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*POGONATUM*  
(POLYTRICHALES,  
BRYOPHYTA) REVISITED<sup>1</sup>

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ABSTRACT

*Pogonatum*, with over 50 species, is the largest genus of the order Polytrichales with four currently distinguished subgenera. The lack of stomata, a deeply pigmented peristome, and papillose exothecium characterize all species that otherwise vary greatly by their size and overall morphology. According to the recent cladistic analyses of the Polytrichales, *Pogonatum* is among the youngest lineages of the order together with *Polytrichastrum*, *Polytrichum*, and *Eopolytrichum*. Phylogenetic analyses of *Pogonatum* were conducted using morphology and sequence data from the chloroplast *rps4* and the *trnL-F* gene regions, plus the nuclear-encoded ITS2. Our analyses included all known species of the genus (16 of them represented only by morphology) plus 18 species from other polytrichalean genera used as outgroups. Phylogenetic trees were constructed with simultaneous parsimony analyses of all sequences, plus morphology. Analyses were performed on a reduced data matrix with ambiguously aligned regions deleted, and using direct optimization using all available data. Our results confirm inclusion of *Pogonatum sinense* and species of *Racelopus*-group (*P. camusii*, *P. iwatsukii*, *P. marginatum*, *P. misimense*, *P. neo-caledonicum*, *P. petelotii*, *P. philippinense*, *P. piliferum*, *P. rutteri*) within *Pogonatum*. Our results, based on the combined data, do not lend support for a division of the genus into four lineages (e.g., subgenera), although some of the species groups are similar to those resolved based on morphological characters alone.

*Key words:* cladistics, direct optimization, moss, phylogeny, *Pogonatum*, Polytrichaceae.

The number of currently accepted genera in the Polytrichales is 19, and they accommodate about 200 species (Hyvönen et al., 1998). One affined genus (and its sole species), *Eopolytrichum antiquum* Konopka, Herendeen, Merrill & Crane, is known only as a fossil from the late Cretaceous (Konopka et al., 1997). Seven of the remaining genera are monotypic, and all the others, with exception of *Polytrichum* Hedw. (ca. 30 species.) and *Pogonatum* P. Beauv. (ca. 50 species), are fairly small. Polytrichales are typically pioneer plants of open, sometimes even dry, habitats. They are largely absent from extremely arid regions, however, and the group exhibits its greatest diversity in areas with humid or moist subtropical and tropical climates (e.g., Hyvönen, 1986, 1989; Gradstein et al., 2001). Polytrichales are probably among the first lineages that diverged from the common ancestor to all mosses (e.g., Mishler & Churchill, 1984; Newton et al., 2000). Earlier cladistic analyses of the group are based on morphology (Hyvönen, 1989; Forrest, 1995); more recent ones also used sequences from the chloroplast genic regions *rbcL*, *rps4*, *trnL-trnF*, ribosomal RNA

gene18S, and mitochondrial *nad5* (Hyvönen et al., 1998, 2004). According to these recent cladistic analyses, *Pogonatum* is among the youngest lineages of the order together with *Polytrichastrum* G. L. Sm., *Polytrichum*, and *Eopolytrichum*.

The genus *Pogonatum*, originally with 15 species, was separated from the complex genus *Polytrichum* by Paulisot de Beauvois (1804). The genus was gradually broadened by several authors, especially Müller (1848), Dozy and Molkenboer (1854–1870), and Mitten (1859, 1869); now it is the largest genus of the order Polytrichales.

The lack of stomata, a deeply pigmented peristome, and mammillose exothecium characterize all species that otherwise vary greatly by their size and overall morphology. Species of *Pogonatum* are epigeic plants confined to more or less disturbed and open habitats. Some species, such as Southeast Asian *P. neesii*, may even be called weeds. It is an aggressive and successful colonizer of open habitats such as roadsides. Consequently, most species of the genus can be assumed to benefit from human disturbance (Fagerstén, 1977).

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Hyvönen (1989) conducted a manual Hennigian analysis based solely on morphology, proposing a division of the genus into four subgenera: *Alienum* Hyvönen, *Dendroidea* (Schimp.) Hyvönen, *Catharinella* (Müll. Hal.) Hyvönen, and *Pogonatum*. In this study we make phylogenetic inferences based on morphology and sequence data from the chloroplast gene *rps4* and the *trnL-trnF* gene region and nuclear-encoded ITS2. These genic regions were chosen based on the results obtained in our former studies of the whole family (Hyvönen et al., 1998, 2004) plus studies of other moss families (e.g., Huttunen & Ignatov, 2004; Quandt et al., 2004). The three gene regions have proved to be variable at and below genus and species level. Our analyses included all species of the genus *Pogonatum* (16 of them represented only by morphology) plus 18 species from other polytrichalean genera used as outgroups. The choice of the outgroup is based on previous analysis of the whole order. In our earlier study, the genus *Pogonatum* was not resolved as a monophyletic lineage but rather as a paraphyletic group grading into *Polytrichum* (Hyvönen et al., 2004). Here we aim to resolve the phylogeny of the most diverse and species-rich apical clade within the Polytrichales.

#### MATERIALS AND METHODS

Our morphological matrix includes 47 characters, and its compilation is based on our earlier analyses of the genus (Hyvönen, 1989) and the whole order (Hyvönen et al., 1998, 2004), plus separate, partly unpublished studies of individual species. This data set is based on an extensive study of specimens from several herbaria during 20 years and includes also the actual DNA voucher specimens. The data matrix and list of characters can be found in Appendices 1 and 2. All species and subspecies included in *Pogonatum* by Hyvönen (1989) and *P. sinense*, a species transferred to *Pogonatum* by Hyvönen and Wu (1993), are included in this analysis. We consider *P. subfuscatum* Broth., a poorly known Chinese species, to belong to *P. contortum*, and thus it is not included. Disagreement also exists on the number of species in Central America. Smith Merrill (1994) recognized 10 species compared to 6 accepted by Hyvönen (1989), with the latter treatment followed here. It should be noted, however, that a separate study with wider population-level sampling is warranted for these species.

DNA was isolated from 58 specimens of *Pogonatum* (representing 56 taxa) and 18 outgroup specimens. The material was either fresh and silica-

dried or originated from the herbarium specimens (see Table 1). We were not able to get sequences of all three loci for all specimens (see Table 1) despite repeated trials. *Pogonatum pensilvanicum* is represented by three terminals for this reason; collections from Brazil (JH6393) and the United States, from Alabama (BG5266) and North Carolina (JWH1843), were included in the analyses (see Table 1). Sequence-level data is totally lacking for 18 species (or subspecies) of *Pogonatum*, plus naturally for the fossil *Eopolytrichum antiquum* that is included in the outgroup.

Three genic regions were chosen for the study: (1) the nuclear ribosomal DNA (rDNA) ITS2, (2) plastid small ribosomal protein 4 (*rps4*), and (3) partial region of the genes for tRNA-leucine and tRNA-phenylalanine, hereafter as *trnL*, including one intron and intergenic spacer encoded by the chloroplast genome.

Two different methods were used to extract DNA (Hyvönen et al., 1998), using DNeasy Plant Mini Kit for DNA isolation from plant tissue (QIAGEN, Valencia, California, U.S.A.).

ITS2, *rps4*, and *trnL* sequences were amplified by the polymerase chain reaction (PCR). PCR procedures for *rps4* and *trnL* are given in Hyvönen et al. (1998). The procedure for ITS2 followed an initial denaturation 94°C for 10 min. to activate *AmpliTaq* Gold DNA polymerase (Perkin-Elmer, Norwalk, Connecticut, U.S.A.), then 35 cycles of denaturation (30 sec. at 95°C), annealing (1 min. at 60°C), and extension (2 min. at 72°C). Primers for ITS2 were ITS3 (forward) and ITS4 (reverse) (White et al., 1990).

PCR and cycle sequencing were done in a Perkin-Elmer 9700 GeneAmp PCR system. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. Sequencing reactions were prepared using the BigDye Terminator Cycle Sequencing Reaction Kit v. 2.0 (PE Applied Biosystems, Foster City, California, U.S.A.) and run on the ABI PRISM 377 DNA sequencing sequencer (Perkin-Elmer). Complementary strands were combined using programs of the Lasergene package (DNASTAR, Inc., Madison, Wisconsin, U.S.A.). All sequences were compared to GenBank, EMBL, and DDBJ databases using BLAST for detection of mistakenly amplified amplicons and, especially in the case of ITS2, to avoid fungal sequences.

Sequence alignment was performed initially with the program ClustalX (Jeanmougin et al., 1998) with default parameters, and sequences were subsequently refined manually in order to maximize positional homology of nucleotides between differ-

ent terminals. For ITS2, this resulted in a matrix of 661 nucleotides and for *trnL* a total length of 665 nucleotides (the original alignments are available from the authors upon request).

The chloroplast gene *rps4* did not pose alignment problems (the AT-rich indel at the 3' was not included in the analysis). The *trnL* gene regions, as well as the nuclear spacer ITS2, however, show considerable length variation and therefore alignment is problematic. There are basically two ways to treat ambiguous alignments of sequences. It has been proposed that all such regions should be excluded from the analyses, a procedure that leads to ignoring part of the data. The logic behind this is, however, that there are portions of the sequences for which we can never be sure (at least with the current technology) that we have found the optimal alignment (i.e., that aligned characters are homologous). If we adopt this approach for the current material, we have to ignore most of the ITS2 region and also a large stretches of the *trnL* sequences. Of the ITS2 only the first 111 nucleotides were aligned unambiguously, but this region was so homogeneous that it included only one parsimony informative site. Of the aligned *trnL* matrix with the length of 665 nucleotides, unambiguous alignment was obtained only for the positions 1–129, 150–194, 345–476, 544–590, and 608–665. Altogether the size of the whole matrix was  $76 \times 1142$  cells for all four partitions. Of the total 1142 characters, 203 were informative and the percentage of informative sites in different partitions is given in Table 2. This data matrix has been submitted to TreeBASE (<http://www.treebase.org>). For the parsimony analyses we used the program Nona (Goloboff, 1993) in conjunction with a Winclada shell (Nixon, 2002). Jackknife support values (Farris et al., 1996) for the nodes were calculated using the program Xac (Farris, 1997).

For the above mentioned analyses we used only part of the available data. Whether sequences showing length variation (i.e., those where assumptions about homology can be challenged) can or should be used in phylogenetic reconstruction is a matter of disagreement. It is quite obvious that these regions, in the same way as reduction and emergence of certain morphological features, can provide valuable information about phylogeny. But how to get hold of this information at sequence level when we have only four (five; A, C, G, T, or a gap) character states that are identical for each character? Homology statements are obviously much more difficult to make than with morphology. It is clear that fast algorithms, for example, those implemented in Clustal and similar programs, do

not evaluate alternative alignments; or if this is done, it is performed only to an extremely small extent as compared to all possible alternatives (Slowinsky, 1998). Different solutions to the problem of ambiguous alignment have been proposed (e.g., Lutzoni et al., 2000; Simmons & Ochoterena, 2000), but either they are not implemented in the programs or the programs where they are included are able to handle only a very limited amount of information. These restrictions do not, however, apply to direct (DO, Wheeler, 1996) and fixed states (FSO, Wheeler, 1999) optimizations. Both of these approaches are implemented in the program POY (Wheeler & Gladstein, 2003), and the limit for the number of taxa or length of the sequences that the program is able to process has not yet been reached. This approach has already been used in many large scale phylogenetic analyses (e.g., Gribet et al., 2001). It does not require manipulation of the primary data before the actual analysis (alignment and phylogenetic analyses are done simultaneously). So, there is no need for the special repository for the analyzed matrices, and it is enough to submit sequences used to GenBank. Ideally intact contiguous sequences can be used, but because the alignment is a computationally demanding task, it is advised to cut sequences into smaller fragments to speed up the procedure. When contiguous sequences are cut, assumptions about homology between different sequences are restricted to smaller areas, which leads to large reductions in computational effort. This should, however, be done with great caution in order to avoid unwarranted and premature assumptions about non-homology. It is advisable to cut sequences within regions that are identical in all terminals, i.e., primer areas or other such highly conserved regions. We did this based on preliminary alignment obtained with ClustalX for ITS2 and *trnL*. We were able to cut ITS2 in two and *trnL* in seven fragments. In the ITS2, the first 111 nucleotides of the Clustal alignment included only one informative position, and it was included in the analysis as a single separate character together with the morphological matrix. Direct optimization was used for the rest of the ITS2 region.

We performed four analyses with POY. All these analyses were made with the regions included in the Nona analyses as prealigned and the problematic regions submitted for direct optimization. Three analyses included 76 terminals while the fourth one included only one composite terminal instead of three for *P. pensilvanicum* (see discussion below). These analyses were performed using the IBMSC parallel supercomputer, located at CSC–

Table 1. Vouchers for taxa sampled in analysis, followed by GenBank accession numbers for DNA sequences. All specimens in H if not stated otherwise.

Ingroup	Taxon	Collection reference	GenBank accession No.		
			<i>rps4</i>	<i>trnL</i>	ITS2
	<i>Pogonatum aloides</i> (Hedw.) P. Beauv.	Sweden. Skåne, <i>Hyvönen 6486</i>	AY137689	AF545016	—
	<i>P. belangeri</i> (Müll. Hal.) A. Jaeger	Tanzania. Tanga Province, <i>Laaka 298</i>	AY396517	AY396499	AY396474
	<i>P. brachyphyllum</i> (Michx.) P. Beauv.	U.S.A. Georgia, <i>Shaw 8304</i>	AY396515	AY396497	AY396473
	<i>P. campylocarpum</i> (Müll. Hal.) Mitt.	Brazil. São Paulo, <i>Hyvönen 6392</i>	AY137690	AF545017	AY396455
	<i>P. cirratum</i> (Sw.) Brid. ssp. <i>cirratum</i>	Taiwan. Taichung, <i>Hyvönen 4008</i>	AY137691	AF545018	AY396448
	<i>P. cirratum</i> ssp. <i>fuscatum</i> (Mitt.) Hyvönen	China. Hunan, <i>Koponen 49039</i>	—	AY396494	AY396470
	<i>P. cirratum</i> ssp. <i>macrophyllum</i> (Dozy & Molk.) Hyvönen	Malaysia. Aug. 1998 <i>Tan s.n.</i>	AY396502	AY396478	AY396443
	<i>P. comosum</i> (Müll. Hal.) Mitt.	Mexico. Oaxaca, <i>Norris 77508</i>	AY396503	AY396481	AY396446
	<i>P. contortum</i> (Brid.) Lesq.	Canada. British Columbia, <i>Hedderson 5803</i>	AF208425	AF545019	—
	<i>P. dentatum</i> (Brid.) Brid.	Finland. Uusimaa, <i>Hyvönen 6169</i>	AY137692	AF545020	AY396454
	<i>P. fastigiatum</i> Mitt.	Taiwan. Nantou, <i>Hyvönen 3556</i>	AY396504	AY396482	AY396447
	<i>P. gracilifolium</i> Besch.	Uganda. Rukungiri, <i>Wigginton 5071a</i>	AY396507	AY396488	AY396462
	<i>P. inflexum</i> (Lindb.) Sande Lac.	Japan. Honshu, <i>Chishiki 1865</i>	—	AY396486	AY396459
	<i>P. japonicum</i> Sull. & Lesq.	Japan. Honshu, <i>Nishimura 10601</i>	AY137693	AF545021	AY396463
	<i>P. microstomum</i> (Schwägr.) Brid.	Taiwan. Taichung, <i>Hyvönen 4087</i>	AY137694	AF545022	AY396450
	<i>P. nanum</i> (Hedw.) P. Beauv.	Sweden. Skåne, <i>Hyvönen 6484</i>	AY396506	AY396484	AY396456
	<i>P. neesii</i> (Müll. Hal.) Dozy	Taiwan. Taichung, <i>Hyvönen 4021</i>	AY137695	AF545023	AY396449
	<i>P. neglectum</i> (Hampe) A. Jaeger	Colombia. Antioquia, <i>Churchill 16370</i>	AY396510	AY396491	AY396466
	<i>P. neo-caledonicum</i> Besch.	New Caledonia. <i>Norris 93319</i>	AY396513	AY396495	AY396471
	<i>P. nipponicum</i> Nog. & Osada	Japan. Honshu, <i>Hayashi 7038</i>	AY137696	AF545024	AY396460
	<i>P. nudiusculum</i> Mitt.	Taiwan. Taichung, <i>Hyvönen 4153</i>	AY396505	—	AY396451
	<i>P. pensilvanicum</i> (Hedw.) P. Beauv.	Brazil. São Paulo, <i>Hyvönen 6393</i>	AY137697	AF545025	—
	<i>P. pensilvanicum</i>	U.S.A. Alabama, <i>Goffinet 5266</i>	—	AY396477	AY396442
	<i>P. pensilvanicum</i>	U.S.A. North Carolina, <i>Horn 1843</i>	—	AY396498	—
	<i>P. pergranulatum</i> P. C. Chen	China. Sichuan, <i>Allen 6501</i>	AY396514	AY396496	—
	<i>P. perichaetiale</i> (Mont.) A. Jaeger	Bolivia. Inquisivi, <i>Lenis 87019 (MO)</i>	—	AY396487	AY396461
	<i>P. piliferum</i> (Dozy & Molk.) Touw	Papua New Guinea, <i>Koponen 36036</i>	AY396512	—	AY396469
	<i>P. procerum</i> (Lindb.) Schimp.	Honduras, La Paz, <i>Liesner 26480</i>	AY396508	AY396489	AY396464
	<i>P. proliferum</i> (Griff.) Mitt.	Uganda. Buhoma, <i>Porley 652a</i>	AY396509	AY396490	AY396465
	<i>P. rufisetum</i> Mitt.	Bhutan. Thimphu, <i>Long 10801</i>	—	AY396479	AY396444
	<i>P. semipellucidum</i> (Hampe) Mitt.	Colombia. Antioquia, <i>Churchill 18679</i>	AY396511	AY396492	AY396467
	<i>P. sinense</i> (Broth.) Hyvönen & P. C. Wu	Bhutan. Mongar, <i>Long 8696</i>	—	AY396480	AY396445
	<i>P. spinulosum</i> Mitt.	Japan. Honshu, <i>Chishiki 1862</i>	AY137698	AF545026	AY396457

Table 1. Continued.

Taxon	Collection reference	GenBank accession No.		
		rps4	trnL	ITS2
<i>P. subortile</i> (Müll. Hal.) A. Jaeger	Papua New Guinea, Koponen 34850	—	AY396493	AY396468
<i>P. subulatum</i> (Brid.) Brid.	Australia, Queensland, Hyönönen 6025	AY137699	AF545027	AY396453
<i>P. tahitense</i> Schimp.	U.S.A. Hawaii, Hyönönen 4904	—	AY396483	AY396452
<i>P. tortile</i> (Sw.) Brid.	Puerto Rico, Goffinet 5067	AY396501	AY396476	AY396441
<i>P. tubulosum</i> Dixon	Papua New Guinea, Hoffmann 89-741	—	AY396485	AY396458
<i>P. umigerum</i> (Hedw.) P. Beauv.	Finland, Uusimaa, Hyönönen 6173	AF208426	AF545028	AY396472
<i>P. usambaricum</i> (Broth.) Paris	Tanzania, Tanga Province, Laaka 3235	AY396518	AY396500	AY396475
Outgroup				
<i>Atrichum androgynum</i> (Müll. Hal.) A. Jaeger	Brazil, São Paulo, Hyönönen 6387	—	AF544999	AY396432
<i>A. angustatum</i> (Brid.) Bruch & Schimp.	U.S.A. Louisiana, Hedderson 10393 (RNG)	AF208417	AF545000	AY396430
<i>A. oerstedianum</i> (Müll. Hal.) Mitt.	Mexico, Veracruz, Hyönönen 6504	AY137680	AF545001	AY396431
<i>A. undulatum</i> (Hedw.) P. Beauv.	Finland, Uusimaa, Hyönönen 6170	AY137681	AF545002	AY396433
<i>Metostrichum lyallii</i> (Mitt.) G. L. Sm.	U.S.A. Colorado, Weber WWB36612	AF208423	AF545011	AY396426
<i>Oligotrichum hercynicum</i> (Hedw.) Lam. & DC.	Finland, Uusimaa, 25 July 1998, Enroth s.n.	AY137688	AF545014	AY396427
<i>O. parallelum</i> (Mitt.) Kindb.	Canada, British Columbia, Hedderson 10043 (RNG)	AF208424	AF545015	AY396428
<i>Polytrichastrum alpinum</i> (Hedw.) G. L. Sm.	Finland, Etelä-Häme, Hyönönen 6204	AY137701	AF545031	—
<i>P. formosum</i> (Hedw.) G. L. Sm.	Finland, Uusimaa, Hyönönen 6197	AY137702	AF545032	AY396435
<i>P. longisetum</i> (Sw. ex Brid.) G. L. Sm.	Finland, Varsinais-Suomi, Hyönönen 6506	AY137703	AF545033	—
<i>Polytrichum brachymitrium</i> Müll. Hall.	Brazil, Minas Gerais, Hyönönen 6230	AY137704	AF545034	AY396436
<i>P. commune</i> Hedw.	Finland, Uusimaa, Hyönönen 6168	AF208428	AF545035	AY396437
<i>P. juniperinum</i> Hedw.	Finland, Uusimaa, Hyönönen 6193	AY137705	AF545036	AY396438
<i>P. piliferum</i> Hedw.	Finland, Uusimaa, Hyönönen 6205	AY137706	AF545037	AY396439
<i>P. subpilosum</i> P. Beauv.	Malawi, Mulanji, Wigginton M1397a	AY137707	AF545038	AY396440
<i>Psilopilum laevigatum</i> (Wahlenb.) Lindb.	Canada, Ellesmere Island, Hedderson 5938 (RNG)	AF208429	AF545039	AY396434
<i>Stereobryon subulirostrum</i> (Schimp.) G. L. Sm.	Mexico, Veracruz, Hedderson 12898	AY137708	AF545040	AY396429

Table 2. Data matrix with the percentage of parsimony informative characters in each partition used in the analyses of static alignments.

Matrix	Characters	Informative	(%)
ITS2	111	1	1
<i>rps4</i>	573	120	21
<i>trnL-F</i>	411	35	9
Morphology	47	47	100
Combined total	1142	203	18

Scientific Computing Ltd., Espoo, Finland. This is an IBM eServer Cluster 1600 system, constructed of 16 pSeries 690 nodes (each equipped with 32 POWER4 processors) and an SP Switch2 (Colony). Sixteen processors of one node were used for our analyses. Command lines used can be found in Appendix 3.

## RESULTS

Simultaneous parsimony analyses of the combined matrix from three gene regions, plus morphology using Nona within Winclada shell, were performed 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), and hold/2 (keeping 2 starting trees for each replication). Using the multiple tree-bisection reconnection algorithm (mult\*max\*) resulted in 225 equally parsimonious trees (EPTs) of 862 steps (CI 0.52, RI 0.67). Only 1% of the initial searches found this minimum length tree, so it was warranted to make a larger analysis. This was performed with mult\*1000 and hold/10, and this time 7113 EPTs were found. Here, the resolution was lost quite dramatically, and because only 28 out of 1000 replicates found shortest trees, we performed also ratchet (Nixon, 1999) searches. These were performed with 1000 iterations per replicate, two trees held per iteration, and reweighting 15%, 20%, and 25% of the total number of characters. Treatment of ambiguous optimization of characters was set as amb = poly-, which means that branches were collapsed only if ancestor and descendant have the same state for all characters. This led to finding 372 (15% reweighting), 469 (20%), and 451 (25%) EPTs with the length of 861 steps. When the results of all these searches were pooled, we had 8556 EPTs. The strict consensus of these trees is illustrated in Figure 1.

Two of the 8556 EPTs were optimal based on morphology, and these trees are illustrated in Figure 2 with unambiguous character state changes mapped on the trees. For discussion of character

evolution and clades found on these two trees, see below.

In the first analysis with POY using direct optimization, 19.5 million (M) trees and over 630 M alignments were examined. This analysis resulted in four equally parsimonious alignments and trees. The second analysis with more thorough heuristics (52.3 M trees, number of performed alignments not recorded because of a mistake) did not find these optimal trees. The third analysis with further expanded heuristics (85.6 M trees and 935 M alignments) resulted in one optimal tree that was five steps shorter than the four trees found in the first analysis. This tree is illustrated in Figure 3.

## DISCUSSION

The clades present in the strict consensus of the 8556 trees (obtained from the analyses based on prealigned sequences) are mostly composed of the species that one would expect to be closely related based on earlier analysis (Hyvönen, 1989) and morphology. The relationships of the outgroups are unaltered from those obtained in the study of the whole family (Hyvönen et al., 2004) with one exception: *Psilopilum* Brid. appears to be within the clade formed by *Atrichum* P. Beauv., *Oligotrichum* Lam. & DC., and *Steereobryon* G. L. Sm., and not sister to the apical clade formed by *Eopolytrichum*, *Pogonatum*, *Polytrichastrum*, and *Polytrichum*. *Pogonatum* is monophyletic but internal resolution is very poor with only small clades of few species present (but without jackknife support). The largest clade is the one formed by nine small species of the *Racelopus* group (Touw, 1986). It should also be noted that *Polytrichastrum alpinum*, a species already treated as a species of *Pogonatum* by some authors (e.g., Osada, 1965; Crum & Anderson, 1981), is within this genus. The present results confirm some conclusions made in earlier studies. Touw (1986) synonymized *Pseudoracelopus* Broth., *Racelopodopsis* Thér., and *Racelopus* Dozy & Molk. with *Pogonatum*, and our results support this. Species of Touw's *Racelopus* group appear again as a monophyletic entity but firmly within *Pogonatum*. Similarly, inclusion of *Microdendron sinense* Broth. within *Pogonatum*, as proposed by Hyvönen and Wu, (1993) is confirmed here.

The small clades present in the strict consensus tree (Fig. 1) are compatible with groupings presented in Hyvönen (1989), with one exception—the pair formed by *Pogonatum tahitense* and *P. campylocarpum*. These two were included in different subgenera in 1989. However, when we examine the two trees that are optimal according to the mor-

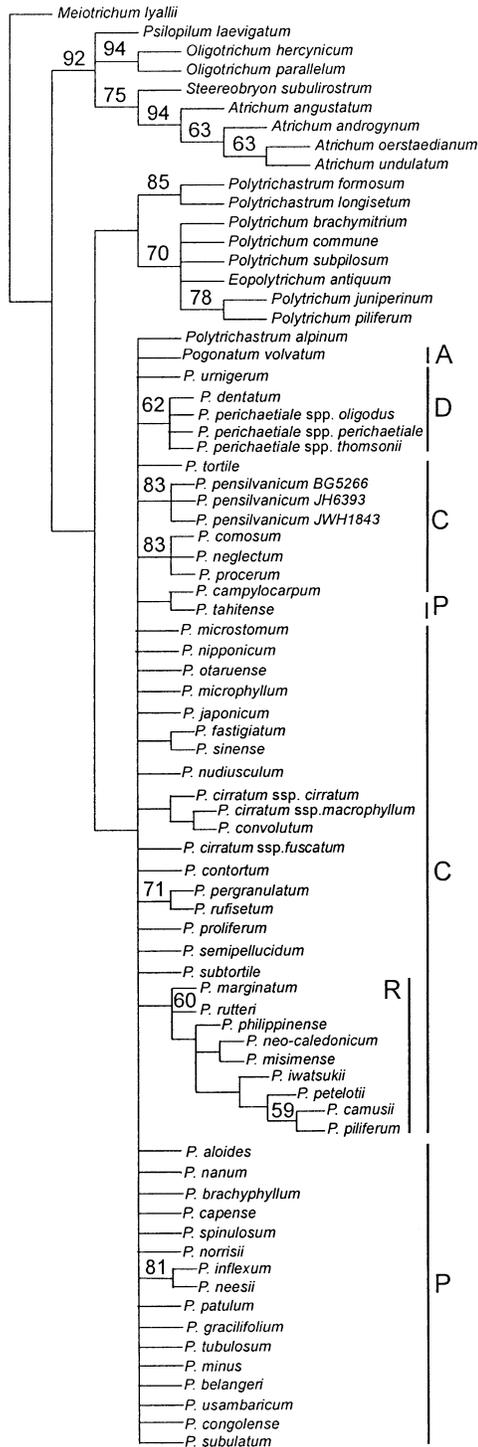


Figure 1. The strict consensus tree based on 8556 equally parsimonious trees (L 862 steps) obtained with simultaneous analysis of three genic regions (ITS2, *rps4*, *trnL-F*), plus morphology. Jackknife support values exceeding 50% are marked by the nodes. Division of the genus *Pogonatum* according to Hyvönen (1989) is shown

phology, it is evident that the present results do not support the subgenera distinguished earlier. Instead of *P. volvatum* (Müll. Hal.) Paris, the basal-most species is *P. microstomum*. All other species forming the basal grade are those of the subgenus *Dendroidea* and *Alienum*, except *P. japonicum*. All these species are, however, plants with fairly thick cell walls in the leaves and many of their features (e.g., large size, dentate leaf margins) are characters that are typical for species of *Polytrichastrum* and *Polytrichum*. The species formerly assigned to the subgenera *Catharinella* and *Pogonatum* (with the exceptions given above) are all included in the large apical clade (Fig. 2). The internal divisions of this clade do not correspond to former subgenera; however, the division into these two subgenera can still be retained, with somewhat altered composition. Based on the results of the present study the smaller group, subgenus *Pogonatum*, around *P. aloides*, is formed of 10 species, and the larger group, subgenus *Catharinella*, is composed of 36 species (Fig. 2).

In the POY analyses, relationships of the outgroups are altered from that of all earlier analyses (Fig. 3). *Oligotrichum* is the first lineage diverging from the common ancestor after *Meiotrichum* G. L. Sm., and after that it is *Atrichum*, *Psilopilum*, and *Steereobryon* that is a sister clade to *Eopolytrichum*, *Polytrichastrum*, and *Polytrichum*, not *Pogonatum* as proposed by our earlier analysis of the whole family (Hyvönen et al., 2004). However, *Pogonatum japonicum* together with *Pogonatum volvatum* are within the latter clade! *Pogonatum microstomum* appears to be the basalmost member of *Pogonatum*, together with *Polytrichastrum alpinum* and *Pogonatum urnigerum*. The position of *P. japonicum* and *P. volvatum* is surprising. For the latter species we did not have any sequence data, only morphology, which certainly resembles more that of *Polytrichastrum* or *Polytrichum* (or *Pogonatum japonicum*) than other species of *Pogonatum* as noted already by Smith (1975) and Hyvönen (1989). Both species, however, have a typical *Pogonatum* capsule—deeply pigmented peristome with 32 compound teeth and mammillose exothecium that lack stomata. Are these two plants possibly intermediates resulting from ancient hybridization between

←

with the letters referring to the species of each subgenera with A for the subgenus *Alienum*, D for *Dendroidea*, C for *Catharinella*, and P for *Pogonatum*. Additionally, the Rancelopus group (Touw, 1986) has been marked with R. Specimen numbers shown reflect collector initials and number (Table 1).

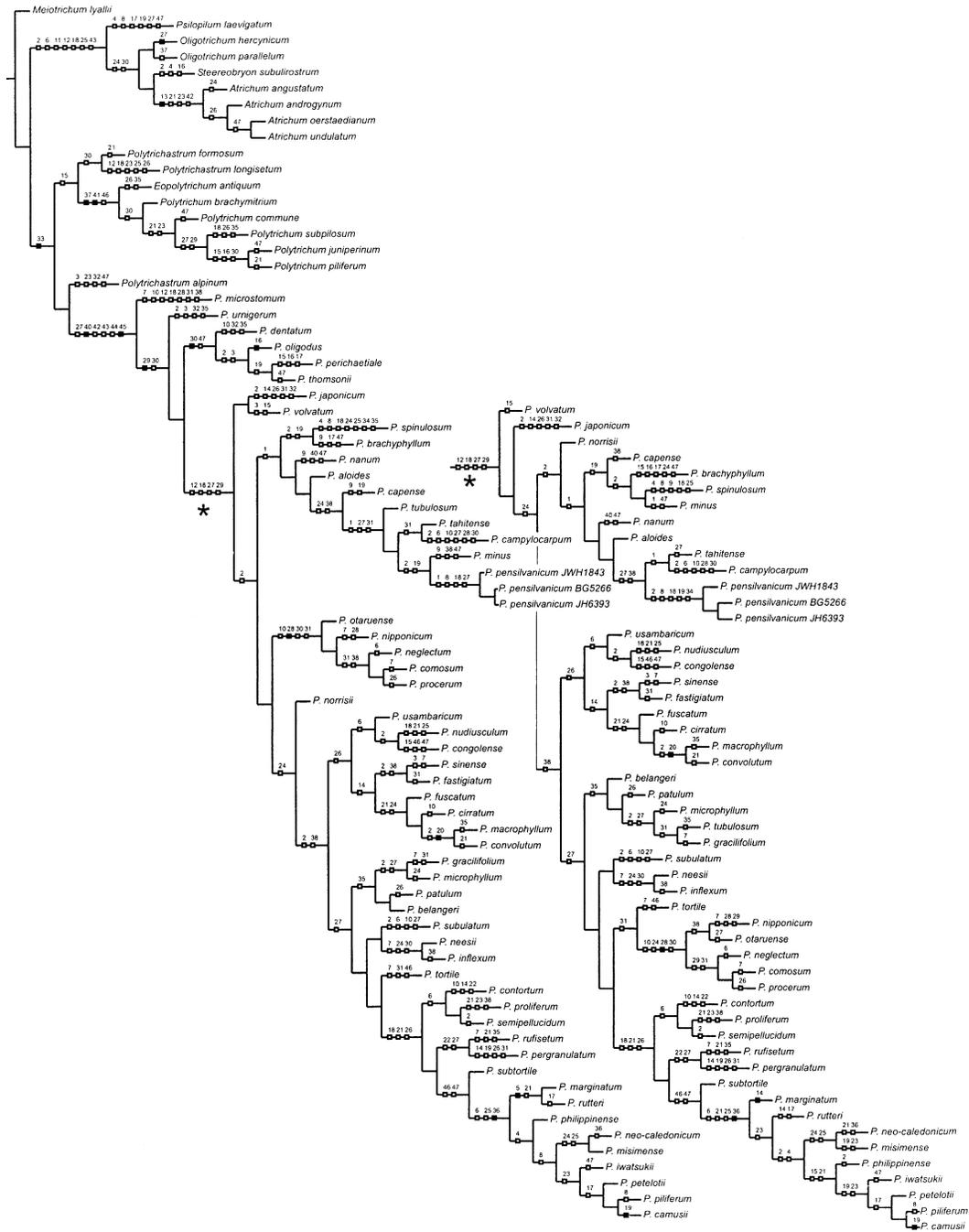


Figure 2. Two equally parsimonious trees (out of 8556) that are optimal based on morphology. Unambiguous character state changes are marked on the nodes and empty nodes are those with support only from sequence-level data. White squares indicate homoplasy (discontinuous states). Asterisks (\*) indicate where the two topologies start to differ.

two lineages? Additional sampling from both chloroplast and nuclear genome is needed to confirm or refute this. As in the analysis based on the static alignment above, the internal division of *Pogona-*

*tum* does not correspond to former subgenera, but this division can be retained with altered composition.

When we examine morphological characters, it



Figure 3. The single parsimonious tree obtained based on direct optimization. Enclosed terminal names indicate those species that were included in the analysis based only on morphology. Branches emphasized with black bars are those that are present on the strict consensus tree based on 8556 obtained through analysis of static alignment.

seems that none of them are very good indicators 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-level data. The combined simultaneous analysis provided results that would have been unexpected based solely on morphology. In the following we will briefly discuss some of the morphological characters based on their distribution on two trees out of 8556 that are optimal based solely on morphology (Fig. 2).

Many species of *Pogonatum* have reduced leaves (char. 8), and it seems that this reduction has taken place independently for *P. spinulosum*, *P. pensilvanicum*, and a third time for the species of the *Racelopus* group. However, the species in the latter group form a monophyletic entity on the other one of these two trees. The other extreme of the leaf development is exemplified by species like *P. cirratum* ssp. *macrophyllum* and *P. convolutum*. They have a secondary ventral stereid band in their leaves (char. 20) like the large species of *Dawsonia* R. Brown, and it seems that this character has developed in *Pogonatum* only once. In some species not only the blade of the leaves but also the number of adaxial lamellae is clearly reduced or they are totally absent (char. 25). These two characters are not, however, coupled with each other. Species like *P. nudiusculum*, *P. proliferum*, and *P. semipellucidum* have otherwise well-developed leaves, but the adaxial lamellae are either restricted to the very central part of the leaf (*P. nudiusculum* and *P. semipellucidum*) or they are present on the ventral side of the very narrow costa only (*P. proliferum*). These three species do not, however, form a monophyletic group. *Pogonatum nudiusculum* is in a clade together with *P. usambaricum* and *P. congolense* Card., and these two species do show fairly wide leaf margins that are devoid of lamellae, although this quantitative difference has not been distinguished as a distinct character state. Such species as *P. spinulosum* and all the species of the *Racelopus* group lack lamellae altogether, or they are very reduced like in *P. neo-caledonicum* and *P. misimense* (Bartr.) Touw. The latter two are, however, nested within the *Racelopus* group (see Fig 1.) and for these two we have to accept a reversal (at least partial) to an earlier character state. Apical cells of lamellae (lamellae marginal cells) are useful characters to distinguish species of *Pogonatum*, and this was considered to be an important taxonomic character by Hyvönen (1989). Apical cell size (compared to other cells of the lamellae) and form, as seen in cross section (chars. 28, 30), were used to distinguish a group of predominantly South

American species as a monophyletic group within *Pogonatum* subg. *Catharinella* in Hyvönen (1989). Although all these species greatly resemble each other in these two characters, this is not without homoplasy on current trees. *Pogonatum microstomum* is consistently in a basal position (Figs. 2, 3), and *P. campylocarpum* also seems to belong to another group of species not closely related to other American species with these characteristic apical cells. These species (*Pogonatum comosum*, *P. neglectum*, and *P. procerum*) share a common ancestor with *P. otaruense* and *P. nipponicum* from Japan (Fig. 2).

When we examine the tree based on direct optimization, there is another unexpected result (in addition to the grouping of *P. japonicum* and *P. volvatum*), namely, *P. pensilvanicum* appearing as polyphyletic. Three specimens of *Pogonatum pensilvanicum* are present as a monophyletic group on the strict consensus based on static alignment. These terminals are similar in the morphological characters included, and this strange result is quite obviously an artifact and not due to the actual phylogeny. We did not have complete sequence-level character partitions for any of these three terminals (see Table 1). They are similar in their *trnL* sequences, differing only by the length so that BG5266 (Alabama) is 18 nucleotides (nts) longer than JWH1843 (North Carolina) and 13 nts longer than JH6393 (Brazil). The terminals from Brazil and North Carolina form a clade and group together with *P. campylocarpum*, while the plant from Alabama appears within the apical clade close to *P. spinulosum* and *P. subulatum*, *P. neesii*, and *P. inflexum*. Which one of these two positions should we consider more reliable? In order to test this we performed direct optimization analysis with the same settings as for the whole material, but now reduced to 74 terminals so that we had a composite of *P. pensilvanicum* specimens with chloroplast sequences of JH6393 from Brazil supplemented with ITS2 sequence of BG5266 from Alabama. With this analysis we obtained two parsimonious alignments and trees, and on both trees (not shown) *P. pensilvanicum* appeared within the clade including *P. campylocarpum* and allies. It seems that *P. pensilvanicum* BG5266 may be drawn out of this clade in the original analysis due only to its lack of *rps4* sequence.

Based on the results of direct optimization and the two trees of the Nona analyses that were optimal and based on morphology, we can draw some conclusions about general trends in the evolution of *Pogonatum*. This is now easier than in former analyses such as that by Hyvönen (1989) because we

now have a robust hypothesis of phylogeny of the whole family (Hyvönen et al., 2004). It appears that the common ancestor of all species of the genus was a fairly large plant with numerous adaxial lamellae on well-developed leaves. In both major clades within the genus, evolution has proceeded towards reduced, and in some cases extremely reduced, structure. It seems that extreme neoteny has been a successful strategy for these plants on almost all continents and in diverse biomes. In future studies it would be interesting to try to estimate the age of these extremely reduced clades and compare them with estimates from other groups of bryophytes growing in similar habitats. At the moment, age estimates for different lineages of bryophytes are still lacking, and different approaches for this kind of age estimation are actively developing (e.g., Sanderson, 2003).

While our results confirm some of the nomenclatural conclusions made in earlier studies (Hyvönen & Wu, 1993; Touw, 1986), we also face some new and complicated challenges. In the tree based on direct optimization, two species of *Pogonatum*, *P. japonicum* and *P. volvatum*, appear clearly outside the genus. Should these two be transferred to another genus? Similarly problematic is the position of *Polytrichastrum alpinum*. It appears nested within *Pogonatum*, but on trees that are optimal based on morphology it can equally well be left out, retaining its generic status. We agree with Wiley (1981) that nomenclature should reflect phylogeny as closely as possible, but at the same time premature alterations should be avoided. This means that we hesitate to create a new genus for *P. japonicum* and *P. volvatum* especially because we did not have sequence data for the latter and because different analyses, including the one based on a larger body of characters but limited taxonomic sampling, do not agree on their position. What seems to be clear, however, is that *Polytrichastrum* as currently delimited is not a monophyletic entity. This pattern has now emerged in several analyses, although it should be noted that the sampling is still far from being comprehensive. If we want to minimize nomenclatural changes and still also present our results as congruent as possible with nomenclature, both *P. formosum* and *P. longisetum* should be assigned to a new genus. If it appears (with extended character sampling) that *Polytrichastrum alpinum* is indeed within *Pogonatum*, this would make *Polytrichastrum* superfluous and available to accommodate both *P. formosum* and *P. longisetum*. This would, however, necessitate a nomenclatural proposal to change the generic type, which is currently *Polytrichastrum alpinum*. Taking all this together,

if we move both *Polytrichastrum formosum* and *P. longisetum* back to *Polytrichum*, then the original names *Polytrichum formosum* Hedw. and *P. longisetum* Sw. ex Brid., respectively, may be applied. Whether *Eopolytrichum antiquum* Konopka et al. (Konopka et al., 1997) should be as well included within *Polytrichum* s.l. remains to be seen. This becomes necessary if *Eopolytrichum antiquum* proves to be nested within *Polytrichum* with more thorough analyses. At the moment, however, our results are indecisive as to its proper nomenclatural treatment.

Future studies should be directed toward expanded character sampling of *Pogonatum*. The obvious choice for this would be the chloroplast gene *rbcL* that has already been sampled for a fair number of species (Hyvönen et al., 2004). When the 14 species of *Pogonatum* (with *Polytrichastrum alpinum* included) are compared, their *rbcL* sequences still include 17% informative sites. However, much more effort is needed to get these sequences because *rbcL* is both longer and not as easy to amplify as the two other chloroplast regions used here. Equally important in future analyses would be the inclusion of the few, as yet, unsampled species, especially species like *P. volvatum*, that appear in a basal or in a very unexpected position on current trees. And, first of all, we should fill in the gaps in our current matrix, as not all sequences were obtained for all terminals for the current analyses.

Our analyses, based on direct optimization, show that sequences with length variation do contain valuable information for phylogeny reconstruction in *Pogonatum*. However, it should be acknowledged that with the analyses presented here we have explored only a tiny fraction of possible alignments and trees, and it would not be surprising that more extensive heuristics will reveal better alignments and shorter trees, possibly with much altered topology. While some guidelines can be given about adequacy of heuristic analyses on static alignments after a decade of experience with different search strategies and algorithms, the same is not true (as yet) when we deal with direct optimization. These methods have not yet been extensively used, and therefore much remains to be learned about their behavior and performance. They are continuously developed, and novel; improved approaches that challenge earlier results are presented almost annually (e.g., Grant & D'Haese, 2003; Wheeler, 2003a, 2003b).

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## Appendix 1. Morphological characters and character states.

- 
1. Protonema fugacious (0); persistent (1).
  2. Size: plants robust > 180 mm (0); large, 50–110 mm (1); small, 10–40 mm (2); depauperate, < 7 mm (3).
  3. Branching: stems unbranched (0); branching common (1); dendroid (2).
  4. Well defined (polytrichoid) central strand present (0); absent (1).
  5. All cauline leaves isophyllous (0); anisophyllous (1).
  6. Sheath distinct and broad (0); not differentiated (1).
  7. Rhizoids on leaves present (0); absent (1).
  8. Leaf blade normal, longer than sheath, ratio > 1.5 (0); reduced, ratio < 1 (1); essentially absent (2).
  9. Leaf apex acute, essentially plane (0); distinctly cucullate (1).
  10. Sheath margins entire or crenate by bulging cells (0); entire or serrate (1); uniformly serrate (2).
  11. Hyaline leaf margins present (0); absent (1).
  12. Hinge tissue between blade and sheath absent (0); present (1).
  13. Leaf border absent (0); *Atrichum*-type (1).
  14. Blade margins unistratose (0); bistratose (1); multistratose (2).
  15. Blade margins dentate (toothed) (0); serrate (1); crenate by bulging cells or entire (2).
  16. Dentation or serration essentially along whole blade (0); serrate portion less than 1/3 of the blade length or entire (1); uniformly entire (2).
  17. Dorsal side of costa serrate (0); entire (1).
  18. Dorsal stereid band almost as wide as blade (0); narrowed (1); present only in costa that is restricted only to central part of leaf (2).
  19. Stereid bands present (0); only dorsal band present (1); absent (2).
  20. Number of stereid bands 2 (0); 3 with secondary ventral stereid band present (1).
  21. Cell walls of stereid bands moderately incrassate (0); dorsal band with pale incrassate walls (1); both bands with incrassate walls (2).
  22. Cell walls of central cells thin (0); distinctly incrassate (1).
  23. Cell walls of dorsal cells of blade extremely incrassate with cell-lumen small and rounded (0); firm, evenly incrassate walls with cell lumen subquadrate (1); thin or firm walls, small thickenings at corners (2); very thin, cells collenchymatous (3).
  24. Outer wall of dorsal cells of costa incrassate, thicker than transverse cell walls (0); thin to firm, similar to transverse cell walls (1).
  25. Ventral lamellae covering practically whole lamina (0); restricted to central part (1); present only on ventral side of narrow costa (2); lamellae absent (3).
  26. Height of lamellae > 4 cells (0); 3 to 4 cells (1); 1 to 3 cells (2).
  27. Lamellae (apical) margins straight or indistinctly crenate as seen in side view (0); distinctly crenate (1); irregularly crenate (2); serrate (3).
  28. Size of LMC (lamellae marginal cell) lumen similar to lower cells of lamellae (0); elongated as seen in side view (1); higher than lower cells (2).
  29. LMC cell walls undifferentiated (0); incrassate (1); only outer wall incrassate (2); outer wall notched (3).
  30. Form of LMCs (as seen in cross section) flattened (0); undifferentiated (1); ovoid to bottle-shaped (2); retuse (3).
  31. LMCs solitary (0); mostly geminate (1); geminate apicals present (2).
  32. LMC apical cell walls smooth or very finely papillose (0); coarsely papillose (1).
  33. Calyptra with ample rhizoids (0); rhizoids sparse (1).
  34. Perigonia elongated (0); short and ellipsoid (1).
  35. Paraphyses (among perigonia) uniseriate (0); at least some paraphyses with club-shaped multiseriate apical portion (1).
  36. Seta smooth (0); scabrose (1).
  37. Apophysis tapering (0); contracted (1); discoid (2).
  38. Angles of capsule absent (0); 4 or very rarely more (1); capsules with 6 to 8 folds (2).
  39. Capsule angle form blunt (0); sharp, knife-edged (1); ribbed (2).
  40. Exothecium smooth or mammillose (0); papillose (1).
  41. Exothecial pitting none (0); thin spots (1); pitted (2).
  42. Stomata on capsules present (0); absent (1).
  43. Peristome teeth 64 (0); 32 (1); 16 (2).
  44. Tooth structure simple (0); compound sinus broad (1); compound, sinus narrow (2).
  45. Peristome pigmentation pale (0); intensely colored (1).
  46. Spore surface ornamentation granular (0); echinoid (1).
  47. Spores large, diameter mostly > 18  $\mu\text{m}$  (0); medium-sized, diameter mostly 8.5–15.5  $\mu\text{m}$  (1); small, diameter mostly < 8.3  $\mu\text{m}$  (2).
-

Appendix 2. Character matrix of morphology. Different character states are coded with (0, 1, 2, and 3) that refer to Appendix 1. Unknown information marked with (?) and inapplicable characters with (-). Specimen numbers shown reflect collector initials and number (see Table 1).

	000000000111111111222222222233333333333344444444 123456789012345678901234567890123456789012345678901234567
<i>Meiotrichum lyallii</i>	01100010000100100000001000002201100011101000001
<i>Oligotrichum hercynicum</i>	02000110101000100200001120300100101000-00012001
<i>O. parallelum</i>	020001100010001002000011200001001??010-00011001
<i>Steereobryon subulirostrum</i>	030101101010002202000011200001001??000-000100?1
<i>Atrichum androgynum</i>	020001100010100002001021220001001??000-00110001
<i>A. angustatum</i>	02000110001010000200102020000100100000-00110001
<i>A. oerstaedianum</i>	02000110001010000200102122000100100000-00110000
<i>A. undulatum</i>	02000110001010000200102122000100100000-00110000
<i>Psilopilum laevigatum</i>	0201011110100020121-001020200200101000-00010000
<i>Polytrichastrum alpinum</i>	011000100001001000000000000220100001000000000
<i>P. formosum</i>	01000010000100000000201000000100000011000000001
<i>P. longisetum</i>	0100001000000000200002011000100000001000000001
<i>Eopolytrichum antiquum</i>	????00?0?0?10??00000?01?00200?01?20-0200??1?
<i>Polytrichum brachymitrium</i>	010000100001000000000010000003000000211020000?1
<i>P. commune</i>	00000010000100000000200000000300000021102000012
<i>P. juniperinum</i>	0100001000010022000020000010320000021102000012
<i>P. piliferum</i>	0200001000010022000010000010320000021102000011
<i>P. subpilosum</i>	000000100001000001002000011033000010211020000?1
<i>Pogonatum aloides</i>	12000010001000100100001000000100000000-10112101
<i>P. belangeri</i>	01000010001000100100001100100100001002210112101
<i>P. brachyphyllum</i>	13000010101000221110001000000100000000-10112100
<i>P. campylocarpum</i>	01000110011000100100001100120210000002210112101
<i>P. camusii</i>	1301011100?00120--2---213-----000102210112112
<i>P. capense</i>	1200001010100010011-001100000100000002210112101
<i>P. cirratum</i> ssp. <i>cirratum</i>	01000010011001100100200002000120000002210112101
<i>P. cirratum</i> ssp. <i>macrophyllum</i>	00000010001001100101201002000120001002210112101
<i>P. cirratum</i> ssp. <i>fuscatum</i>	01000010001001100100200002000100000002210112101
<i>P. comosum</i>	01000000011000100100001000113210000002210112101
<i>P. congolense</i>	02000110001000200100001102000100000002210112112
<i>P. contortum</i>	01000110011001100200111102100100000002210112101
<i>P. convolutum</i>	00000010001001100101101002000100000002210112101
<i>P. dentatum</i>	01000010011100100000001000101001001000-10112100
<i>P. fastigiatum</i>	00000010011001100100001102000110000000-10112101
<i>P. gracilifolium</i>	02000000001000100100001100200120001002210112101
<i>P. inflexum</i>	01000000001000100100001000100300000000-10112101
<i>P. iwatsukii</i>	1301011100?00020021-10213-----0??102210112111
<i>P. japonicum</i>	00000010001001100100001001000111000000-10112101
<i>P. marginatum</i>	?100111000?00210020000113-----000102210112112
<i>P. microphyllum</i>	02000010001000100100001000200100001002210112101
<i>P. microstomum</i>	01000000011000100100001000122210000002210112101
<i>P. minus</i>	0300001010?00010011?0011002001200??000-101121?2
<i>P. misimense</i>	1301011100?00010021-0010221--100000102210112112
<i>P. nanum</i>	12000010101000100100001000000100000000-00112100
<i>P. neesii</i>	01000000001000100100001000100300000002210112101
<i>P. neglectum</i>	02000110011000100100001000113210000002210112101
<i>P. neo-caledonicum</i>	1301011100?000100200203022100100000002210112112
<i>P. nipponicum</i>	02000000011000100100001000122220000000-10112101
<i>P. norrisii</i>	020000100010001001000011000001000??000-10112101
<i>P. nudiusculum</i>	02000110001000100200201112000100000002210112101
<i>P. otaruense</i>	02000010011000100100001000210220000000-10112101
<i>P. patulum</i>	01000010001000100100001101100100001002210112101
<i>P. petelotii</i>	1301011100?00020121-10213-----0??1022101121?2
<i>P. pensilvanicum</i> BG5266	13000-1100100010021-001100100120010002210112101

Appendix 2. Continued.

	0000000001111111112222222222333333333344444444 12345678901234567890123456789012345678901234567
<i>P. pensilvanicum</i> JH6393	13000-1100100010021-001100100120010002210112101
<i>P. pensilvanicum</i> JWH1843	13000-1100100010021-001100100120010002210112101
<i>P. pergranulatum</i>	0100001000?00110021-11110020012000000??101121??
<i>P. perichaetiale</i> ssp. <i>perichaetiale</i>	02100010001100221010001000101000000000-10122100
<i>P. perichaetiale</i> ssp. <i>oligodus</i>	01200010001100110000001000101000000000-10122100
<i>P. perichaetiale</i> ssp. <i>thomsonii</i>	02100010001100100010001000101000000000-10112101
<i>P. philippinense</i>	1201011000100020020010313-----000102210112112
<i>P. piliferum</i>	1301011200?-----121-1--13-----000102210112112
<i>P. procerum</i>	00000010011000100100001001113210000002210112101
<i>P. proliferum</i>	010001100010001002002031221001000000000-10112101
<i>P. rufisetum</i>	010000000?00010020021110220010000100??10112101
<i>P. rutteri</i>	?100111000100110120000313-----0001022101121?2
<i>P. semipellucidum</i>	00000110001000100200101112100100000002210112101
<i>P. sinense</i>	001000000210011001000011020001000??000-10112101
<i>P. spinulosum</i>	13010-12001-----021-00-13-----011000-10112101
<i>P. subtortile</i>	0100001000?000100200101102100100000002210112112
<i>P. subulatum</i>	02000110011000100100001100000100000002210112101
<i>P. tahitense</i>	02000010001000100100001100200110000002210112101
<i>P. tortile</i>	01000000001000100100001100100120000002210112111
<i>P. tubulosum</i>	02000010001000100100001100200120000002210112101
<i>P. urnigerum</i>	00100010000100100000001000101101001000-10112101
<i>P. usambaricum</i>	01000110001000100100001102000100000002210112101
<i>P. volvatum</i>	01100010001000000100001000000100000000-10112101

Appendix 3. Command lines used for direct optimization with POY.

First analysis used the following commands:  
poy -parallel -solospawn 15 mo.ss IT2a.1.ss IT2b t2 t4 t6 t8 -prealigned rp t1 t3 t7 t9  
-noprealigned -molecularmatrix 111 -maxtrees 3 -holdmaxtrees 20 -random 10  
-multibuild 15 -ratchettbr 3 -ratchettrees 2 -treefuse -fuselimit 25  
-fusingrounds 1 -slop 1 -checkslop 5 -seed -1 -fitchtrees -noleading  
-norandomizeoutgroup > ibp.out  
mo.ss IT2a.1.ss IT2b t2 t4 t6 t8 rp t1 t3 t5 t7 t9 input filenames, ibs.out output filename

In the second analysis, random value was 20, mutibuild 16, ratchettbr 5, and checkslop 8, otherwise as above.  
In the third analysis, random and fuselimit was increased to 30, otherwise as above.  
Fourth analysis was made with the same settings, but with 74 terminals