like *Polytrichum juniperinum* Hedw., are almost cosmopolitan, while others, like the Macaronesian *Alophosia azorica* (Ren. & Card.) Card., are narrow endemics and possibly

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TABLE 1. The approximate number of species in the genera of Polytrichales. †–fossil.

Alophosia	1	*Meiotrichum*	1
Atrichopsis	1	*Notoligotrichum*	10
Atrichum	15	*Oligotrichum*	10
Bartramiopsis	1	*Pogonatum*	50
Dawsonia	10	*Polytrichadelphus*	10
Dendroligotrichum	2	*Polytrichastrum*	10
†*Eopolytrichum*	1	*Polytrichum*	30
Hebantia	1	*Psilopilum*	2
Itatiella	1	*Steereobryon*	1
Lyellia	4		

even threatened by extinction. Ecologically, the Polytrichales range from xerophytes like *Polytrichum piliferum* Hedw. to species of peaty, wet habitats like *P. commune* Hedw. Although their structure appears so obviously adapted to dry environments, the Polytrichales are largely absent from extremely arid regions, and the group exhibits greatest diversity in areas with humid or moist climates like SE Asia and Central America-northern South America.

Phylogenetic relationships of the Polytrichales are particularly relevant to considerations of moss evolutionary history since the group is probably among the first of the lineages that diverged from the common ancestor of all mosses (Mishler & Churchill 1984). Smith (1971) presented a dendrogram with assumed phylogenetic trends for Polytrichales, and these ideas were further developed in his study of epiphragm structure and spore ornamentation (Smith 1974). Hyvönen (1989), in a revision of *Pogonatum* that included cladistic analysis based on manual Hennigian argumentation, tentatively distinguished three entities in the Polytrichales as an unresolved basal trichotomy. These, and many other authors, viewed *Polytrichastrum* G. L. Sm. as the closest extant approximation to the common ancestor of Polytrichales.

Forrest (1995) presented the first computer-aided analyses of Polytrichalean phylogeny based on a matrix of 50 characters compiled from the literature. The cladogram from her successively weighted analysis of these data is presented in Figure 1 (fig. 3b in Forrest 1995). In contrast to the views noted above, the position of *Atrichum* as sister to the remaining Polytrichales suggests that relatively simple members of the group more closely approximate the ancestral condition. This is congruent with ideas presented previously by Fleischer (1923).

Forrest's (1995) analyses resolved both strongly and weakly supported groups as implied by the number of characters supporting each clade. To test the strength of the phylogenetic hypotheses based on morphology and to determine whether they are congruent with other sources of data we explored

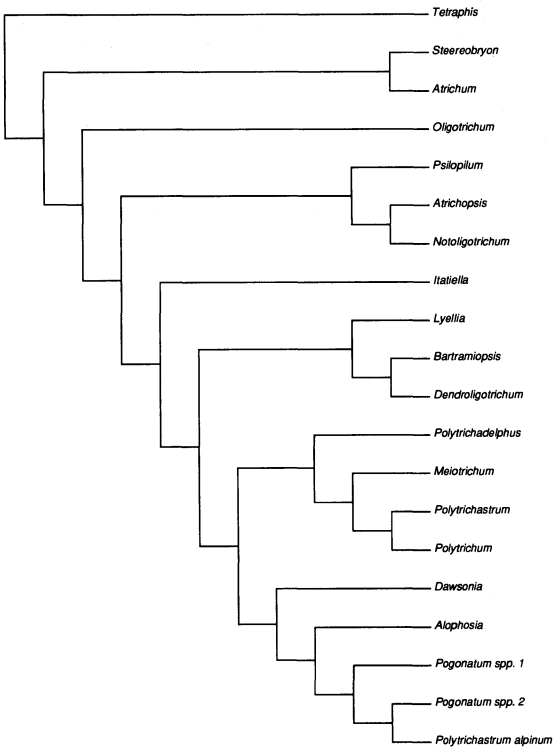


FIGURE 1. The cladogram obtained by Forrest (1995) based on a data set of 50 morphological characters (successively approximated weighting applied).

the historical information residing in DNA sequences of different genes. Our goal was to obtain sequences for three genes (the chloroplast-encoded *rbc*L and rps4 loci and the nuclear-encoded 18S rRNA gene) from species representing all currently accepted genera of Polytrichales. The chloroplast gene *rbc*L is used widely to infer phylogenetic relationships among families and genera of angiosperms. Current geographical distributions of Polytrichalean genera suggest that most of them are at least as old as angiosperm families, and therefore it should be possible to study their phylogeny using this gene. Although less widely used, rps4 varies at approximately the same level as *rbc*L. It has proven informative at generic and familial levels in mosses (Cox & Hedderson 1998) and therefore provides a second independent marker for phylogeny reconstruction. The 18S gene is considerably more conserved than either of the chloroplast genes (e.g., Hedderson et al. 1996, 1998) and therefore provides data that are likely to be useful at deeper phylogenetic levels.

MATERIAL AND METHODS

Our analyses include 22 species, representing all known genera of Polytrichales. Our attempts to PCR amplify DNA from material of three extant monotypic genera

TABLE 2. The specimens/source for the gene sequences used in the analyses.

POLYTRICHALES			
Alophosia azorica	Azores	*Rumsey* 18.3.1997	(F, H, RNG)
Atrichum angustatum[2,3]	U.S.A. Louisiana	*Hedderson 10393*	(F, H, RNG)
A. undulatum	Capesius (1995)		
Bartramiopsis lescurii	Canada. British Columbia	*Hedderson 10044*	(F, H, RNG)
Dawsonia papuana	Papua New Guinea	*Baker 662*	(F, H, RNG)
Dendroligotrichum dendroides	New Zealand. North Island	*Stenroos 4677*	(F, H, RNG, TUR)
Itatiella ulei	Brazil. Saõ Paulo	*Ahti 51824*	(F, H)
Lyellia aspera	Canada. Ellesmere Island	*Hedderson 6825*	(F, H, RNG)
Meiotrichum lyallii	U.S.A. Colorado	*Weber B36612*	(COLO, F, H)
Notoligotrichum australe	New Zealand. South Island	*Hyvönen 6069*	(F, H)
Oligotrichum parallelum	Canada. British Columbia	*Hedderson 10043*	(F, H, RNG)
Pogonatum contortum	Canada. British Columbia	*Hedderson 5803*	(F, H, RNG)
P. urnigerum	Finland. Uusimaa	*Hyvönen 6173*	(F, H, RNG)
Polytrichadelphus magellanicus	Chile. X Region de Los Lagos	*Hyvönen 5865*	(F, H, RNG, TUR)
Polytrichastrum formosum[1]	Finland. Uusimaa	*Hyvönen 6197*	(F, H, RNG, TUR)
P. longisetum	Finland. Uusimaa	*Hyvönen 6172*	(F, H, RNG, TUR)
Polytrichum commune[2,3]	Finland. Uusimaa	*Hyvönen 6168*	(F, H, RNG, TUR)
Psilopilum laevigatum	Canada. Ellesmere Island	*Hedderson 5938*	(F, H, RNG)
OUTGROUP TAXA[2,3]			
Andreaea rupestris			
Buxbaumia aphylla	Canada. Nova Scotia	*Belland 16889*	(DUKE)
Diphyscium foliosum[5]	U.S.A. North Carolina	*Goffinet 4492*	(DUKE)
Funaria hygrometrica[1,4]			
Oedipodium griffithianum	U.S.A. Alaska	*Schofield 98670*	(DUKE)
Sphagnum palustre			
Tetraphis pellucida[5]	U.S.A. North Carolina	*Goffinet 4542*	(DUKE)
Timmia sibirica[4,5]			

[1] 18S ribosomal DNA sequences from Capesius 1995; [2] *rbc*L sequences from Mishler *pers. comm.*; [3] 18S sequences from Hedderson et al. 1996; [4] rps4 sequences from Cox & Hedderson 1998; [5] 18S sequences from Cox & Hedderson 1998.

(*Atrichopsis, Hebantia,* and *Steereobryon*) failed for all genes. Accordingly, these taxa are represented only by their morphology, as is the fossil taxon *Eopolytrichum. Andreaea rupestris* Hedw., *Buxbaumia aphylla* Hedw., *Diphyscium foliosum* (Hedw.) Mohr, *Funaria hygrometrica* Hedw., *Oedipodium griffithianum* (Dicks.) Schwaegr., *Sphagnum palustre* L., *Tetraphis pellucida* Hedw., and *Timmia sibirica* Lindb. & Arn. were used as outgroup taxa based on previous higher-level analyses (Cox & Hedderson 1998; Hedderson et al. 1996, 1998). Specimen data or citation of the original source of the sequences used in our analyses are given in Table 2 and the morphological matrix used is shown in Appendix 1.

Morphological characters.—Different character states are coded with (0), (1), (2). These codes do not, however, designate *a priori* which of the states is plesiomorphic or apomorphic.

1. *Branching.* Most Polytrichales are unbranched or only sparingly branched, or branching only by subfloral innovations from beneath female inflorescences. *Dendroligotrichum* is dendroid (branches forming a distinct apical cluster), however, and similar branching is also seen in *Pogonatum sinense* (Broth.) Hyvönen & Wu. While other species of *Pogonatum* might be regularly branched (Hyvönen & Wu 1993), this is not interpreted as a distinct character state from unbranched stems. Hyvönen (1989) suggested that the "dendroid Polytrichaceae" may form a monophyletic unit. *Sphagnum* is unique among mosses in having branches in several fascicles along the stem. Branching is treated as an unordered multi-state character with three states: not or sparingly branched (0); dendroid (1); branches in fascicles (2).

2. *Sheath type.* The leaves of many Polytrichales (*Dawsonia Polytrichum*) are differentiated into a broadened, membranous sheathing base and a divergent, firm-textured blade. Others (*Atrichum*) have the sheath scarcely differentiated. The differentiated sheath is not, however, a polytrichalean character as exemplified for example by the outgroup *Timmia,* an arthrodont moss, that also has a differentiated sheath. Sheath type is treated as a binary character: sheath differentiated, broad (0); sheath not differentiated (1).

3. *Hyaline sheath margin.* The sheathing base of the leaf in some Polytrichales has a scarious margin composed of several rows of thin-walled, colorless cells. Other members of the family with differentiated sheaths (*Dawsonia*) lack such a margin. In most species of *Pogonatum* it is lacking as well, with *P. urnigerum* (Hew.) P. Beauv. being an exception. Hyaline margin is treated as a binary character: present (0); absent (1).

4. *Sheath/leaf base margin.* The leaf margins of *Bartramiopsis* are ciliate near the base. Among the outgroups, *Buxbaumia* and *Oedipodium* are also scored in this way. All other taxa are either toothed, serrate, or entire. Sheath margin is treated as a binary character (toothed or serrate margins are not present in the species included in this analysis): entire (0); ciliate (1).

5. *Hinge tissue.* Where a distinct sheath is present, a "swelling-tissue" or hinge is sometimes present (*Dawsonia, Polytrichadelphus, Polytrichum*), extending laterally from the margins toward the nerve (Smith 1971). *Pogonatum urnigerum* differs from most species of the genus in having hinge tissue. The fossil, *Eopolytrichum,* also appears to have had this character, although only surface details can be seen (the leaves cannot be viewed by transmitted light). Hinge tissue is treated as a binary character: present (0); absent (1).

6. *Leaf (blade) margin.* The leaves of some taxa (*Atrichum, Dawsonia, Polytrichum,* some species of *Pogonatum*) have sharp, unicellular teeth at intervals along the margins, whereas the leaves of most other taxa are merely serrate, the serrations made up of several to many cells, or entire. Leaf margin is treated as an unordered multi-state character with three states: serrate (0); toothed (1); entire (2).

7. *Leaf border.* In *Atrichum,* the leaf margins have a thickened border of linear, thick-walled cells, and are spinosely toothed. Other taxa lack this distinctive type of differentiated border. Leaves of *Steereobryon* are bordered by a single row of lax, papillose cells in a single layer (Smith 1971), and in *Psilopilum* by short, rhomboidal cells with slightly projecting ends. However, the latter two, less clearly differentiated types are not distinguished as distinct character states in the present analysis. Leaf border is treated as a binary character: absent (0); *Atrichum*-type (1).

8. *Thickness of leaf margin.* The leaf margins of most Polytrichaceae are unistratose. The differentiated border of *Atrichum* leaves is typically 1–3 stratose. Several rows of cells along the blade margin in *Alophosia, Bartramiopsis, Dendroligotrichum,* and *Lyellia* are bistratose. Margin thickness is treated as a binary character: unistratose (0); two- or more stratose (1).

9. *Adaxial lamellae.* Photosynthetic lamellae borne on the upper (adaxial) leaf surface are a hallmark of members of the Polytrichales. A few members of the family (*Alophosia, Atrichopsis,* a few *Pogonatum* species) lack lamellae, and it has been generally assumed that they have been lost. In *Atrichopsis,* for example, lamellae are present on the perigonial bracts. A few other mosses, such as *Aloina* (Pottiaceae) and *Aligrimmia* (Grimmiaceae), not represented in this data set, have adaxial lamellae which are almost certainly independently derived; none of the included outgroups has lamellae. Adaxial lamellae is treated as a binary character: lamellae present (0); lamellae absent (1).

10. *Extent of adaxial lamellae.* In *Atrichum, Bartramiopsis, Hebantia, Lyellia, Oligotrichum, Psilopilum,* and *Steereobryon* the lamellae are restricted to the median longitudinal portion of the leaf, flanked by a broad lamella-free lamina. This feature appears in some *Pogonatum* species [*P. proliferum* (Griff.) Mitt.], but the two *Pogonatum* species included in the analysis [*P. contortum* (Brid.) Lesq., *P. urnigerum*] are broadly lamellate. Smith (1971) referred to this character state as the "*anomalum* phenomenon" after a variety of *Polytrichastrum longisetum* (Brid.) G. L. Sm. by that name, originally described as a species of *Atrichum.* Extent of adaxial lamellae is treated as a binary character: numerous, occupying full width of lamina (0); restricted to median strip (1). Taxa lacking lamellae were scored as "–" (inapplicable).

11. *Thickness of lamella-free lamina.* In *Atrichopsis, Bartramiopsis, Dendroligotrichum,* and *Lyellia* the lamella-free portion of the leaf blade is bistratose; in *Alophosia* the corresponding portion of the leaf is also bistratose and almost identical in appearance in transverse sections, but lamellae are lacking from the median portion of the leaf as well. In other taxa with lamellae restricted to the median portion of the blade (*Atrichum*), the elamellate portion is unistratose. In taxa with broadly lamellate leaves (*Dawsonia, Polytrichum*), this character refers to the narrow lamella-free strip along the leaf margin, which is unistratose. Lamella-free lamina is treated as a binary character: unistratose (0); bistratose (1). Taxa lacking lamellae were scored as inapplicable.

12. *Lamella marginal cells (apical cells of lamellae).* The marginal row of cells of the lamellae in the Polytrichales may be undifferentiated, or variously modified in size and/or shape (in cross-section appearing pyriform, retuse, subquadrate, etc.) The form of these marginal cells is often sufficient to distinguish between species in some genera (*Pogonatum, Polytrichastrum,* and *Polytrichum*). Other genera (*Polytrichadelphus*) have a uniform type of lamellar marginal cell. Lamella marginal cells is treated as an unordered multi-state character with three states: undifferentiated (0); differentiated but single (1); geminate (2). Taxa lacking lamellae were scored as inapplicable.

13. *Lamella cuticle.* Most members of the Polytrichales have smooth lamellae, but several of the taxa in the data set [*Meiotrichum lyallii* (Mitt.) G. L. Merr., *Notoligotrichum australe* (Hook. f. & Wils.) G. L. Sm. *Pogonatum urnigerum*] have lamellae with coarsely papillose margins. Lamella cuticle is treated as a binary character: smooth (0); papillose (1). Taxa lacking lamellae were scored as inapplicable.

14. *Paraphyses.* Paraphyses associated with the antheridia are absent in *Andreaea* but present (or information is lacking) in all other taxa. Paraphyses is treated as a binary character: present (0); absent (1).

15. *Calyptra.* All taxa in the data set except *Sphagnum* have an "aerial calyptra". Calyptra is treated as a binary character: present (0); absent (1).

16. *Calyptra hair.* The characteristic hair-like outgrowths of the calyptra not only account for the vernacular name, "hair-cap mosses", for the Polytrichales as a whole, but their presence or absence has traditionally been used as a major character in the delimitation of genera. The names of many of the genera reflect these distinctions, as *Atrichum, Oligotrichum,* or *Polytrichum.* In most Polytrichales the axial strands of the calyptra (when present) are uniseriate, but in *Dawsonia* they are stout, multiseriate, and often barbed by the projecting ends of the cells. The calyptra of *Alophosia* has occasional multiseriate strands mixed with uniseriate strands. Calyptra hair is treated as an unordered multi-state character with three states: uniseriate (0); multiseriate (1); sparse or none (2).

17. *Pseudopodium.* In *Sphagnum* and *Andreaea,* a true seta does not develop, and the capsule is elevated on a leafless elongation of the shoot, the so-called pseudopodium. In *Andreaeobryum* and *Takakia* (taxa related to *Andreaea*), a true seta is present. Pseudopodium is treated as a binary character: absent (0); present (1).

18. *Seta.* This character is linked to the previous character (17) in the sense that when a pseudopodium is present (*Andreaea, Sphagnum*), a true seta is not developed. However, the assumption is that these two characters arose independently in the gametophyte and sporophyte generations. Seta is treated as a binary character: present (0); absent (1).

19. *Seta surface.* A "rough" seta is one in which the surface layer of cells bears projecting papillae, one to a cell, like the exothecium of *Pogonatum.* For example, *Dawsonia longifolia* (Bruch & Schimp.) Zant., has a rough seta, and this feature is characteristic of *Plagioracelopus, Pseudoracelopus, Racelopodopsis,* and *Racelopus,* all of which are included in *Pogonatum s. lat.* by Hyvönen (1989) and Touw (1986). The outgroup taxon *Buxbaumia aphylla* also has a rough seta. Seta surface is treated as a binary character: smooth (0); papillose (1).

20. *Capsule transverse section.* Capsules of Polytrichaceae have considerable variation in shape, ranging from terete to strongly bilaterally or dorsiventrally symmetric. Angled capsules are characteristic of many genera, the number of angles ranging from from two to eight. Capsules of *Alophosia, Dawsonia, Lyellia,* and some species

of *Polytrichadelphus* are dorsiventrally flattened and lunate in transverse section with two prominent angles; Smith (1971) gave a discussion of similarities in capsule shape in these taxa. Capsules of *Buxbaumia* and *Diphyscium* are also dorsiventrally flattened. Longitudinally 6–8-angled capsules (3) are characteristic of many *Pogonatum* species. *Atrichopsis* and *Notoligotrichum* have capsules which are strongly bilaterally compressed, but not angled. Capsule section is treated as an unordered multistate character with five states: terete (0); 2-angled, dorsiventral (1); 4(6)–angled (2); 6– to 8–angled (3); bilaterally compressed (4).

21. *Capsule angles.* In *Alophosia, Dawsonia, Lyellia, Meiotrichum,* and *Polytrichum* the capsules are sharply angled, particularly when old and empty. The longitudinal ribbing of *Pogonatum* spp. is treated as a distinct state. Capsule angles is treated as an unordered multi-state character with four states: none (0); blunt (1); sharp, knife-edged (2); ribbed (3).

22. *Capsule dehiscence.* All taxa in the data set have operculate capsules except *Andreaea,* where the capsule dehisces by four longitudinal slits. Capsule dehiscence is treated as a binary character: longitudinal slits (0); operculum (1).

23. *Exothecium.* Most of the taxa included in the analysis have a smooth exothecium i.e., the exposed face of the exothecial cell is essentially flat. In *Polytrichum commune* and in the fossil *Eopolytrichum* the exothecial cells are bulging convex, involving the full surface of the cell. In most species of *Pogonatum* the exothecial cells are papillose (with a single, papillar projection from the center of the cell, on an otherwise flat surface; Smith 1971, fig. 19, called mammillose in Hyvönen 1989). Exothecium is treated as an unordered multi-state character with three states: smooth (0); mamillose (1); papillose (2).

24. *Exothecial pitting.* The exothecial cells in *Polytrichum* have prominent, sharply defined "pits", which appear like holes or perforations in the center of the cell, an optical effect caused by the abrupt thinning of the wall from the inside (Ignatov & Merrill 1995). The exothecial cells of *Eopolytrichum* (Konopka et al. 1997, fig. 4) would also have appeared pitted in transmitted light. No other members of the family have this feature, nor do any of the outgroup taxa. *Meiotrichum* has the central portion of the exterior wall thinner, appearing as a diffuse "thin-spot" by transmitted light; thin-spots also occur in some *Polytrichastrum* species. Exothecial pitting is treated as an unordered multi-state character with three states: none (0); thin-spots (1); pitted (2).

25. *Apophysis.* A distinct constriction appears above the base of the capsule in *Eopolytrichum* and *Polytrichum,* delimiting a discoid apophysis. In other taxa [*Hebantia, Oligotrichum parallelum* (Mitt.) Kindb, *Polytrichastrum formosum* (Hedw.) G. L. Sm.] the basal portion of the capsule is clearly defined, but typically only moderately contracted. Apophysis is treated as an unordered multi-state character with three states: tapering (0); contracted (1); discoid (2).

26. *Stomata.* Stomata are present in all genera of Polytrichales, the exceptions being *Atrichum, Itatiella,* and *Pogonatum.* Hyvönen (1989) cited absence of stomata as a synapomorphy of the genus *Pogonatum,* and regarded their absence in the other two genera as an independent reduction. Among the outgroups, stomata are present in all but *Andreaea* and *Tetraphis.* Stomata is treated as a binary character: present (0); absent (1).

27. *Stomata type.* This character refers to the level of the guard cells in relation to the surrounding exothecial cells. Typically, these are flush with the surface (most Polytrichales, as well as the outgroups; Smith 1971). In *Atrichopsis, Notoligotrichum,* and *Buxbaumia aphylla* the guard cells are sunken and lie in a pit and are partially hidden by the overarching exothecial cells (Smith 1971). In *Polytrichum,* the stomata lie within the deep constriction at the base of the capsule, but the individual stomata are flush with the surface. Stomata level is treated as a binary character: superficial (0); cryptopore (1).

28. *Stomata extent.* In *Dendroligotrichum, Hebantia,* and some species of *Polytrichadelphus* [but not *P. magellanicus* (Hedw.) Mitt.], stomata are not restricted to the capsule base, but are dispersed over the capsule wall (Smith 1971). Stomata extent is treated as a binary character: restricted to base (0); dispersed (1).

29. *Peristome.* In most mosses some form of peristome is present. The question of homology between the various major types of peristomes ultimately hinges upon developmental studies as well as on the form of the mature peristome. In a series of papers (Shaw et al. 1987, 1989*a,b*) on development of moss peristomes, Shaw, Anderson and Mishler have developed the thesis that all moss peristomes arise from a common pattern of concentric peristomial layers, designated the IPL (inner), PPL (primary), and OPL (outer) peristomial layers, which constitute the innermost layers of the exothecium. Developmental studies of Polytrichales (*Atrichum, Pogonatum,* and *Polytrichum*) have been completed by these authors, but are as yet unpublished. *Alophosia, Bartramiopsis* and *Lyellia,* lack a peristome, as do the outgroups *Sphagnum* and *Oedipodium. Andreaea* is also scored as lacking a peristome. The fossil *Eopolytrichum* is scored as peristome present (0) and all other characters pertaining to teeth are scored as inapplicable. This follows the interpretation of Konopka et al. (1997, figs. 6–8) that *Eopolytrichum* had a polytrichoid "basal membrane" but lacked teeth. This should not be confused with the basal membrane of the arthrodont endostome, which is a different structure. Peristome is treated as a binary character: present (0); absent (1).

30. *Peristome type.* By definition, nematodontous peristomes are composed of whole cells, rather than consisting of adhering wall thickenings from adjoining cells as in arthrodontous mosses (*Funaria, Timmia*). More importantly, however, the "polytrichoid" peristome differs fundamentally from that of arthrodontous mosses in being composed of fascicles of horseshoe-shaped (or 'J'-shaped) cells, which arrive at their final shape by a process of symplastic growth and elongation similar to that of libriform fibers (Smith 1971). By contrast, peristome development in arthrodontous mosses is static, and does not involve growth.

The peristomes of *Dawsonia* and *Tetraphis* have been scored as two separate states. *Tetraphis* has a unique type of peristome consisting of four massive teeth. The cells of the outer "casing" layer(s) are slightly elongated longitudinally, suggesting a comparison with the peristomes of Polytrichales. The brush-like peristome of *Dawsonia* is also unique, but a homology with the polytrichoid peristome is likely due to what appear to be 'U'-shaped structures at the base of the bristles (Smith 1971). The peristomes of *Buxbaumia* and *Diphyscium* have been regarded as intermediate between arthrodont and nematodont types. Shaw et al. (1987) have, however, shown that the peristome of *Diphyscium* arises from the same basic pattern of cell layers as other arthrodonts, and these are scored accordingly.

Peristome type is treated as an unordered multi-state character with four states: polytrichoid (0); dawsonioid (1); tetraphid (2); arthrodont (3).

31. *Tooth structure.* Polytrichoid peristome teeth are of two types, "simple" and "compound" (Smith 1971). In the first of these, "a single, vertical divisural line bisects the mature tooth, the arms of the U-shaped cells to either side arching upward and inward towards it" (Smith 1971). Viewed from the outside, the compound tooth appears double, since the outlines of two teeth are visible on its face (Smith 1971). Compound teeth do not result from the fusion of teeth in pairs, but from the fact that the layer(s) of cells which form the outer face of each tooth are made up of twice the number of cells as those in the inner layers. Viewed from the inside, the teeth appear single. Simple peristome teeth may be 64 (*Polytrichum*) or 32 (*Atrichum*) in number. Compound teeth are usually 32 in number (some *Oligotrichum* spp., *Pogonatum*), but in *Polytrichastrum* peristomial development is sometimes irregular and the number of teeth variable, with at least some teeth double. In *Pogonatum* species, the median sinus on the face of the tooth is typically narrowed to almost obliterated. Tooth structure is treated as an unordered multi-state character with three states: simple (0); compound, sinus broad (1); compound, sinus narrow (2).

32. *Tooth number.* Peristome tooth number is treated as an unordered multi-state character with four states: 32 (0); 64 (1); 4 (2); 16 (3).

33. *Peristome pigmentation.* Hyvönen (1989) listed compound teeth with intense pigmentation as a synapomorphy of the genus *Pogonatum.* Other taxa with a deeply pigmented deep orange or reddish-orange peristome are *Dendroligotrichum* and *Hebantia,* but in both of these the teeth are simple. Peristome pigment is treated as a binary character: pale (0); intensively colored (1).

34. *Epiphragm type.* Members of most Polytrichales are distinctive in having a membranous epiphragm (tympanum) formed from the flared columella near the capsule mouth. In immature capsules the epiphragm is discoid and fleshy and typically shrinks to a thin membrane by the time the operculum is shed (Smith 1974). In taxa with a peristome, the rim of the epiphragm is either loosely (*Polytrichastrum*) or firmly attached (*Pogonatum, Polytrichum*) to the tips of the peristome teeth.

The capsule mouth in *Alophosia* and *Lyellia* is surrounded by a prominent broad disc. The "stopper"-like flared apex of the columella protrudes from a small circular opening at the center of the disc (Smith 1971; Konopka et al. 1997). In recently deoperculate capsules, the rim of the stopper is attached to the edge of the central opening in the disc; in *Bartramiopsis* (Konopka et al. 1997) it appears to be attached directly to the narrow capsule rim. *Eopolytrichum* (Konopka et al. 1997) has a broad, flared stopper that is strikingly similar to that of *Bartramiopsis.* These taxa were all scored as having an epiphragm of the "stopper" type.

In *Dawsonia longifolia* the operculum is greatly elongated and narrowly conic. In intact capsules the peristome bristles are spirally wound around a stout central rod which extends the full length of the operculum and is attached at the upper end. When the operculum is shed, the rod is withdrawn from the peristome "sleeve", and falls with the operculum. Epiphragm type is treated as an unordered multi-state character with four states: discoid (0); absent (1); stopper (2); cylindric (rod) (3).

35. *Capsule rim (disc).* The conspicuous, broad, indurated disc surrounding the capsule mouth in *Alophosia* and *Lyellia* is unique to these two genera. This structure was misinterpreted by Smith (1971), who regarded it as possibly homologous with the pad of tissue beneath the *Dawsonia* peristome. Examination of operculate capsules of *Alophosia* and *L. aspera* (I. Hagen & C. Jensen) Frye has since shown that the disc is actually the broad contact face between the operculum and the capsule proper. In other words, it corresponds to the narrow ledge-like space between the exothecium and the base of the peristome in other Polytrichales. *Bartramiopsis* is similar to *Alophosia* and *Lyellia* in having a broad "stopper" at the capsule mouth, but differs in the absence of a disc. Capsule rim is treated as a binary character: narrow (0); broad (disc) (1).

36. *Spore sac.* In most mosses the columella penetrates the archesporium and the resulting spore sac, so that the resulting spore sac is cylindric in form. In *Andreaea* and *Sphagnum,* however, the archesporium overarches the columella apex. Spore sac is treated as a binary character: overarching columella (0); cylindric (1).

37. *Spore origin.* In *Sphagnum,* spores originate from the exothecium, whereas in all other mosses they are endothecial in origin. Spore origin is treated as a binary character: from endothecium (0); from exothecium (1).

38. *Spore surface.* Variations in spore surface ornamentation in Polytrichales (seen with the SEM) were described by Smith (1974), who suggested these as a possible basis for major taxa within the family. In most genera, the spores are papillose. In *Polytrichastrum* these papillae take the form of rather tall and thick-set tubercules which are smooth or irregularly papillose at the apex (Smith 1974); in others (*Atrichum, Notoligotrichum*) the papillae are shorter and less distinctly papillose at the apex. In *Alophosia, Dawsonia, Lyellia,* and *Polytrichum* the spore surface is echinulate, with distinct, ± equally-spaced conic "Christmas tree"-like projections on an essentially smooth ground (Smith 1974). Hyvönen (1989) suggested that the smaller, echinulate spores of these genera might represent an apomorphic character state. The fossil *Eopolytrichum* has strikingly similar spores, 7–8 μm in diameter, with an echinulate surface (Konopka et al. 1997). *Bartramiopsis* has a unique type of spore surface ornamentation resembling a rough-plastered wall, with coarse, spheric bodies resting on the spore surface (Smith 1971). All the outgroup taxa have papillose spores except *Oedipodium* that has a spore surface with irregular, "corrugate" ornamentation (Dickson 1973) somewhat reminiscent of the spores of *Bartramiopsis.* Spore surface is treated as an unordered multi-state character with four states: papillose (0); echinulate (1); *Bartramiopsis*-type (2); *Oedipodium*-type (3).

39. *Brood-bodies.* The distinctive flattened-lenticular brood-bodies of *Alophosia* were described and illustrated by Smith (1971). These propagules are strikingly similar to the brood-bodies of *Oedipodium,* and somewhat less so to the "gemmae" of *Tetraphis.* Similarities with *Oedipodium* include the presence of distinct growing points (each consisting of an apical cell surrounded by its derivatives, on the edges, giving rise to leafy shoots). The brood-bodies of these three genera were treated as homologous. All other taxa in the data set lack any specialized means of vegetative reproduction. Brood-bodies is treated as a binary character: absent (0); present (1).

Gene sequences.—Total DNA was extracted from fresh, herbarium (oldest specimen 25 years) or silica-dried specimens. The extraction protocol used was either the slightly modified standard hot CTAB (Doyle & Dickson 1987) as described in Lewis et al. (1997) or the method given by Edwards et al. (1991). Specimens were ground using either a mortar and pestle, or in a 1.5 ml microcentrifuge tube using plastic micropestles. Liquid nitrogen was used for the hot CTAB method, while for the Edwards et al. (1991) protocol material was ground directly in 200–300 μl of the extraction buffer. After extraction, the resulting

pellet was dissolved in 100 µl of $2dH_20$, purified with the Wizard DNA Clean-Up Kit (Promega), and stored at −20°C.

Template DNA suitable for cycle sequencing was prepared via the PCR. Amplification was done using either the DynaZyme DNA Polymerase Kit (Finnzymes Oy) and Personal Minicycler (MJ Research) or with the *Taq* DNA Polymerase Kit (Promega) and DNA Thermal Cycler 480 (Perkin-Elmer). For the first alternative the programme comprised a 95°C initial melting step (2 min. followed by 35 cycles of 95°C melting (70 sec.), 50°C annealing (1 min.), and 72°C extension (80 sec.) with a final annealing step at 72°C for 7 minutes. Reaction volumes ranged from 30–100 µl. For the second alternative the cycling programme included a 97°C initial melting step (1 min.) followed by 30 cycles of 97°C melting (1 min.), 52°C annealing (1 min.), and 72°C extension (1 min.) with a final annealing step of 72°C for 7 minutes. All reactions were done in 100 µl volumes. 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. Sequences were visualized using ABI 373 or 377 automated sequencers. The PCR primers used for 18S were NS1 and B and the internal sequencing primers were ERC, G, H, KRC, and Q respectively (see Hedderson et al. 1998 for primer details). Primers rps5 and trnas were used both for PCR amplification and sequencing of rps4 (Cox & Hedderson 1998). For *rbc*L, primers M28 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 PCR reactions. The primer sequences for *rbc*L were provided by L. Lewis (Department of Biology, University of New Mexico) and S. Schaffer and B. D. Mishler (Department of Integrative Biology, University of California, Berkeley).

The individual sequencing products from different primer reactions were aligned as a composite strand using either programs of the Lasergene package (DNASTAR Inc.) or with the aid of Sequence Navigator version 1.0 (ABI, Perkin-Elmer). Five sequencing primers were used for 18S, four for *rbc*L and two for rps4. Portions of the completed sequence for each gene are therefore based on reads in only one direction and the extent to which this is true varies somewhat among taxa. Discrepancies between reads were solved manually by inspection of the original electropherograms. In doubtful cases, IUPAC ambiguity codes were assigned.

Sequence alignment was performed initially with the Clustal algorithm as implemented in the Sequence Navigator v.1 package of programs (ABI, Perkin-Elmer). The final alignment was done visually with the aid of Sequence Navigator using a color coded font. The protein coding genes rps4 and *rbc*L did not pose any serious alignment problems. Similarly the RNA-coding 18S gene was not particularly length-variable over the range of taxa in this study and thus was easily aligned. Some difficulty was experienced with aligning the non-coding region at the 3′ end of the rps4 sequences, so these positions were excluded from all taxa. Besides this, 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. The sequences obtained and used are indicated in Table 3. The complete data matrix used in the analyses is available from the first author upon request.

Analyses.—Parsimony analyses were conducted using PAUP* version 4.0d60 (Swofford 1997) and PAUP 3.1.1 (Swofford 1993). The heuristic search algorithm was employed with the options TBR and MULPARS in effect and using 300 or 1000 random addition replicates. In the analyses all characters were weighted equally with no distinction between transitions and transversions and no differentation between morphological characters of sporophyte and gametophyte generation. All morphological characters were treated as unordered. Traditionally more "weight" has been given to sporophytic characters in studies of moss phylogeny. However, we prefered to avoid additional assumptions which *a priori* character weighting necessitates (Kluge 1997). We performed simultaneous analyses (Nixon & Carpenter 1996) including data of all genes plus morphology based on inclusion of all taxa plus also analyses leaving out taxa with high proportion of missing entries. We performed also parsimony analyses based on three gene sequences. See below under Results for more detailed description of different analyses.

Different methods and indices have been proposed to study "reliability" or "strength" of different phylogenetic hypotheses included in each cladogram. To discuss pros and cons of these methods is beyond the scope of this paper; relevant discussion of the topic is provided for example by Carpenter (1992, 1996), Felsenstein and Kishino (1993), and Sanderson (1989, 1995).

We used two of the currently most widely used indices: bootstrap (Felsenstein 1985) and jackknife values (Farris et al. 1996). Bootstrap analysis was performed as implemented in the program PAUP* version 4.0d60 (Swofford 1997). The search strategy employed was "fast" stepwise-addition with 10,000 replicates and uninformative characters excluded. Jackknife values were calculated using the Parsimony Jackknifer version 4.22 (Farris 1995) using 10,000 replicates. The values for both indices are presented in Figure 4. The results of the jackknife analysis and bootstrapping indicate that the best support is given for only very few clades in addition to the trivial cases of species pairs of the same genera.

However, some authors have challenged the use of these metrics (Kluge 1997) altogether arguing convincingly that comparisons of the most 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.

Results

Our matrix included 3,808 characters. 1,835 nucleotides were obtained from the 18S sequences and 4% of these were phylogenetically informative. The figures for *rbc*L and rps4 were 1,354 (15%) and 580 (17%), respectively. The total number of phylogenetically informative sites from the sequence data were 383. Of the informative sites those from 18S account for 21%, from *rbc*L 53% and from rps4 26%.

We performed simultaneous parsimony analysis of the total matrix with all of the 30 taxa included with 1,000 random addition replicates. This analysis, based on 383 informative characters, yielded a

TABLE 3. The gene sequences available of each taxa for the analyses. The X in the first column (comp. = composite) means that sequences are not all from the same specimen. For taxa in boldface complete sequences were available. Cells with a small x mean that sequencing reactions for a particular primer failed, but the data were available from the reactions of the adjacent primers.

		Gene	18s					*rbc*L				rps4	
Comp.	Taxa	Primers	ERC	G	H	KRC	Q	M28	M740r	M1010r	M1390r	rps5	trnas
	Alophosia azorica		X	X		X	X	X	X	X	X		
	Atrichum angustatum		X	X	X	X	X	X	X	X	X	X	X
	undulatum		X	X	X	X	X						
	Bartramiopsis lescurii				X	X	X	X	X	X	X	X	X
	Dawsonia papuana		X	X	X	X	X	X	X	X	X	X	X
	Dendroligotrichum dendroides		X	X	X	X	X	X	X	X	X	X	X
	Itatiella ulei		X		X			X	X	X	X	X	X
	Lyellia aspera		X	X	X	X	X	X	X	X	X	X	X
	Meiotrichum lyallii			X			X	X	X			X	X
	Notoligotrichum australe		X	X	X	X	X	X	X	X	X		
	Oligotrichum parallelum					X	X	X	X	X	X	X	X
	Pogonatum contortum			X	X							X	X
	Pogonatum urnigerum		X	X	X	X	X	X	x	X	X	X	X
	Polytrichadelphus magellanicus		X	X	X	X	X	X	X	x	X	X	X
X	*Polytrichastrum formosum*		X	X	X	X	X	X	X	x	X		
	Polytrichastrum longisetum							X	X	X	X		
X	***Polytrichum commune***		X	X	X	X	X	X	X	X	X	X	X
	Psilopilum laevigatum		X	X	X	X		X	X	X	X	X	X
	Andreaea rupestris		X	X	X	X	X	X	X	X	X		
X	***Buxbaumia aphylla***		X	X	X	X	X	X	X	X	X	X	X
X	***Diphyscium foliosum***		X	X	X	X	X	X	X	X	X	X	X
X	***Funaria hygrometrica***		X	X	X	X	X	X	X	X	X	X	X
	Oedipodium griffithianum		X	X	X	X	X					X	X
X	*Sphagnum palustre*		X	X	X	X	X	X	X	X	X		
X	***Tetraphis pellucida***		X	X	X	X	X	X	X	X	X	X	X
	Timmia sibirica		X	X	X	X	X					X	X

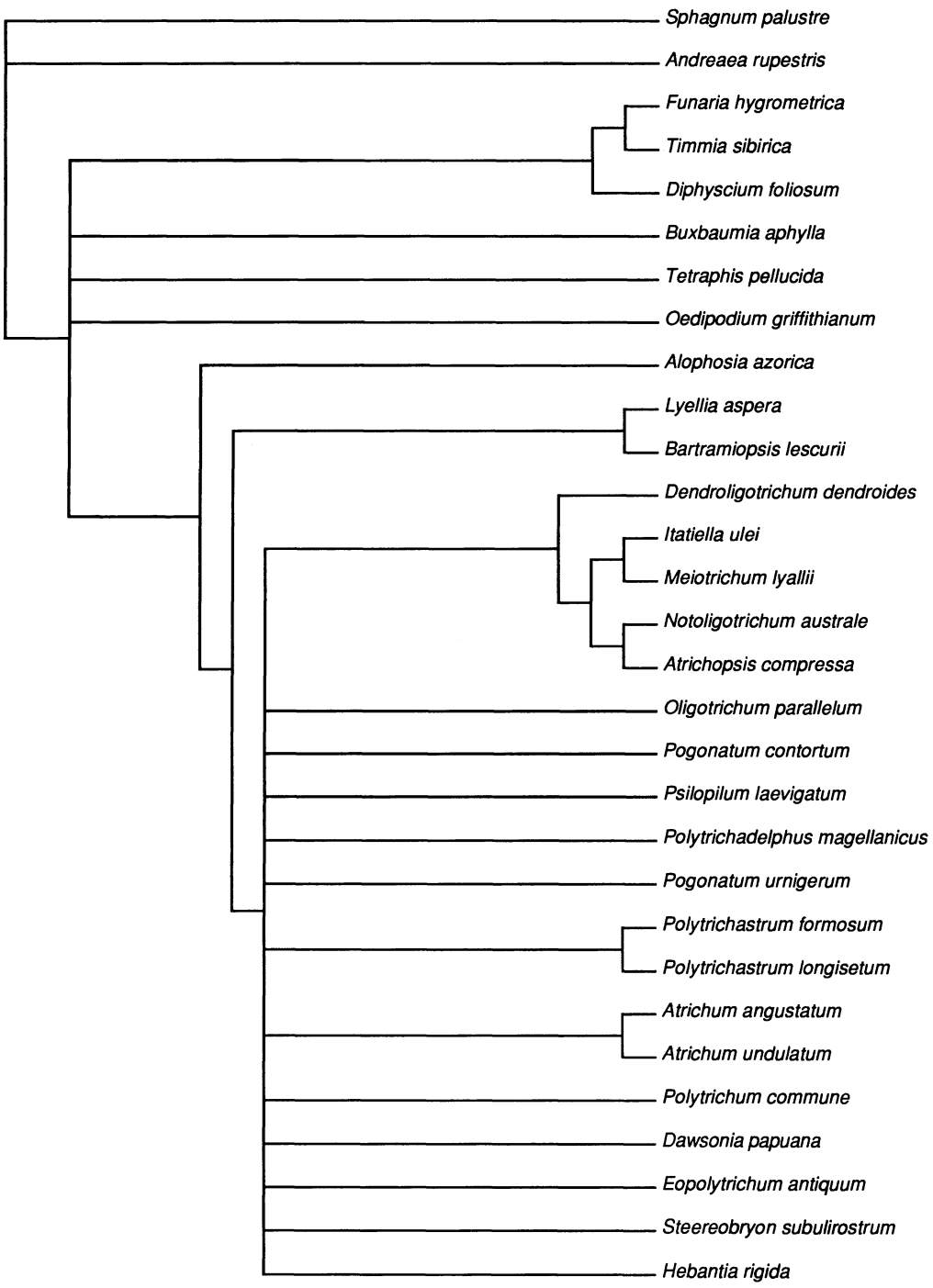


FIGURE 2. The strict consensus tree based on 228 minimum length trees of 1,232 steps, with a consistency index (CI) of 0.459 and retention index (RI) of 0.478 (uninformative characters excluded) based on parsimony analysis of the total matrix.

single island of 228 minimum length trees of 1,233 steps, with a consistency index (CI) of 0.460 and retention index (RI) of 0.478 (uninformative characters excluded). The strict consensus tree based on this set of trees is illustrated in Figure 2. After one round of successively approximated weighting (Farris 1969) the number of equally parsimonious trees was reduced to seven. The number of trees did not change after the second round of weighting, nor did their length after the third round. The seven

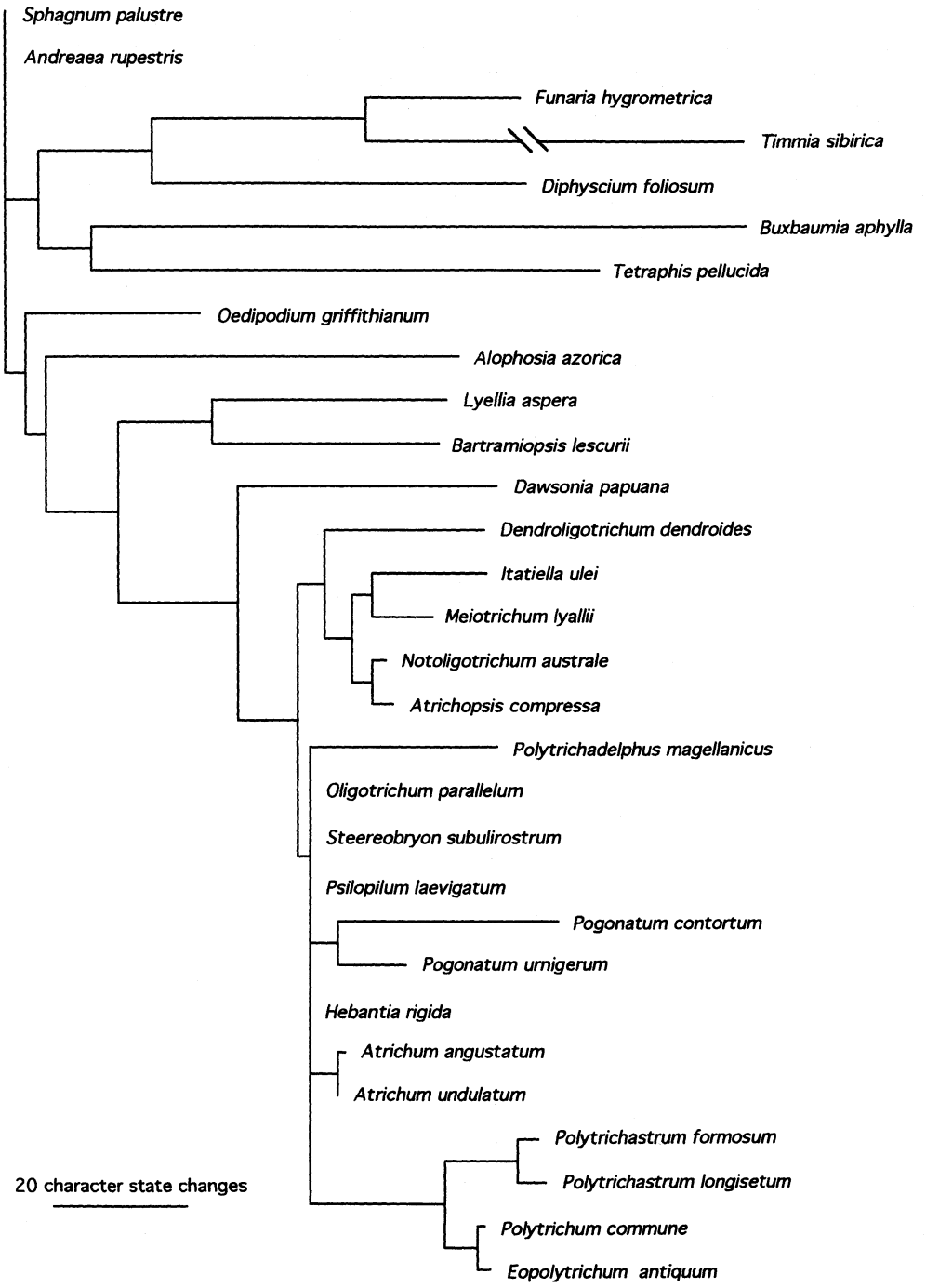


FIGURE 3. The strict consensus tree based on seven trees obtained from the analysis of 30 taxa after successively applied weighting. Branch lengths proportional to character state changes except for highly autapomorphic *Timmia sibirica*.

trees represent a subset of the original 228 trees and the strict consensus based on them is illustrated in Figure 3.

The large proportion of missing values in parsimony analysis is a problem that has been dealt with in several papers dealing with fossils and their importance for phylogenetic analysis (e.g., Donoghue et al. 1989; Novacek 1992; Wilkinson 1995). Our material includes a large proportion of missing entries for taxa where we had only morphological

data (or part of it in the case of the fossil *Eopolytrichum antiquum*). We have no doubt that we will be able to obtain sequence data for the three extant taxa currently lacking this information. Prospects for obtaining there data for the fossil, *E. antiquum* are, however, meagre and therefore the existence of a problem with many missing entries will continue to be a problem in future phylogenetic analyses of the Polytrichales.

Unfortunately our matrix did not include any taxa that could be safely removed according the rules presented by Wilkinson (1995). Other strategies have also been proposed (e.g., Rowe 1988) but as discussed by Novacek (1992) and Wilkinson (1995) these do not offer safe rules for exclusion of taxa. Despite all these uncertainties we wanted to explore what would be the effect of removing the four taxa represented only by morphological data. The parsimony analysis of 26 taxa resulted in 32 equally parsimonious trees. After three rounds of successively approximated weighting the analysis yielded one most parsimonious tree. It is almost identical with the strict consensus tree based on the successively weighted set of seven trees that resulted from the analyses of 30 taxa. The phylogenetic position of the four taxa (except *Steereobryon subulirostrum*) represented only by their morphology appears to be unambiguous, despite the large number of missing entries. The differences regarding the topological position of taxa in the results of these analyses are illustrated in Figure 4.

Parsimony analyses of 18S sequences from of 25 taxa yielded 5,668 minimum length trees, of *rbc*L sequences from 22 taxa – 17 trees, and the one based on rps4 sequences from 19 taxa 66 equally parsimonious trees. The analysis based on 39 morphological characters of 30 taxa resulted in 57 minimum length trees. The strict consensus trees from each of these analyses are given in Figures 5–7. The strict consensus of the 66 rps4 trees is totally without resolution and therefore it is not illustrated.

Discussion

Our analysis provides substantial support for the monophyly of the Polytrichales, with *Oedipodium griffithianum* (formerly included in the Bryales) as a sister-taxon. At the same time, the earlier division of the order into two families, Dawsoniaceae and Polytrichaceae (e.g., Brotherus 1925) is unacceptable and this strengthens the views presented by Smith (1971). *Alophosia azorica* appears to be sister to all other Polytrichales. With the species of *Bartramiopsis, Lyellia,* and finally *Dawsonia* and the rest of Polytrichales we have a full series of more elaborate development of photosynthetic adaxial lamellae from the species without them, *A. azorica,* to species with numerous, high lamellae with specialized enlargened apical cells having an ornamented outer wall (e.g., *Dendroligotrichum*). The lack of adaxial lamellae is a feature that distinguishes *A. azorica* from other Polytrichales and it is most parsimonious to interpret the current results so that this species never had them while in some other taxa such as *Atrichopsis compressa* or some species of *Pogonatum* they are lacking due to reduction.

Leaf margins in the Polytrichales vary from essentially entire to distinctly toothed. Taxa with sharp, unicellular teeth by the blade margins do not form a monophyletic group and therefore one must assume that this type of teeth was developed independently at least three times. However, it is possible that sharp unicellular teeth seen in some species of *Pogonatum* are homologous with the teeth in *Atrichum, Polytrichastrum,* and *Polytrichum.* Species such as *Pogonatum volvatum* (C. Müll.) Par. and *P. japonicum* Sull. & Lesq. with large unicellular teeth by their blade-margins (dentate margins) were postulated to be among the most basal members of the genus by Hyvönen (1989). However, contrary to assumptions made by Hyvönen (1989) it seems now that this kind of dentation might be a plesiomorphic feature in these taxa.

Polytrichales also show variation in thickness of leaf margins, but this seems to be a highly homoplasious character. One can assume that thick margins of the basal most three genera (*Alophosia, Bartramiopsis,* and *Lyellia*) are homologous but other occurrences of this feature in *Atrichum, Dendroligotrichum* and also some species of *Pogonatum* seem to have evolved independently.

The hairy calyptra, a structure typical for the Polytrichales, is present in three different groups. It seems that the calyptra of *Alophosia,* with both uni- and multiseriate hairs, predates those with exclusively multiseriate (*Dawsonia*) or uniseriate hairs (*Polytrichum* and allies). At the moment it is more parsimonious to assume the independent origin of profusely hairy calyptrae three times rather than to assume them to be homologous and that the development of hairs has been suppressed in taxa with naked or sparsely hairy calyptrae. It is, however, evident that further, more elaborate studies are needed to determine homologies and ontogeny of the different hair-like appendices of the calyptrae.

It is also evident from our results that peristomes of all mosses are not homologous structures despite their positional similarity. The difference between alternative scenarios is, however, only two steps. The lack of a peristome in all the basal Polytrichales appears to be a primitive feature, and its development in more "advanced" Polytrichales and in other mosses can be interpreted as parallellism. Given the

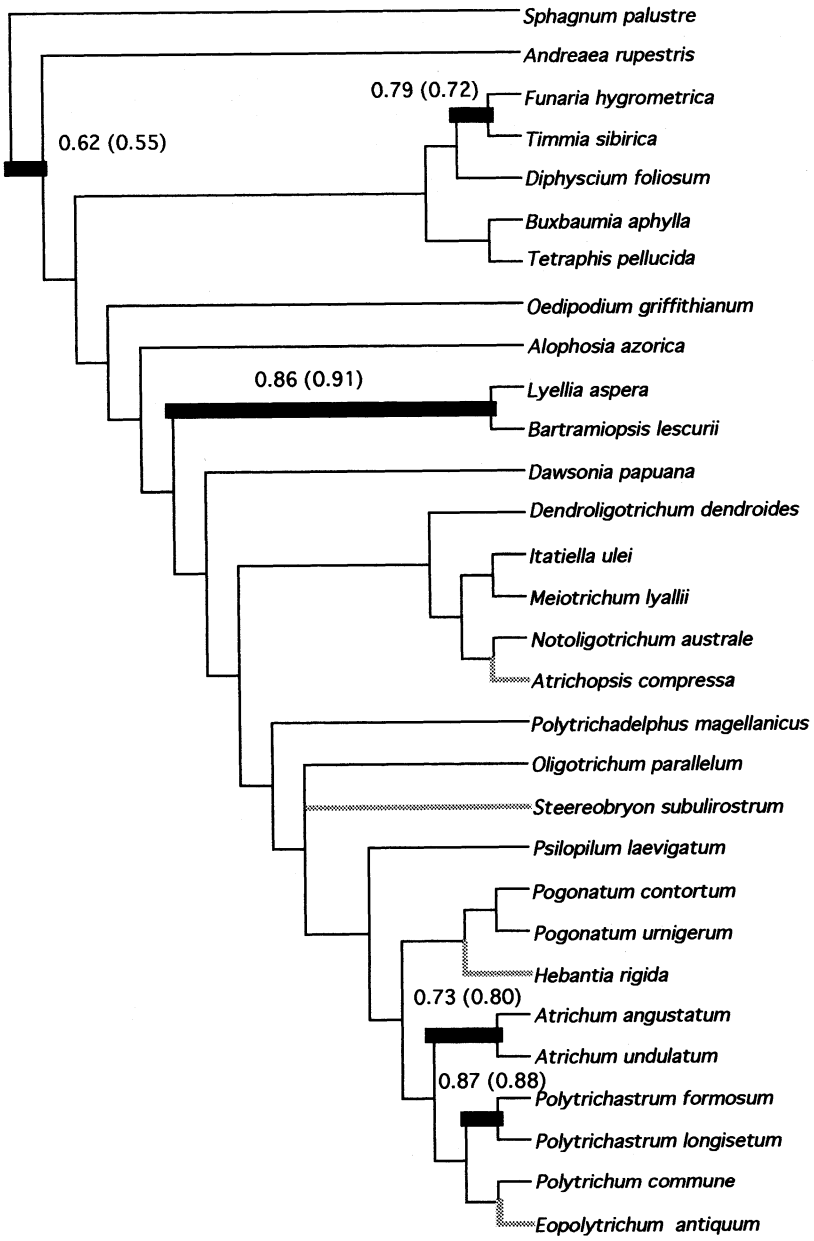
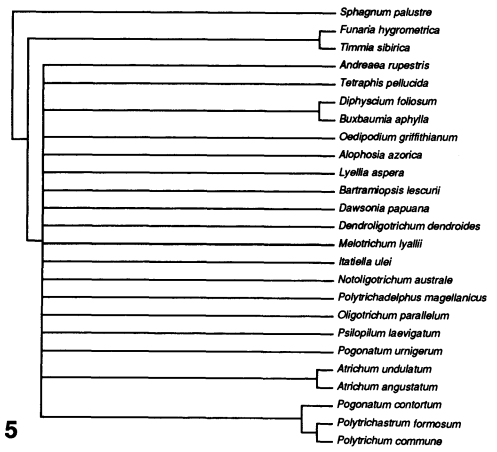
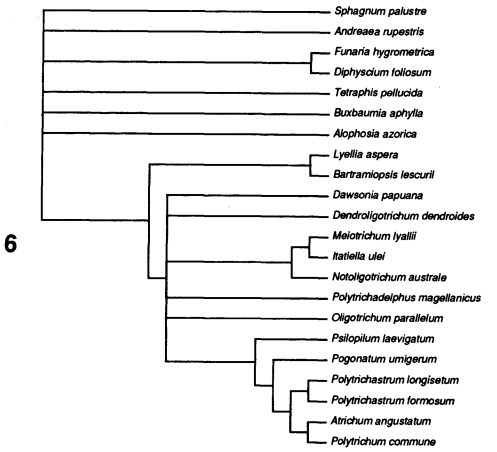
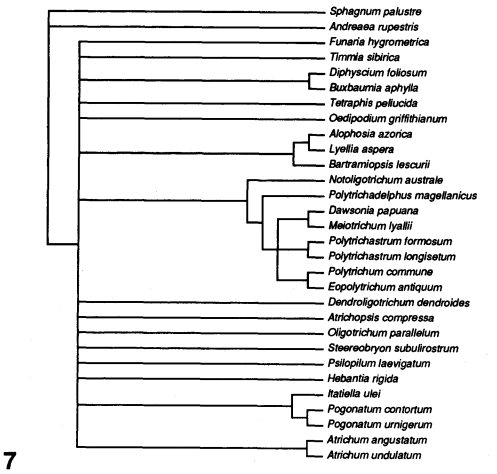


FIGURE 4. The single most parsimonious tree after successively applied weighting based on the analysis of 26 species. Shaded branches indicate positions of the four species based on the analysis including all species. Thickened branches imply bootstrap and/or jackknife values for the clades that exceed 50%. The values are based on the analysis of all species, indices are given above the branches; those for bootstrap in parentheses.

position of *Dawsonia* as sister to the remaining peristomate species in most of our analyses, it appears that the bristle-like teeth of the peristome in this genus are not deformed from the basic polytrichoid peristome, but represent an independent evolutionary innovation, though it is more parsimonious to assume that they share a common ancestor, one in which growth and elongation of the peristomial initials was involved. The basal most Polytrichales possess a prominent disc surrounding the capsule mouth (lacking in *Bartramiopsis*) and all three have a broadly flaring, stopper-like epiphragm. The intensive coloration of peristome teeth which supposedly unites *Dendroligotrichum* and *Hebantia* plus *Pogonatum* (Hyvönen 1989; Merrill 1996) seems to be due to parallel evolution and is not inherited from a common ancestor, since these two groups appear to be quite unrelated to each other.







FIGURES 5–7. Strict consensus trees based on separate parsimony analyses of gene sequences and morphology.—5. The strict consensus tree based on 5 668 ML trees obtained from analysis of the 18S sequences of 25 taxa.—6. The strict consensus tree based on 17 ML trees obtained from analysis of the *rbc*L sequences of 22 taxa.—7. The strict consensus tree based on 57 ML trees obtained from analysis of the 39 morphological characters of 30 taxa.

The weakly differentiated, tapering, basal part of the capsule is typical for all the outgroup taxa and most of the Polytrichales as well. The contracted apophysis seems to have originated several times independently. A discoid apophysis, on the other hand, has originated only once and it is a synapomorphy uniting *Eopolytrichum* and *Polytrichum.*

Stomata are present in almost all outgroup taxa. In the Polytrichales they are present in all genera but *Atrichum, Itatiella,* and *Pogonatum.* Hyvönen (1989) postulated that reduction of stomata has taken place independently in these three genera—they do not form a monophyletic group and our results lend support for this view.

Whether the brood-bodies in *Alophosia, Oedipodium,* and *Tetraphis* are homologous remains to be seen. At the moment it seems that in the latter two genera they may be homologous. It is, however, equally parsimonious to assume that they have developed independently as if they were inherited from the common ancestor of *Alophosia* and *Oedipodium* and then subsequently lost in other Polytrichales, a view already presented by Smith (1971). The position of *Oedipodium* remains ambiguous; in our analyses it was either resolved as sister to the Polytrichales, or was unresolved. In part this results from the fact that one whole gene region (*rbc*L) was unsampled for this taxon, and some morphological characters (protonema, many sporophyte characters) were also not available.

At the moment it seems that none of the morphological characters is a very good indicator of phylogeny i.e., all of them show considerable homoplasy. In the seven trees resulting from successive weighting the consistency index based on informative morphological characters is only 0.417. This is lower than the value of the ensemble CI for the whole data set, however, not by a very wide margin. The combined simultaneous analysis provided results that would have been unexpected based solely on morphology and this is quite evident when we compare the cladograms of Figures 3 and 7. For example, the close relationship of *Atrichum* and *Polytrichum* is something that one would not have expected based on their morphological dis-similarities. Equally surprising is that plants such as *Dawsonia, Polytrichadelphus* and *Polytrichum,* all with large, well-developed gametophytes and leaves with differentiated hinge-tissue and numerous adaxial lamella with specialized marginal cells appear to be quite unrelated to each other.

When we examine the current geographical distributions of the taxa it is evident that no clear biogeographic pattern emerges from the current results. There is a clade of taxa with their distribution exclusively restricted to the southern hemisphere

with the taxonomically problematic *Meiotrichum lyallii* being the only exception among them.

The virtue of simultaneous (Nixon & Carpenter 1996) or total evidence analysis as compared to partitioning of data has been explored both with simulations (Hillis 1996) and empirically (Chase & Cox 1998; Soltis et al. 1997). The theoretical advantages over the other alternatives have been equally well presented (e.g., Brower et al. 1996; Kluge & Wolf 1993). Our results are congruent with these findings. None of the gene sequences alone was able to resolve relationships of the genera to the same extent as the simultaneous analysis of them all. The number of most parsimonious trees obtained when the rps4 and morphological data were analyzed separately was much lower than the one obtained from the simultaneous analysis, but the results cannot be considered reliable because of the small size of the data matrix regarding either number of sampled taxa or characters.

Although the data set that we analyzed here is limited in some respects (e.g., parts of the data are missing for several taxa), it provides resolution of several features of Polytrichalean phylogeny and suggests how others might eventually be resolved. We are confident that as our sampling of both taxa (especially the larger genera such as *Atrichum, Pogonatum, Polytrichastrum,* and *Polytrichum*) and characters continues to improve much new light will be shed on the evolution of this intriguing group of mosses.

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APPENDIX 1. The morphological matrix used in the analysis. Polymorphism (0, 1) marked with m, (0, 2) with r, (0, 3) with w and (0, 4) with z. - = Inapplicable character; ? = character state unknown.

Taxon	0000000001 1234567890	1111111112 1234567890	2222222223 1234567890	333333333 123456789
Alophosia azorica	001010011-	1--00m0001	210000001-	---211011
Atrichopsis compressa	011010001-	1--0020004	1100001000	000001000
Atrichum angustatum	0110111m01	0000020000	010001--00	000001000
Atrichum undulatum	0110111101	0000020000	010001--00	000001000
Bartramiopsis lescurii	0111100101	1000020000	010000001-	---201020
Dawsonia papuana	0010010000	0100010001	2100000001	--0301010
Dendroligotrichum dendroides	10101m0100	1000020000	0100000100	011001000
Eopolytrichum antiguum	?0?0000000	0100???000	0112200000	---201?10
Hebantia rigida	01101m0001	0000020000	0100100100	001001000
Itatiella ulei	0110100000	0000020000	010?01--1-	---001000
Lyellia aspera	0010100101	1r00020001	210000001-	---211010
Meiotrichum lyallii	m000010000	0110020002	2101100000	010001000
Notoligotrichum australe	0000120000	011002000z	1100001000	000001000
Oligotrichum parallelum	0110100001	0000020000	0100100000	100001000
Pogonatum contortum	0110100000	000000000w	312001--00	201001000
Pogonatum urnigerum	m000000000	011000000w	w12001--00	201001000
Polytrichadelphus magellanicus	0000000000	0100020001	1100000000	010001000
Polytrichastrum formosum	0000010000	0000000002	1100100000	010001000
Polytrichastrum longisetum	0000010000	0000000002	1100000000	m10001000
Polytrichum commune	0000010000	0100000002	2112200000	010001010
Psilopilum laevigatum	0110120001	0000020000	0100000000	000001000
Steereobryon subulirostrum	0110100001	0000020000	0100000000	000001000
Andreaea rupestris	011012001-	0--1021100	000001?01-	---1-0000
Buxbaumia aphylla	0--110001-	0--?020001	11??001?03	-30101000
Diphyscium foliosum	011012001-	0--?020001	110?000?03	-30101000
Funaria hygrometrica	011010001-	0--0020000	010?00??03	-30101000
Oedipodium griffithianum	011112001-	0--?020000	010?00?01-	---20?031
Sphagnum palustre	211012001-	0--01-11-0	0100000?1-	---100100
Tetraphis pellucida	011012001-	0--0020000	010001??02	-20301001
Timmia sibirica	001010001-	0--0020000	0100000003	-30101000