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Phylogeny of the moss class Polytrichopsida (BRYOPHYTA): Generic-level structure and incongruent gene trees

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ABSTRACT

Analysis of an extensive new molecular dataset for the moss class Polytrichopsida provides convincing support for many traditionally recognised genera and identifies higher level phylogenetic structure with a strong geographic component. A large apical clade that is most diverse in the northern hemisphere is subtended by a grade of southern temperate and tropical genera, while the earliest diverging lineages have widely separated relictual distributions. However, there is strongly supported topological incongruence between the nuclear 18S rRNA gene tree and the chloroplast and mitochondrial data for the positions of some taxa and notably for the status of *Pogonatum*. While *Pogonatum* is unambiguously paraphyletic in the 18S tree, it is well supported as monophyletic by the combined chloroplast and mitochondrial data, this being corroborated by several distinctive morphological synapomorphies and a 51–53 bp deletion in the *rps4-trnS* spacer. We explore various reticulate historical processes and methodological issues as possible explanations for incongruence, and suggest that either (1) the 18S topology is an artefact created by convergence of substitutions at specific sites due to functional and/or molecular-structural constraints not accounted for by the model, or (2) the incongruence is a product of ancient hybridization events. Under the latter scenario, incongruent topologies for *Pogonatum* are parsimoniously explained if *Polytrichum* (including *Polytrichastrum* sect. *Aporotheca*) is ultimately descended from a hybridization event involving an extinct maternal taxon derived from the branch ancestral to the combined *Pogonatum*/*Polytrichum s.l.* clade, and a paternal taxon belonging to (or ancestral to) the apical *Pogonatum* group to which the majority of extant species belong. Numerous novel relationships of taxonomic and evolutionary significance are supported. Notably, both *Polytrichastrum* and *Oligotrichum* are polyphyletic. While *Polytrichastrum* sect. *Aporotheca* is closely related to *Polytrichum*, other species, including the type, are not. The large majority of *Oligotrichum* species sampled occur in one of two distantly related clades with predominantly northern and southern hemisphere distributions, respectively, implying convergent evolution of this morphology in each of the two temperate zones.

1. Introduction

One function of phylogenetics is to highlight entrenched perspectives within systematics that misrepresent the evolutionary significance of particular taxa. This is a prerequisite for informing conservation decisions using indices such as phylogenetic diversity (Faith, 1992; Faith and Baker, 2006) or evolutionary distinctiveness (Isaac et al., 2007) that take account of the phylogenetic uniqueness of taxa but are widely neglected in favour of ecologically derived measures such as species diversity. Recent progress in the phylogenetics of land plants, or embryophytes (see Qiu, 2008 for a review) now provides confidence in the relationships of the major lineages, of which the "vascular plants" (Tracheophyta) are only one example. We strongly support the use of the alternative term "polysporangiophytes" (Kendrick and Crane, 1997) for the clade of non-bryophyte land plants and the recognition of division Polysporangiophyta, for two reasons. Firstly, water conducting cells (WCCs) have arisen independently in some bryophyte lineages as well as in polysporangiophytes (Ligrone et al., 2000; Carafa et al., 2005), with vascularization being well-developed in many mosses, particularly the Polytrichopsida (Smith, 1971; Ligrone et al., 2000). Both the imperforate WCCs (hydroids) in peristomate mosses and the perforate WCCs (tracheids and vessels) in polysporangiophytes undergo cytoplasmic lysis (Ligrone et al., 2000) to form true vascular tissue. Although the name "Tracheophyta" strictly refers only to the presence of lignified tracheids, the occurrence of this particular type of WCC is an obscure way to define the most prominent group of land plants, while to non-specialists the terms "tracheophyte"

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and "vascular plant" are interchangeable. Indirectly characterising the Bryophyta as non-vascular encourages the popular erroneous assumption that they are intrinsically primitive and ancestral to polysporangiophytes, i.e. it implies orthogenic rather than cladogenic views of macroevolution and is complicit in a general undervaluing of bryophyte diversity. Secondly, the transition from a gametophyte-dominant lifestyle with a dependent monosporangiate sporophyte to a sporophyte-dominant one in which multiple sporangia occur on free-living sporophytes appears to be the definitive innovation of the polysporangiophyte clade.

Liverworts are now recognised as a monophyletic group sister to the rest of the land plants. Within the latter, mosses are the sister group of an apical clade in which hornworts are sister to polysporangiophytes (Malek and Knoop, 1998; Kelch et al., 2004; Wolf et al., 2005; Qiu et al., 2006; Groth-Malonek et al., 2005; Qiu, 2008). Of the three bryophyte (i.e. monosporangiophyte) lineages, the mosses (division Bryophyta) are the most diverse and the most strongly adapted to terrestrial environments. They have highly developed mechanisms of desiccation tolerance (e.g. Oliver et al., 2005; Proctor et al., 2007) and, unlike liverworts, show considerable diversity in their sporophytes; these are relatively long-lived and have structurally complex peristomes for regulating the release of spores over extended periods of time in response to environmental conditions.

The Polytrichopsida are now recognised as a class within the mosses and can be viewed as a significant group of land plants in their own right. They have a position strikingly analogous to that of the conifers within the seed plant clade; an apparently ancient group with considerable variety of form but relatively few species compared to a more prominent related group (approximately 200 accepted names next to more than 12,000 for arthrodontous mosses; Crosby et al., 1999), they include the largest extant mosses (*Dawsonia* R.Br., *Dendroligotrichum* (Müll.Hal.) Broth.) as well as some highly abundant taxa that may dominate certain communities, especially in the northern boreal zone (e.g. certain species of *Polytrichum* Hedw.). The structure that regulates the release of spores from the sporangium in the Polytrichopsida, the peristome, only superficially resembles the peristomes found in other groups of mosses. Most mosses have arthrodontous peristomes – one or more rings of membranous projections formed from the cell walls of amphithecial tissue at the apex of the sporangium that are often highly mobile in response to atmospheric moisture and facilitate the release of spores in particular conditions. In the Polytrichopsida the peristome, when present, is nematodontous – also of amphithecial origin but composed of solid tooth-like processes – and attaches to a disk-like "epiphragm", a development of the central (endothecial) columella. Spores are released through the gaps between the peristome teeth, possibly influenced by subtle changes in the shape of the epiphragm. Recent work (Bell and Hyvönen, 2008) supports the view that the polytrichopsid nematodontous peristome is not homologous with arthrodontous peristomes nor with the nematodontous peristome of Tetraphidopsida, and thus is part of a novel sporangial dehiscence apparatus in land plants.

The gametophytes of most Polytrichopsida have a closely set series of photosynthetic adaxial lamellae on the leaf lamina, forming a "pseudo-mesophyll" (Smith, 1971) that considerably increases the area available for CO_2 uptake and thus the potential rate of photosynthesis (Proctor, 2005). Additionally, most species have a basally sheathing leaf base well differentiated from the lamina. These features, in conjunction with a particularly well-developed vasculature based on hydroids (WCCs) and leptoids (food-conducting cells), apparently not homologous with the vasculature of polysporangiophytes (Ligrone et al., 2000; Carafa et al., 2005), may be key to the large size of some species. Although the class is often associated with robust plants of open habitats, it also includes very small plants (e.g. the neotenous *Pogonatum pensilvanicum*) and a number of taxa adapted to shaded forest environments (e.g. *Dawsonia* and many tropical species of *Pogonatum* P.Beauv.). Truly epiphytic species are conspicuously lacking, even compared with other acrocarpous moss groups.

The group has been the subject of two major phylogenetic analyses in the past few years (Hyvönen et al., 1998, 2004), although many internal groupings remained poorly resolved. In addition, Bell and Hyvönen (2008) recently obtained strongly supported resolution of the earliest dichotomies within the class and summarised previous molecular phylogenetic research. In this study we undertook a comprehensive molecular phylogenetic analysis of the Polytrichopsida using more than twice the number of terminal nodes included in the largest previous study (Hyvönen et al., 2004). Five DNA regions from all three genomes were sampled, including for the first time the entire *nad5* group I intron as well as previously excluded non-coding chloroplast areas (the *rps4-trnS* spacer and large parts of the *trnL* intron and the *trnL-F* intergenic spacer). One obstacle to including these data in previous analyses was the difficulty of obtaining credible hypotheses of primary homology (alignment) for outgroup taxa, which due to the phylogenetically isolated nature of the Polytrichopsida have diverged considerably in non-coding regions. However, we believe that our recent work (Bell and Hyvönen, 2008) reliably establishes the mono-specific genus *Alophosia* Cardot as sister to the remainder of the class and allows rooting of the phylogeny in the absence of more distantly related outgroups. Identifying well-supported major groupings within the Polytrichopsida will be an important step towards a natural classification at the generic and familial levels, interpretation of the strong phylogeographic signal within the class, and an understanding of the evolution of poorly understood morphological variation such as that found within the peristome–epiphragm complex.

2. Materials and methods

2.1. Taxon sampling

We sampled 120 specimens in total representing approximately 90 currently recognised species, about half of the estimated number in the class (Table 1). All 18 currently accepted extant genera (Hyvönen et al., 2004) were represented, seven of which are mono-specific (*Alophosia, Atrichopsis* Cardot, *Bartramiopsis* Kindb ., *Hebantia* G.L.Merr., *Itatiella* G.L.Sm ., *Meiotrichum* (G.L.Sm.) G.L.Merr. *and Steereobryon* G.L.Sm.). Multiple accessions were sampled for some widespread, variable and/or taxonomically ambiguous taxa, notably *Polytrichastrum alpinum*, *P. norwegicum* (= *P. alpinum* var. *septentrionale* (Brid.) G.L.Sm.), *P. sphaerothecium* (= *P. sexangulare* var. *vulcanicum* (C.E.O.Jensen) G.L.Sm.), *P. sexangulare*, and *Pogonatum urnigerum*. Although pilot studies suggested that the common and widespread species *Polytrichum commune* is not monophyletic, the systematics of this important taxon will be the subject of further study and here we have included only two genetically dissimilar representatives. In selecting exemplars we concentrated on sampling as much morphological variation as possible, especially where we considered that this might represent unrecognised generic-level diversity. Extensive sampling within very well-defined genera (e.g. *Atrichum* P.Beauv., *Dawsonia*) was a lower priority. Geographic representation was wide-ranging and care was taken to equally represent both northern and southern temperate as well as tropical diversity. The genus *Notoligotrichum* G.L.Sm. occurs in South Africa in the form of a species or species complex that has not yet been validly described. As with *Polytrichum commune* this will be the subject of a future publication, and the entity is represented here by a single exemplar, *Notoligotrichum* TH14147.

Table 1
Terminals in the analyses with vouchers and GenBank accession numbers. Accession numbers beginning "GU" are newly published. Numbers in italics represent sequences derived from different vouchers (see Genbank record and/or original publication). Where two numbers are provided for *nad5* sequences, these represent a combination of 3′ segments from the voucher with 5′ segments from a different specimen (the italicised number represents the 5′ segment). Asterisks denote *nad5* sequences lacking data for the 5′ segment. All vouchers are held at H unless otherwise indicated.

Taxon and voucher	18S	*rbcL*	*rps4-trnS*	*trnL-F*	*nad5*
Alophosia azorica (Renauld & Cardot) Cardot Rumsey s.n. 1998 ("Pico: Bocas da Fogo") (Azores)	GU569586	GU569408	GU569762	–	GU569491
Atrichopsis compressa (Hook.f. & Wilson) G.L.Sm. Bell 1615 (Chile)	EU927319	EU927307	EU927332	GU569669	GU569492
Atrichum androgynum (Müll.Hal.) A.Jaeger Hyvönen 6387 (Brazil)	AY126952	AY118234	GU569763	AF544999	AY137714*
Atrichum androgynum (Müll.Hal.) A.Jaeger Bell 29.02.08 #1 (New Zealand)	GU569587	GU569409	GU569764	GU569670	GU569493
Atrichum angustatum (Brid.) Bruch & Schimp. Norris 83235 (U.S.A.)	GU569588	GU569410	*AF208417*	GU569671	GU569494
Atrichum flavisetum Mitt. Ignatova 06–02 (Russia)	GU569589	GU569411	GU569765	GU569672	GU569495
Atrichum oerstedianum (Müll.Hal.) Mitt. Hyvönen 6504 (Mexico)	AY126953	AY118235	AY137680	AF545001	AY137716*
Atrichum tenellum (Röhl.) Bruch & Schimp. Bell 02.07.06 #1 (Norway)	EU927320	EU927308	EU927333	GU569673	GU569496
Atrichum undulatum (Hedw.) P.Beauv. Hyvönen 6170 (Finland)	*X85093*	AY118236	AY137681	AF545002	*AJ001229*
Bartramiopsis lescurii (James) Kindb. Hedderson 10044, RNG (Canada)	AY126954	AF208409	AF208418	AF545003	AY137718, *AY908800*
Dawsonia beccarii Broth. & Geh. Bell 31.07.07 #35 (Sabah, Malaysia)	GU569590	GU569412	GU569766	GU569674	GU569497
Dawsonia papuana F.Muell. *ex* Geh. Baker 662, RNG. (Papua New Guinea)	AF208405	AF208410	AF208419	AF246704	AY150372*
Dawsonia polytrichoides R.Br. Schulman 125 (Australia)	AY126956	AY118238	AY137683	AF545005	AY137720*
Dawsonia superba Grev. Bell 31.07.07 #24 (Sabah, Malaysia)	GU569591	GU569413	GU569767	GU569675	GU569498
Dawsonia superba Grev. Stenroos 4677 (New Zealand)	AY126955	AY118237	AY137682	AF545004	AY137719*
Dendroligotrichum dendroides (Brid. ex Hedw.) Broth. Bell 1955 (Chile)	EU927321	EU927309	EU927334	GU569676	GU569499
Dendroligotrichum microdendron (Müll.Hal.) G.L.Sm. Hyvönen 6083 (New Zealand)	AF208402	AF208411	AF208420	AF545006	AY137721*
Dendroligotrichum microdendron (Müll.Hal.) G.L.Sm.. Bell 29.02.08 #2 (New Zealand)	GU569592	GU569414	GU569768	GU569677	GU569500
Dendroligotrichum squamosum (Hook.f. & Wilson) Cardot Bell 1358 (Chile)	GU569593	GU569415	GU569769	GU569678	GU569501
Hebantia rigida (Lorentz) G.L.Merr. Kelt 26.V.86 (Chile)	GU569594	GU569416	GU569770	GU569679	GU569502
Itatiella ulei (Broth. *ex* Müll.Hal.) G.L.Sm. Marcelli, Ahti & Yano 51824 (Sao Paulo, Brazil)	GU569595	GU569417	GU569771	GU569680	GU569503
Itatiella ulei (Broth. *ex* Müll.Hal.) G.L.Sm. Buck 27004 (Minas Gerais, Brazil)	GU569596	GU569418	GU569772	GU569681	GU569504
Lyellia aspera (I.Hagen & C.E.O.Jensen) Frye Hedderson 6825, RNG (Canada)	AF208403	AF208413	AF208422	AF545010	AY137725*
Lyellia aspera (I.Hagen & C.E.O.Jensen) Frye Afonina 8/3, 10.07.06 (Russia)	GU569597	GU569419	GU569773	GU569682	GU569505
Lyellia crispa R.Br. Shevock 23078, UC (China)	EU927322	EU927310	EU927335	GU569683	GU569506
Meiotrichum lyallii (Mitt.) G.L.Sm. Weber WWB36612 (U.S.A.)	EU927331	AY118241	AF208423	AF545011	AY137726, *AY908802*
Notoligotrichum angulatum (Cardot & Broth.) G.L.Sm. Bell 1630 (Chile)	GU569598	GU569420	GU569774	GU569684	GU569507
Notoligotrichum australe (Hook.f. & Wilson) G.L.Sm. Bell 02.03.08 #8 (New Zealand 1)	GU569599	GU569421	GU569775	GU569685	GU569508
Notoligotrichum australe (Hook.f. & Wilson) G.L.Sm. Bell 12.03.08 #3 (New Zealand 2)	GU569600	GU569422	GU569776	GU569686	GU569509
Notoligotrichum australe (Hook.f. & Wilson) G.L.Sm. Bell 12.03.08 #6A (New Zealand 3)	GU569601	GU569423	GU569777	GU569687	GU569510
Notoligotrichum bellii (Broth.) G.L.Sm. Bell 03.03.08 #1 (New Zealand)	GU569602	GU569424	GU569778	GU569688	GU569511
Notoligotrichum crispulum (Hook.f. & Wilson) G.L.Sm. Bell 02.03.08 #2 (New Zealand)	GU569603	GU569425	GU569779	GU569689	GU569512
Notoligotrichum minimum (Cardot) G.L.Sm. Bell 1581 (Chile)	EU927323	EU927311	EU927336	GU569690	GU569513
Notoligotrichum sp. nov. (TH14147) Hedderson 14147 (South Africa)	GU569604	GU569426	GU569780	GU569691	GU569514
Notoligotrichum tapes (Müll.Hal.) G.L.Sm. Bell 1631 (Chile 1)	GU569605	GU569427	GU569781	GU569692	GU569515
Notoligotrichum tapes (Müll.Hal.) G.L.Sm. Bell 1632 (Chile 2)	EU927324	EU927312	EU927337	GU569693	GU569516
Notoligotrichum trichodon (Hook.f. & Wilson) G.L.Sm. Bell 1493 (Chile)	GU569606	GU569428	GU569782	GU569694	GU569517
Oligotrichum afrolaevigatum (Dixon) G.L.Sm. Magill 4340 (South Africa)	GU569607	GU569429	GU569783	GU569695	GU569518
Oligotrichum austroaligerum G.L.Sm. Bell 1629 (Chile)	GU569608	GU569430	GU569784	GU569696	GU569519
Oligotrichum canaliculatum (Hook. & Arn.) Mitt. Hyvönen 6528 (Chile XII)	GU569609	GU569431	GU569785	GU569697	GU569520
Oligotrichum canaliculatum (Hook. & Arn.) Mitt. Schaumann 01–308, MO (Chile X)	GU569610	GU569432	GU569786	GU569698	GU569521
Oligotrichum canaliculatum (Hook. & Arn.) Mitt. Hyvönen 5625 (Argentina)	AY126961	AY118241	AY137687	AF545013	AY137728*
Oligotrichum hercynicum (Hedw.) Lam. & DC. Enroth 25.07.98 (Finland)	AY126962	AY118243	AY137688	AF545014	AY137729*
Oligotrichum obtusatum Broth. Hyvönen 3469 (Taiwan)	GU569611	GU569433	GU569787	GU569699	GU569522
Oligotrichum parallelum (Mitt.) Kindb. Hedderson 10043, RNG (Canada)	AY126963	AF208415	AF208424	AF545015	AY137730, *AY908805*
Oligotrichum riedelianum (Mont.) Mitt. Schäfer-Verwimp & Verwimp 13321 (Brazil)	GU569612	GU569434	GU569788	GU569700	GU569523
Oligotrichum suzukii (Broth.) C.C.Chuang Hyvönen 3985 (Taiwan)	GU569613	GU569435	GU569789	GU569701	GU569524
Oligotrichum tenuirostre (Hook.) A.Jaeger Bell 07.03.08 #2 (New Zealand)	GU569614	GU569436	GU569790	GU569702	GU569525
Pogonatum aloides (Hedw.) P.Beauv. Bell 04.01.07 #1 (U.K.)	GU569615	GU569437	GU569791	GU569703	GU569526
Pogonatum belangeri (Müll.Hal.) A.Jaeger Hedderson 16289 (Réunion)	GU569616	GU569438	GU569792	GU569704	GU569527
Pogonatum campylocarpum (Müll.Hal.) Mitt. Hyvönen 06392 (Brazil)	GU569617	GU569439	GU569793	GU569705	GU569528
Pogonatum cirratum (Sw.) Brid. Bell 31.07.07 #6 (Sabah, Malaysia 1)	GU569618	GU569440	GU569794	GU569706	GU569529
Pogonatum cirratum (Sw.) Brid. Bell 01.08.07 #1 (Sabah, Malaysia 2)	GU569619	GU569441	GU569795	GU569707	GU569530
Pogonatum cirratum (Sw.) Brid. Hyvönen 4008 (Taiwan)	AY126966	AY118246	AY137691	AF545018	AY137733*
Pogonatum contortum (Menzies ex Brid.) Lesq. Hedderson 5803 (Canada)	AY126967	AY118247	AF208425	AF545019	AY137734*
Pogonatum convolutum (Hedw.) P.Beauv. Hedderson 16265 (Réunion)	GU569620	GU569442	GU569796	GU569708	GU569531
Pogonatum dentatum (Menzies ex Brid.) Brid. Bell 10.09.05 #2 (Finland)	GU569621	GU569443	GU569797	GU569709	GU569532
Pogonatum japonicum Sull. & Lesq. Nishimura 10601 (Japan)	GU569622	GU569444	GU569798	GU569710	GU569533
Pogonatum macrophyllum Dozy & Molk. Bell 30.07.07 #19 (Sabah, Malaysia)	GU569623	GU569445	GU569799	GU569711	GU569534
Pogonatum microstomum (R.Br. ex Schwägr.) Brid. Shevock 22895 (China)	GU569624	GU569446	GU569800	GU569712	GU569535
Pogonatum neesii (Müll.Hal.) Dozy Shevock 22891 (China)	GU569625	GU569447	GU569801	GU569713	*GU569536*

(*continued on next page*)

Table 1 (*continued*)

Taxon and voucher	18S	*rbcL*	*rps4-trnS*	*trnL-F*	*nad5*
Pogonatum nipponicum Nog. & Osada Hayashi 7038 (Japan)	GU569626	GU569448	GU569802	GU569714	GU569537
Pogonatum perichaetiale (Mont.) A.Jaeger Hyvönen 3478 (Taiwan)	GU569627	GU569449	GU569803	GU569715	GU569538
Pogonatum proliferum (Griff.) Mitt. Bell 31.07.07 #9 (Sabah, Malaysia)	GU569628	GU569450	GU569804	GU569716	GU569539
Pogonatum spinulosum Mitt. Chishiki 1862 (Japan)	AY126974	AY118254	AY137698	AF545026	AY137741*
Pogonatum subulatum (Menzies ex Brid.) Brid. Bell 02.03.08 #1 (New Zealand)	GU569629	GU569451	GU569805	GU569717	GU569540
Pogonatum subulatum (Menzies ex Brid.) Brid. Streimann 58707 (Australia)	GU569630	GU569452	GU569806	GU569718	GU569541
Pogonatum urnigerum (Hedw.) P.Beauv. Hyvönen 6173 (Finland)	AF208406	AY118256	AF208426	AF545028	*AJ291554*
Pogonatum urnigerum (Hedw.) P.Beauv. Bell 01.08.07 #94 (Sabah, Malaysia)	GU569631	GU569453	GU569807	GU569719	GU569542
Pogonatum urnigerum (Hedw.) P.Beauv. Shevock 23397 (China)	GU569632	GU569454	GU569808	GU569720	GU569543
Pogonatum urnigerum (Hedw.) P.Beauv. Hyvönen 4087 (Taiwan)	AY126970	AY118250	AY137694	AF545022	AY137737*
Pogonatum usambaricum (Broth.) Paris Hedderson 16241 (Réunion)	GU569633	GU569455	GU569809	GU569721	GU569544
Polytrichadelphus ciliatus (Hook. & Wilson) Mitt. Churchill, Rengifo & Arbeláez 17195 (Columbia)	GU569634	GU569456	GU569810	GU569722	GU569545
Polytrichadelphus giganteus (Hook.) Mitt. Churchill & Betancur 18057 (Columbia)	GU569635	GU569457	GU569811	GU569723	GU569546
Polytrichadelphus innovans (Müll.Hal.) A.Jaeger Hyvönen 06072 (New Zealand)	EU927325	EU927313	EU927338	GU569724	GU569547
Polytrichadelphus innovans (Müll.Hal.) A.Jaeger Bell 04.03.08 #4 (New Zealand)	GU569636	GU569458	GU569812	GU569725	GU569548
Polytrichadelphus longisetus (Brid.) Mitt. Churchill, Franco & Parra 18869 (Columbia)	GU569637	GU569459	GU569813	GU569726	GU569549
Polytrichadelphus peruvianus Broth. Holz CR99–504 (Costa Rica)	EU927326	EU927314	EU927339	GU569727	GU569550
Polytrichadelphus pseudopolytrichum (Raddi) G.L.Sm. Hyvönen 6276 (Brazil)	AY126976	AF261074	AY137700	AF545030	AY137745*
Polytrichadelphus purpureus Mitt. Churchill Arbeláez & Rengifo 16296 (Columbia)	GU569638	GU569460	GU569814	GU569728	GU569551
Polytrichastrum alpinum (Hedw.) G.L.Sm. Bell 30.04.06 #1 (U.K.)	EU927327	EU927315	EU927340	GU569729	GU569552
Polytrichastrum alpinum (Hedw.) G.L.Sm. Bell 1788 (Chile)	*GU569639*	GU569461	GU569815	GU569730	GU569553
Polytrichastrum alpinum (Hedw.) G.L.Sm. Bell 02.03.08 #5 (New Zealand)	GU569640	GU569462	GU569816	GU569731	GU569554
Polytrichastrum alpinum (Hedw.) G.L.Sm. Bell 01.07.06 #13 (Norway)	GU569641	GU569463	GU569817	GU569732	GU569555
Polytrichastrum alpinum (Hedw.) G.L.Sm. Bell 02.07.06 #3 (Finland)	GU569642	GU569464	GU569818	GU569733	GU569556
Polytrichastrum altaicum Ignatov & G.L.Merr. Ignatov 36/361 (Russia)	GU569643	GU569465	GU569819	GU569734	GU569557
Polytrichastrum appalachianum (L.E.Anderson) G.L.Merr. Anderson 27559, DUKE (U.S.A.)	GU569644	GU569466	GU569820	GU569735	GU569558
Polytrichastrum emodi G.L.Sm. G. & S. Miehe 00–381-22 (Bhutan)	GU569645	GU569467	GU569821	GU569736	GU569559
Polytrichastrum formosum (Hedw.) G.L.Sm. Bell 05.01.07 #1 (U.K.)	EU927328	EU927316	EU927341	GU569737	GU569560
Polytrichastrum formosum (Hedw.) G.L.Sm. Hyvönen 6197 (Finland)	*X80982*	AY118259	AY137702	AF545032	*AJ001228*
Polytrichastrum longisetum (Sw. ex Brid.) G.L.Sm. Hyvönen 6506 (Finland)	AY126978	AY118260	AY137703	AF545033	AY137748*
Polytrichastrum longisetum (Sw. ex Brid.) G.L.Sm. Bell 1717 (Chile)	GU569646	GU569468	GU569822	GU569738	GU569561
Polytrichastrum norwegicum (Hedw.) Schljakov Bell 02.07.06 #8 (Finland 2)	GU569647	GU569469	GU569823	GU569739	GU569562
Polytrichastrum norwegicum (Hedw.) Schljakov Bell 03.07.06 #7 (Finland 1)	GU569648	GU569470	GU569824	GU569740	GU569563
Polytrichastrum norwegicum (Hedw.) Schljakov Hedderson 1877 (Canada)	GU569649	GU569471	GU569825	GU569741	GU569564
Polytrichastrum ohioense (Renauld & Cardot) G.L.Sm. Nelson 24122, DUKE (U.S.A)	GU569650	GU569472	GU569826	GU569742	GU569565
Polytrichastrum pallidisetum (Funck) G.L.Sm. Ignatova 06–03 (Russia)	GU569651	GU569473	GU569827	GU569743	GU569566
Polytrichastrum papillatum G.L.Sm. G. & S. Miehe 00–183-17 (Bhutan)	GU569652	GU569474	GU569828	GU569744	GU569567
Polytrichastrum sexangulare (Flörke ex Brid.) G.L.Sm. Bell 03.07.06 #4 (Finland)	GU569653	GU569475	GU569829	GU569745	GU569568
Polytrichastrum sexangulare (Flörke ex Brid.) G.L.Sm. Belland 5727 (Canada)	GU569654	GU569476	GU569830	GU569746	GU569569
Polytrichastrum sphaerothecium (Besch.) J.-P.Frahm Ignatov 06- (14.IX.06) (Russia, Kuril Islands)	GU569655	GU569477	GU569831	GU569747	GU569570
Polytrichastrum sphaerothecium (Besch.) J.-P.Frahm Nishimura Naoki 11697 (Japan)	GU569656	GU569478	GU569832	GU569748	GU569571
Polytrichastrum sphaerothecium (Besch.) J.-P.Frahm Blockeel 34/418, E. (Iceland)	GU569657	GU569479	GU569833	GU569749	GU569572
Polytrichastrum tenellum (Müll.Hal.) G.L.Sm. Churchill, Magombo & Price 19894 (Bolivia)	GU569658	GU569480	GU569834	GU569750	GU569573
Polytrichastrum torquatum Mitt. ex Osada & G.L.Sm. Shevock 23201 (China)	GU569659	GU569481	GU569835	GU569751	GU569574
Polytrichastrum xanthopilum (Wilson ex Mitt.) G.L.Sm. G. Miehe 05–052-05:1 (China)	GU569660	GU569482	GU569836	GU569752	GU569575
Polytrichum brachymitrium Müll.Hal. Hyvönen 6230 (Brazil)	AY126979	AY118261	AY137704	AF545034	AY137749*
Polytrichum commune Hedw. var. *perigoniale* (Michaux) Hampe Hyvönen 6890 (Finland)	GU569661	GU569483	GU569837	GU569753	GU569576
Polytrichum commune Hedw. var. *perigoniale* (Michx.) Hampe Anderson 27751, DUKE (U.S.A)	GU569662	GU569484	GU569838	GU569754	GU569577
Polytrichum commune Hedw. *var. commune* Hyvönen 6168 (Finland)	GU569663	GU569485	AF208428	AF545035	GU569578
Polytrichum ericoides Hampe Churchill, Sastre-De Jesús & Acosta 13325 (Columbia)	GU569664	GU569486	GU569839	GU569755	GU569579
Polytrichum hyperboreum R.Br. Bell 02.07.06 #2 (Finland)	GU569665	GU569487	GU569840	GU569756	GU569580
Polytrichum juniperinum Hedw. Bell 29.06.07 #1 (Finland)	EU927329	EU927317	EU927342	GU569757	GU569581
Polytrichum piliferum Hedw. Hyvönen 6205 (Finland)	AY126981	AY118263	AY137706	AF545037	AY137752*
Polytrichum strictum Menzies ex Brid. Bell 30.06.06 #11 (Finland)	GU569666	GU569488	GU569841	GU569758	GU569582
Polytrichum strictum Menzies ex Brid. Bell 1775 (Chile)	GU569667	GU569489	GU569842	GU569759	GU569583
Polytrichum subpilosum P.Beauv. Hedderson 16281 (Réunion)	GU569668	GU569490	GU569843	GU569760	GU569584
Psilopilum cavifolium (Wilson) I.Hagen Bell 05.07.06 #2 (Finland)	EU927330	EU927318	EU927343	GU569761	GU569585
Psilopilum laevigatum (Wahlenb.) Lindb. Hedderson 5938, RNG (Canada)	AY126983	AF208416	AF208429	AF545039	AY137754*
Steereobryon subulirostrum (Schimp. ex Besch.) G.L.Sm. Hedderson 12898 (Mexico)	AY126984	AY118265	AY137708	AF545040	AY137755*

2.2. Character sampling and rooting

While the earliest dichotomies in the Polytrichopsida are likely to be very ancient we also wished to resolve species-level relationships within some recent lineages, and thus characters with an assumed wide range of evolutionary rates were sampled. The nuclear 18S and mitochondrial *nad5* regions (and to some extent the chloroplast *rbcL* gene) are relatively conserved and likely to provide signal for ancient dichotomies, while the *rps4* gene, the *rps4-trnS* spacer and the *trnL-F* region (including a group I intron and the *trnL-F* spacer) provide faster-evolving characters for resolving generic and species-level relationships. Although the *rps4-trnS* spacer

and extensive non-coding areas of the *trnL-F* region were excluded from the analysis of Hyvönen et al. (2004) due to alignment difficulties, these regions may actually be aligned relatively unambiguously across the entire class and are highly informative. The problem lies in alignment with outgroup taxa (Hyvönen et al., 2004 used a number of outgroups including *Oedipodium griffithianum* (Dicks.) Schwägr. and species of *Andreaea* Hedw., *Sphagnum* L., *Tetraphis* Hedw., and several arthrodontous mosses). However, in our recent investigation of the earliest dichotomies within the Polytrichopsida (Bell and Hyvönen, 2008) we resolved *Alophosia* as the sister lineage to the remainder of the class with considerable confidence, with a clade comprising *Lyellia* R.Br. and *Bartramiopsis* diverging next. We were thus able to dispense with more distant outgroups in the current analysis and all topologies are rooted with *Alophosia*. A further addition to the character sampling of Hyvönen et al. (2004) was the inclusion of the entire *nad5* region, including the group I intron, for the majority of taxa (Hyvönen et al., 2004 used only the 3′ end of the conserved coding region). As the 3′ end of the *rbcL* gene has proved difficult to amplify in most Polytrichopsida, in this study we included only the first 700–800 base pairs (bp) at the 5′ end.

The study of Hyvönen et al. (2004) had some problems with contamination of PCRs due to the unavailability of high quality material for extraction (see, for example, Bell and Hyvönen, 2008) and with misidentification of vouchers. Sequences for all such exemplars have been newly generated from fresh extractions (Table 1). A number of other sequences have also been freshly generated due to missing data and multiple ambiguous base calls in previously existing sequences, such as some of those used in Hyvönen et al. (2004) and Koskinen and Hyvönen (2004).

2.3. DNA extraction, amplification and sequencing

Shoots were selected individually for extraction using a dissecting microscope to screen for contaminants. For robust plants two or three shoot tips were generally used. Many Polytrichopsida show clear annual growth patterns from the shoot apex, and shoot tips represent the most recently metabolically active parts of the plant as well as having the least soil and epiphyte contamination. For extractions from older herbarium material the greenest shoot tips were selected. Extraction of genomic DNA was carried out using the Invisorb spin plant mini kit (Invitek) and eluted DNA stored in the supplied buffer. A few samples were further purified using the Wizard DNA clean-up kit (Promega).

PCR amplifications were performed in 50 μl reactions with 1.25 U *Taq* DNA polymerase (Fermentas, native), 1× Tris–HCl/$(NH_4)_2SO_4$ buffer, 2 mM $MgCl_2$, 200 μM dNTPs, 0.3 μM of each primer, and 5 μg BSA. Standard PCR protocols all included an initial melting step of either 94 °C or 97 °C for 3 min and a final extension period of 72 °C for 7 min. For the iterated parts of amplifications (30 or 35 cycles), protocols were as follows: 18S: 94 °C (30 s), 52 °C (30 s), 72 °C (3 min). *rps4*: 94 °C (30 s), 50 °C or 52 °C (30 s), 72 °C (2 min or 2 min 30 s). *rbcL*: 94 °C (30 s), 48 °C (30 s), 72 °C (1 min 30 s). *trnL*: 94 °C (30 s), 50 °C (30 s), 72 °C (1 min 30 s). *nad5*: 94 °C (30 s), 52 °C (30 s), 72 °C (2 min 30 s). Occasionally *trnL* products were amplified using the *rbcL* protocol and *nad5* products using the 18S protocol. Primers used for *rps4*, *trnL*, *nad5* and *rbcL* were as reported in Bell and Newton (2005), except that only the primer pairs for the first half (5′ end) of *rbcL* were used here[1]. The *nad5* region was amplified in two parts as in Bell and Newton (2005). 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 and gel purification kit, the Qiagen QIAquick PCR purification kit or the Qiagen MinElute PCR purification kit, while others were cleaned prior to sequencing by Macrogen Inc., South Korea (www.macrogen.com). All products were sequenced by Macrogen Inc. using primers supplied by the authors. For *rps4*, *trnL* and *rbcL* 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) and for *nad5*, Ki was also used (Beckert et al., 1999).

2.4. Alignment

All sequences were edited and assembled into consensus files using Seqman II v4.00 (LaserGene, DNAStar Inc.). Sequences were aligned manually using PhyDE v0.992–v.0.995. (Müller et al., 2005) in order to maximise positional homology, with areas of ambiguity and significant missing data excluded where necessary. The *trnL-F* region was aligned with reference to the major structural elements described by Quandt and Stech (2004). Specific details of alignment in the length-variable regions are provided in the results and the matrix is available from TreeBASE (accession number M5008).

2.5. Parsimony analysis

Under parsimony, separate analyses were performed for the 18S dataset alone, the combined *rps4/trnL-F/rbcL/nad5* data alone (the chloroplast and mitochondrial data, henceforth referred to as the c/m data), and the combined matrix of all gene regions together. Pilot studies had confirmed that there was no significantly supported incongruence between any of the individual regions within the c/m dataset. Searches for most parsimonious trees were undertaken using PAUP* v.4.0b10 (Swofford, 2002) with stepwise random taxon addition and TBR branch swapping. Branches were collapsed when the minimum length was zero and all character transitions were equally weighted. Initial searches of 10,000 replications were performed saving only one tree of length $\geqslant 1$ on each replication (nchuck = 1, chuckscore = 1). 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 step. This procedure simulates the highly effective heuristic search strategy (e.g. Davis et al., 2005) normally used with Nona (Goloboff, 1999) inside the Winclada shell (Nixon, 2002). Non-parametric bootstrap analyses were conducted on each dataset with 1000 replications of two full heuristic searches (settings as above) and maxtrees fixed at 3000. Where analyses indicated the paraphyly of well-established genera, constrained searches were conducted on some datasets to assess the extent to which monophyletic solutions were less parsimonious. To assess congruence of the 18S and c/m data under parsimony we searched for nodes supported in one topology that were contradicted by well-supported nodes in the other. Although metrics such as the incongruence length difference test (Farris et al., 1994, 1995) exist for assessing congruence under parsimony, the inappropriate use of these has been widely criticised (Siddall, 1997; Reeves et al., 2001; Yoder et al., 2001; Barker and Lutzoni, 2002).

2.6. Bayesian analysis

Heterogeneous Bayesian analysis was performed using MrBayes 3.1.2. (Ronquist and Huelsenbeck, 2003). Complexity of partitioning was restricted to some extent by available computing resources, as well as by the results of pilot studies suggesting that

[1] Codes for *rbcL* primers refer to positions in the chloroplast genome of the reference taxon *Marchantia polymorpha* L. (L. Lewis, pers. comm.). The code consistent with this usage for the primer NM34 (Cox et al., 2000) is M28 (Hyvönen et al., 1998, 2004; García-Avila et al., 2009). This was originally designed in 1995 by S. Schaffer at UC as an improved, more specific alternative to M34.

several parameters in very complex models were highly unstable even after many millions of generations. A very complex model in which each molecular sub-region as well as different codon positions within each protein coding gene were compartmentalised resulted in some parameters having a very large potential scale reduction factor (PSRF) even after 1×10^7 iterations. Instability was corroborated by examining traces of individual parameters with Tracer v.1.4 (Rambaut and Drummond, 2007). For the final analyses we used five compartments, one for each gene region, except that we combined the *rps4-trnS* spacer with the *trnL-F* region rather than with the *rps4* protein coding gene. Nucleotide substitution models selected by the AIC criterion as implemented within MrModeltest 2.2 (Nylander, 2004) were applied to all of the compartments individually with parameters unlinked across partitions.

As with the parsimony runs we performed separate analyses on the 18S data, the combined c/m data, and the total combined data. For all analyses we conducted two simultaneous independent runs each with 12 chains (temp parameter = 0.092). These were run for as long as necessary to ensure convergence as measured by an average standard deviation of split frequencies <0.01, a meaningful effective sample size (ESS) for each parameter, and accurate sampling from the posterior probability distribution as assessed by examination of raw trace files within Tracer and PSRF values approaching 1.00. Majority rule consensus trees were created within MrBayes, while maximum clade credibility (MCC) topologies (estimates of the single maximum total probability tree out of all trees sampled) were identified using TreeAnnotator v.1.4.7, part of the BEAST v.1.4.7 package (Drummond and Rambaut, 2007).

2.7. Testing for incongruence using Bayes factors

As well as searching for well supported topological incongruence between the 18S and c/m datasets based on the results of the separate analyses, we used Bayes factors to assess incongruence using the method of Nylander et al. (2004) and the recommendations of Kass and Raftery (1995). Bayes factors can be used to test for statistically significant differences in the strength of evidence in favour of two competing models. In this case, the models differ in whether the topologies for the 18S and the chloroplast/mitochondrial data are linked or unlinked, i.e. whether the analysis attempts to find a single topology for all of the data or permits different topologies for different compartments within the same model. If the strength of evidence is significantly greater for the unlinked model, the data partitions are assumed to be incongruent. Thus we repeated the analysis of the total combined dataset using separate topology parameters for the 18S data and the combined c/m data (by unlinking the topology parameter across all partitions and then linking it across the c/m partitions only). Estimated marginal likelihoods for the linked and unlinked analyses were then used to calculate and assess Bayes factors according to the table on p.777 of Kass and Raftery (1995). Marginal likelihoods were estimated using the method of Newton and Raftery (1994) with the modifications proposed by Suchard et al. (2001), as implemented within Tracer. The smoothed estimate method was used with 1000 bootstrap replicates (this provides a more accurate estimation of marginal likelihoods than the harmonic means reported by the MrBayes sump command).

2.8. Construction of consensus and hybridization networks

We used SplitsTree 4.10 (Huson and Bryant, 2006) to construct a consensus network to display incompatible splits between Bayesian 95% p.p. consensus trees for the 18S data and the combined c/m data. This produces an unrooted network in which "splits" (bipartitions) represented in one tree that are not incompatible with splits in the other tree are represented by single lines, and incompatible topologies by parallel lines (parallel "edges"; each set of two or more parallel lines represents a split). Ninety-five percent p.p. consensus trees were imported directly into SplitsTree and the consensus network option was used with the standard settings. We subsequently used the hybridization network algorithm within SplitsTree (Huson et al., 2005) to calculate the most parsimonious resolution of the 95% supported incompatible splits under the assumption that they are explained by reticulation events (i.e. the solution that assumes the smallest number of hybridization events).

3. Results

3.1. Alignment and model specification

There was no alignment ambiguity in the protein coding regions (*rbcL*, the *rps4* gene, and the *nad5* exons), the only length variation being a single codon insertion (CAA = glutamine) near the 5′ end of *rps4* in all *Polytrichadelphus* (Müll.Hal.) Mitt. species other than *P. innovans* (≡ *P. magellanicus* subsp. *innovans* (Müll.Hal.) M.Stech, T.Pfeiff. & W.Frey). *Polytrichadelphus magellanicus* (Hedw.) Mitt. *s.s.* (not included in the analyses) also lacks this insertion. In the 18S region a small CT-rich area of nine positions was excluded due to alignment ambiguity. All other indels in 18S and in the *nad5* intron were small, embedded within more conserved regions, and alignable without ambiguity. In the *rps4-trnS* spacer, a small hyper-variable area was excluded, as was the short region of seven base pairs identified by Quandt and Stech (2004) towards the end of the *trnL-F* spacer corresponding to a frequently inverted terminal loop in a hairpin structure formed by two putative promoter elements. Other indels in these non-coding regions were alignable without significant ambiguity.

In the *rps4-trnS* spacer, a 51–53 bp deletion uniquely characterises all *Pogonatum* exemplars with the exception of the four *P. urnigerum* specimens, which were sampled from widely separated geographical areas. The deletion is identical in all species, excepting a 2 bp length difference caused by the size of an A-repeat sequence immediately preceding it (6–8 bp). Another large deletion of ±113 bp characterises the three *Pogonatum cirratum* exemplars (including an unusual dendroid form collected on Mt. Kinabalu), and is also found in a shorter form in *P. dentatum* (±104 bp), although it is absent in *P. macrophyllum* (≡ *P. cirratum* subsp. *macrophyllum* (Dozy & Molk.) Hyvönen).

The aligned *trnL-F* region comprised the *trnL* intron, the 3′ exon of the *trnL* transfer RNA gene, the *trnL-F* intergenic spacer, and part of the *trnF* exon. Substitutions occur at six positions in the highly conserved 3′ *trnL* exon and at one position in the small part of the *trnF* exon we included. Length variation is restricted to the intron (264–386 bp) and spacer (44–110 bp).

Despite the significant length variability of the spacer, alignment was unproblematic, as variation is mostly in the form of large, isolated indels, either autapomorphic or shared by a few taxa only. Of particular note is a 43 bp deletion uniquely shared by all *Polytrichadelphus* species other than *P. innovans* (and *P. magellanicus s.s.*; the character is congruent with the *rps4* codon insertion described above). Most of the length variation in the *trnL* intron is in the highly variable P8 loop (see Quandt and Stech, 2004). In nearly all of the *Pogonatum* species and in five *Oligotrichum* DC. species (*O. austroaligerum, O. hercynicum, O. obtusatum, O. parallelum* and *O. suzukii*) P8 is highly reduced at only 56–59 bp, while in most other taxa the region is 160–170 bp length. In *Polytrichastrum sphaerothecium,* as well as in many of the *Notoligotrichum* species (all except *O. tapes, O. trichodon, O. tetragonium* and TH14147), it is 72–73 bp, while *Pogonatum japonicum* has a partially reduced P8 at 136 bp. Interestingly, the four exemplars of *P.*

urnigerum vary greatly in the length of P8, with the specimens from Taiwan and Borneo (Mt. Kinabalu) having a highly reduced (59 bp) P8 in common with the other *Pogonatum* species, while the Himalayan and Finnish specimens are only partially reduced (151 and 120 bp, respectively). A parsimony search based on the *trnL-F* region alone nonetheless resolves these four specimens as a clade. Finally, a group of *Polytrichastrum* G.L.Sm. species (*P. alpinum, P. papillatum, P. emodii, P. tenellum, P. altaicum, P. sexangulare* and *P. norwegicum*), together with *Meiotrichum lyallii*, share a distinctive deletion of ±17 bp near the 5′ end of P8.

After exclusion of the aforementioned areas of ambiguity and of regions of missing data at the ends of sequences, the aligned matrix comprised 5842 characters, of which 944 were variable and 613 parsimony informative. Parsimony informative characters were distributed as follows; 18S: 78, *rbcL*: 81, *rps4* gene: 97, *rps4-trnS* spacer: 85, *nad5*: 151, *trnL-F*: 121.

The general time reversible model with a proportion of invariable sites and gamma-distributed rate variation across sites (GTR + I + G) was selected for all of the partitions individually by the AIC criterion as implemented in MrModeltest.

3.2. Bayesian

Stationarity of sampling from the posterior probability distribution and convergence of runs was confirmed for the 18S dataset by the methods described after 8.5×10^6 replications, the c/m dataset after 7.5×10^6 replications, and the total combined dataset after 1×10^7 replications. In discussing Bayesian consensus topologies, "strongly supported" refers to nodes supported at 95% or greater posterior probability.

Fig. 1 shows the 50% majority rule consensus topology for the c/m analysis and the maximum clade credibility (MCC) phylogram. The only conflict between these (i.e. where the 50% majority consensus tree differs from the MCC topology after branches with <50% p.p. are collapsed) relates to very weakly supported apical relationships within *Atrichum*. The relationships of the earliest diverging lineages are strongly supported and are consistent with the results of Bell and Hyvönen (2008). *Lyellia* and *Bartramiopsis* (which are eperistomate, in common with *Alophosia*) occur as a clade outside of the large group of peristomate Polytrichopsida, within which *Dawsonia* is sister to the group of taxa having the classic polytrichoid peristome (i.e. with teeth attached to a more or less membranous epiphragm). A notably long branch is associated with the node representing the peristomate clade. Within the polytrichoid peristome group, a strongly supported clade of predominantly southern temperate and southern tropical *Oligotrichum* species, together with *Notoligotrichum* and *Itatiella,* is sister to the other taxa. *Atrichopsis* is shown as derived from within *Notoligotrichum*, as in Bell and Hyvönen (2008). The Brazilian endemic *Itatiella* is sister to *Oligotrichum riedelianum*, while the New Zealand endemic *Oligotrichum tenuirostre* is sister to the *Notoligotrichum* clade.

There is strong support for a clade comprising the three remaining predominantly southern hemisphere genera (*Polytrichadelphus*, *Dendroligotrichum* and *Hebantia*) together with the predominantly northern hemisphere genera, and also for the latter collectively forming a large monophyletic group. *Hebantia* is strongly supported as sister to *Dendroligotrichum* (a relationship found in Hyvönen et al., 2004). As the suggested grouping of the *Hebantia*/*Dendroligotrichum* clade with the northern hemisphere taxa is effectively unsupported (p.p. = 61%) with an extremely short branch, the sister group of the northern hemisphere clade is uncertain. Within the northern hemisphere clade, many major groupings and isolated smaller entities are well supported, although relationships between them frequently are not.

A large, strongly supported monophyletic group includes all *Pogonatum* and *Polytrichum* species as well as the *Polytrichastrum* species belonging to sect. *Aporotheca* (Limpr.) G.L.Merr. *Polytrichastrum alpinum, P. tenellum, P. emodi, P. papillatum, P. altiacum* and *P. norwegicum* are not closely related to this group and form a strongly supported clade with *Meiotrichum lyallii* (henceforth referred to as the *Polytrichastrum s.s.* clade). *Polytrichastrum sexangulare* and *P. sphaerothecium* do not appear in either of these groups, although only very weakly supported nodes separate them from the *Polytrichastrum s.s.* clade. The three exemplars of *Polytrichastrum sphaerothecium*, representing the breadth of the unusual disjunct distribution of this species, form a strongly supported clade distinct from *P. sexangulare* (within which *P. sphaerothecium* has recently been treated, e.g. Smith Merrill, 2007a). Within the *Polytrichastrum s.s.* clade, *P. tenellum* and *Meiotrichum lyallii* are outside of an apical group, within which *P. alpinum* is strongly supported as monophyletic, quite distinct from *P. norwegicum*. *Polytrichastrum altaicum* appears as derived from within *P. norwegicum*.

The remainder of the *Oligotrichum* exemplars, all northern hemisphere species with the notable exception of the Fuegian *O. austroaligerum*, form a strongly supported monophyletic group. *Steereobryon* is sister to *Atrichum* as in Hyvönen et al. (2004), while *Psilopilum* Brid. is strongly supported as sister to this larger clade in turn.

Within the large *Polytrichum*/*Polytrichastrum* sect. *Aporotheca*/*Pogonatum* clade, *Pogonatum* is strongly supported as monophyletic, sister to an equally well supported *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade. Within the latter, both *Polytrichum* sect. *Polytrichum* and the *Polytrichastrum* sect. *Aporotheca* exemplars are strongly supported as monophyletic. Within *Pogonatum*, an apical clade is strongly supported as monophyletic to the exclusion of *P. japonicum, P. perichaetiale, P. dentatum* and a monophyletic *P. urnigerum*.

A search of the .parts file produced by the MrBayes sumt command (using the displaygeq = 0 option) showed that the bipartitions representing a monophyletic *Polytrichastrum* and a monophyletic *Oligotrichum* were never sampled after the burnin, indicating that these hypotheses have a probability of zero (based on the data and the model).

The majority consensus topology for the results of the 18S Bayesian analysis (not shown, but see Fig. 2) is generally poorly resolved at the infra-generic level and also often at higher levels, with many groupings supported by very low p.p. scores. We will restrict description of the topology to nodes with support values of 90% p.p. or above (the 95% p.p. subset of these is included in Fig. 2).

Many nodes appearing in the results of the c/m analysis are also strongly supported at >90% p.p. in the 18S analysis, including the monophyly of *Atrichum*, *Polytrichadelphus*, the clade containing *Polytrichum s.s.* and *Polytrichastrum* sect. *Aporotheca,* the *Hebantia*/*Dendroligotrichum* clade, the clade containing *Polytrichum commune* var. *perigoniale*, *P. subpilosum, P. brachymitrium* and *P. ericoides* to the exclusion of *P. commune var. commune* (all supported at 100% p.p.), the *Lyellia*/*Batramiopsis* clade (92% p.p.), and the apical *Pogonatum* clade, i.e. excluding *P. urnigerum, P. dentatum, P. perichaetiale* and *P. japonicum* (98% p.p.). However, a number of nodes with strong or moderate support values conflict with the c/m topology. A group containing the *Polytrichum* s.s./*Polytrichastrum* sect. *Aporotheca* clade and the apical *Pogonatum* clade to the exclusion of the other *Pogonatum* species has a p.p. support value of 100%, implying a paraphyletic *Pogonatum*. *Polytrichadelphus* appears as sister to the *Polytrichum*/*Polytrichastrum*/apical *Pogonatum* clade (supported at 87% p.p., although a more inclusive group also including one of the *P. urnigerum* exemplars is supported at 91% p.p.), while *Hebantia* and *Dendroligotrichum* appear in a clade with *Notoligotrichum*, *Oligotrichum riedelianum*, and *Itatiella* (95% p.p.). *Polytrichum juniperinum* and *P. strictum* form a clade with the other *Polytrichum* species, without *P. hyperboreum* and *P. piliferum*.

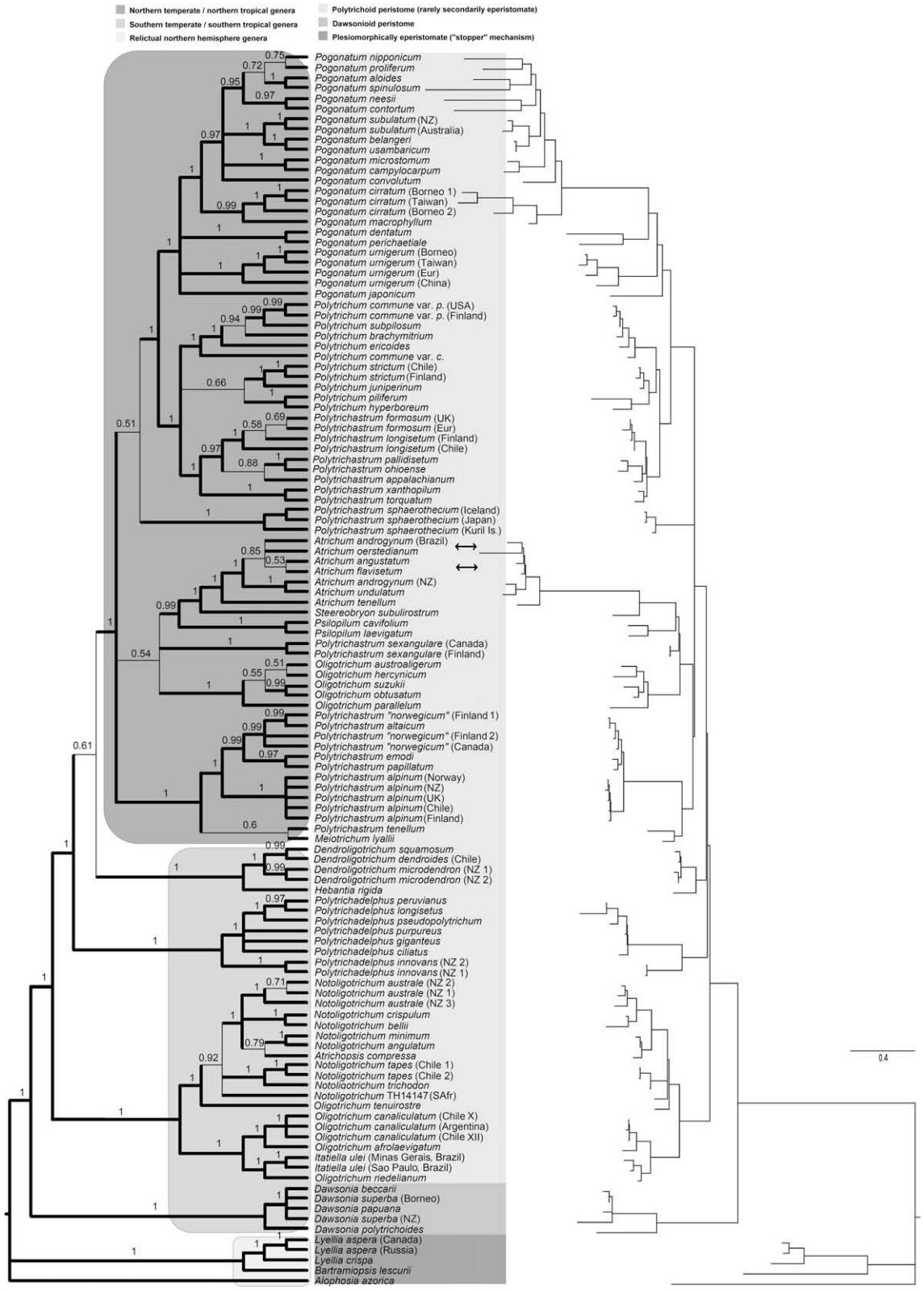

Fig. 1. Bayesian 50% majority rule consensus tree (left) and maximum clade credibility (MCC) phylogram (right) from analysis of the combined chloroplast and mitochondrial dataset. Numbers on branches are posterior probabilities (p.p.) for clades. Resolution supported at $\geqslant$95% p.p. is represented by heavy lines on the consensus tree. Arrows indicate areas where the consensus tree continues to differ from the MCC tree after nodes in the latter supported at <50% p.p. are collapsed. Shaded boxes on the left indicate general geographic distribution of extant members of genera (or in the case of *Oligotrichum*, generic level groups). Shaded boxes on the right indicate major categories of capsule dehiscence mechanism.

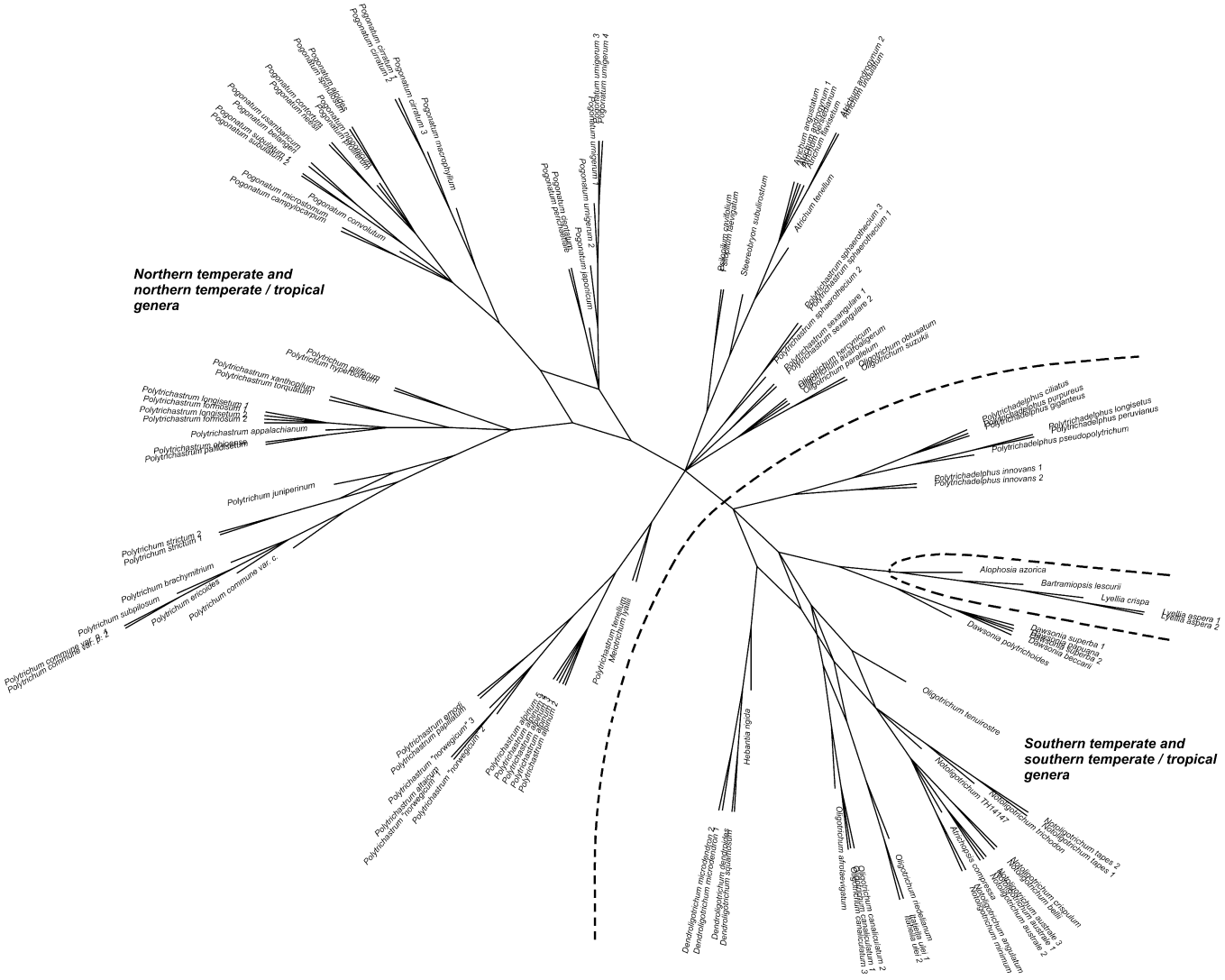

Fig. 2. SplitsTree consensus network of all splits (bipartitions) supported at ⩾95% Bayesian p.p. in the results of either the analysis of the chloroplast and mitochondrial dataset or of the 18S dataset. Boxes indicate incongruence. Major groups of generic level clades with different geographic distributions of extant members are separated by dashed lines.

While phylogenetic incongruence between 18S and the organellar data makes the results of the combined Bayesian analysis (not shown) difficult to interpret, the majority of the relationships resolved are congruent with those revealed by analysis of the c/m data alone. The most notable exception is that the paraphyletic *Pogonatum* scenario is supported at 100% p.p., as in the 18S topology. Thus the signal from 18S entirely overrides the c/m signal with regard to the status of *Pogonatum* when the data are combined, despite the conflicting topologies each having 100% p.p. in separate analyses. Other groupings in the 18S results that conflict with the c/m results are generally not found in the combined topology, e.g. the positions of *Polytrichadelphus* and *Dendroligotrichum* in the combined consensus tree are the same as in the c/m topology, with comparable p.p. scores. Support values for clades are generally comparable in the combined topology and in the c/m topology, although in a few cases p.p. scores are improved, as would be expected for parts of the tree where the signals from the datasets are congruent.

3.3. Incongruence test (Bayes factors)

In the analysis of the total combined dataset with topology parameters unlinked the two runs did not begin to converge on comparable posterior distributions until after nearly 2×10^7 iterations, while the chains were run for a total of 4×10^7 iterations. Sampling frequency was set to 100. Observation of the Ln likelihood samples in Tracer revealed that while one run had reached stationarity fairly rapidly, the other had sampled from a temporarily stable island with a considerably lower Ln likelihood score for nearly half of the run, before jumping rapidly to one having the same mean Ln likelihood score as the first run's samples. Using a burnin of 1×10^7 for the first run and 2×10^7 for the second produced a combined sample with a normal distribution for the Ln Likelihood parameter. The estimated mean marginal Ln likelihood for this sample was −20,307.439 (± 0.216), while the figure for the analysis of the combined dataset with a single topology parameter (burnin = 3×10^6) was −20,460.484 (± 0.132), producing a value of 306.09 for $2 \times \text{Ln}(B_{10})$. This is very strong evidence (Kass and Raftery, 1995) for assuming that the unlinked topology model best fits the combined data, and thus that the c/m and18S data are strongly topologically incongruent.

3.4. Parsimony

The number of equally parsimonious trees (EPTs) and other statistics for the parsimony analyses are summarised in Table 2.

Apart from relationships within *Pogonatum* (see below), the results of the parsimony analyses of the c/m dataset (Fig. 3) are congruent with the Bayesian analysis, other than that a few unsupported or weakly supported nodes in the Bayesian results do not appear in the parsimony strict consensus. Additionally, the grouping of *Meiotrichum lyallii* and *Polytrichastrum tenellum* with the majority of the other *Polytrichastrum* sect. *Polytrichastrum* species (to form the *Polytrichastrum s.s.* clade referred to above) receives no bootstrap support, despite this node being supported at 100% p.p. in the Bayesian analysis. We will not describe poorly supported disagreement at the subgeneric level.

One clear difference between the parsimony strict consensus and the Bayesian majority consensus is the lack of resolution in the former between early-diverging elements in the northern hemisphere clade, although most such relationships are in any case very weakly supported in the Bayesian topology. The clade grouping *Polytrichastrum sexangulare*, the predominantly northern hemisphere *Oligotrichum spp.,* and the *Atrichum/Steereobryon/Psilopilum* group is not resolved under parsimony, while neither is the putative relationship of *Polytrichastrum sphaerothecium* to the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca*/*Pogonatum* clade (as well as having a very low p.p., the relevant branch in the Bayesian phylogram is so short as to be invisible in Fig. 1). *Meiotrichum* and *Polytrichastrum tenellum* are not resolved as sisters, and *Atrichopsis* is not resolved as sister to *Notoligotrichum minimum* and *N. angulatum*.

Within *Pogonatum*, the parsimony analysis of the c/m data resolved *P. urnigerum* as sister to the remainder of the genus, with *P. japonicum* and the *P.dentatum/P. perichaetiale* clade, respectively, forming a grade between *P. urnigerum* and the apical *Pogonatum* clade, although with low bootstrap values. The Bayesian MCC phylogram places *P. japonicum* as sister to the rest of the genus, although none of the relationships among the three "basal" *Pogonatum* entities and the apical group have p.p. >50%. The parsimony strict consensus differs from the Bayesian topology in relations within the apical *Pogonatum* group, the most significant of these being the failure to place the *Pogonatum cirratum s.l.* clade as sister to the remainder of the species (the relevant node has 97% p.p. in the Bayesian analysis).

Constrained analyses of the c/m dataset showed topologies in which *Polytrichastrum* was monophyletic to be 16 steps longer (15 steps when *Meiotrichum* was included in *Polytrichastrum*), and topologies with a monophyletic *Oligotrichum* to be 43 steps longer (28 steps if *Itatiella* was included, 21 steps including *Itatiella* and excluding *Oligotrichum tenuirostre*).

The strict consensus of the parsimony analysis of the 18S dataset (not shown) did not resolve any nodes absent from the Bayesian consensus, although the topology is in general less resolved and a number of nodes with moderate support under Bayesian analysis do not occur. Significantly, although the position of *Polytrichadelphus* is the same as in the Bayesian tree, the node does not have a bootstrap value over 50%, while the grouping of *Dendroligotrichum* and *Hebantia* with *Notoligotrichum*, *Itatiella* and *Oligotrichum riedelianum* has only 62% bootstrap support. However, the node implying a paraphyletic *Pogonatum* has a high support value (97% bootstrap), as in the Bayesian results.

The results of the parsimony analysis of the total combined dataset were very similar to the Bayesian results based on the same matrix. As with the c/m dataset, the parsimony strict consensus did not resolve relations amongst the early-diverging northern hemisphere entities. Perhaps significantly, there was relatively low bootstrap support (72%) for the node grouping the apical *Pogonatum* clade with the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* group. A novel resolution of relationships between the other *Pogonatum* species appears in the strict consensus ((*P. japonicum*, (*P. perichaetiale P. dentatum*)) (*P. urnigerum, apical clade*)), but without a bootstrap support value >50%.

3.5. Networks

The SplitsTree consensus network of all splits supported at $\geqslant$ 95% p.p. in either the c/m or 18S Bayesian analyses is shown in Fig. 2, providing a visual representation of the strongly supported incongruence described above. The hybridization network derived from this is shown in Fig. 4.

Under the assumption of hybridization, strongly supported incongruence between the 18S and c/m data for the status of *Pogonatum* is most parsimoniously explained as caused by an ancient reticulation event involving a member of (or ancestor of) the apical *Pogonatum* clade (i.e. excluding *P. urnigerum, P. dentatum, P. perichaetiale* and *P. japonicum*), and an extinct species derived from the ancestral lineage of the combined clade that contains all extant members of *Pogonatum* and *Polytrichum*. This event has given rise to the common ancestor of all members of the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade (Fig. 4). As the chloroplast and mitochondrial genomes are thought to be maternally inherited in mosses (Duckett et al., 1983), and only splits derived from the nuclear 18S data group the apical *Pogonatum* clade with the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade, it is likely that the ancestor of (or from) the apical *Pogonatum* group is the paternal progenitor and the ancestor representing the earlier diverging lineage is the maternal one.

Three other reticulation events are hypothesised from the hybridization network (Fig. 4). *Polytrichum strictum* appears as the product of hybridization between the *P. juniperinum* lineage and a taxon basal to the *P. juniperinum*/*Polytrichum* sect. *Polytrichum* clade. *Dendroligotrichum*, together with the closely related *Hebantia rigida*, is hypothesised as descended from an ancient reticulation event involving a maternal ancestor at the base of the clade including the "northern hemisphere" Polytrichopsida together with *Polytrichadelphus*, and a paternal progenitor in (or ancestral to) the *Notoligotrichum* clade. Finally, the clade containing *Itatiella ulei* and *Oligotrichum riedelianum* appears as the product of hybridization between an extinct member or ancestor of the austral *Oligotrichum* clade on the maternal side, and (just as with *Dendroligotrichum*) a paternal *Notoligotrichum* progenitor.

4. Discussion

4.1. Incongruence of 18S gene tree with chloroplast and mitochondrial data

Topological incongruence between the 18S gene tree and the c/m data is apparent from both the parsimony and Bayesian analyses and is associated with high bootstrap and p.p. values. Incongruence supported at >95% p.p. applies to the relations of some taxa in the

Table 2
Statistics for each of the independent parsimony analyses.

Analysis	Alignment length	Informative sites	Tree length	Tree number	CI (Inc./exc. uninf.)	RI	RC
Chloroplast and mitochondrial data	4121	519	1664	1536	0.556/0.470	0.850	0.473
Nuclear 18S data	1694	77	266	21721	0.639/0.495	0.937	0.599
Combined	5815	596	1967	384	0.557/0.462	0.865	0.482

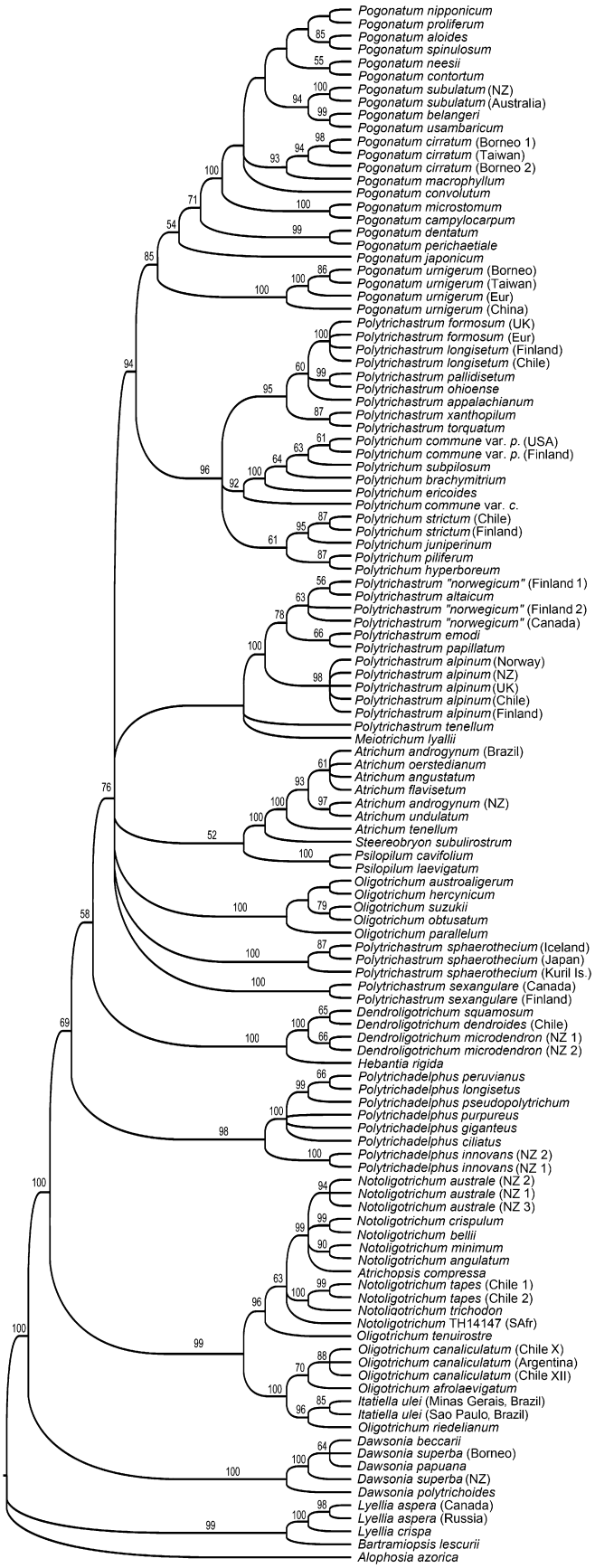

Fig. 3. Strict consensus of 1536 equally parsimonious trees found in the parsimony analysis of the combined chloroplast and mitochondrial dataset. Numbers above branches are bootstrap percentages.

austral *Oligotrichum*/*Notoligotrichum* clade, the positions of *Dendroligotrichum* and *Polytrichum strictum*, and to the status of *Pogonatum* (Fig. 2). Incongruence is corroborated by the Bayes factor test, in which the evidence very strongly favours independent topologies for the c/m data and the 18S data when these are combined.

Results from 18S gene trees that conflict with other molecular data have been observed many times in diverse organismal groups and have been attributed to various factors (e.g. Soltis et al., 1997; Duval, 2000; Duval and Bricker Ervin, 2004; Xia et al., 2003). Duval and Bricker Ervin (2004) found that 18S trees were misleading for phylogeny reconstruction of major angiosperm groups, uniquely suggesting that monocots were paraphyletic, and attributed this to differential lineage sorting after consideration of other possibilities, including methodological error. In the current study we discount poor sequence quality as a primary source of error, as care was taken to resequence poor quality data and a number critical taxa (e.g. *Pogonatum urnigerum*) have multiple exemplars. Similarly, alignment cannot be an issue, as there are only a few short indels in 18S across the Polytrichopsida and the individual sites supporting the paraphyly of *Pogonatum* are not subject to alignment ambiguity.

Lineage sorting must be considered as a possible explanation for incongruence, although we consider this to be less likely than other explanations for the following reasons. In order to explain the 18S gene tree by differential lineage sorting we would need to assume the persistence of ancestral polymorphism in 18S over a length of time spanning both the node on the c/m topology corresponding to the divergence of the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade from the *Pogonatum* clade, and the node representing the origin of the apical *Pogonatum* clade (with subsequent selective loss in the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade, the apical *Pogonatum* clade, and the basal *Pogonatum* lineages). Although these nodes are not dated it is clear that this might be credible if *Pogonatum urnigerum*, *P. japonicum*, *P. dentatum* and *P. perichaetiale* form a monophyletic group, but much less so if they form a grade, as in the maximum clade credibility phylogram and the parsimony topology (Figs. 1 and 3). In the latter case, polymorphism would need to persist at each node in the grade leading up to the common ancestor of the apical *Pognatum* clade, with the same version of 18S retained in each of the basal *Pogonatum* lineages when sorting was complete. It seems unlikely that an ancient grade with sufficiently short temporal internodes to retain ancestral polymorphism in 18S would survive in the form of multiple extant and morphologically highly differentiated lineages. Nonetheless, if the basal *Pogonatum* lineages form a clade it is feasible that a common ancestor could have retained a different version of a polymorphic 18S from the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* group and the ancestor of the apical *Pogonatum* clade. As the relevant nodes are poorly supported, we cannot decisively reject this scenario, but currently we consider it to be the least likely of several possibilities.

Several independent lines of evidence corroborate the combined c/m signal in supporting a monophyletic *Pogonatum* and suggest that the 18S topology alone is unlikely to represent the phylogeny. The presence of a 51–53 bp deletion in the *rps4-trnS* spacer for all *Pogonatum* species other than *P. urnigerum* may be viewed as a unique synapomorphy in the c/m topology, providing that *P. urnigerum* is assumed to be sister to the remainder of the genus as hypothesised under parsimony (Fig. 3; although *P. japonicum* is the first diverging lineage in the Bayesian MCC tree, the relevant nodes appear in less than 50% of the topologies sampled, Fig. 1). However, the 18S topology would require either that the deletion had occurred independently in a *Pogonatum perichaetiale*/*P. dentatum*/*P. japonicum* clade, or else that it had been reversed on at least one occasion. The latter scenario in particular seems highly unlikely for a character of this type. Although morphological

Fig. 4. Diagram based on SplitsTree hybridization network generated from all splits (bipartitions) supported at ⩾95% Bayesian p.p. in the results of either the analysis of the chloroplast and mitochondrial dataset or of the 18S dataset. Clades potentially hypothesised to derive from reticulation events are shifted right, with dashed lines indicating the putative phylogenetic origins of progenitors.

characters were not coded in this study, several distinctive putative morphological synapomorphies, particularly of the sporophyte, additionally support the monophyly of *Pogonatum*, most notably the absence of stomata, a mamillose exothecium, and a peristome composed of 32 large compound teeth that are usually strongly coloured (Hyvönen, 1989; Merrill, 1992). The combination of these features makes *Pogonatum* immediately recognisable when mature sporophytes are present.

A number of reticulate processes could have resulted in the 18S gene tree tracking a different phylogenetic history from the other regions sampled. Firstly, as allopolyploids are known to occur in *Polytrichastrum* (Derda and Wyatt, 2000; van der Velde and Bijlsma,

2001), and both the chloroplast and mitochondrial genomes are thought to be maternally inherited in mosses (Duckett et al., 1983), relatively recent inter-generic hybridization between *Pogonatum* species and other genera is a possible source of incongruence between historical signals from different genomes. Based on electrophoretic data Derda and Wyatt (2000) concluded that *Polytrichastrum pallidisetum*, *P. ohioense* and *P. sexangulare* are all allopolyploids and suggested that parents from different genera might be involved in each case, although in the light of the relationships revealed by the current study and of the limited generic-level sampling of Derda and Wyatt (2000), true inter-generic hybridization need not be assumed. Derda and Wyatt (2000) concluded that both *Polytrichastrum pallidisetum* and *P. ohioense* shared many alleles with *P. longisetum*, *P. formosum* and *Polytrichum commune*. The closest candidates for progenitors of *Polytrichastrum pallidisetum* were *P. appalachianum* and *Polytrichum commune*, while for *Polytrichastrum ohioense* they were *Polytrichastrum formosum* (or *P. longisetum*) and *Polytrichum commune*. However, in our analyses the *Polytrichastrum* clade in which the type, *P. alpinum,* occurs is not closely related to these taxa. This is consistent with the findings of Derda et al. (1999), who found that *Polytrichastrum alpinum* and *P. sexangulare* were distant from other *Polytrichastrum* exemplars (which grouped with *Polytrichum*). Thus the results of Derda and Wyatt (2000) and Derda et al. (1999) are best interpreted as further evidence in support of the congeneric status of *Polytrichum* combined with *Polytrichastrum* sect. *Aporotheca* (within which allopolyploidy has occurred), rather than of true inter-generic hybridization between *Polytrichastrum* s.s. and *Polytrichum*.

In the case of *Polytrichastrum sexangulare*, Derda and Wyatt (2000) concluded that *P. sphaerothecium* was one likely progenitor of the allopolyploid *P. sexangulare* and that the other "possessed alleles that are common in *Pogonatum*". In the light of our phylogeny, in which *Polytrichastrum sexangulare* and *P. sphaerothecium* appear as isolated elements of ambiguous association within the large clade of predominantly northern hemisphere taxa, it is likely that these alleles are not definitive of *Pogonatum* but rather are plesiomorphic, not necessarily providing evidence of a close relationship between *Pogonatum* and *Polytrichastrum sexangulare*. Nonetheless our results are entirely compatible with *Polytrichastrum sphaerothecium* being one progenitor of an allopolyploid *P. sexangulare*. We anticipate that this allopolyploidy event, as well as the others identified by Derda and Wyatt (2000) and van der Velde and Bijlsma (2001), might produce well-supported incongruence between selected c/m and nuclear data evolving at a faster rate.

Can, then, the strong incongruence between the 18S gene tree and the c/m data for the status of *Pogonatum* be explained by relatively recent inter-generic allopolyploidy events? Of the four *Pogonatum* species appearing outside of the *Polytrichum s.l.*/*Pogonatum* clade in the 18S gene tree, the ploidy status of two (*P. urnigerum* and *P. dentatum*) is known; they are not polyploids (Derda et al., 1999). A more plausible and parsimonious explanation for the pattern observed is the scenario suggested by the SplitsTree hybridization network (Fig. 4), as described in the results. Presumably the maternal progenitor of the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade possessed the plesiomorphic morphology of *Polytrichastrum*, this being largely retained (perhaps aided by introgression) along with the maternally inherited organellar genomes, while the paternal progenitor resembled extant *Pogonatum spp.* and contributed the apomorphic version of 18S. *Polytrichum s.s.* then represents a derived lineage, and both the *Polytrichum* and *Pogonatum* morphologies are likely to be derived from a plesiomorphic *Polytrichastrum*-like form.

Turning briefly to the origin of *Polytrichum strictum* (results, Fig. 4), the very similar morphology shared by this species and *P. juniperinum* increases confidence in proposing the latter as a maternal ancestor. Given the lack of well supported resolution for the position of *P. hyperboreum* and *P. piliferum*, one of these species, or a related extinct taxon, could easily be the paternal progenitor (all of these species are placed in *P.* sect. *Juniperifolia*).

It should be stressed that these scenarios simply represent parsimonious resolutions of the observed strongly supported ($\geqslant$ 95% p.p.) incongruence under the assumption of hybridization based on the available data and taxon sampling, and may not be robust against altered data and/or taxon sampling.

It is striking that in all four of the hypothesised reticulation events displayed in Fig. 4, the morphological affinities of the resultant clade or species are clearly closest to the supposed maternal progenitor. This is a function of the c/m tree (Fig. 1) being far more plausible as a species tree than the 18S topology from a morphological perspective. While effectively ruling out organellar capture as a source of the observed incongruence, this further casts doubt on the hitherto assumed validity of the apparent phylogenetic signal in the 18S data for the disputed nodes. An alternative explanation for the incongruence is homoplasy, magnified by possible close linkage of substitutions at a small number of specific sites. As a functional RNA gene, the secondary and tertiary structure of the 18S molecule may impose strong constraints on the manner in which viable substitutions may occur, i.e. changes at one site may strongly favour compensatory substitutions at a range of other distant sites. Adequately exploring and modelling such dependencies for phylogenetic analysis is beyond the scope of this study. Although it is possible to apply specific models to RNA stem and loop regions (e.g. Savill et al., 2001) and to exclude subsets of data that are found to be saturated (transitions within loop regions for example), this requires robust knowledge of secondary structure, which for 18S is currently lacking in this group or in any close relatives. We attempted, but were unable, to construct a credible secondary structure (or set of closely similar structures) for 18S using published structures from angiosperms combined with appropriately constrained analyses based on free-energy minimisation. Furthermore, such models would not allow for linkage of substitutions due to three-dimensional structure and functional constraints, and it is unlikely that saturation of independently evolving sites alone would produce the very strong signal in the18S data that is the source of the incongruence. Nonetheless, indirect evidence of pronounced non-independence of substitutions at specific sites is provided by the observation that many of the individual apomorphies in 18S linking the apical *Pogonatum* clade with *Polytrichum s.l.* are also definitive of *Polytrichadelphus*. A wealth of evidence from the c/m dataset, morphology and biogeography supports the distinctness of *Polytrichadelphus* from *Pogonatum*, *Polytrichum* and *Polytrichastrum* and suggests that it shares a common ancestor with these groups prior to the origin of the large northern hemisphere clade. Nonetheless, these shared substitutions are sufficient to place *Polytrichadelphus* as sister to an apical *Pogonatum*/*Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade in the analyses of the 18S data alone, albeit with a probability of <95%. If this scenario is rejected, then multiple linked substitutions at specific sites in 18S shared by *Polytrichadelphus*, the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade and the apical *Pogonatum* clade have occurred *en masse* at least once, undermining the phylogenetically fundamental assumption that these sites are predominantly evolving independently.

Future studies may determine whether 18S provides a reliable phylogenetic signal within the Polytrichopsida; at this stage, it is only possible to provide alternative phylogenetic scenarios for each of two possibilities. If the signal in 18S is artefactual, then the c/m topology (Fig. 1) provides the current best estimate of phylogeny for the group. If it is sound, then the hybridization network (Fig. 4) is a more realistic portrayal of relationships, and it is further possible that future studies will provide evidence of other reticulate historical events.

4.2. Phylogeny and evolution

4.2.1. General patterns and earliest dichotomies

Despite a lack of high support values associated with resolution of the initial divergences in the northern hemisphere clade and uncertainty surrounding the interpretation of statistically supported incongruence between datasets for the origins of a few key taxa, the results collectively provide a robust phylogenetic hypothesis for the majority of generic and higher level relationships within the class.

The strong phylogeographic pattern observed (Figs. 1 and 2) was also obtained by Hyvönen et al. (1998, 2004) based on much smaller datasets. The earliest diverging terminals (*Alophosia, Bartramiopsis* and *Lyellia*) show highly disjunct distributions that are parsimoniously interpreted as relictual and are separated from the large, predominantly northern hemisphere clade by an exclusively southern temperate/southern tropical grade (Fig. 1). The phylogenetically isolated nature of *Alophosia*, *Bartramiopsis* and *Lyellia* is further indicated by the very long branch associated with the node representing the most recent common ancestor of the peristomate clade, and is consistent with their unique eperistomate capsules in which dehiscence is controlled by a "stopper" formed from endothecial (columellar) tissue abutting against a disk formed from amphithecial cells (Fig. 5A). The first dichotomy in the peristomate clade also corresponds to major differences in the spore dispersal mechanism (Fig. 5B–D), *Dawsonia* having a "brush-like" peristome formed of long filaments derived from the inner cells of the amphithecium and lacking a functional component derived from the columella, while the remainder of the class possesses the classic polytrichoid peristome consisting of short tooth-like processes joined to the edge of a more or less membranous disk (the epiphragm), formed from the expanded apex of the columella and presumably homologous at some level with the "stopper" of the eperistomate taxa.

Alophosia has by far the longest branch of any terminal in the analysis. This mono-specific genus is endemic to Madeira and the Azores, which, given its highly isolated phylogenetic position, is significant in the light of recent work on the origins of the Macaronesian flora, some of which has wholly or partially focussed on bryophytes (Vanderpoorten et al., 2007; Huttunen et al., 2008; Rycroft et al., 2004; Vanderpoorten and Long, 2006). The traditional view (Engler, 1879) of the Macaronesian flora as a relict of a previously much more widespread Tertiary European and North African element has been challenged in these studies and in those of selected angiosperm taxa (e.g. Emerson, 2002; Carine et al., 2004), which have suggested that the origins of at least some groups are best explained by complex dispersal patterns between the various Macaronesian islands and continental areas over a considerable period of time. In the case of the moss flora in particular (Vanderpoorten and Long, 2006; Vanderpoorten et al., 2007), it seems that many endemics may have evolved in situ following discrete long-distance dispersal events from various continental areas, thus explaining the weaker phytogeographic associations with Europe and North Africa compared with angiosperms. The position of *Alophosia* as sister to the rest of the Polytrichopsida, however (Bell and Hyvönen, 2008), given the considerable age of this node (estimated at 253–207 mya by Newton et al., 2007, much older than any of the Macaronesian islands), is clearly only explicable in terms of relictualism (inclusive of classic vicariance scenarios as well as the possibility that *Alophosia* occurred elsewhere until relatively recently and reached Macaronesia via long-distance dispersal). The conservation value of this taxon cannot be over-emphasised. Although we have not quantified evolutionary distinctiveness in this study (Isaac et al., 2007), it seems highly probable that by this measure, *Alophosia* would be by far the most important single species of land plant in the entire Macaronesian flora. It appears to be quite common in the Azores, although much less so on Madeira (Rumsey, pers. comm.).

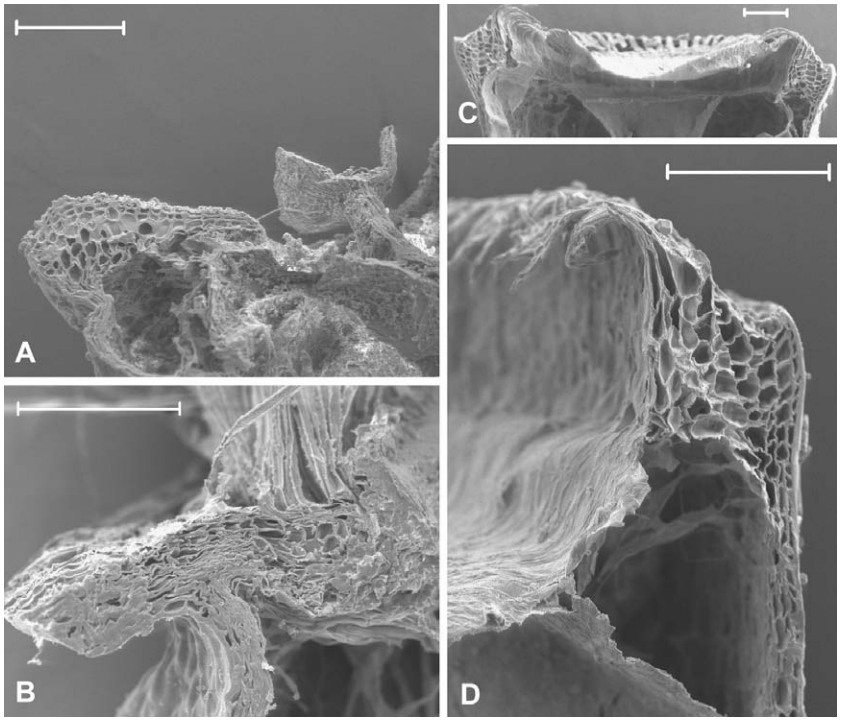

Fig. 5. SEM micrographs showing longitudinal sections of apical parts of sporophytes in the Polytrichopsida. All scale bars indicate 200 μm. (A) *Lyellia crispa*. The "stopper" formed from the expanded apex of the columella is on the right. (B) *Dawsonia papuana*. The bases of the peristome bristles can be seen at top right. (C and D) *Dendroligotrichum dendroides*. In C, the epiphragm (which is formed from the expanded columellar apex but becomes detached from the rest of the columella when mature) is seen, while in D the epiphragm was detached during preparation of the specimen.

Lyellia occurs as three species, one of which, *L. aspera* (previously *Philocrya* I.Hagen & C.E.O.Jensen) has a circum-high arctic distribution, the other two being found only in the Himalayas and in Yunnan, China. The fruit of *L. aspera* was discovered and described relatively recently (Afonina and Andrejeva, 1993). *Bartramiopsis* is a monotypic genus found only in the highly oceanic fringes of NW North America, Japan, and Eastern Siberia (Smith Merrill, 2007b).

Incongruence between datasets (Fig. 2) and lack of strongly supported resolution in the c/m topology (Fig. 1) makes it uncertain whether *Polytrichadelphus* or the *Dendroligotrichum*/*Hebantia* group is sister to the large northern hemisphere clade, a further possibility being that *Dendroligotrichum* and *Hebantia* derive from an ancient reticulation event (Fig. 4, discussed above). Although sampling is limited within these clades, our results support those of Stech et al. (2008) in suggesting that the New Zealand *D. microdendron* should be recognised separately from the South American *D. dendroides*. Although we did not include *Polytrichadelphus magellanicus s.s.* in this study due to unreliable data for some gene regions, pilot studies additionally indicated the distinctness of this species from the New Zealand *P. innovans*, further supporting the findings of Stech et al. (2008).

4.2.2. Oligotrichum and Notoligotrichum

A significant finding of this study is strong support for the monophyly of a southern hemisphere clade comprising *Notoligotrichum* (including *Atrichopsis*), *Itatiella*, and some *Oligotrichum* species, this being sister to a clade containing all other peristomate Polytrichopsida. Smith (1971) separated the exclusively southern hemisphere *Notoligotrichum* from *Psilopilum* (the two remaining species of which have northern arctic distributions) based partially on the distinctly different peristome teeth, and also noted that the peristomes of most southern hemisphere *Oligotrichum* species resemble those of *Notoligotrichum* more than those of northern *Oligotrichum*. He did not go as far as to split *Oligotrichum*, however, despite clearly having reservations about this genus as well as *Psilopilum*:

"The type elements of both *Psilopilum* and *Oligotrichum* appear to be more closely related to one another than to many of the species in their respective genera, and these species, in turn, are evidently more closely related to one another than to the type species of the genus in which they now reside." (Smith, 1971, pp. 49–50).

Our molecular results entirely corroborate this observation based on morphology. In practice, *Oligotrichum* is defined largely by the reduction of adaxial lamellae combined with a lack of the defining features of other genera. It seems to be the "dustbin" genus of the Polytrichopsida, home particularly to small plants that are possibly gametophytically neotenous to varying extents and thus lack morphological features that might otherwise offer clues to their relationships. Our results suggest that this reduced morphology has arisen at least twice; once as the probable plesiomorphic condition in a southern hemisphere group (from which *Notoligotrichum* is derived), and once in the ancestor of a distinct clade within the large northern hemisphere group (including the type, *O. hercynicum*), that is indeed more closely related to *Psilopilum* s.s. Tentative results (unpublished) based on limited data suggest possible further independent origins of the *Oligotrichum* morphology in isolated single taxa.

There are several characters distinguishing the northern and southern *Oligotrichum* species, many of which were highlighted by Smith (1971). In the southern clade, peristome teeth are usually single (see Smith, 1971, for the distinction between single and double teeth in Polytrichopsida; in practice, peristomes in both *Oligotrichum* clades can be rather irregular, with some single teeth and some double), narrow, pointed, and well separated (Fig. 6A). The teeth often project strongly inwards towards the center of the capsule rather than being upright and have an extended basal membrane, so the entire peristome/epiphragm complex appears relatively flat. This is characteristic of most southern hemisphere Polytrichopsida, particularly *Polytrichadelphus* and *Dendroligotrichum*, and may represent a plesiomorphic condition closer to the eperistomate disk of *Alophosia* and *Lyellia*. In the northern clade, peristomes are composed of mostly double teeth that are less narrowed and acute, generally more crowded, and more upright (Fig. 6B). In the southern clade stomata are highly condensed in a prominent and well-defined narrow band at the base of the capsule, while in the northern clade they are more dispersed, although still usually concentrated at the base. Finally, the southern taxa always lack abaxial lamellae, while the northern species nearly always have some indication of these (in addition to the normal adaxial lamellae).

Currently 28 species of *Oligotrichum* are recognised, although some are very poorly known. Based on the specimens we have been able to examine the majority have the morphology of the northern type and probably occur in that clade, although one or two may have independent origins. We estimate that nine species occur in the southern clade, four of which we have been able to sample for molecular characters (*O. tenuirostre, O., canaliculatum, O. afrolaevigatum*, and *O. riedelianum*). The others are *O. novae-guineae* (E.B.Bartram) G.L.Sm., *O. wageri* (Broth.) G.L.Sm., *O. tristaniense* Dixon, *O. erosum* (Hampe) Lindb., and *O. denudatum* G.L.Merr. The group as a whole is characterised by a very scattered southern hemisphere distribution and by narrow endemics, some of which

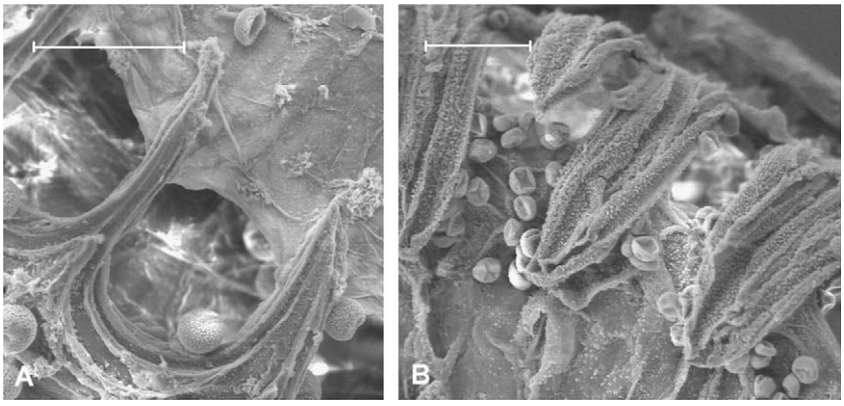

Fig. 6. SEM micrographs of peristome teeth and epiphragm margins in representatives of southern hemisphere and northern hemisphere clades of *Oligotrichum*. Scale bars indicate 50 μm. (A) *O. canaliculatum*. (B) *O. hercynicum*.

are very rare. *Oligotrichum canaliculatum, O. riedelianum, O. denudatum* and *O. erosum* occur in various regions of South America, *O. afrolaevigatum* and *O. wageri* are endemic to South Africa, and *O. tenuirostre* to New Zealand. *Oligotrichum novae-guineae* is known only from the type, while *O. tristaniense* is endemic to Tristan da Cunha. The latter shares with *O. canaliculatum* a broad nerve and fairly numerous lamellae that are more typical of *Notoligotrichum*, although both lack the *Notoligotrichum* sporophyte morphology.

Notoligotrichum shares the above mentioned features of the southern *Oligotrichum* species and is additionally defined by other characters, including distinctly 2–3 angled capsules that are usually curved and narrowed towards the apex, a generally even more prominent band of stomata at the broad capsule base, and a wider nerve that usually supports a greater number of adaxial lamellae (these are reduced in *N. tapes* and absent in *Atrichopsis*). Additionally the lamellae, and often also the abaxial side of the lamina and the adaxial side towards the margin above, are papillose to varying degrees in mature examples of all species other than the exclusively South American clade that includes *N. tapes* and *N. trichodon*. The New Zealand endemic *O. tenuirostre* appears as the sister to *Notoligotrichum* in our analyses, and indeed has a morphology consistent with this. Many specimens (including the type) are distinctly papillose at the leaf margin towards the apex, a character we have not definitively observed in any of the other southern *Oligotrichum* species, and the capsule, while more or less straight and cylindrical, often has two ridges and a slight curvature (see illustration in Frye, 1947). Further, while the nerve is narrow and only 5–10 true lamellae are present (absent entirely on the lower leaves), there are often areas of bistratose lamina elsewhere on the leaf (apparently first observed by Frye, 1947, and not in the original description). These could be interpreted as the vestiges of a fully bistratose lamina (as in *Atrichopsis*), or of more numerous lamellae extending over a wider area of the leaf surface. Our sampling includes all species of *Notoligotrichum* other than *N. mexicanum* (G.L.Sm.) G.L.Sm. and two species endemic to Tristan da Cunha. At least one of the latter, *N. laxifolium* (Dixon) G.L.Sm., is very distinct, although by virtue of the sort of extreme morphology typical of island endemics that have evolved in situ; it is a very lax plant of grasslands with stems up to 10 cm long, a habit completely at odds with *Notoligotrichum*, although in other respects it has the typical morphology of the genus. *Notoligotrichum* occurs in South Africa (represented by TH14147 in our analysis), but descriptions of two species (under *Oligotrichum*) were not validly published (Fanshawe, 1980). The African plants are quite variable and it is not clear from the limited material we have seen whether one or more species occur there.

The eperistomate *Itatiella ulei* appears to be derived from within the southern *Oligotrichum* group and is sister to *O. riedelianum* in our analysis. Alternatively, both of these species may be descended from an early reticulation event involving extinct members of the austral *Oligotrichum*/*Notoligotrichum* clade (Fig. 4), probably prior to its subsequent diversification. These two morphologically rather different species, together with the elamellate *O. denudatum*, share a sympatric distribution on mountains in S.E. Brazil. *Itatiella* has uniquely derived sporophytes that lack both a peristome and stomata (Smith, 1971).

4.2.3. Polytrichastrum

Polytrichastrum was particularly well sampled in our analyses, which included all currently recognised species and multiple exemplars from problematic taxa. Peristome morphology, character evolution and taxonomy within this group are the subject of a separate study (Bell and Hyvönen, 2010). Including *Polytrichastrum sexangulare* and *P. sphaerothecium*, there are four distinct lineages (not all of which are necessarily separate) within extant *Polytrichastrum*, only one of which (*P.* sect. *Aporotheca*) is closely related to *Polytrichum*. Another approximately corresponds to *Polytrichastrum* sect. *Polytrichastrum* (including the mono-specific *Meiotrichum* but not *P. sexangulare* or *P. sphaerothecium*). Consisting exclusively of plants of montane, cold-temperate or arctic environments, from the Andes (*Polytrichastrum tenellum*) to the Himalayas (*P. emodi, P. papillatum*) and the circum-arctic (*P. norwegicum*), it is likely to represent a plesiomorphic morphology within the northern hemisphere clade. *Polytrichastrum alpinum* s.s. has a remarkably widespread bipolar distribution in arctic, sub-arctic and montane temperate regions, and our five exemplars from both hemispheres are well supported as monophyletic. *Polytrichastrum norwegicum* (= *P. alpinum var. septentrionale* according to Smith, 2007a) seems to be a distinct species that includes *P. altaicum*, although its circumscription is highly confused and requires further study.

The strongly supported monophyly of *P. sphaerothecium* (= *P. sexangulare var. vulcanicum*, Smith, 2007a) in our results is significant given its unusual distribution and controversial taxonomic status. The plant occurs on volcanic rock in northern oceanic areas, with one center of distribution in Japan, the Kuril Islands and the Aleutians (possibly also mainland Alaska and British Columbia), and another in Iceland. We have also seen two specimens collected at altitude on Mt. Changbai (an active volcano) in the Jilin provence of N.E. China near the North Korean border (*Koponen* 36797, 36662, H). Based on the limited evidence available, the Icelandic plants are very close to the Northern Pacific ones, suggesting that the species is able to disperse within the northern sub-arctic and cool temperate regions and has a distribution that is limited by the co-occurrence of its particular substrate and climatic requirements. Intriguingly, it may be particularly adapted to tectonically derived oceanic islands associated with sea floor spreading and subduction. It is possible that the species is more widespread, and that sterile collections have been misidentified as *P. sexangulare*. As mentioned above, according to Derda and Wyatt (2000) this species is likely to be one of the progenitors of the allopolyploid *P. sexangulare*, which is considerably more widely distributed and associated with arctic-alpine late snow zones. Although these taxa superficially appear not to be closely related on the c/m phylogeny (Fig. 1), this is due to very weakly supported and probably misleading associations with larger clades. We suspect that both of these species have close affinities to the *Polytrichastrum* s.s./*Meiotrichum* group; they have occurred at the base of this clade with weak support in some of our early analyses using incomplete datasets, while *P. sexangulare* additionally shares the otherwise unique deletion in the P8 loop of the *trnL* intron that characterises this clade. Given that allopolyploidy is known to be a factor in the origins of at least *P. sexangulare*, it is possible that reticulate historical processes are responsible for the ambiguous relationships of these species, as well as for the general lack of resolution between major entities in the northern hemisphere clade.

4.2.4. Psilopilum

The strong support obtained here for the grouping of *Psilopilum* as sister to the *Steereobryon*/*Atrichum* clade is a result not found in previous studies. Hyvönen et al. (1998, 2004) and Bell and Hyvönen (2008) all either failed to convincingly resolve the position of *Psilopilum* or else found evidence to suggest a relationship with the *Pogonatum*/*Polytrichum s.l.* clade (in the case of Hyvönen et al., 1998, including *Atrichum* and *Hebantia*). Bell and Hyvönen (2008) even found strong support (98% Bayesian p.p.) for this relationship, although taxon sampling was very limited and characters were not specifically selected to be informative for this part of the phylogeny. The signal implying this position for *Psilopilum* appears to derive from 18S, and indeed in this study we obtained 88% p.p. for such a relationship using 18S alone. If we had considered less strongly supported incongruence between 18S and the other data

to be significant, the position of *Psilopilum* would have been discussed in this context. However, its position as sister to *Atrichum* and *Steereobryon* is very strongly supported by the c/m data, and the additional implication that all of these taxa may be close to the northern *Oligotrichum* clade is corroborated by a number of morphological characters (see Smith, 1971, discussed above in relation to the southern *Oligotrichum*/*Notoligotrichum* group). All of these groups often have highly undulate lamellae restricted to the nerve and 32 peristome teeth that are relatively broad and crowded. Smith (1971) noted the similarity of the leaf form of *Atrichum subulirostrum* Schimp. ex Besch. (≡ *Steereobryon subulirostrum*) to *Psilopilum*, and our work on peristome morphology (Bell and Hyvönen, in prep.) suggests that *Psilopilum* may represent an intermediate stage between an *Oligotrichum* type, in which teeth are attached to the side of the epiphragm or to the extreme edge on the dorsal surface (Fig. 6B), and an *Atrichum* type, in which teeth appear to be very broadly attached to the dorsal epiphragm surface due to differences in the way that the mature epiphragm develops.

4.2.5. Future directions

A robust hypothesis of phylogeny is a tool for exploring wider historical questions, evolutionary processes, and phytogeography, as well as the starting point for a natural taxonomy. This study lays the foundations for research into specific aspects of phenotype evolution in the Polytrichopsida, which are of particular interest due to the phylogenetically isolated nature of the class. Because major structural features such as the peristome have arisen independently (and differently) in the Polytrichopsida and in the much more numerous arthrodontous mosses, the group provides an invaluable window into a different region of viable morphospace, and hence an additional set of coordinates for exploring the extent and meta-form of this space. Unfortunately we cannot "re-run" evolution from a given common ancestor in order to study the extent to which certain morphologies arise stochastically within an extensive and easily traversed viable morphospace (i.e. representing just a few out of many possibities), or alternatively are strongly determined by small and tightly constrained regions of viability within conceivable morphospace. Hence we need to rely on "re-runs" that have occurred historically, to the extent that these are accessible. The extreme poverty of the fossil record for mosses compared with polysporangiophytes necessarily focuses this endeavour on comparative studies of extant groups (and underlines the importance of their conservation).

We intend to use our phylogenetic hypothesis to study the evolution of the peristome within the Polytrichopsida (Bell and Hyvönen, 2010, in prep.). In addition to the major types of sporophyte dehiscence apparatus in the class (basal non-peristomate, dawsonioid peristomate and polytrichoid peristomate), there is much under-described variation in the polytrichoid peristome–epiphragm complex. Traditional classifications in the Polytrichopsida have over-emphasised gametophytic characters, creating artificial groups such as *Oligotrichum*, *Psilopilum s.l.* (i.e. including *Notoligotrichum*), and *Polytrichum s.l.* (the artificiality of *Polytrichastrum* as currently circumscribed descends from that of *Polytrichum s.l.* and the splitting of this group in what now appears to have been the wrong place). In each of these cases a re-examination of sporophyte characters in particular provides a way to circumscribe groups that correspond to clades. Smith (1971) began this process with the description of *Notoligotrichum* and the identification of peristome features that define northern and southern groups of *Oligotrichum* species, and there is further work still to be done.

In order to create a classification of the Polytrichopsida that is compatible with our current understanding of phylogeny it is clear that a number of nomenclatural changes are required. These will be the subjects of separate studies, including examination of type material as well as expanded sampling in some cases. The low support values obtained for some nodes further highlight areas (Grant and Kluge, 2003) where additional character and/or taxon sampling is required to provide a fully comprehensive robust hypothesis of phylogeny for this ancient and highly distinct group of mosses.

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