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ROOTING THE POLYTRICHOPSIDA: THE PHYLOGENETIC POSITION OF *ATRICHOPSIS* AND THE INDEPENDENT ORIGIN OF THE POLYTRICHOPSID PERISTOME

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SUMMARY

The class Polytrichopsida are a diverse but phylogenetically isolated lineage of mosses, and hence putative homologues of many molecular characters that are informative within the group cannot be confidently identified in outgroup taxa. Here we use a small dataset of predominantly slowly evolving nuclear and chloroplast DNA characters to identify the earliest dichotomy within the class and provide an internal root for our ongoing larger scale analyses including more rapidly evolving regions. The peristomate *Atrichopsis compressa*, previously hypothesised to form the sister group to the rest of the class together with the eperistomate *Alophosia azorica*, is shown to be derived from within *Notoligotrichum*. *Alophosia* alone is sister to all other polytrichopsid taxa while the next dichotomy within the class involves a second lineage of eperistomate taxa and the peristomate clade, strongly supporting the hypothesis that the structurally unique polytrichopsid peristome is a parallel evolutionary development analogous with the peristomes of other mosses.

KEYWORDS: Polytrichopsida, Polytrichales, *Atrichopsis*, moss, bryophyte, *Alophosia*, phylogeny, peristome

INTRODUCTION

Rooting is a perennial problem in the phylogenetic analysis of relationships within well circumscribed groups that are likely to be evolutionarily isolated. Our recent work (Bell & Hyvönen, unpublished) on relationships within the Polytrichopsida has revealed that some relatively rapidly evolving non-coding regions, such as the *rps*4-trnS intergenic spacer, the *trn*L intron and the *trn*L-*trn*F intergenic spacer, can be aligned across the class and are highly informative, allowing hypotheses of primary homology to be tested by congruence under parsimony or by conformity to optimal topologies derived from model-based methods. However, variation in these regions is too great for credible primary homologies with outgroup taxa to be identified. Potential approaches to circumventing this problem include direct optimization (optimization alignment, Wheeler, 1996), which does not require primary homology assumptions prior to analysis, and non-outgroup rooting methods such as the

molecular clock within a model-based framework (e.g. Drummond *et al*., 2006), both of which we intend to use in future analyses to test the results obtained here. Another solution, and the one we currently employ, is simply to use a higher-level phylogenetic analysis to identify the earliest dichotomy within the ingroup based on a dataset that is informative for the nodes of interest. The first-diverging ingroup taxon then serves as the outgroup in subsequent analyses. Resolution of the most basal relationships within Polytrichopsida is a question with wide ranging evolutionary implications in its own right; previous analyses (Forrest, 1995; Hyvönen *et al*., 1998; Hyvönen *et al*., 2004) have conflicted in suggesting whether the peristome is a plesiomorphic or a derived feature within the class and thus whether it is homologous with the very different peristomes found in other mosses. Note that throughout this paper the term "peristome" is used in the modern sense to refer to one or more rings of projecting, linear and/or membranous processes surrounding the capsule opening (however see discussion of this below).

Pre-cladistic concepts of higher-level relationships within Polytrichopsida predominantly emphasised gametophytic characters. Smith (1971) referred to an "ancient polytrichoid alliance" of which he thought *Polytrichum* Hedw., *Polytrichastrum* G.L.Sm., *Polytrichadelphus* (Müll.Hal) Mitt. and *Dawsonia* R.Br. were surviving descendants. These are all relatively robust genera with abundant adaxial lamellae, features he considered primitive. Although Smith included sporophytic as well as gametophytic characters in his list of primitive and derived features, the latter clearly have priority in determining higher-level grouping in his diagrammatic "phyletic arrangement" (Smith, 1971).

Hyvönen *et al.* (1998) used the molecular 18S, *rps*4 and *rbc*L regions combined with a morphological matrix of 30 characters for 22 species of Polytrichopsida to investigate relationships under parsimony. Although it was necessary to use successively applied weighting to achieve a resolved topology and very few nodes had bootstrap or jackknife support values > 50%, an interesting pattern emerged in which *Alophosia azorica* (Renauld & Cardot) Cardot was sister to the remainder of the class, with a clade comprising *Lyellia aspera* (I. Hagen & C.E.O. Jensen) Frye and *Bartramiopsis lescurii* (James) Kindb. forming the next earliest diverging lineage. All of these taxa lack a peristome, instead releasing spores through an entire-margined circular opening formed by amphithecial tissue, which takes the shape of a broad disk in *Lyellia* R.Br. and *Alophosia* Card. The columella protrudes through this opening as an expanded plug or "stopper" that controls dehiscence. The authors concluded that the peristomes of all mosses are not homologous structures and that the polytrichoid peristome found in putatively derived taxa is a parallelism. However, alternative topologies were only two steps less parsimonious, not all taxa were sampled for molecular characters (most notably *Atrichopsis* Card.) and the most critical basal nodes lacked high jackknife support values.

The study of Hyvönen *et al.* (2004) expanded both taxon and character sampling, including 46 species and adding the chloroplast *trn*L-F region and part of the mitochondrial *nad*5 gene to the molecular matrix (although approximately 50% of the *trn*L-F region and the *rps*4-*trn*S intergenic spacer were excluded due to alignment problems). A surprising result was that a high support value (jackknife = 99%) was obtained for the sister group relationship of the

peristomate *Atrichopsis compressa* (Hook.f. & Wilson) G.L.Sm. to *Alophosia azorica* and that this clade was sister to the rest of the order, implying that the polytrichoid peristome is a plesiomorphic feature within the class. In this context, the question of whether it is most parsimonious to interpret the peristomes in Polytrichopsida and other mosses as homologous hinged on the position of *Oedipodium griffithianum* (Dicks.) Schwägr., which appeared as sister to the Polytrichopsida in the analysis, although with a low jackknife support value. Although surprising, the novel position of *Atrichopsis* was credible, as both it and *Alophosia* are narrow endemics lacking adaxial leaf lamellae (although male plants of *Atrichopsis* have some weakly developed lamellae). However, in the early stages of the current project it became apparent that this position of *Atrichopsis* is artefactual and due to contamination of PCR reactions for both *Atrichopsis* and *Alophosia,* for which only limited and poor quality material had been available for DNA extraction. Organismal representation in the NCBI databases has expanded considerably during the last three years, and recent BLAST searches suggest that the 18S sequence of *Atrichopsis* is contaminated with fungal DNA while the *rps*4 sequence of *Alophosia,* as well as the *trn*L sequences of both *Atrichopsis* and *Alophosia,* have partial hepatic contamination. Consequently both taxa appeared outside of the clade containing all of the remaining Polytrichopsida in Hyvönen *et al.* (2004) and were grouped together on the basis of the shared hepatic contamination of *trn*L.

In this study we aim to: 1) resolve the current ambiguity surrounding the early phylogeny of the Polytrichopsida in order to identify an outgroup for future analyses 2) clarify the position of *Atrichopsis compressa* 3) address the issue of the origin and homology of the polytrichoid and dawsonioid peristomes. Infrageneric taxon sampling is intentionally limited, as characters best suited to resolve basal and outgroup relations are less informative at generic and species levels, while the detailed phylogeny of the group is the subject of future publications in preparation. New sequences have been generated for a number of taxa including *Atrichopsis compressa*, fresh material of which was obtained during fieldwork by the first author in southern Chile in 2005. We employ a heterogeneous Bayesian approach in addition to standard parsimony. Although the assumptions underlying these techniques are fundamentally different, any conflict between the results may be mutually informative, while the use of parsimony facilitates comparison with the results of previous studies.

MATERIALS AND METHODS

Sampling

Twenty seven species of Polytrichopsida and 10 outgroup taxa were sampled for the nuclear 18S ribosomal RNA gene, the gene coding for the large subunit of the RuBisCO enzyme (*rbc*L, chloroplast) and the small ribosomal protein 4 (*rps*4, chloroplast). Forty sequences from 14 taxa were newly generated for this study (Table 1). Due to the difficulty of amplifying the 3' end of *rbc*L in most Polytrichopsida, only the first 700 bp (approx.) were amplified for newly generated sequences. All genera of Polytrichopsida were represented other than the monospecific *Hebantia* G.L.Sm. and *Steereobryon* G.L.Sm., known to be closely related to *Dendroligotrichum* (Müll.Hal.) Broth. and *Atrichum* P.Beauv. respectively (Hyvönen *et al.*, 2004; Bell & Hyvönen, unpublished). As the object

Table 1. *Taxa sampled with GenBank accession numbers. Voucher details are provided in the GenBank records and/or in original publications. Sequences marked with an asterisk were newly generated for this study.*

Taxon	18S	*rbc*L	*rps*4
Alophosia azorica (Renauld & Cardot) Cardot	AY126951	AY312924	AY330476
Andreaea wilsonii Hook.f.	AY330416	AY312925	AY330477
Atrichopsis compressa (Hook.f. & Wilson) G.L.Sm.	*EU927319	*EU927307	*EU927332
Atrichum tenellum (Röhl.) Bruch & Schimp.	*EU927320	*EU927308	*EU927333
Atrichum undulatum (Hedw.) P.Beauv.	X85093	AY118236	AY137681
Bartramiopsis lescurii (James) Kindb.	AY126954	AF208409	AF208418
Bryoxiphium norvegicum (Brid.) Mitt.	AF223008	AF231294	AF223037
Buxbaumia aphylla Hedw.	Y17603	AF478212	AF306959
Dawsonia papuana F.Muell. *ex* Geh.	AF208405	AF208410	AF208419
Dawsonia polytrichoides R.Br.	AY126956	AY118238	AY137683
Dendroligotrichum dendroides (Brid. *ex* Hedw.) Broth.	*EU927321	*EU927309	*EU927334
Diphyscium foliosum (Hedw.) D.Mohr	AJ275008	AY312928	AF223034
Funaria hygrometrica Hedw.	X80212	AF226818	AJ845203
Hymenodontopsis vallis-gratiae (Hampe *ex* Müll.Hal.) N.E.Bell, A.E.Newton & D.Quandt	AF023695	AY631202	AY631167
Itatiella ulei (Broth. *ex* Müll.Hal.) G.L.Sm.	AY126959	AF208412	AF208421
Lyellia aspera (I.Hagen & C.E.O.Jensen) Frye	AF208403	AF208413	AF208422
Lyellia crispa R.Br.	*EU927322	*EU927310	*EU927335
Meiotrichum lyallii (Mitt.) G.L.Sm.	*EU927331	AY118241	AF208423
Notoligotrichum australe (Hook.f. & Wilson) G.L.Sm.	AF208404	AF208414	AY137686
Notoligotrichum minimum (Cardot) G.L.Sm.	*EU927323	*EU927311	*EU927336
Notoligotrichum tapes (Müll.Hal.) G.L.Sm.	*EU927324	*EU927312	*EU927337
Oedipodium griffithianum (Dicks.) Schwägr.	AF228668	AY312923	AF306968
Oligotrichum hercynicum (Hedw.) Lam. & DC.	AY126962	AY118243	AY137688
Oligotrichum parallelum (Mitt.) Kindb.	AY126963	AF208415	AF208424
Pogonatum spinulosum Mitt.	AY126974	AY118254	AY137698
Pogonatum urnigerum (Hedw.) P.Beauv.	AF208406	AY118256	AF208426
Polytrichadelphus magellanicus (Hedw.) Mitt.	*EU927325	*EU927313	*EU927338
Polytrichadelphus peruvianus Broth.	*EU927326	*EU927314	*EU927339
Polytrichadelphus pseudopolytrichum (Raddi) G.L.Sm.	AY126976	AF261074	AY137700
Polytrichastrum alpinum (Hedw.) G.L.Sm.	*EU927327	*EU927315	*EU927340
Polytrichastrum formosum (Hedw.) G.L.Sm.	*EU927328	*EU927316	*EU927341
Polytrichum brachymitrium Müll.Hal.	AY126979	AY118261	AY137704
Polytrichum juniperinum Hedw.	*EU927329	*EU927317	*EU927342
Psilopilum cavifolium (Wilson) I.Hagen	*EU927330	*EU927318	*EU927343
Sphagnum palustre L.	Y11370	AF231887	AF231892
Tetraphis geniculata Girg. *ex* Milde	AY126950	AF478204	AF306955
Timmia megapolitana Hedw.	AY330423	DQ778620	AY908619

of the study was to identify the earliest bifurcations in the ingroup, taxon sampling was highly selective below the generic level, where the data was not anticipated to be highly informative. Outgroup taxa included exemplars from all major lineages of mosses other than Takakiopsida.

DNA extraction, PCR amplification and sequencing

Extraction of genomic DNA was carried out using the Invisisorb spin plant mini kit (Invitek). Some samples were further purified using the Wizard DNA clean-up kit (Promega).

PCR amplifications were performed in 50 ml reactions with 1.25 U *Taq* DNA polymerase (Fermentas, native), 1X Tris-HCL / $(NH_4)_2SO_4$ buffer, 2 mM $MgCl_2$, 200 mM dNTPs, 0.3 mM of each primer, and 5 mg BSA. Standard PCR protocols all included an initial melting step of 94 °C for three minutes and a final extension period of 72°C for seven minutes. For the iterated parts of amplifications (30 or 35 cycles), protocols for the three regions were as follows: 18S: 94°C (30 sec), 52°C (30 sec), 72°C (3 min). *rps*4: 94°C (30 sec), 50°C or 52°C (30 sec), 72°C (2 min or 2 min 30 sec). *rbc*L: 94°C (30 sec), 48°C (30 sec), 72°C (1 min 30 sec). Primers used for *rps*4 and *rbc*L were as reported in Bell & Newton (2005), except that only the primer pairs for the first half (5′ end) of *rbc*L were used here (see above). Primers for 18S were NS1 and PCRB (Hedderson, pers. comm. in Cox *et al*., 2000).

Some PCR products were cleaned using either the GFX PCR DNA & gel purification kit, the Qiagen QIAquick PCR purification kit or the Qiagen MinElute PCR purification kit. All products were sequenced by Macrogen Inc., South Korea (www.macrogen.com) using primers supplied by the authors. For *rps*4 and *rbc*L these were the same as the PCR primers. For 18S, the following additional sequencing primers were used: 18G, 18GRC, 18J, 18KRC (Hamby *et al*., 1998 in Cox *et al*., 2000).

Alignment and Phylogenetic Analysis

Sequences were aligned manually using PhyDE v0.992 (Müller *et al*., 2005). Alignment of the protein coding regions (*rbc*L and *rps*4) was unambiguous. In the 18S region all indels were autapomorphic and/or represented only single bp insertion/deletions, other than two short CT rich areas of approximately 10-15 bp each that could not be aligned unambiguously and were excluded.

For all analyses *Sphagnum palustre* L. was used to root topologies. Heterogeneous Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) running on a Windows PC. Each of the three genomic regions was defined as a separate compartment. The AIC criterion as implemented in MrModeltest 2.2 (Nylander 2004) was used to select the best fitting model for each region. Compartments were unlinked to allow parameters to vary independently. For each analysis, three independent runs using the default prior settings, each with five chains (temp parameter = 0.15), were run simultaneously with trees sampled every 100 generations until the runs had converged. The results from one of the runs were summarized as a maximum clade credibility tree using TreeAnnotator v1.4.6 after discarding the trees sampled during the "burn-in" phase (3000 out of 10000 sampled trees). The final tree for publication was produced using FigTree v.1.0. The TreeAnnotator and FigTree programs are distributed as part of the BEAST v.1.4 package (Drummond & Rambaut, 2006). The maximum clade credibility tree is the single tree with the maximum product of clade posterior probabilities and an estimate of the tree with the maximum

total probability (Drummond, http://beast.bio.ed.ac.uk/). This is a better summary of the results than the more commonly presented 50% majority rule tree, which potentially may represent a topology that has not actually been sampled.

Parsimony analyses were performed using PAUP* 4.0b10 (Swofford, 2001) on a Windows PC. Searches for most parsimonious trees were undertaken using stepwise random taxon addition and TBR branch swapping with all characters unordered and equally weighted. Initial searches of 50,000 replications were performed saving only one tree of length >= 1 on each replication. A second search was performed with no limits on the number of trees saved (nchuck = 0) starting with the trees held in memory from the first search. This strategy is effectively equivalent to the heuristic search strategy implemented within NONA (Goloboff, 1998) and WINCLADA (Nixon, 1999). Bootstrap values were obtained using 1,000 replications each of 10 full (random addition) standard heuristic searches.

RESULTS

The GTR + Γ + I model was selected as the best fit to the data for all three genomic compartments individually. The Bayesian analysis was aborted after 1 x 10^6 generations when it was apparent that all three runs had converged according to the standard deviation of split frequencies and were at equilibrium according to the potential scale reduction factors (PSRF) calculated for all parameters (this being consistent with a manual examination of the distribution of posterior probability values in the output files). The maximum clade credibility tree is shown in Fig. 1. The Polytrichopsida are monophyletic with a posterior probability (PP) of 1.00. The earliest split is between *Alophosia azorica* and the remainder of the class, the latter also having maximum probability (PP = 1.00). The other eperistomate genera, *Lyellia* and *Bartramiopsis* Kindb., form a monophyletic group (PP = 1.00) that is sister to the large clade of peristomate taxa (PP = 1.00), within which *Dawsonia* and the genera characterised by polytrichoid peristomes form separate clades (PP = 1.00 and 0.97 respectively). *Atrichopsis compressa* has a relatively derived position within the peristomate clade as a member of a clearly monophyletic group (PP = 1.00) that includes *Notoligotrichum australe* (Hook.f. & Wilson) G.L.Sm. and *N. minimum* (Cardot) G.L.Sm. A more inclusive clade of *Notoligotrichum* G.L.Sm. species with an equally high probability (PP = 1.00) also includes the Brazilian monospecific endemic *Itatiella* G.L.Sm.

The parsimony analysis resulted in 36 equally parsimonious trees of 1872 steps, consistency index (CI, Kluge & Farris, 1969) = 0.54, retention index (RI, Farris, 1989) = 0.56, rescaled consistency index (RC, Farris, 1989) = 0.31. The strict consensus with bootstrap support values (BS) is shown in Fig. 2. The topology of the earliest diverging lineages within the Polytrichopsida is identical to that found in the Bayesian analysis, with a BS of 100% obtained for the monophyly of the peristomate clade. The clade including all polytrichopsid taxa to the exclusion of *Alophosia* also has a high support value (BS = 88%) although for the Polytrichopsida itself the bootstrap value is significantly lower (BS = 75%). There is strong support (BS = 94%) for *Atrichopsis* forming a monophyletic group with *Notoligotrichum australe* and *N. minimum*. While the larger *Notoligotrichum* clade also has a fairly high support value (BS = 85%), it does not include *Itatiella*, which in the most parsimonious topologies forms a clade with *Dendroligotrichum* and *Polytrichadelphus* (although without bootstrap support).

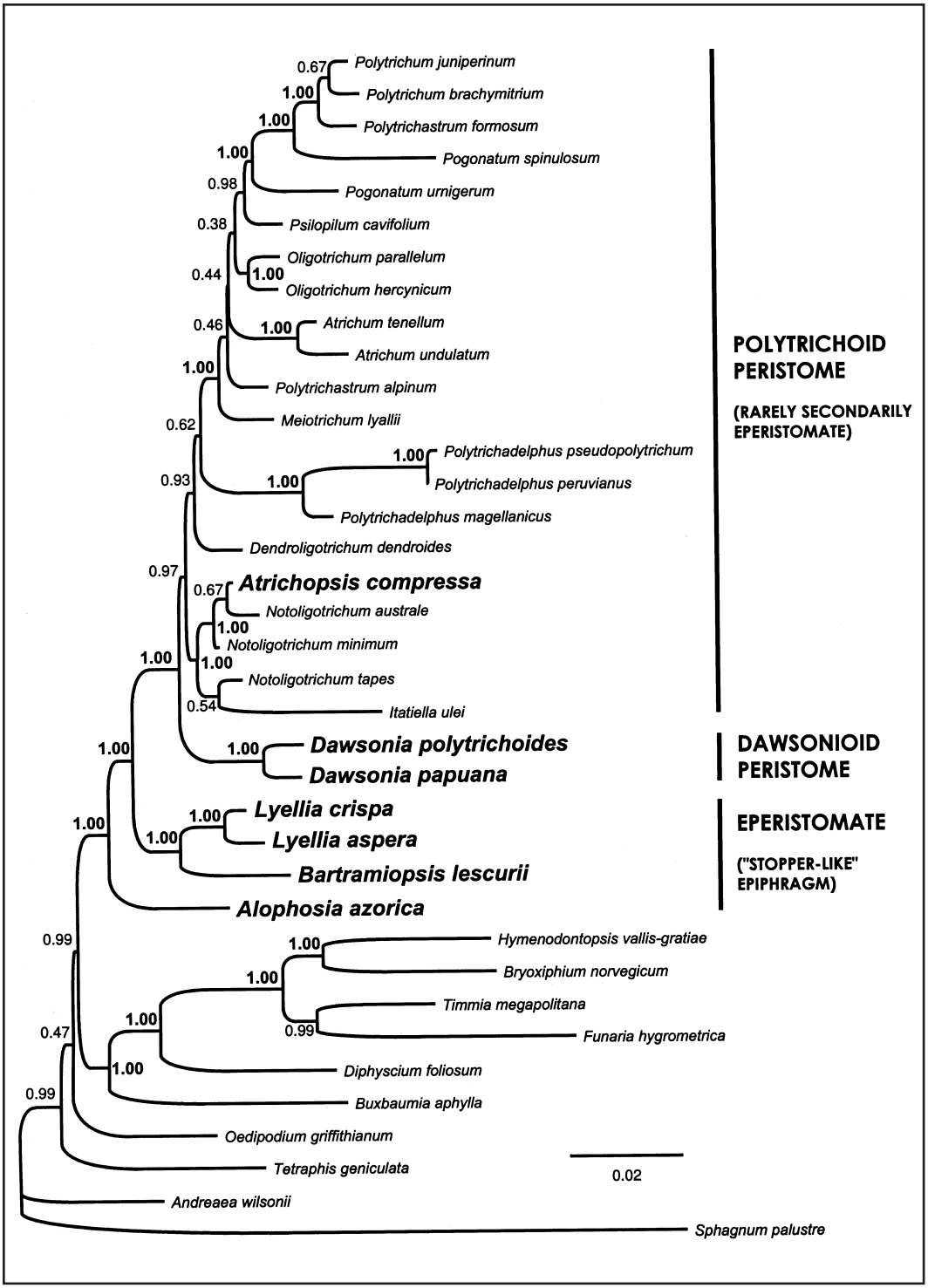

Fig. 1. *Bayesian maximum clade credibility tree derived from 7000 trees sampled during one of three convergent MCMC runs. Numbers on branches are clade posterior probabilities.*

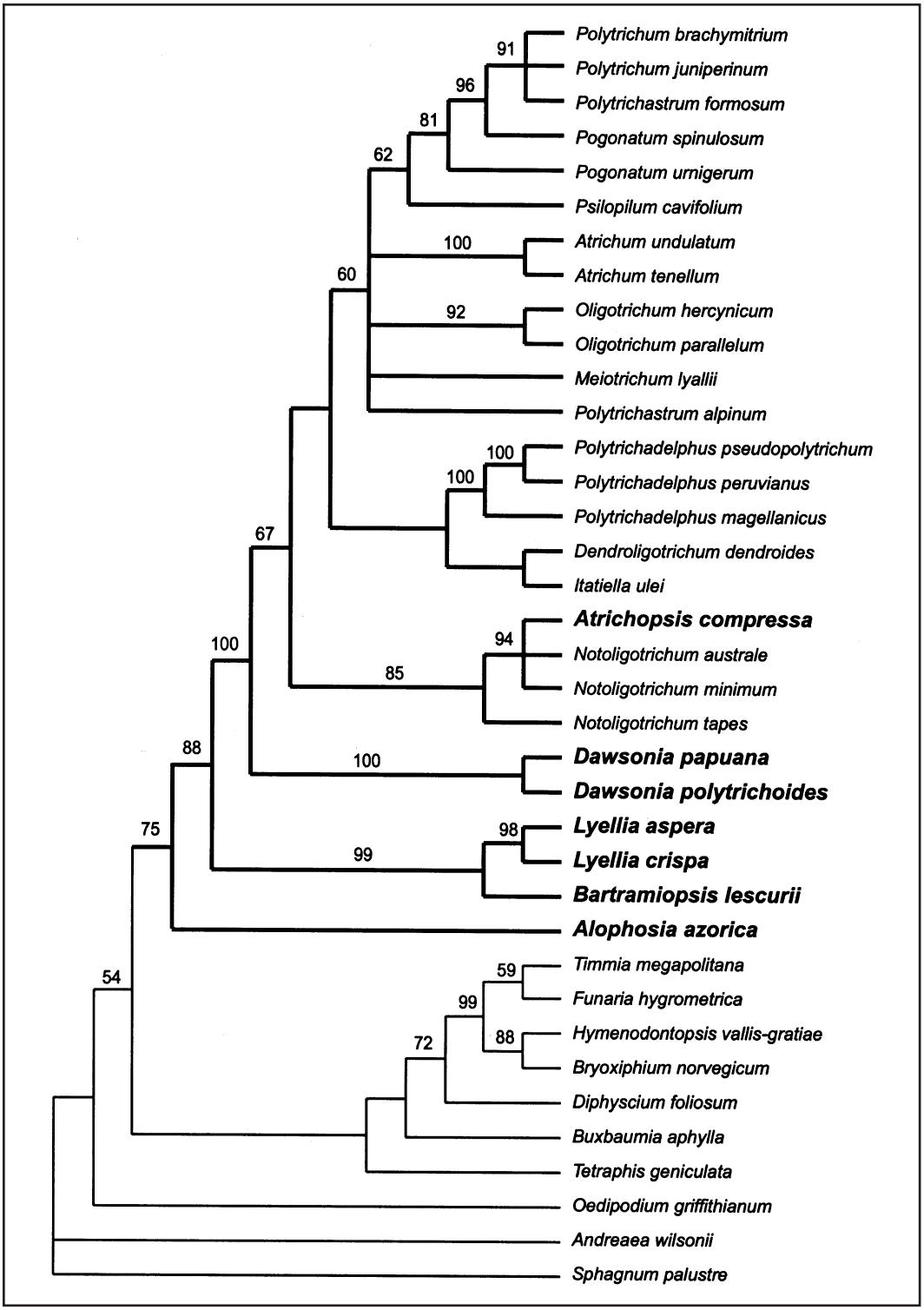

Fig. 2. *Strict consensus of 36 equally parsimonious trees of 1872 steps found during the parsimony analysis. Numbers on branches are bootstrap percentages.*

Amongst the outgroup taxa the Bayesian and parsimony analyses agree in placing *Oedipodium griffithianum* sister to the combined clade of the Polytrichopsida and the arthrodontous mosses, although they conflict on the position of *Tetraphis geniculata* Girg. *ex* Milde, which is sister to the arthrodontous mosses in the parsimony analysis and to the combined *Oedipodium* Schwägr. / Polytrichopsida / arthrondontous clade in the Bayesian topology. In the latter there is no credible support value (PP = 0.47) for the grouping of *Oedipodium* with the Polytrichopsida / arthrodontous clade to the exclusion of *Tetraphis* Hedw., although there is a high probability value for *Tetraphis* lying outside of the larger clade (PP = 0.99). In the parsimony analysis BS values for resolution among the outgroup taxa are all < 50%, other than for and within the clade including the diplolepideous and haplolepideous exemplars.

DISCUSSION

The results strongly support *Alophosia azorica* as the sister taxon to the remainder of the Polytrichopsida and are consistent with interpreting the nematodontous peristome as it is found within the class (i.e. excluding the very different structure found in Tetraphidopsida) as a synapomorphy for the clade that includes *Dawsonia* and the group of genera possessing polytrichoid peristomes. However, the apparent reciprocal monophyly of the latter two groups, combined with the very different structures of the dawsonioid and polytrichoid peristomes, also lends credibility to the hypothesis that the peristome itself (as a collection of linear processes surrounding the capsule mouth) has entirely independent origins within these two clades. Resolving this question is likely to depend less on ancestral character state reconstruction and more on whether the dawsonioid and polytrichoid peristomes can regarded as positionally and developmentally homologous (see Edwards, 1984; Smith, 1971 for discussion and references).

Contrary to previous results (Hyvönen *et al.*, 2004) *Atrichopsis compressa* is clearly shown to be derived from within *Notoligotrichum*, the latter genus being a segregate from *Psilopilum* Brid. created by Smith (1971). The specific epithet has previously been recognised under *Psilopilum*, as *P. compressum* (Hook.f. & Wilson) Mitt. The morphology of *Atrichopsis* is entirely consistent with a position within *Notoligotrichum* (Smith, 1971; and pers. comm.), despite the general lack of lamellae (see below).

Topological congruence and phylogenetic confidence

The branching order of the earliest lineages in the Polytrichopsida as revealed here (and consequently the relationships of the groups representing the major peristome types) is identical to the topology that Hyvönen *et al.* (1998) found after successively applied weighting. However, whereas in that study the only node in this region with a BS value > 50% corresponded to the clade uniting *Lyellia* and *Bartramiopsis*, the current results provide statistical certainty under Bayesian assumptions (given the data and the model) for all nodes in the Polytrichopsida up to and including the peristomate clade. Under parsimony all of these nodes with the exception of the one supporting the Polytrichopsida itself have BS values >= 88%. The results of Hyvönen *et al.* (2004) differ principally in the position of *Atrichopsis* and in lower support values for comparable clades.

We explored relevant alternative topologies under parsimony by constraining the non-monophyly of the

peristomate clade and also of the peristomate clade + *Lyellia* and *Bartramiopsis*. In the first instance maximally parsimonious solutions were 13 steps longer and in all optimal trees the respective positions of *Dawsonia* and the *Lyellia* + *Bartramiopsis* clade were reversed. In the second case trees were 7 steps longer, with *Alophosia* forming a clade with *Bartramiopsis* and *Lyellia* in 86% of topologies.

Although sampling amongst outgroup taxa was insufficient to allow sound conclusions to be drawn, it is worth noting the incongruence between the Bayesian and parsimony topologies with regard to the position of *Tetraphis*. The parsimony trees agree with Cox *et al*. (2004) in placing *Tetraphis* sister to the arthrodontous mosses, while the Bayesian topology seems to offer statistically significant support (PP = 0.99) for the monophyly of the arthrodontous mosses + Polytrichopsida to the exclusion of *Tetraphis*, which is more compatible with the results of Magombo (2003). Goffinet *et al*. (2005) revealed that *Tetraphis* shares the loss of the chloroplast *rpo*A gene with the arthrodontous mosses, although the presence of this gene in *Buxbaumia* Hedw. requires the assumption of an independent loss in *Tetraphis* (or other equally parsimonious scenarios).

Atrichopsis

When Smith (1969) made the combination *Atrichopsis compressa* on the grounds that the specific epithet of *Polytrichum compressum* Hook.f. & Wilson had priority over that of *Atrichopsis magellanica* Card., he was explicit in noting the similarities of the plant to several austral *Psilopilum* species (now placed in *Notoligotrichum*, Smith 1971). In particular he highlighted features of the sporophyte, including the form of the exothecial cells, the form and position of the stomata and features of the peristome and calyptra. Preliminary observations suggest that the microarchitecture of the epiphragm / peristome junction is also similar (Bell & Hyvönen, unpublished). The general lack of adaxial lamellae is easily interpreted as a secondary loss, especially as these are present in a vestigial form in male plants. Furthermore, extreme lamellar reduction often also characterises *Notoligotrichum tapes* (Müll.Hal.) G.L.Sm., a plant with a similar geographical distribution that occurs in similar microhabitats. These plants may appear similar in the field, although under magnification the bistratose lamina and unistratose, pigmented border of *Atrichopsis* are highly distinctive. It is an unfortunate coincidence that *Atrichopsis* and *Alophosia* share a bistratose lamina and a lack of lamellae (as originally noted by Cardot, 1912 when he described *Atrichopsis*), thus supporting the artefactual position of *Atrichopsis* in Hyvönen *et al.* (2004).

Atrichopsis compressa should almost certainly be placed in *Notoligotrichum*, although we prefer to defer taxonomic changes until after publication of analyses including greater sampling from *Notoligotrichum*.

What is a peristome anyway?

"Peristome" simply means "around the mouth", from the Greek "peri" and "stoma". There is no explicit reference to "teeth", and indeed the disk surrounding the capsule opening in *Lyellia* was originally described as a peristome (Brown, 1819 in Smith, 1971). By a process of semantic change the word has come to refer to one or more rings of tooth-like processes and is now usually defined as such (e.g. Malcolm & Malcolm, 2000; Smith, 2004; the definition in Magill, 1990 is slightly more conservative). Not only does this concept not refer to homologous structures, but

even as a descriptive term it is vague and unhelpful in its inclusivity.

Tetraphis has traditionally been described as having a "nematodontous peristome", thus implying a link with the "peristomate" Polytrichopsida, although the quadripartite division of the entire apical part of the sporangium into four massive "teeth" has little structural or developmental similarity to the spore releasing apparatus of the derived Polytrichopsida, which relies on separate elaboration of the endothecial columella and the apical, inner layers of the amphithecium (although see Taylor, 1962; Edwards, 1984 for discussion of potential homologies of the outer layer of the peristome teeth in *Tetraphis*). Developmentally, *Tetraphis* is more similar to the arthrodontous mosses in the early anticlinal divisions of the peristomal layers (Shaw & Anderson, 1988) and further lacks the lysigenous abscission layer that surrounds the polytrichoid peristome (Lantzius-Beninga, 1850 in Edwards, 1984). Indeed, while the earliest diverging Polytrichopsida are not usually referred to as peristomate in the modern sense because they do not have "teeth", it is clear that their spore releasing apparatus, in relying on the interaction of an expanded endothecial columella with an elaborated inner amphithecial structure, has much in common with the more derived polytrichoid peristome. Hence while the polytrichoid peristome in the sense of a ring of linear processes is merely analogous to similar structures in other mosses, under a more inclusive (and traditional) definition of the term, peristomal development itself may yet have a single origin (i.e. deep homology). In any case, more critical interpretation of the micro-architecture and development of these structures will be a more meaningful way to explore processes of evolutionary development and homology than analytically complex ancestral-state reconstruction based on artificial and biologically meaningless characters. Particularly when coding morphological characters for phylogenetic analysis, it is necessary to challenge traditionally accepted vocabulary where necessary and make unbiased observations of structural variation. The validity of arguments against using morphology (as opposed to, for example, protein-coding molecular characters) for phylogeny reconstruction (e.g. Scotland *et al.*, 2003) partially rests on whether it is possible to do this in practice, i.e. on whether it is possible to arrive at an unbiased categorisation of morphological variation.

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