

POLARITY OF PEATMOSS (*SPHAGNUM*) EVOLUTION: WHO SAYS BRYOPHYTES HAVE NO ROOTS?¹

A. JONATHAN SHAW,² CYMON J. COX, AND SANDRA B. BOLES

Department of Biology, Duke University, Durham, North Carolina 27708-0338 USA

The class Sphagnopsida (Bryophyta) includes two genera: *Ambuchanania* and *Sphagnum*. *Ambuchanania* contains just one rare species known from two Tasmanian localities, but *Sphagnum* comprises a speciose clade of mosses that dominates many wetland ecosystems, especially in the boreal zone of the Northern Hemisphere. Recent phylogenetic analyses have resolved well-supported clades within *Sphagnum*, but polarizing *Sphagnum* evolution has been problematic because the genus is so isolated that it is difficult to determine homologies between morphological and/or molecular traits within *Sphagnum* with those of any potential outgroup. DNA sequences from 16 genomic regions representing the mitochondrial, chloroplast, and nuclear genomes (ca. 16 kilobases) were obtained from 24 species of *Sphagnum* plus one species each from *Takakia* and *Andreaea* in order to resolve a rooted phylogeny. Two tropical species, *S. sericeum* and *S. lapazense*, were resolved as sister to the rest of the genus and are extremely divergent from all other sphagna. The main *Sphagnum* lineage consists of two clades; one includes the sections *Sphagnum*, *Rigida*, and *Cuspidata*, and the other includes *Subsecunda*, *Acutifolia*, and *Squarrosa*. The placement of section *Subsecunda* is weakly supported, but other nodes are strongly supported by maximum parsimony, maximum likelihood, and Bayesian analyses. In addition to homogeneous Bayesian analyses, heterogeneous models were employed to account for different patterns of nucleotide substitution among genomic regions.

Key words: Bayesian inference; bryophytes; peatmoss; phylogenetic reconstruction; *Sphagnum*.

The peatmosses (*Sphagnum* L.) are literally in a class of their own (i.e., Sphagnopsida, Bryophyta). These are the only mosses with direct and substantial economic value, and peatmosses have been used by people for centuries and even millennia (Williams, 1982; Crum, 1992a; Turner, 1993). Many of their uses derive from the extraordinary absorptive capacity of peatmoss (as, for example, in bandages, diapers, filtration materials, feminine hygiene products), which in turn derives from the unique morphology of *Sphagnum* plants.

Sphagnum-dominated peatlands cover extensive areas in the boreal zone of North America, Europe, and Asia (Vitt, 2000). These peatlands constitute an important reservoir for global carbon and currently function as a carbon sink (Gorham, 1991). *Sphagnum*-dominated peatlands also have profound effects on gas fluxes that are determinants of global climate (e.g., CO₂, N₂O, NH₃, H₂S, COS, and DMS), nutrient cycling, regional patterns of hydrology, and biodiversity (Gorham, 1991; Moore, 1994; Wieder and Yavitt, 1994; Camill and Clark, 1998). As is true of their anthropocentric utility, the ecological significance of boreal peatlands is directly related to the physical and chemical characteristics of peatmosses.

Gorham (1994) outlined an agenda for future research on peatland ecology with particular reference to global change and stressed the importance of understanding how *Sphagnum* species differ in biological characteristics that affect community and ecosystem processes. Morphological, chemical, and life history features of different sphagna have demonstrable effects on their species-specific ecologies, including rates of photosynthesis, decomposition, vegetative growth, competitive ability, and reproductive biology (Clymo, 1963; Clymo and Heywood, 1982; McQueen, 1987; Cronberg, 1993; Johnson and Damman, 1993; Rydin, 1993; Rice, 1995, 2000; Rice and Schuepp, 1995; Rice and Giles, 1996). Correlations between habitat and species traits are informative, but a phylogenetic context is essential for rigorous comparative ecological studies

so that the contributions of shared ancestry and similar (or differing) ecology can be differentiated (Harvey and Pagel, 1991).

Several phylogenetic hypotheses have been put forward for the genus *Sphagnum* (Eddy, 1977, 1979; He and Aur, 1991; Shaw, 2000a). Rooting a phylogeny for the genus, however, presents exceptional difficulties. The isolated phylogenetic position of the peatmosses has made it difficult to interpret the polarity of evolutionary change within *Sphagnum* (Shaw, 2000a). The problem is a familiar one in phylogenetic analyses dealing with isolated groups: morphological characters that distinguish species of *Sphagnum* have no clear homologs outside *Sphagnum*, so outgroup rooting of a generic phylogeny based on morphology is difficult or impossible. The same problem exists with regard to molecular data; nucleotide sequences for genomic regions that are variable enough to resolve phylogenetic relationships within *Sphagnum* cannot be aligned with homologous sequences from potential outgroups, including *Andreaea*, *Takakia*, or species of Bryopsida (Shaw, 2000a). Genes that are conserved enough to align between *Sphagnum* and outgroups, on the other hand, are nearly invariant within *Sphagnum* and offer little with regard to phylogenetic topology among peatmoss species.

We sequenced eight conserved genes representing all three genomes for a sample of 24 *Sphagnum* species and two outgroups (*Takakia* and *Andreaea*), in an effort to root the phylogeny of *Sphagnum*. Although even eight genes (ca. 9 kilobases [kb]) provided too few informative characters to resolve relationships among most sphagna, two species, *S. sericeum* C. Muell. and *S. lapazense* Crum, were strongly supported as sister to the rest of the genus. This permitted a second tier of analyses in which *Takakia* and *Andreaea* were deleted from the analyses, *S. sericeum* and *S. lapazense* were coded as outgroups, and more variable genes were added to the data set. This approach yielded a resolved phylogeny rooted to *S. lapazense* and *S. sericeum*.

Sphagnum is distinct from all other mosses in numerous aspects of both sporophyte and gametophyte morphology. In-

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² E-mail: shaw@duke.edu.

deed, Crum (2001a) recently elevated peatmosses to the level of division, as the Sphagnophyta. For purposes of this paper, the peatmosses are classified as one of four classes within the division Bryophyta: Takakiopsida, Andreaeopsida, Sphagnopsida, and Bryopsida. The Sphagnopsida include two orders, Ambuchananiales and Sphagnales. The Ambuchananiales contain only one species, *A. leucobryoides*, which was described from Tasmania in its own section of *Sphagnum* (Yamaguchi et al., 1990). Crum and Seppelt (1999) described the new genus *Ambuchanania* for *Sphagnum leucobryoides* and placed it in its own family and order. Shaw (2000a) reported that 26S rDNA sequence data support the separation of *Ambuchanania* from *Sphagnum*. *Sphagnum*, in contrast, includes approximately 250–400 species and has a worldwide distribution. The informal term “peatmoss” is used in this paper with reference to members of the genus *Sphagnum*, which, unlike *Ambuchanania*, form extensive peat deposits in many parts of the world (Vitt, 2000).

Morphological and developmental features that set *Sphagnum* apart from other mosses pertain to practically every stage of the life cycle. *Sphagnum* spores germinate to produce a protonemal stage that is filamentous only for the first few cell divisions, after which a two-sided apical cell is differentiated and the protonema becomes thalloid (illustrated in Ruhland, 1924; von Goebel, 1930; Anderson and Crosby, 1965). Thalroid protonemal morphology is shared by the Sphagnopsida and Andreaeopsida and is probably a plesiomorphic character within the Bryophyta (Mishler and Churchill, 1985). *Sphagnum* protonema are remarkably similar to the gametophytes of *Coleochaete* (Chlorophyta).

The leafy gametophore has an apical cell with three cutting faces, as in most other mosses, but the stems also have a sub-apical group of meristematic cells that contributes to growth in length (Ligrone and Duckett, 1998). *Sphagnum* is generally easy to recognize in nature because mature gametophores have fasciculate branching, typically with 3–5 branches per fascicle, although sometimes with more (e.g., *S. wulfianum* Girg.) and occasionally with fewer or even none (in simplex forms such as *S. cyclophyllum* Sull. & Lesq. in Sull.). Branches are more or less dimorphic, with 1–2 so-called pendent branches that extend down the stem, effective for external capillary movement of water, and 1–2 “spreading” branches that diverge more widely from the stem. Branches near the stem apices are clustered and form a more or less distinct capitulum. The shape and prominence of capitula are often useful in determining species of *Sphagnum* (e.g., Eddy, 1977; Andrus, 1980; Flatberg, 2002). The main stems of peatmosses consist of a central region of more or less homogeneous, thin-walled cells surrounded by a “wood cylinder” of thicker-walled, generally pigmented cells, and (0–)1–4 layers of enlarged, thin-walled, so-called hyalodermal cells. The number of hyalodermal layers, and also the degree of size differentiation between hyalodermal and internal cells, vary across the genus. In species of the section *Sphagnum*, the hyalodermal cells have fibrils deposited on the inside surfaces of the cell walls. These layers, and especially the strongly differentiated superficial layer(s), make the cross sectional anatomy of *Sphagnum* stems unlike that of any other moss.

The stem and branch leaves are usually differentiated in size and shape, but both are characterized by dimorphic leaf cells in which large, empty hyaline cells are enclosed in a network of narrower, chlorophyllous cells. The degree of differentiation in shape between the two cell types is variable, but the exist-

tence of dimorphic cells is one of the “hallmarks” of peatmosses. Holcombe (1984) described the complex pattern of cellular morphogenesis, involving a unique series of asymmetric cell divisions, that gives rise to the dimorphic leaf cells in *Sphagnum*. The hyaline cells are involved in water absorption and storage and are variously perforated by pores and ornamented with cell wall fibrils. Fibrils are absent on the stem leaf hyaline cells of many species, but they are absent from the hyaline cells of branch leaves in only a few taxa (Warnstorf, 1911). Cell wall ornamentation (i.e., fibrils) is another of the unique and characteristic features of peatmosses, so the absence of these fibrils in a few taxa has led to the obvious question of which, if any, cases represent a primitive absence. That question will be addressed in this paper.

Unlike those of “true” mosses (Bryopsida), the sporophytes of *Sphagnum* consist of a capsule (sporangium) and foot, with little or no development of a seta. The sporophyte is raised on a pseudopodium of gametophyte origin. It has an operculum like members of the Bryopsida, but there is no peristome, and dehiscence of the capsule occurs through a unique “pop-gun” mechanism that involves increasing internal air pressure as the columnella breaks down and the capsule dries (Ingold, 1965). Development of the *Sphagnum* sporophyte is fundamentally different from that in other mosses in that sporogenous tissue originates from the amphithecium and the endothecium gives rise to the massive columnella. In the Bryopsida, both columnella and spores originate from endothecium. The capsule wall is solid in *Sphagnum* (i.e., without air spaces as in most Bryopsida), and there are numerous pseudostomata which may or may not be homologous with the stomata of true mosses (Boudier, 1988).

These numerous unique morphological features suggest that the Sphagnopsida are isolated from all other mosses. Molecular phylogenetic analyses have consistently indicated that the Sphagnopsida constitute one of the earliest diverging lineages of bryophytes (Hedderson et al., 1998; Newton et al., 2000; Yatsentyuk, 2001; Cox et al., in press). These studies suggest that the Sphagnopsida and Takakiopsida (one genus: *Takakia*) may form a monophyletic group that is sister to the Andreaopsida plus Bryopsida, although morphologically, *Takakia* and *Sphagnum* have little in common. Indeed, Newton et al. (2000) were not able to identify a single morphological synapomorphy that unites *Sphagnum* and *Takakia*. The placement of these two morphologically divergent groups in one clade could be an analytical artifact such as so-called “long branch attraction.” Cox et al. (in press) found that statistical support for monophyly of *Sphagnum* plus *Takakia* disappeared in Bayesian analyses utilizing increasingly complex models of substitution. This observation may support the view that their monophyly in other studies is artifactual.

Both *Andreaea* and *Takakia* were included in the present analyses as outgroups. The aims of this study were to resolve phylogenetic relationships among the major lineages of peatmosses and to clarify evolutionary polarity within the genus by outgroup rooting. The results of these analyses provide the framework for future investigations of ecological, morphological, chemical, and molecular evolution in peatmosses.

MATERIALS AND METHODS

Taxon sampling—Twenty-four species of *Sphagnum* were included in the analyses, with *Takakia lepidozoides* S. Hatt. & Inoue and *Andreaea rothii* Web. & Mohr. as outgroups. The 24 *Sphagnum* species were selected to rep-

resent the major lineages resolved by Shaw (2000a). Sampling included four species of section *Sphagnum*, two *Rigida*, four *Cuspidata*, three *Subsecunda*, four *Acutifolia*, and two *Squarrosa* (for Supplemental Data, see the online version of this article). *Sphagnum wulfianum* was included with the aim of clarifying its relationship to the sections *Acutifolia* and *Squarrosa*. This species has generally been segregated in the monospecific section *Polyclada* (e.g., Isoviita, 1966; Andrus, 1980; Crum, 1984; Flatberg, 2002), but Shaw (2000a) suggested that it might be nested within the *Acutifolia*. Analyses of molecular diversity across the genus *Sphagnum* indicated that two species, *S. sericeum* and *S. lapazense*, are extremely divergent from all other peatmoss species (Shaw et al., 2003). Warnstorf (1911) classified *S. sericeum* in the subsection *Sericea* with *S. macrophyllum* Brid., because these species lack fibrils on the branch leaf hyaline cell walls. While *S. macrophyllum* is clearly nested within the section *Subsecunda* (Shaw, 2000a), *S. sericeum* may be a phylogenetically critical species (Eddy, 1977) and was therefore included in the current analyses. Crum (2001b) included *S. lapazense* in the section *Sphagnum* when he recently described the species from Bolivia, but like *S. sericeum*, *S. lapazense* was surprisingly divergent from all other sphagna based on two genomic regions sequenced for a study of global peatmoss biodiversity patterns (Shaw et al., 2003).

Genomic sampling—Nucleotide sequences were obtained from 16 genomic regions. Mitochondrial intron sequences were obtained from NADH protein-coding subunits 1, 5, and 7, yielding 3220 nucleotides of mtDNA (hereafter, *nad1*, *nad5*, and *nad7*, respectively). From the chloroplast genome, we sequenced two photosystem II proteins (*psbA*, *psbT*), ribulose bis-phosphate carboxylase (*rbcL*), chloroplast ribosomal small protein 4 (*rps4*), transfer RNA^{Gly} (UCC) (*trnG*), and the *trnL* (UAA) 5' exon-*trnF*(GAA) intergenic spacer (hereafter, *trnL*). Nuclear sequences were obtained from the ITS1–5.8S-ITS2 region (hereafter, *ITS*), and 5' segment of the large subunit (26S) ribosomal RNA gene. Additional nuclear sequences were obtained for two introns in the *LEAFY/FLO* gene (hereafter, *LEAFY1* and *LEAFY2*).

Three anonymous regions, assumed to be nuclear, were sequenced using primers designed for regions identified from random amplified polymorphic DNA (RAPDs). The RAPDs were generated for a sample of nine divergent *Sphagnum* species, including two representatives from each of the four major sections and a single representative from section *Squarrosa*. Two primers from the Operon 10-mer Kit A (OPA-1 and OPA-3) (QIAGEN Operon, Alameda, California, USA) were used in a standard RAPD amplification. Using a Qiagen *Taq* DNA Polymerase Kit (QIAGEN Operon), 50 μ L reactions contained 1 \times buffer (with 1.5 mmol/L MgCl₂), 200 μ mol/L dNTPs, 4 μ mol/L primer, 1 unit *Taq*, and 5–10 ng template DNA. Cycling conditions were 45 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C. Amplified products were concentrated to 18–20 μ L and run entirely on a 1.5% agarose gel at 6 V/cm for 4.5 h. Bands that appeared to be monomorphic in size for three or more of the nine representatives were excised from the agarose gel and cleaned with a Qiagen Gel Extraction Kit (QIAGEN Operon). Fragments were then cloned using a TA Cloning Kit (Invitrogen, Carlsbad, California, USA) and sequenced with M13 primers and the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California, USA). Sequencing was accomplished using an ABI 3700 automated sequencer. Sequences found to be homologous were aligned, and primers were designed within conserved regions at the 5' and 3' ends of the fragments. After an accumulation of sequences generated with these primer sets, additional primers were designed so that a nested polymerase chain reaction (PCR) protocol might be pursued. None of the three “RAPD” regions identified in this manner was found to BLAST to a homologous sequence in GenBank, and they are here utilized as anonymous markers (designated RAPDa, RAPDb, and RAPDf).

Primer sequences for amplifying and sequencing all genomic regions utilized in this study are provided in Table 1. The PCR amplification was accomplished in several ways. An initial attempt was made to amplify fragments in 25- μ L reaction volumes containing 1 \times buffer, 2.5 mmol/L MgCl₂, 200 μ mol/L dNTPs, 0.5 μ mol/L each primer, 5% “Q” solution, 1 unit Qiagen *Taq* polymerase, and 0.3 μ L template DNA (stock DNA or first round product; see below). Where possible (see Table 1), a nested protocol was utilized,

in which the reaction just described was preceded with a 5 μ L amplification with flanking primers, performed with the same reaction conditions and designated “first round product.” When this regime failed to yield adequate amplification for sequencing, a similar procedure was followed using the Advantage-GC cDNA Polymerase Kit (Clontech, Palo Alto, California, USA). Here reaction conditions were 1 \times buffer, 1 \times GC Melt, 800 μ mol/L dNTPs, 0.2 μ mol/L each primer, 0.4 \times Advantage-GC cDNA polymerase mix, and 0.3 μ L template DNA (stock DNA or first round product). All amplifications were accomplished using a similar thermocycling regime: 3 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 50°C, and 45 (+5 s/cycle) at 72°C. A final extension of 7 min at 72°C preceded a 4°C hold. Reactions were screened on 1% agarose gels and successful amplifications cleaned using a QIAquick PCR Purification Kit (QIAGEN Operon). The pair of bands resulting from amplification with the *LEAFY/FLO* primers were excised individually from agarose gels and cleaned with the Qiagen Gel Extraction Kit. Sequencing was accomplished as with the RAPD clones above, using amplification primers and where available, additional sequencing primers (see Table 1).

Phylogenetic analyses—Analyses of the 26-taxon data set were conducted under maximum parsimony (MP), maximum likelihood (ML), and Bayesian statistical inference. Preliminary parsimony analyses of each of the 16 genomic regions did not reveal incongruent relationships that were supported with bootstrap values greater than 70%, so all sequences from each accession were combined for subsequent analyses. Analyses employing maximum parsimony and maximum likelihood optimality criteria were conducted using PAUP4.0b–10 (Swofford, 2001). Bayesian analyses were performed using MrBayes3 (Huelsenbeck and Ronquist, 2002) and P4 (Foster, 2002).

The phylogenetic analyses were performed in a two-tiered fashion. The most conserved genes (mtDNA: *nad1*, *nad5*, *nad7*; cpDNA: *psbA*, *psbT*, *rbcL*, *rps4*; nrDNA: 26S) were used for an initial analysis of the 26-taxon data set containing 24 sphagna plus *Takakia* and *Andreaea*. Sequences from the more variable genes could not be aligned with those from *Takakia* and *Andreaea*. Although the conserved genes are not variable enough within *Sphagnum* to resolve sectional relationships, the eight-gene analysis indicated that two *Sphagnum* species, *S. sericeum* and *S. lapazense*, are early diverging taxa outside (and sister to) the main *Sphagnum* clade. Because that inference was strongly supported by MP, ML, and Bayesian analyses, a second data set was constructed in which *Takakia* and *Andreaea* were removed and additional sequence data from less conserved regions were added to the eight conserved genes for the 24 *Sphagnum* species. In the second analysis, *S. lapazense* and *S. sericeum* were coded as outgroups in order to clarify relationships among the remaining *Sphagnum* species. The most variable genes (RAPDa, RAPDb, RAPDf) could not be unambiguously aligned between “mainstream” *Sphagnum* species and the two *Sphagnum* outgroups, so these were coded as missing data for *S. lapazense* and *S. sericeum*. All of the phylogenetic analyses described below were conducted on both data sets.

Equally weighted parsimony analyses were conducted with 300 random taxon-addition replicates with tree bisection and reconnection (TBR) branch swapping. The “steepest descent” option was turned off but the “collapse branches when maximum length is equal to zero” option was invoked. Support for nodes was assessed by 300 nonparametric bootstrap replicates each with 10 random taxon-addition replicates. For maximum likelihood analyses, the best substitution model, namely the general time-reversible model with a proportion of invariant characters and other site rates modeled by a discrete gamma distribution (GTR + I + G), was determined by hierarchical likelihood ratio tests with the aid of MrModeltest 1.1b (Nylander, 2002). Heuristic searches under maximum likelihood were conducted with 100 replicates of random taxon addition to the starting tree.

Maximum parsimony and maximum likelihood analyses implicitly or explicitly employ a single substitution model for the combined multigenic, multigenomic data set, although it is to be expected that different genes and regions are subject to different evolutionary processes and therefore evolve according to heterogeneous patterns of nucleotide substitution. It is possible to apply relatively crude but heterogeneous substitution models in MP analyses (e.g., first and second vs. third codon positions for protein-coding genes),

TABLE 1. Primers used for amplifying and sequencing genomic regions used in the analyses described in this paper. * = primer used in a single amplification reaction and in sequencing; \$ = primer used in the first step of a nested amplification (flanking primers); ** = primer used in the second step of a nested amplification (internal primers) and in sequencing; *** = primer used in sequencing only.

Region	Primer	Use	Sequence 5'-3'	Direction	Reference	Primer design based on	
Mitochondrial							
<i>nadI</i>	nad1B	*	GCATTACGATCTGCAGCTCA	forward	Demesure et al., 1995		
	nad1C	*	GGAGCTCGATTAGTTTCTGC	reverse	Demesure et al., 1995		
	nad5F4	*	GAAAGGATAGGTTCTCGCTTCA	forward	C. J. Cox, unpublished	±20 available moss sequences	
	nad5R3	*	AAAAAGCTGCTGTTACCAT	reverse	C. J. Cox, unpublished	±20 available moss sequences	
	nad5F3	***	AGGATCTCAGAGACTGTACGTTG	forward	C. J. Cox, unpublished	±20 available moss sequences	
	nad5R2	***	GGTCTCAGGTACAGTCTCTGAGG	reverse	C. J. Cox, unpublished	±20 available moss sequences	
	nad7F2	*	GTGGCTCTGTTATCCACACT	forward	C. J. Cox, unpublished	±5 available moss sequences	
	nad7R2	*	ACCCATCACACGGCTCTC	reverse	C. J. Cox, unpublished	±5 available moss sequences	
	nad7F4	***	AGAGATCCAGCGGAATG	forward	C. J. Cox, unpublished	±5 available moss sequences	
	nad7F5	***	GAAAGTCAAGTCAAGGGAGG	forward	S. B. Boles, unpublished	available <i>Sphagnum</i> sequences	
	nad7R1m	***	CCTCCCTGACTTGACCTTC	reverse	S. B. Boles, unpublished	available <i>Sphagnum</i> sequences	
	nad7R5	***	ACACACAAGGAAAAGACGTTG	reverse	S. B. Boles, unpublished	available <i>Sphagnum</i> sequences	
	Chloroplast	<i>psbA1</i>	tmK2F	GACGAGTTCGGGTTTCGA	forward	C. J. Cox, unpublished	<i>Marchantia</i> cpDNA sequences
			psbA576R	TGGAATGGTGCATAAGG	reverse	C. J. Cox, unpublished	<i>Marchantia</i> cpDNA sequences
			psbA501F	TTTCACAGACGGTATGCC	forward	C. J. Cox, unpublished	<i>Marchantia</i> cpDNA sequences
tmHR			GAAACGCGGAAATGAAAC	reverse	C. J. Cox, unpublished	<i>Marchantia</i> cpDNA sequences	
psbT			ATGGAAGCWTAGTWTATACWTT	forward	Krellwitz et al., 2001		
<i>rbcL</i>		psbH	*	GTHCCCCARCCDDGDRVHACTTT	reverse	Krellwitz et al., 2001	
		SrbcLF	*	GCAGCATTTCCRAATGACTC	forward	S. B. Boles, unpublished	available <i>Sphagnum</i> , <i>Takakia</i> , & <i>Andraea</i> sequences
		SrbcLJF	***	TATTRGGATGTACTATTAAACC	forward	S. B. Boles, unpublished	available <i>Sphagnum</i> , <i>Takakia</i> , & <i>Andraea</i> sequences
		SrbcLIR	***	TCTCGCRATAAATFRAGC	reverse	S. B. Boles, unpublished	available <i>Sphagnum</i> , <i>Takakia</i> , & <i>Andraea</i> sequences
		SrbcLJR	*	CTCATTACGGGCTTGTACAC	reverse	S. B. Boles, unpublished	available <i>Sphagnum</i> & <i>Andraea</i> sequences
<i>rps4</i>		TbrcLR	*	ATGTCCCGTTATCGAGGACCT	forward	S. B. Boles, unpublished	available <i>Takakia</i> sequences
		rps5	*	TACCGAGGGTTCGAATC	reverse	Nadot et al., 1994	
		trnas	*	CCGAGTTATCGGTGTAACCC	forward	Souza-Chies et al., 1997	
		rpsi	***	CCGAGTTATCGGTGTAACCC	forward	S. B. Boles, unpublished	available <i>Sphagnum</i> sequences
		trmGF	*	ACCCGCATCGTTAGCTTG	forward	Pacak and Szweykowska-Kulinska, 2000	
<i>trnG</i>	trnGR	*	GCGGGTATAGTTTAGTGG	reverse	Pacak and Szweykowska-Kulinska, 2000		
	trnA	\$	CATTACAAAATCGATGCTCT	forward	Taberlet et al., 1991		
	trnF	\$	ATTTGAACCTGGTGACACGAG	reverse	Taberlet et al., 1991		
	trnC	**	CGAAATCGGTAGACGCTACG	forward	Taberlet et al., 1991		
	trnLIR	**	TACCAACTGAGCTATCCCGG	reverse	S. B. Boles, unpublished	available <i>Sphagnum</i> sequences	
Nuclear	<i>26S</i>	LS0F	ACCCGCTGTTTAAAGCATAT	forward	Shaw, 2000a		
		LS12R	ATCGCCAGTTCGTCTTACCA	reverse	Shaw, 2000a		
		LS8R	TGCTCAGACTCGAACCCTTC	reverse	Shaw, 2000a		
		BMBC-R	GTACACACCCCGCTCG	forward	Shaw, 2000b		
		LS4R	TGAAGCACTTTTGACTCTC	reverse	Shaw, 2000b		
	<i>ITS</i>	its1	**	TCCGTAGGTGAACCTGCGG	forward	Shaw, 2000b	
		its4	**	TCTCCGTTATGATATGC	reverse	Shaw, 2000b	
		5.8S	***	CGTGCCTTTCATCG	reverse	Shaw, 2000b	
		5.8SR	***	TCCGATGAAGAACGCGG	forward	Shaw, 2000b	
		1428F	*	GGAAGAAGTGGATGGACTA	forward	C. J. Cox, unpublished	<i>Arrichum</i> sequences
	2327R	*	TCTCATCTTGGCTTGTATTG	reverse	C. J. Cox, unpublished	<i>Arrichum</i> sequences	

TABLE 1. Continued.

Region	Primer	Use	Sequence 5'-3'	Direction	Reference	Primer design based on
RAPDa	A-F	\$	AACCAAGTGAATTTGGAAATGC	forward	S. B. Boles, unpublished	see Materials and Methods
	A-R	\$	AGGAGCGGAAGCCAAAATG	reverse	S. B. Boles, unpublished	see Materials and Methods
	AiF2	**	GCTGAGCTTCKTCTTGAT	forward	S. B. Boles, unpublished	see Materials and Methods
	AiR2	**	ACGTGAGGTGACYGTTATCC	reverse	S. B. Boles, unpublished	see Materials and Methods
	AiF	***	CAGCATTTTGGCTTCCAAAG	forward	S. B. Boles, unpublished	see Materials and Methods
RAPDb	AiR	***	CAGAATGGCGAGCTTCCT	reverse	S. B. Boles, unpublished	see Materials and Methods
	B-F	\$	CCTGTTTACTCTGGTTTGTG	forward	S. B. Boles, unpublished	see Materials and Methods
	B-R	\$	GCARAGATTTGAACATCATC	reverse	S. B. Boles, unpublished	see Materials and Methods
	BiF	**	TGTACTCAATACCTCAATATG	forward	S. B. Boles, unpublished	see Materials and Methods
	BiR	**	CCCTCAACCAYGAATCAYAAGG	reverse	S. B. Boles, unpublished	see Materials and Methods
RAPDf	F-F	\$	CCACTGGACGAGGATATGA	forward	S. B. Boles, unpublished	see Materials and Methods
	F-R	\$	CACTACCTGATTGGGATGAG	reverse	S. B. Boles, unpublished	see Materials and Methods
	FiF	**	CTAGCTCACCAAGDTKCCVTCTC	forward	S. B. Boles, unpublished	see Materials and Methods
	FiR	**	TAAACACATTTTGTGTGAAT	reverse	S. B. Boles, unpublished	see Materials and Methods

TABLE 2. Optimal substitution models, total number of nucleotides included in the phylogenetic analyses, and numbers of variable sites for each of eight data partitions used in heterogeneous Bayesian analyses of 24 *Sphagnum* species plus *Andreaea* and *Takakia*.

Region	Model	Nucleotides	Autapomorphic	Informative
<i>nad1</i>	HKY	155	9	14
<i>nad5</i>	GTR + G	1166	114	109
<i>nad7</i>	GTR + G	1980	181	246
<i>psbA</i>	GTR + G	1637	88	119
<i>psbT</i>	HKY + G	470	39	64
<i>rbcL</i>	GTR + G	1107	90	134
<i>rps4</i>	HKY + G	1016	109	96
<i>nuc26S</i>	GTR + I + G	2109	82	57

but ML analyses under heterogeneous substitution models, while theoretically possible, are too computationally intensive to be practical. Recent advances in Bayesian approaches to phylogenetic inference permit separate models to be applied to different regions and subregions of DNA sequences (Foster, 2002; Huelsenbeck and Ronquist, 2002). Heterogeneous Bayesian approaches were employed here.

Best-fit models of nucleotide substitution were determined for each of the eight genomic regions for the first analysis (including *Andreaea* and *Takakia*) and for the 15 regions used in the second analysis (excluding the non-*Sphagnum* outgroup taxa) using MrModeltest, as above. The optimal substitution models for each region are listed in Tables 2 and 3. In the second analysis (excluding *Andreaea* and *Takakia*), the *nad1* intron included only three variable sites, insufficient to estimate an optimal likelihood model. This region was excluded from the heterogeneous Bayesian analyses of the second data set.

Bayesian analyses were conducted with six runs, each with 2000000 generations, using default, uniform priors. Model parameters including trees were sampled every 100th generation. The number of trees needed to reach stationarity (i.e., the “burnin”) in the MCMC algorithm was estimated by visual inspection of the plot of ML score at each sampling point using Excel (Microsoft, Seattle, Washington, USA). The trees of the burn-in (i.e., those before the chain reached stationarity) for each run were excluded from the tree set, and the trees from each run combined to form the full sample of trees assumed to be representative of the posterior probability (p.p.) distribution.

RESULTS

Phylogenetic reconstruction I: including *Takakia* and *Andreaea*—The aligned data matrix with 24 *Sphagnum* species plus *Andreaea* and *Takakia* included a total of 9718 nucleotide characters. Seventy-eight characters were excluded because of ambiguities in alignment so that 9640 were included in the analyses, of which 8089 were constant, 839 were autapomorphic, and 712 were parsimony informative. The breakdown of autapomorphic and informative characters among the eight genomic regions, along with optimal substitution models used in the likelihood and Bayesian analyses, are provided in Table 2. The eight-partition substitution model used in the heterogeneous Bayesian analyses provided a better fit to the data than did the homogeneous model (−ln 23 742.69 173 vs. 23 390.264 27 for the homo- and heterogeneous models, respectively; df = 103, *P* < 0.001).

Parsimony, likelihood, and Bayesian analyses all converge on three inferences (Fig. 1): (1) *Sphagnum* is monophyletic, (2) *S. lapazense*, from Bolivia, and *S. sericeum*, from the Old World tropics, comprise early diverging lineages that are outside the main *Sphagnum* clade, and (3) these two species are highly divergent from all other *Sphagnum* species. Note branch lengths in Fig. 1 leading to these two species, relative to the branches within *Sphagnum* sensu stricto (s.s.). Figure 1

TABLE 3. Optimal substitution models, total number of nucleotides included in the phylogenetic analyses, and numbers of variable sites for each of 15 data partitions used in heterogeneous Bayesian analyses of 24 *Sphagnum* species.

Region	Model	Nucleotides	Autapomorphic	Informative
<i>nad5</i>	GTR + G	1159	40	38
<i>nad7</i>	GTR + G	1911	69	70
<i>psbA</i>	GTR + I + G	1638	68	71
<i>psbT</i>	GTR	474	17	30
<i>rbcL</i>	HKY + G	1107	46	19
<i>rps4</i>	HKY + G	1019	68	53
<i>trnG</i>	GTR + G	750	78	53
<i>trnL</i>	GTR + G	634	69	54
<i>LEAFY</i> (long)	HKY + G	534	86	62
<i>LEAFY</i> (short)	HKY	396	42	53
<i>nrITS</i>	GTR + G	655	94	69
<i>nr26S</i>	GTR + I + G	2098	31	33
RAPDa	K80 + G	1166	82	122
RAPDb	HKY + G	751	37	113
RAPDf	HKY + G	536	37	71

also shows how remarkably distinct *Sphagnum* is at the molecular level from the outgroups *Takakia* and *Andreaea*. *Sphagnum lapazense* and *S. sericeum* to some extent break up the extremely long branch leading to *Sphagnum*, but the genus is still highly divergent from its closest relatives. It appears from this analysis that *S. lapazense* is sister to *Sphagnum* (s.s.) plus *S. sericeum*, with the latter being sister to the remaining taxa of *Sphagnum*.

Phylogenetic reconstruction II: rooting the main *Sphagnum* clade with *S. sericeum* and *S. lapazense*—Sequences from eight additional genomic regions were added to the data set from which *Takakia* and *Andreaea* were deleted. These regions were not alignable with homologous sequences from *Takakia* and *Andreaea*, but with three exceptions, the sequences could be aligned across the 24 *Sphagnum* species, including *S. lapazense* and *S. sericeum*. Only the three anonymous regions—RAPDa, RAPDb, and RAPDf—could not be aligned between the two outgroup taxa, *S. sericeum* and *S. lapazense*, and species in the main *Sphagnum* clade. These regions were scored as missing data for *S. lapazense* and *S. sericeum* (although sequences were obtained for these species). The data matrix contained 15 422 characters, of which 439 were excluded because of alignment ambiguity. Of the 14 983 nucleotides included in the analyses, 13 204 were constant, 912 were autapomorphic, and 867 were parsimony informative. The breakdown of variable and informative characters by region, with optimal substitution models, is shown in Table 3. The 15-partition substitution model used in the heterogeneous Bayesian analyses provided a better fit to the data than did the homogeneous model ($-\ln 35\,555.62442$ vs. $35\,757.4258889$ for the homo- and heterogeneous models, respectively; $df = 154$, $P < 0.001$).

With *Takakia* and *Andreaea* deleted from the data set and more variable regions included in the analyses, more resolution was obtained among species within the main lineage of *Sphagnum* (Fig. 2). All four of the large sections of *Sphagnum* (*Sphagnum*, *Cuspidata*, *Subsecunda*, *Acutifolia*), as well as *Rigida* and *Squarrosa*, are strongly supported as monophyletic. Two major clades are resolved within *Sphagnum* s. s. One includes the sections *Sphagnum*, *Cuspidata*, and *Rigida*. The position of section *Rigida* relative to *Sphagnum* and *Cuspidata*

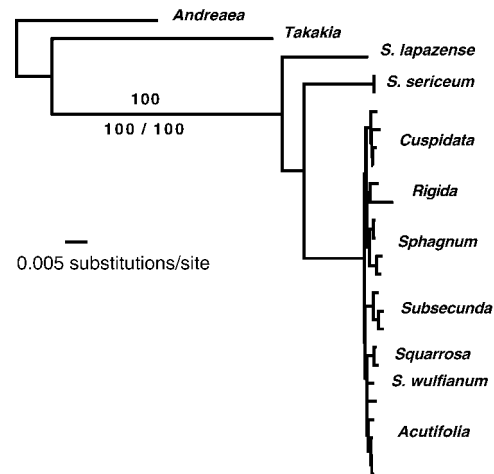


Fig. 1. Phylogram showing phylogenetic relationships among the major clades of *Sphagnum*, rooted with sequences from *Andreaea* and *Takakia*. The aligned matrix included 9640 nucleotides representing eight genes. See text for additional details of the analysis. Note the very long branches separating *S. lapazense* and *S. sericeum* from all other *Sphagnum* species.

was ambiguous in previous analyses (Shaw, 2000a). Parsimony bootstrap support for the sister group relationship between sections *Rigida* and *Cuspidata* is moderate (69%), but the posterior probabilities from the homogeneous and heterogeneous Bayesian analyses are 100% for this relationship.

The second major clade includes the sections *Subsecunda*, *Squarrosa*, and *Acutifolia*, but support for this clade is weak. Parsimony bootstrap support is less than 50% (and is therefore not shown on Fig. 2), and posterior probabilities are well below 95% in both the homogeneous and heterogeneous analyses. Basically, the position of section *Subsecunda* should be considered unresolved; some maximum parsimony trees, and

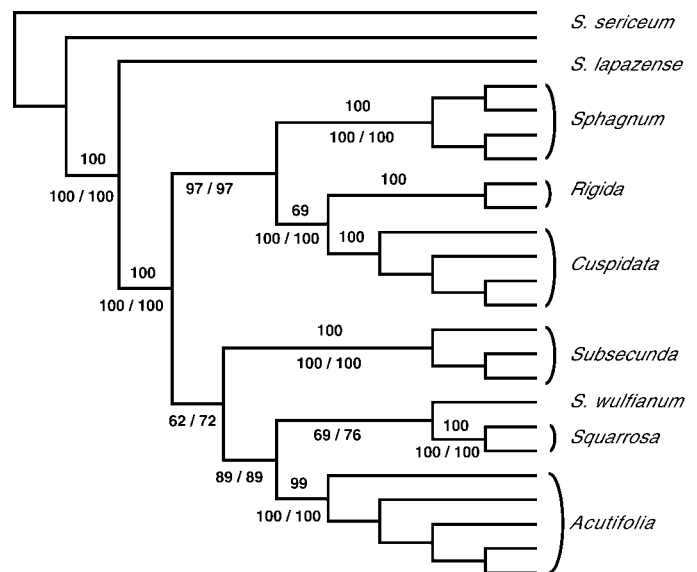


Fig. 2. Cladogram showing phylogenetic relationships among the sections of *Sphagnum*, rooted with *S. lapazense* and *S. sericeum*. Parsimony bootstrap percentages are shown above branches; posterior probabilities from Bayesian analyses using a homogeneous model of substitution (left) and a 15-partition heterogeneous model (right) are shown below the branches. See text for additional details of the parsimony and Bayesian analyses.

some in the 95% Bayesian posterior probability space, placed the *Subsecunda* as sister to the first clade (*Sphagnum* + *Rigida* + *Cuspidata*). The heterogeneous Bayesian analysis provided the strongest support for a sister group relationship between the *Subsecunda* and *Acutifolia* plus *Squarrosa*, but the posterior probability level was not compelling (Fig. 2).

Although the *Acutifolia* were strongly supported as monophyletic, the sister group relationship between *Acutifolia* and *Squarrosa*, strongly supported by previous analyses (Shaw, 2000a), was not overwhelming. Furthermore, the precise relationships of *S. wulfianum*, a species often segregated as its own section (*Polyclada*), is still ambiguous. Shaw (2000a) suggested that *S. wulfianum* may be nested within the *Acutifolia*, whereas the current analyses suggest a closer relationship to the *Squarrosa*. *Sphagnum wulfianum* may be sister to the *Squarrosa*, as shown in Fig. 2; it may be sister to *Squarrosa* plus *Acutifolia*, or it may be sister to the *Acutifolia*.

DISCUSSION

Perhaps the most surprising result of this study is the phylogenetic position of two tropical species of *Sphagnum*: *S. lapazense* and *S. sericeum*. These two species are highly divergent in terms of DNA sequences from all other species of *Sphagnum* and are also strongly differentiated from one another. Their position sister to the main *Sphagnum* clade is highly supported by multiple genes sampled from all three genomic compartments. Eddy (1977) called attention to *S. sericeum* as possibly primitive within *Sphagnum*, but Crum (2001b) classified *S. lapazense* in section *Sphagnum* when he described it as new from Bolivia. *Sphagnum lapazense* and *S. sericeum* are highly differentiated from other sphagna for every one of the 16 genes used in this study, and their isolated position in the combined analysis is therefore not the result of conflicts among different genes or genomic compartments. There is no molecular (or morphological) evidence that either species is of hybrid origin. In an analysis of global biodiversity patterns in *Sphagnum* based on chloroplast and nuclear DNA sequences from 436 accessions, *S. lapazense* and *S. sericeum* accounted for almost 20% of peatmoss molecular diversity even though they were represented by only three accessions (0.7% of the sample). Notwithstanding the rather unusual morphology of *S. sericeum* (Andrews, 1911; Warnstorf, 1911; Eddy, 1977), this level of molecular differentiation could not have been predicted from morphological observations. The significance of these species to peatmoss phylogeny is comparable to the resolution of *Amborella* at or near the base of the angiosperms (Mathews and Donoghue, 1999; Qui et al., 1999) and to the identification of *Oedipodium griffithianum* (Dicks.) Schwaegr. as sister to all other "true" mosses (Bryopsida) (Newton et al., 2000; Cox et al., in press).

Warnstorf (1911) classified *S. sericeum* in its own subsection, *Sericea*, a taxonomic level equivalent to the groups referred to here as sections (*Sphagnum*, *Cuspidata*, *Acutifolia*, etc.). The primary morphological feature that forms the basis for segregating *Sericea* is the absence of fibrils on the hyaline cells of branch leaves (Fig. 3B, F). Fibrillose hyaline cells are almost universal in *Sphagnum* and are lacking in only a few species, including *S. macrophyllum*, *S. cribosum* Lindb., and *S. splendens* Maass from eastern North America, and *S. efi-brillosum* A.L. Andrews and *S. novo-caledoniae* Paris & Warnstorf from Oceania. DNA sequence data clearly indicate that *S. macrophyllum* and *S. cribosum* are nested within the

section *Subsecunda* (Shaw, 2000a; A. J. Shaw, unpublished data). Warnstorf (1911), Eddy (1977), and Crum (1992b) argued for a similar placement for *S. efi-brillosum* and *S. novo-caledoniae* in section *Subsecunda*, based on morphology. *Sphagnum splendens* appears to be an aberrant phenotype in the section *Cuspidata*, doubtfully worth recognizing at the specific level. It is known from only two or three localities in an area that has been relatively thoroughly collected for *Sphagnum*. These phylogenetic and taxonomic hypotheses imply that the absence of hyaline cell wall fibrils in these species represents a secondary loss. The absence of fibrils on the hyaline cells of *S. sericeum*, in contrast, appears to be primitive, as argued by Eddy (1977). The single apical pore on each branch leaf hyaline cell of *S. sericeum* distinguishes this species from all other sphagna, including those few that lack fibrils on the hyaline cell walls (Fig. 3B).

Eddy (1977) further argued that the lack of differentiation between spreading and pendent branches, the relative similarity in size and structure of stem and branch leaves, and the weak differentiation in shape between hyaline and chlorophyllose cells of both stem and branch leaf cells, characteristic of *S. sericeum* (Fig. 3), also appear to be plesiomorphic. The arrangement of hyaline and chlorophyllose cells is rather irregular in the stem leaves of *S. sericeum* (Fig. 3F), an unusual feature that warrants developmental investigation given the highly regular pattern characteristic of most sphagna. Adjacent clusters of hyaline and chlorophyllose cells cannot reflect the kind of regular development described by Holcombe (1984) and *S. sericeum* provides an opportunity to study the evolutionary origin of the complex developmental program that results in the regular differentiation of hyaline and chlorophyllose leaf cells in other sphagna. The apiculate leaf apices of stem and branch leaves in *S. sericeum* (Fig. 3A, B, E) constitute an additional feature that sets this species apart from almost all other species of *Sphagnum*. Most species of *Sphagnum* have extensive cell wall resorption near the leaf apices, and Eddy (1977) considered the cuspidate apices of *S. sericeum* leaves, lacking wall resorption, as plesiomorphic. The apiculae of stem leaves consist of uniformly thick-walled cells in *S. sericeum* (Fig. 3B). It is noteworthy, on the other hand, that the cortical cells of *S. sericeum* branches include well-differentiated retort cells, a uniquely *Sphagnum* characteristic (Fig. 3C).

Sphagnum lapazense is less morphologically distinctive than is *S. sericeum*. When Crum (2001b) recently described *S. lapazense* as a new species from Bolivia, he classified the species in section *Sphagnum*. Crum did note the absence of fibrils in the cortical cells of branches and stems of *S. lapazense*, but these fibrils are poorly developed or nearly absent in some other species of the section, especially in those from South America. The branch leaves are ovate (Fig. 4A), typical of section *Sphagnum*, and the stem leaves are smaller and triangular, also not noteworthy. The hyaline cells of branch leaves are relatively broad, as is typical of section *Sphagnum* (Fig. 4C), and the branch leaves have a marginal resorption furrow (not shown), a feature characteristic of sections *Sphagnum* and *Rigida*. The chlorophyllose cells are elliptical in cross section, similar to such section *Sphagnum* species as *S. magellanicum* Brid. One morphological character that does appear to be unique for the section *Sphagnum* (and not mentioned by Crum, 2001b) is the absence of extensive cell wall resorption near the apices of branch leaves. Species of section *Sphagnum* regularly have the cell walls on adaxial branch leaf surfaces so

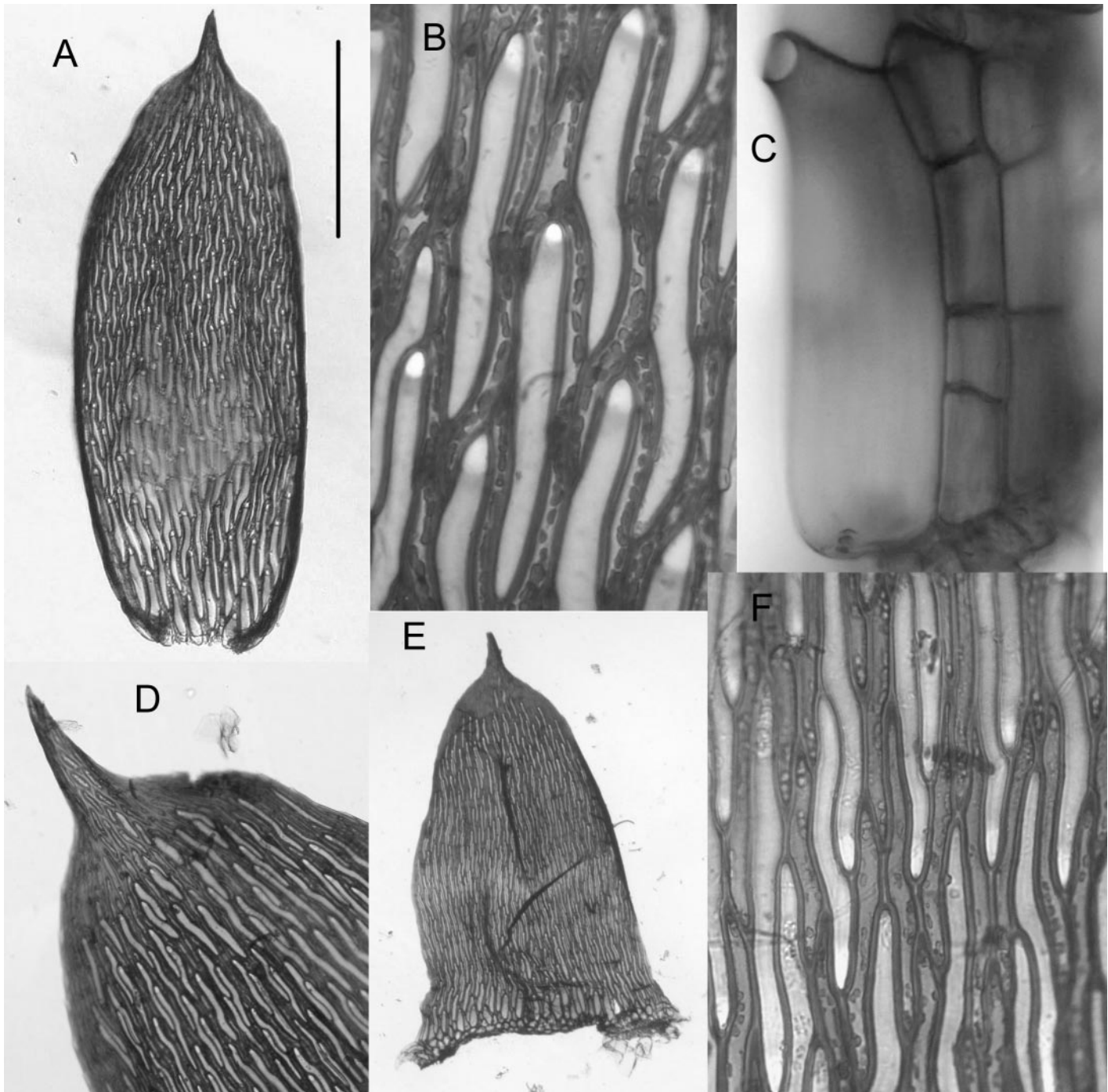


Fig. 3. *Sphagnum sericeum*. (A) Branch leaf. (B) Branch leaf cells. (C) Retort (branch cortical) cell. (D) Stem leaf apex. (E) Stem leaf. (F) Stem leaf cells. Scale bar A and F = 375 μm , B = 42 μm , C = 80 μm , D = 25 μm .

extensively resorbed that the leaf surface has a roughened texture that derives from projecting wall remnants. This feature, so characteristic of section *Sphagnum*, is conspicuously absent from the branch leaves of *S. lapazense* (Fig. 4B).

There is no evidence that *S. lapazense* and *S. sericeum* form a monophyletic group, nor do the two species share morphological similarities. While morphological observations had previously suggested that *S. sericeum* could be phylogenetically critical, the position of *S. lapazense* was completely unexpected. Indeed, both *S. sericeum* and *S. lapazense* are so

distinct on a molecular level that all three accessions (two of *S. sericeum*) were reextracted and resequenced twice, with identical results. Moreover, the two accessions of *S. sericeum* are very similar. The only alternative interpretation of these results, other than being sister to the rest of *Sphagnum*, is that one or both is characterized by exceptionally high mutation rates. The mutation rate would have to be extremely high, however, to erase any signal of a relationship to *Sphagnum* species within the main peatmoss clade.

Bayesian, ML, and MP analyses resolve two major clades

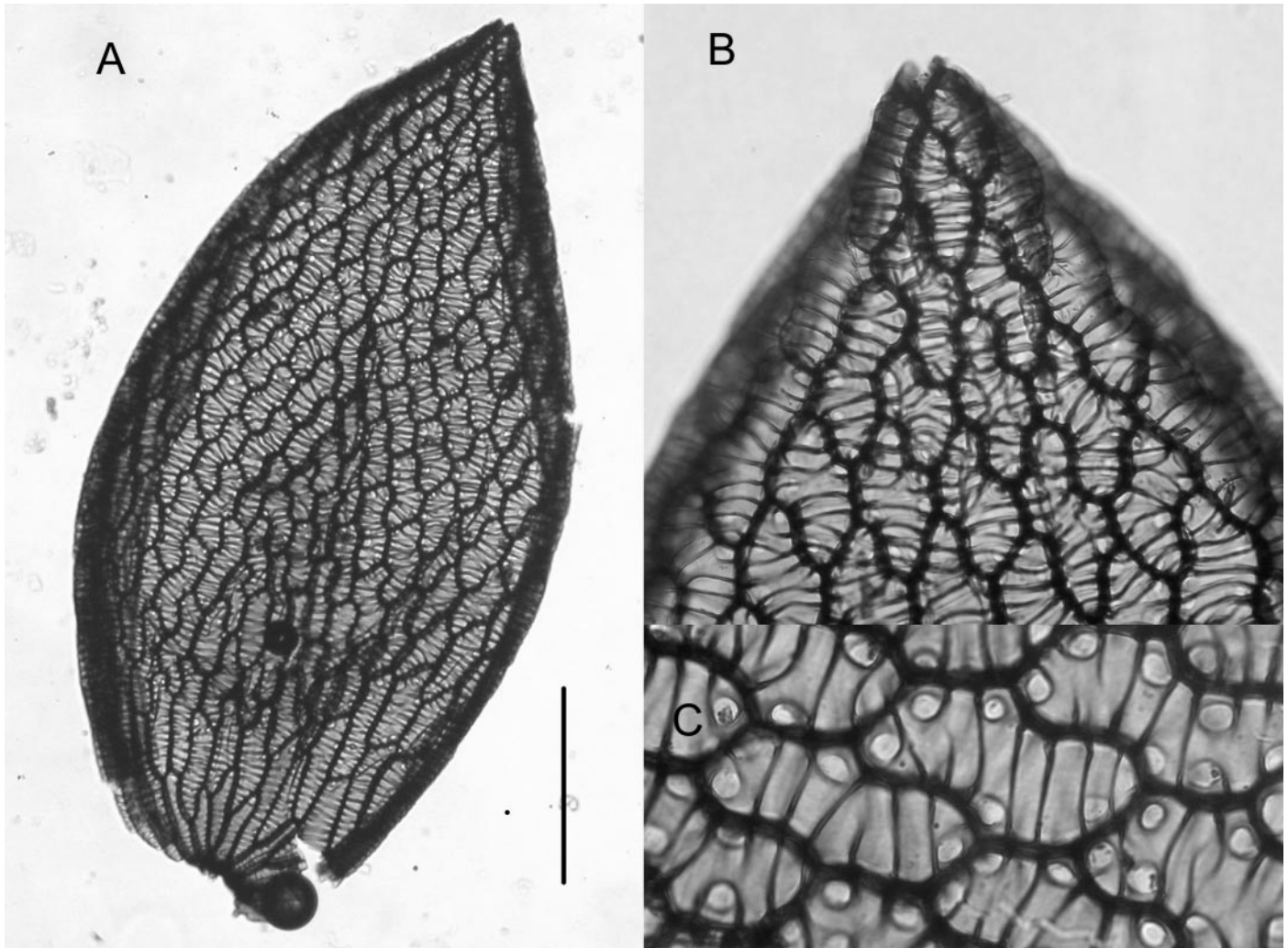


Fig. 4. *Sphagnum lapazense*. (A) Branch leaf. (B) Branch leaf apex. (C) Branch leaf cells. Scale bar A = 345 μm , B = 310 μm , C = 165 μm .

within the main *Sphagnum* lineage, one including sections *Sphagnum*, *Rigida*, and *Cuspidata* and the other including the *Acutifolia*, *Subsecunda*, and *Squarrosa*. This topology is unlike any previous hypothesis for *Sphagnum* phylogeny. Eddy (1977) provided a diagram (not a tree) illustrating his phylogenetic concepts for *Sphagnum* and indicated that *S. sericeum* is most similar to a hypothetical ancestral peatmoss. He further indicated that section *Subsecunda* includes primitive *Sphagnum* taxa derived from something like *S. sericeum* and that sections *Cuspidata*, *Sphagnum*, and *Acutifolia* are independently derived from the section *Subsecunda*, implying paraphyly of the *Subsecunda*. More derived species of section *Subsecunda* are hypothesized to have given rise to section *Rigida*. Eddy (1977) specifically pinpointed *S. robinsonii* Warnst., a species of section *Subsecunda*, as a possible sister taxon to section *Rigida*. The results of the current analyses suggest that the *Rigida* are sister to the section *Cuspidata*.

The phylogenetic hypothesis of Eddy (1977) implies that branch leaf marginal resorption furrows, shared by species of sections *Sphagnum* and *Rigida*, evolved independently. The current topology suggests that the resorption furrow might have evolved once in the ancestor of sections *Sphagnum* and *Rigida* plus *Cuspidata*, but this implies a loss of resorption

furrows in the *Cuspidata*. Although resorption furrows characterize all species of sections *Sphagnum* and *Rigida*, morphologically indistinguishable furrows occur in the section *Acutifolia* (in *S. molle* Sull. from the Northern Hemisphere and in *S. costae* Crum from Brazil [Crum and Da Costa, 1994]). Resorption furrows do not occur in other species of section *Acutifolia*, so any phylogenetic hypothesis requires homoplasious origins and/or losses of these features.

Eddy (1977, p. 367) argued that the section *Subsecunda* "is usually considered to be the most primitive group among the Sphagna." He bases this contention on the relatively low degree of (spreading and pendent) branch dimorphism within branch fascicles, the commonness of stem and branch leaf isophylly in the *Subsecunda*, and the cosmopolitan distribution of the section. Most authors (e.g., Andrews, 1911; Eddy, 1977) argue that the *Subsecunda* and *Cuspidata* are most closely related among the large sections, although one of the examples used by Andrews (1911) to argue for actually combining the two sections (*S. mendocinum* Sull. & Lesq. in Sull. from the American Pacific Northwest) turns out to likely be an inter-sectional hybrid (Shaw and Goffinet, 2000). The phylogenetic topology presented here is ambiguous on this point. The relationship between the *Subsecunda* and *Acutifolia* plus *Squar-*

rosa is weakly supported, and it is possible that the *Subsecunda* are sister to the other clade (*Sphagnum* + *Rigida* + *Cuspidata*). The *Subsecunda* do not appear to be the sister group to the *Cuspidata* alone, however, although the topology presented here does not exclude the possibility that morphological similarities shared by *Cuspidata* and *Subsecunda* are plesiomorphic rather than convergent.

The lack of support for a sister group relationship between the sections *Acutifolia* and *Squarrosa* is surprising as this relationship was supported by previous analyses (Shaw, 2000a) and is generally argued from morphological considerations (Eddy, 1977; Crum, 1984; Daniels and Eddy, 1990). *Sphagnum wulfianum* is often segregated as the section *Polyclada*, but the section is usually thought to be closely related to the *Squarrosa*, *Acutifolia*, or both. Shaw (2000a) argued that *S. wulfianum* is nested within the *Acutifolia*, along with *S. girgensohnii* Russ. and *S. fimbriatum* Wils. However, several additional populations of *S. wulfianum* now included in a more extensive data set (A. J. Shaw, unpublished data) appear more closely related to the *Squarrosa* than to the *Acutifolia*. Nevertheless, the precise position of *S. wulfianum* is still without strong support; *S. wulfianum* is either sister to the *Squarrosa* (which includes just *S. squarrosom* and *S. teres*), sister to the *Acutifolia*, or possibly sister to the *Squarrosa* plus *Acutifolia*. None of these hypotheses agree with the phylogenetic topology for Chinese species of *Sphagnum* presented by He and Aur (1991). According to their tree, the *Squarrosa* are sister to section *Sphagnum*, *S. wulfianum* is sister to *S. subsecundum* Nees, and the *Acutifolia* are sister to the *Cuspidata*. Clearly, their hypothesis is inconsistent with the topology presented here in just about every way.

This study has provided the most fully resolved and reliably polarized phylogeny for *Sphagnum* available to date. Nevertheless, it is remarkable that even with almost 15 000 nucleotides from all three genomes and more than 850 parsimony informative characters, several nodes remain weakly supported. The early divergence of *S. sericeum* and *S. lapazense* is likely, although these two species do not appear to be at all closely related. The absence of hyaline cell fibrils is probably a plesiomorphic feature of *S. sericeum*, as hypothesized by Eddy (1977), and the lack of cell wall resorption on the adaxial leaf surfaces of *S. lapazense* may also be primitive. *Sphagnum lapazense* otherwise shares morphological features with members of the section *Sphagnum*, and it is therefore surprising that it does not share more with that group at the DNA level. *Sphagnum lapazense*, but not *S. sericeum*, has a marginal resorption furrow on the branch leaves, so the origin of this feature during the course of *Sphagnum* evolution is unclear. Well-differentiated retort cells occur in *S. sericeum* but not *S. lapazense*, so the origin(s) of these uniquely peatmoss features is likewise ambiguous. The phylogenetic topology presented here suggests that resorption furrows may have evolved early in *Sphagnum*, were retained in the sections *Sphagnum* and *Rigida*, lost independently in the *Cuspidata* and in the clade(s) including *Subsecunda*, *Acutifolia*, and *Squarrosa*, and regained in the *Acutifolia*.

It is clear that the sections *Sphagnum*, *Rigida*, and *Cuspidata* form a well-supported clade, but an outstanding question that remains is the relationship of the *Subsecunda* to this group. The results of this study strongly support monophyly of all six sections of *Sphagnum* and therefore do not support paraphyly of the *Subsecunda* as proposed by Eddy (1997). Analyses of a much more taxon-extensive data set also support

monophyly of all the sections, including *Subsecunda*. The present results do not provide compelling evidence to negate Eddy's view that the *Subsecunda* comprise an early diverging lineage within the peatmosses. The status of section *Polyclada* (*S. wulfianum*) is still in question, although the *Polyclada* are probably worth segregating taxonomically only if *S. wulfianum* is ultimately resolved as sister to the *Acutifolia* plus *Squarrosa*. The current results strongly support the conclusion of Shaw (2000a), that the monotypic section *Insulosa* (*S. aongstroemii* Hartm.) is part of the larger section *Acutifolia*, although *S. aongstroemii* does appear to be an early diverging lineage within that section.

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