

Phylogeny, Species Delimitation, and Recombination in *Sphagnum* Section *Acutifolia*

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ABSTRACT. Nucleotide sequences for six nuclear loci and one chloroplast region were used to reconstruct phylogenetic relationships in *Sphagnum* section *Acutifolia*. The combined data matrix, which includes 136 accessions (129 ingroup taxa and seven outgroups) and 5126 nucleotide sites, was analyzed using Bayesian inference. Most of the individual morphospecies commonly recognized in the section were represented by multiple populations, in some cases by up to 16 accessions from throughout the Northern Hemisphere. Results of the combined seven-locus analysis resolved many of the species as monophyletic, but the deeper nodes were generally without support. Separate analyses of single-locus data sets revealed significant conflicts, indicating gene flow among both closely and more distantly related species within the section. The sequence data allowed likely parentage to be identified for several species of hybrid origin, and identified individual accessions that appear to be genetic admixtures. Taxonomic conclusions that can be made from the analyses include: 1. *Sphagnum wulfianum* and *S. aongstroemii* should both be included in section *Acutifolia*, 2. *S. subtile* cannot be separated from *S. capillifolium*, and the two should be synonymized, 3. *S. capillifolium* and *S. rubellum* each contain a monophyletic core of populations and should be retained as separate species, but 4. *S. rubellum* cannot be separated from *S. andersonianum* and *S. bartlettianum* and the three should be merged, 5. *S. tenerum* is highly differentiated from *S. capillifolium* and should be treated as a separate species, 6. interspecific mixed ancestry is demonstrated for *S. russowii* (a likely allopolyploid), *S. skyense*, *S. arcticum*, and *S. olafii*. Interspecific recombination appears to be rather common in section *Acutifolia*, yet species, for the most part, maintain cohesiveness.

With about 150–250 species (Isoviita 1966; Crum 2001), the genus *Sphagnum* (peatmosses) is one of the most speciose genera of mosses. *Sphagnum* dominates many habitats in which it occurs, forming deep peat deposits in boreal regions of the Northern Hemisphere (Halsey et al. 2000). Boreal peatlands currently function as a net sink for atmospheric CO₂ (Gorham 1991) and have profound impacts on regional patterns of hydrology owing to the unique moisture-holding capacity of *Sphagnum* gametophytes (Clymo and Heywood 1982). In terms of its ecological dominance, the genus is without parallel among plants; Clymo and Heywood (1982) commented that there is probably more biomass currently bound up in *Sphagnum* than in any other living plant genus.

Class Sphagnopsida includes *Sphagnum* and the monospecific genus, *Ambuchanania* (Crum and Seppelt 1999; Shaw 2000). *Ambuchanania* has been collected only at two relatively inaccessible, high elevation localities in western Tasmania (Yamaguchi et al. 1990). *Sphagnum*, in contrast, occurs worldwide. The genus is diverse in boreal regions of the Northern Hemisphere (Crum 1984; Ignatov and Afonina 1992; He 1998; Flatberg 2002a), but appears to be less species-rich at high latitudes of the Southern Hemisphere despite fairly extensive peatlands (Fife 1996; He 1998; Seppelt 2000).

There is much disagreement about the delimitation of species in *Sphagnum*. At one extreme, Warnstorf (1911) applied narrow species concepts and recognized 340 species of peatmosses worldwide. At the other end of the spectrum, Andrews (1913) recognized only 39 species in North America and argued (An-

draws 1947) that many or most of the taxa described from South America are in fact conspecific with broadly distributed northern species. In his revision of North American sphagna, Crum (1984) found middle ground by recognizing 51 North American species, but also included 15 subspecific taxa. On the other hand, Crum (e.g., 1984, 1987a,b, 1989, 1990a,b, 1992a,b, 1993a,b, 1994, 1995a,b) described more than 50 new species of *Sphagnum* from South America. In South America, Crum recognized species that he synonymized in his North American treatment (1984), along with numerous new taxa segregated from these species. Clearly, consistent delimitation of morphospecies in this difficult group is challenging.

There are probably a number of reasons why species delimitation in *Sphagnum* is especially difficult. Aquatic and semi-aquatic mosses are notoriously variable in morphology because of phenotypic plasticity related to water level and other environmental factors (Loeske 1907, 1928; Vitt and Glime 1984; Hedenäs 1996). At least some of the confusing morphological variation within and among putative species of *Sphagnum* has been interpreted as plastic responses to environmental gradients (Flatberg 1985; Daniels 1993; Săstad 1998). Another possible factor underlying morphological variation and taxonomic confusion is hybridization. Allopolyploid origins have been proposed, and supported by isozyme data, for several species: *S. jensenii* H. Lindb. (Săstad et al. 1999), *S. majus* (Russ.) C. Jens. (Săstad et al. 2000), and *S. troendelagicum* Flatb. (Săstad et al. 2001) in section *Cuspidata*, and *S. russowii* Warnst. (Cronberg 1996) in section *Acutifolia*. Cronberg

and Natcheva (2002) documented hybridization between the closely related species *S. capillifolium* (Ehrh.) Hedw. and *S. quinquefarium* (Lindb. ex. Braithw.) Warnst. in three out of four populations they sampled where the two species were sympatric. Cronberg (1997, 1998) found isozyme evidence of crossing between *S. capillifolium* and *S. rubellum* Wils. Shaw and Goffinet (2000) showed that several species of *Sphagnum* appear to be the result of intersectional hybridization. Hybridization, possibly exacerbated by backcrossing to one or both parents (Cronberg and Natcheva 2002), may help explain high levels of variation and frequent interspecific intermediacy, as well as the lack of correlation between different supposedly diagnostic morphological characters for distinguishing species.

Species of *Sphagnum* have been classified in subgeneric groups according to various systems since the time of Müller (1848), Schimper (1876), and Braithwaite (1880). The early history of *Sphagnum* taxonomy was thoroughly reviewed by Isoviita (1966).

Most modern classifications of *Sphagnum* divide the genus into 6–8 sections or subgenera (Isoviita 1966; Crum 1984; Flatberg 2002a). Four large sections include over 90% of all *Sphagnum* species: *Acutifolia*, *Cuspidata*, *Sphagnum*, and *Subsecunda*. Monophyly of two smaller sections, namely *Rigida* and *Squarrosa*, is supported by molecular data, but the monospecific sections *Insulosa* (for *S. aongstroemii* C. Hartm.), *Isocladius* (for *S. macrophyllum* Brid.), *Hemitheca* (for *S. pylaisii* Brid.), *Mollusca* (for *S. tenellum* (Brid.) Bory), and *Polyclada* (for *S. wulfianum* Girg.) are each nested within the larger sections (Shaw 2000; Shaw et al. 2003). Based on nucleotide sequences from 16 genes, Shaw et al. (2003) examined phylogenetic relationships among the sections of *Sphagnum*. Two major clades were resolved in the genus, one including sections *Sphagnum*, *Cuspidata*, and *Rigida*, and the other sections *Acutifolia*, *Squarrosa*, and *Subsecunda*. Only the position of section *Subsecunda* lacked strong support from Bayesian phylogenetic analyses (Shaw et al. 2003), and its phylogenetic position should be considered unresolved.

This paper describes phylogenetic analyses at the population and species levels within *Sphagnum* section *Acutifolia*. The section includes important peat-forming species in boreal regions; for example, *S. fuscum* (Schimp.) Klinggr. Section *Acutifolia* forms a monophyletic group sister to the smaller section, *Squarrosa* (Shaw 2000; Shaw et al. 2003). Taxonomic concepts in *Acutifolia* have been controversial, especially with regard to closely related species in the so-called “*S. capillifolium* complex” (MQueen 1989; Cronberg 1994). In particular, the taxonomic status of *S. rubellum* and *S. capillifolium* has engendered much discussion and disagreement (Hill 1976; Crum 1984; Andrus 1980; MQueen 1989; Daniels and Eddy 1990). In North America, the relationships of these taxa to *S. tenerum*

Sull. & Lesq. and *S. bartlettianum* Warnst. are also unclear (Andrus 1980; Crum 1984).

For purposes of the present study, *S. wulfianum*, sometimes segregated as the monospecific section *Polyclada*, is included in *Acutifolia*. Nucleotide sequence data indicate that *S. wulfianum* is nested within the *Squarrosa* plus *Acutifolia* clade (Shaw 2000; Shaw et al. 2003), although its precise relationship to the two sections has not been strongly supported by measures of clade confidence. *Sphagnum aongstroemii* is also included in section *Acutifolia* (section *Insulosa* of most authors) based on previous molecular results.

Although systematic relationships in the *Acutifolia* have been controversial, the section is easier to deal with on a global scale than other sections because, unlike sections *Sphagnum*, *Cuspidata*, and *Subsecunda*, *Acutifolia* is not diverse in tropical regions outside Central and South America. In his unpublished manuscript for the Flora Neotropica, Crum (personal communication) recognized 34 species of *Acutifolia*, but Eddy (1985) included only three species in the African flora (one being the widespread *S. fimbriatum* Wils. in Wils. & Hook f.) and one in tropical Asia (Eddy 1977). Similarly, only one species of *Acutifolia*, *S. fimbriatum*, is known from southern South America (He 1998), no *Acutifolia* are included in the most recent revision of Australian sphagna (Seppelt 2000), and only one species is found in New Zealand (Fife 1996; the widespread Northern Hemisphere species, *S. subnitens* Russ. & Warnst. in Warnst.). Fife (1996) suggested that *S. subnitens* is a recent introduction to New Zealand. The other three large sections of *Sphagnum* are relatively diverse in these same regions. Our population sampling of *Acutifolia* is not exhaustive at the species level, but almost all Northern Hemisphere species are represented, some by up to 16 populations. A few northern species are not included in the analyses because sequences were available for only one to several genes: *S. quinquefarium*, *S. wilfii* Crum, *S. schofieldii* Crum, and *S. junghunianum* Dozy & Molk.

H. Crum (University of Michigan) allowed tissue samples to be taken from many of his Neotropical specimens, including nomenclatural types, so the relationship between South American and northern taxa could be explored. Generation of molecular data from type collections provides a unique opportunity to annotate these specimens with a molecular “fingerprint.” For purposes of the phylogenetic analyses, these specimens were supplemented with additional collections from South and Central America.

MATERIALS AND METHODS

Population Sampling. A total of 136 samples were included in the molecular analyses, 129 representing sect. *Acutifolia* plus seven outgroup accessions from sect. *Squarrosa* (four of *S. teres* and three of *S. squarrosium*). Of the 136 samples, 66 (49%) are from North

TABLE 1. Total length, numbers of autapomorphic and parsimony-informative sites, and optimal substitution model for the seven genomic regions included in the phylogenetic analyses of *Sphagnum* section *Acutifolia*. GTR: General time reversible, Rodriguez et al. 1990; HKY: Hasegawa-Kishino-Yano, Hasegawa et al., 1985; K80: Kimura, 1980; G: a discrete gamma distribution of among-site rates.

Region	Aligned length	Autapomorphic	Informative	Model
ITS	781	33	87	GTR+G
RAPDA	580	41	76	HKY+G
RAPDB	1125	62	168	K80
RAPDF	870	46	152	HKY+G
LEAFY1	713	34	115	HKY+G
LEAFY2	348	17	46	HKY
<i>trnL-trnF</i>	759	23	48	HKY+G
Total	5176	256	691	

America, 30 (22%) from Europe, 23 (17%) from South or Central America (including Mexico), eight (6%), from northern Asia (Russia), eight from Japan (6%), and one from India. Each population is represented in the data set by a single plant; thus, "population" and "plant" and "specimen" are used interchangeably below.

All specimens were identified to morphospecies by L. E. Anderson and J. Shaw and vouchers are accessioned in the Duke University herbarium under the binomials used in this paper. Individual stems from which DNA was extracted were placed in small packets within the herbarium specimen and can be checked in cases where the specimen consists of an interspecific mixture. Such mixtures are very common in *Sphagnum* and identifications reported here reflect examinations of the actual gametophyte that was sequenced. Specimen information, voucher location, and Genbank accession numbers are provided in Appendix 1. The data matrix used for these analyses has been submitted to TreeBase (study accession number S1195; matrix accession number M2064).

Nucleotide Sequencing. Analyses are based on nucleotide sequences from seven genomic regions. From the chloroplast genome, we sequenced 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 two introns in the *LEAFY/FLO* gene (hereafter, *LEAFY1* and *LEAFY2*). In addition, three anonymous regions, assumed to be nuclear, were sequenced using primers designed for the regions identified from RAPDs (random amplified polymorphic DNA) as described in Shaw et al. (2003). Shaw et al. (2003) provided primer sequences and described protocols for amplifying and sequencing the seven genomic regions used in this paper.

For each taxon and sequenced DNA region, forward (5'–3') and reverse (3'–5') sequences were assembled and checked using Sequencher (vers. 4.1, Gene Codes Corp.). Consensus sequences were aligned manually using Se-Al (vers. 2, <http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html>) and regions of ambiguous alignment and incomplete data (i.e., at the beginning and end of sequences) were excluded from the analyses.

Phylogenetic Methods. The data were analyzed by Bayesian inference implemented with MrBayes3 (Huelsenbeck and Ronquist 2002). Best-fit models of nucleotide substitution were determined for each of the seven genomic regions by hierarchical likelihood ratio tests with the aid of MrModeltest 1.1b (Nylander 2002). The optimal substitution model for each region is listed in Table 1. Heterogeneous Bayesian analyses were conducted with each genomic region having its own model of substitution. Homogeneous Bayesian analyses with a single substitution model specified for the seven-gene combined data matrix were also performed. The optimum substitution model for the combined data, determined by likelihood ratio tests (as above), was GTR+I+G. Optimal trees under heterogeneous models had significantly higher likelihood (LRT = 2(-ln null—ln alternative)); 2(19790.04667–19836.85444) = 93.61554; degrees of freedom = 290; p << 0.001) and the topo-

logical results of homogeneous analyses were completely congruent with those from the heterogeneous analyses, so only the latter are presented.

Bayesian analyses were conducted with six runs, each with 8,000,000 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 likelihood scores at each sampling point using GnuPlot (Williams and Kelley 1999). Trees of the burnin for each run were excluded from the tree set, with remaining trees from each run combined to form the full sample assumed to be representative of the posterior probability (p.p.) distribution.

In addition to analyses of the combined 7-gene data set, Bayesian analyses were conducted separately for each gene. Optimal substitution models for each gene were as in the combined heterogeneous analysis (Table 1). Sampling from the posterior probability distribution was as described above for the combined analyses.

Forty-one indels were scored across the seven loci. These were not included in the formal phylogenetic analyses, but indels that provided phylogenetic information or corroborated relationships supported by nucleotide substitutions are described in Table 2.

Maximum parsimony analyses were run on the combined data set with and without indel scores, and on the separate single-gene data sets, also with and without indel scores included. The parsimony results differed in minor topological details from those obtained in the Bayesian analyses (trees not presented). However, the same patterns of incongruence among genes were observed, branches that were well-supported by Bayesian posterior probabilities were also supported by the parsimony bootstrap, and deep nodes in the combined tree were similarly unsupported in the parsimony analysis. The parsimony results are therefore not described here.

RESULTS

Combined Analyses. The combined data matrix consisted of 5126 sites, of which 256 were autapomorphic for single accessions and 691 were parsimony-informative. The breakdown of variation by genomic region is shown in Table 1. The anonymous RAPD regions were highly variable both within and between species and were therefore valuable markers of genetic affinity.

Three fixed synapomorphic insertions support the monophyly of *S. wulfianum*, one supports *S. teres*, one unique deletion is fixed in *S. subnitens*, and one deletion is fixed in the clade that includes *S. molle* Sull. and *S. angermanicum* Melin. The three populations of *S. aongstroemii* share a 93 base insertion in the RAPDB locus (Table 3). Indels that support population-level relationships are discussed below.

The all-compatible splits tree from the posterior probability distribution identified by Bayesian analyses of the combined data is shown in Fig. 1. *Sphagnum wulfianum* is sister to section *Acutifolia* with $\geq 95\%$ Bayesian posterior probability. Although the backbone of interspecific relationships within the *Acutifolia* has little or no support, monophyly of many of the traditional morphospecies is strongly supported. In some species, a "core" of populations forms a monophyletic group, but one or more populations appear to be admixtures of different morphospecies (see below). The

TABLE 2. Informative insertions and deletions in *Sphagnum* section *Acutifolia*. "Positions" refers to nucleotides (nts) in the edited data matrix (with ambiguous regions deleted).

Positions	Locus	Marker	Pattern
153-159	ITS	7 nts deletion	Fixed in <i>S. aongstroemii</i>
173	ITS	1 nts insertion	Fixed in <i>S. wulfianum</i>
523-524	ITS	2 nts insertion	Fixed in <i>S. wulfianum</i>
589-591	ITS	3 nts insertion	Fixed in <i>S. squarrosom</i>
674	ITS	1 nts insertion	Shared by <i>S. olafii</i> , one pop. of <i>S. arcticum</i> , & some <i>S. fimbriatum</i> , <i>S. girgensohnii</i>
1562-1579	RAPDA	18 nts deletion	Shared by two of three <i>S. squarrosom</i> pops.
2619-2711	RAPDB	93 nts insertion	Fixed in <i>S. aongstroemii</i>
2712-2881	RAPDB	170 nts insertion	Shared by <i>S. rubiginosum</i> & 2 pops. of <i>S. girgensohnii</i>
3012-3017	RAPDB	6 nts deletion	Shared by two pops. of <i>S. girgensohnii</i> & one <i>S. russowii</i>
3390-3394	RAPDF	6 nts deletion	Shared by two pops. of <i>S. girgensohnii</i>
3521-3523	RAPDF	3 nts deletion	Shared by three pops. of <i>S. rubellum</i> & 3 pops. of <i>S. bartlettianum</i>
905-910	LEAFY1	7 nts deletion	Synapomorphic for two pops. of <i>S. fuscum</i>
1146-1147	LEAFY1	2 nts insertion	Fixed in <i>S. wulfianum</i>
1209-1220	LEAFY1	12 nts insertion	Synapomorphic for two pops. of <i>S. molle</i>
4267-4277	LEAFY2	11 nts deletion	Fixed in <i>S. subnitens</i>
4973-4976	trnL	4 nts insertion	Fixed in <i>S. teres</i>
4979-4982	trnL	4 nts deletion	Shared by <i>S. molle</i> & <i>S. angermanicum</i>
5057-5061	trnL	5 nts duplication	In some Neotropical populations

combined analysis resolves populations of the following species as monophyletic: *S. fuscum* (10 of 11 populations with > 95% support), *S. capillifolium* (including *S. subtile*; see below), *S. tenerum*, *S. subfulvum*, *S. subnitens*, *S. angermanicum*, *S. aongstroemii*, and *S. wulfianum* (Fig. 1). Two populations of *S. rubellum* form a paraphyletic grade leading to a group of Neotropical accessions. All other populations of *S. rubellum* form a group (without support) that includes populations of the morphospecies, *S. andersonianum* and *S. bartlettianum*. The name *S. rubellum* is hereafter used for the clade that includes these three morphospecies. Some individual genes resolve *S. rubellum* (including *S. andersonianum*, and *S. bartlettianum*) as monophyletic. A clade of Neotropical populations is resolved with a single outlier population that appears most closely related to *S. flavicomans*, *S. subnitens*, and *S. subfulvum* (Fig. 1). Two well-supported groups of populations are resolved within that clade.

Several morphospecies appear to be paraphyletic or

polyphyletic. *Sphagnum capillifolium* is monophyletic only if populations often assigned to *S. subtile* are included. Two clades are resolved within *S. capillifolium*, but populations that conform to the morphology of *S. subtile* occur in both clades. Hereafter, the name *S. capillifolium* is used for the clade that includes both morphospecies. *Sphagnum olafii* and *S. arcticum* form a well-supported clade, but *S. olafii* is sister to one of the two samples of *S. arcticum*, making the latter paraphyletic (Fig. 1). *Sphagnum girgensohnii* and *S. fimbriatum* may be sister taxa, but this relationship is not supported. However, a clade including these two species plus three populations of *S. russowii*, *S. rubiginosum*, and one population of *S. squarrosom* is supported by 93% Bayesian posterior probability (Fig. 1). *Sphagnum fimbriatum* itself is resolved as monophyletic with 92% support. In addition, *S. rubiginosum* is resolved as nested within *S. girgensohnii*. While *S. angermanicum* is strongly supported as monophyletic, this species is part of a clade that includes populations of *S. molle*.

TABLE 3. Genealogical relationships among loci and accessions of *S. russowii*. All relationships are supported at > 95% Bayesian posterior support. *S. cap* - *S. rubellum* = a clade containing these two species plus *S. tenerum*; *S. girg* - *S. fim* = a clade including *S. girgensohnii*, *S. fimbriatum*, and *S. rubiginosum*. *S. cap* - *S. rub* - *S. fusc* = an inclusive clade within the *Acutifolia* including these species and several others, but excluding *S. girgensohnii* and *S. fimbriatum*.

Locus	Russia (885)	New York (322)	Washington (588)	Norway (577)
ITS	<i>S. cap</i> - <i>S. rub</i>	<i>S. cap</i> - <i>S. rub</i>	<i>S. cap</i> - <i>S. rub</i>	<i>S. cap</i> - <i>S. rub</i>
RAPDA	<i>S. girg</i> - <i>S. fim</i>	<i>S. girg</i> - <i>S. fim</i>	<i>S. rubellum</i>	<i>S. girg</i> - <i>S. fim</i>
RAPDB	<i>S. girgensohnii</i>	unresolved	unresolved	<i>S. girg</i> - <i>S. fim</i>
RAPDF	<i>S. girg</i> - <i>S. fim</i>	<i>S. girg</i> - <i>S. fim</i>	<i>S. rubellum</i>	<i>S. girg</i> - <i>S. fim</i>
LEAFY1	unresolved	unresolved	unresolved	unresolved
LEAFY2	unresolved	unresolved	unresolved	unresolved
trnL	<i>S. cap</i> - <i>S. rub</i> - <i>S. fusc</i>	<i>S. cap</i> - <i>S. rub</i> - <i>S. fusc</i>	<i>S. warnstorffii</i>	<i>S. cap</i> - <i>S. rub</i> - <i>S. fusc</i>

7 genes

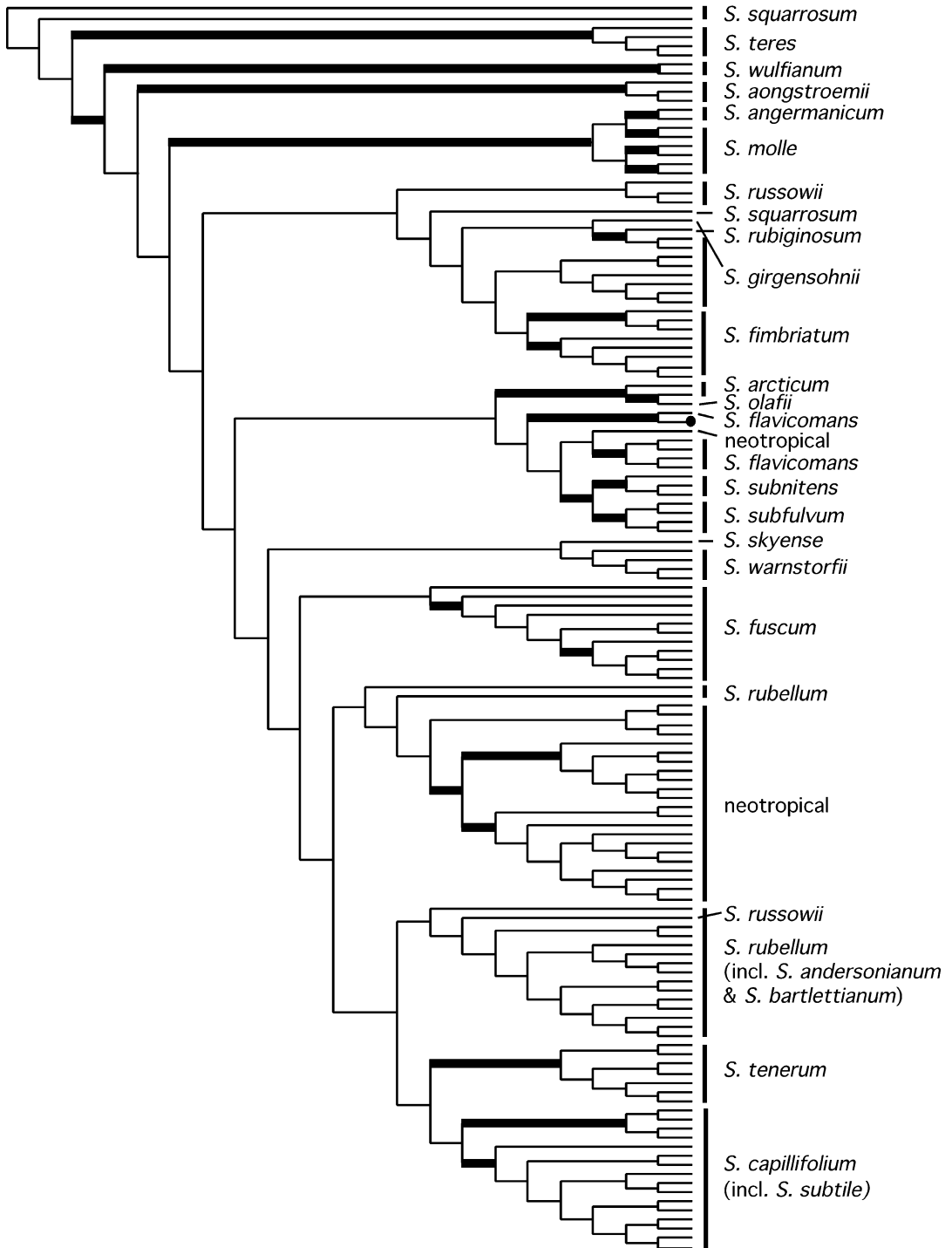


FIG. 1. Phylogenetic reconstruction for *Sphagnum* sect. *Acutifolia*, based on heterogeneous Bayesian analyses of seven genomic regions. Thick branches are supported at $\geq 95\%$ Bayesian joint posterior probability. The accession of *S. subtile* (no. 1281, identified by J. Shaw and L. E. Anderson) indicated by a solid black circle does not group with other accessions of the morphospecies.

The latter do not form a monophyletic group. Rather, pairs of populations within *S. molle* are well-supported.

Sphagnum russowii, a species thought to be of allopolyploid origin (Cronberg 1996), is not resolved as monophyletic. Three accessions show a relationship to *S. girgensohnii* and *S. fimbriatum* whereas the fourth is most closely related to *S. rubellum* based on combined data (Fig. 1). Relationships in the rest of the tree were unaffected when analyses were run with *S. russowii* excluded.

Single Gene Analyses. Deep nodes antedating the origins of extant morphospecies were largely unsupported in the combined analysis, hence single gene analyses were conducted to test the hypothesis that the ambiguity is due to conflicts among loci.

ITS. ITS sequences resolve *S. teres*, *S. wulfianum*, *S. aongstroemii*, a core of *S. fimbriatum* populations, *S. angermanicum*, *S. subnitens*, and *S. tenerum* individually with strong support (Fig. 2). *Sphagnum wulfianum* is part of a well-supported clade that includes all of section *Acutifolia*. Two major clades are resolved within *Acutifolia*. One includes *S. aongstroemii*, *S. skyense*, *S. girgensohnii*, *S. fimbriatum*, *S. rubiginosum*, *S. molle*, *S. angermanicum*, *S. subfulvum*, *S. subnitens*, *S. flavicomans*, *S. olafii*, *S. arcticum*, some (but not all) populations of *S. warnstorffii*, and the Neotropical accessions. The other includes *S. fuscum*, *S. capillifolium*, *S. tenerum*, *S. rubellum*, one of the *S. warnstorffii* populations, and all four populations of *S. russowii*. The Neotropical accessions included in this clade have a well-supported relationship to *S. fuscum*. *Sphagnum capillifolium*, *S. tenerum*, and *S. rubellum* form a clade that is sister to *S. fuscum* plus the Neotropical group (in part). ITS is the only genomic region that provides significant support for some of the deeper nodes.

RAPDA. A population of the outgroup taxon, *S. squarrosom*, is nested within *Acutifolia* in a strongly supported clade with two populations of *S. girgensohnii* and *S. rubiginosum* (Fig. 2). Section *Acutifolia*, including *S. wulfianum* and the one accession of *S. squarrosom*, forms a monophyletic group with strong support. Within *Acutifolia*, two populations of *S. wulfianum* are monophyletic. *Sphagnum molle* and *S. angermanicum* form a monophyletic group, as do *S. girgensohnii* plus *S. fimbriatum* plus *S. rubiginosum* (plus three populations of *S. russowii* and one of *S. squarrosom*). *Sphagnum aongstroemii* appears to be sister to a clade that includes the remaining species of *Acutifolia*. The Neotropical taxa again do not form a monophyletic group. One population is resolved near the base of this large clade with a population of *S. rubellum*, one groups with *S. flavicomans*, and two divergent groups of Neotropical populations are resolved in a clade that includes *S. capillifolium*, *S. tenerum*, *S. fuscum*, *S. rubellum*, and one population of *S. russowii*. One of these Neotropical clades is strongly supported as monophyletic. Popu-

lations of *S. rubellum* are resolved in three different clades on the tree, two of them with strong support. There is no indication of a close relationship between Neotropical accessions with *S. fuscum*, as for ITS.

RAPDB. A population of *S. fuscum* is sister to the rest of section *Acutifolia*. At the level of morphospecies, *S. tenerum*, *S. aongstroemii*, *S. fuscum* (minus the basal population), *S. capillifolium*, *S. wulfianum*, *S. angermanicum*, and four (of five) populations of *S. warnstorffii* are resolved, with strong support, as monophyletic. The fifth *S. warnstorffii* population is nested within a strongly supported clade that is otherwise composed of *S. capillifolium*. A well-supported clade includes some populations of *S. flavicomans* plus *S. molle* and *S. angermanicum*. Most of the Neotropical populations are related to *S. rubellum*, but one population is resolved outside that clade, with a clade that includes populations of *S. fuscum*, *S. subnitens*, and *S. skyense*. Most groupings of morphospecies are not supported.

RAPDF. *Sphagnum aongstroemii*, *S. wulfianum*, and *S. tenerum* are resolved as monophyletic species, but most other relationships are without support (Fig. 3). A well-supported clade that includes four populations of *S. capillifolium* also includes two populations of *S. rubellum*. All but one of the Neotropical populations are resolved with strong support in a clade with some populations of *S. capillifolium*. One Neotropical population is resolved with *S. flavicomans*, without support. A well-supported clade includes *S. girgensohnii*, *S. fimbriatum*, and *S. rubiginosum* (plus a population of *S. russowii* and one *S. squarrosom*). Unlike the topology from RAPDB, RAPDF sequences resolve *S. skyense* with some populations of *S. warnstorffii*.

LEAFY1. *Sphagnum wulfianum*, *S. angermanicum*, *S. subfulvum*, *S. tenerum*, and *S. capillifolium* are resolved as monophyletic species (Fig. 4). In addition, eight of the ten *S. fuscum* populations form a well-supported monophyletic group. The remaining *S. fuscum* population groups with populations of *S. rubellum*. Deeper relationships are not well-supported by *LEAFY1* sequences. Twenty-one of the Neotropical populations fall out as a monophyletic group (without support) related to several populations of *S. capillifolium* and *S. fuscum* (with support). The other two populations are most closely related to *S. flavicomans*.

LEAFY2. Few relationships are well-supported by *LEAFY2* sequences alone, although *S. wulfianum* and *S. tenerum* are strongly supported as monophyletic (Fig. 4). One of the Neotropical populations is part of a well-supported clade with two populations of *S. aongstroemii*. As with other loci, *LEAFY2* sequences support a close relationship between *S. molle* and *S. angermanicum*, but neither species is resolved as monophyletic.

TRNL. *Sphagnum teres*, *S. angermanicum*, *S. rubellum*, six populations of *S. girgensohnii*, and nine populations of *S. fuscum* are supported as monophyletic (Fig. 5). A

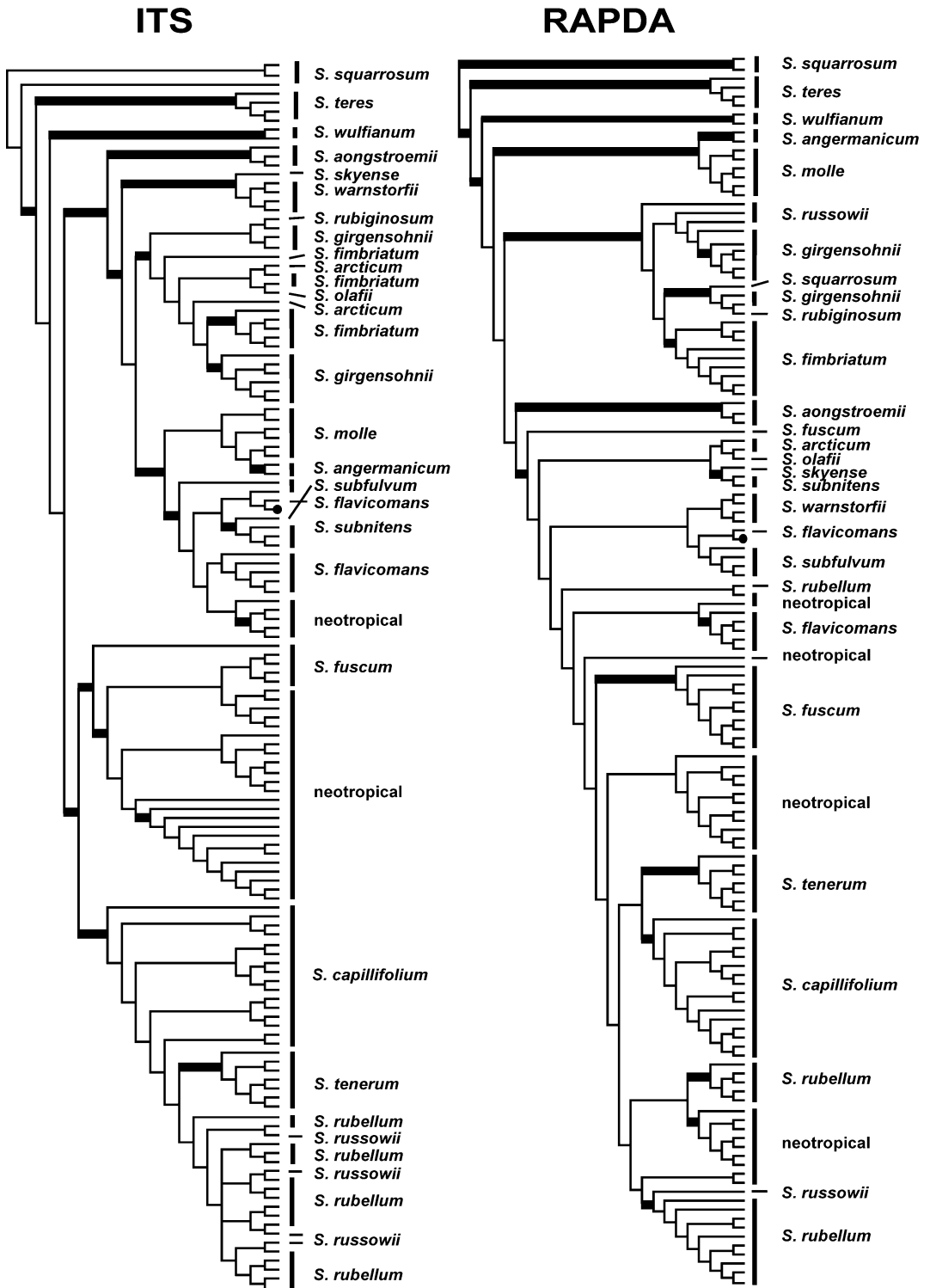


FIG. 2. Phylogenetic reconstruction for *Sphagnum* section *Acutifolia*, based on heterogeneous Bayesian analyses of ITS (nrDNA) and anonymous RAPDA sequences. See text for details concerning the amplification and sequencing of the anonymous region. Thick branches are supported at $\geq 95\%$ Bayesian joint posterior probability. The accession of *S. subtile* (no. 1281, identified by J. Shaw and L. E. Anderson) indicated by a solid black circle does not group with other accessions of the morphospecies.

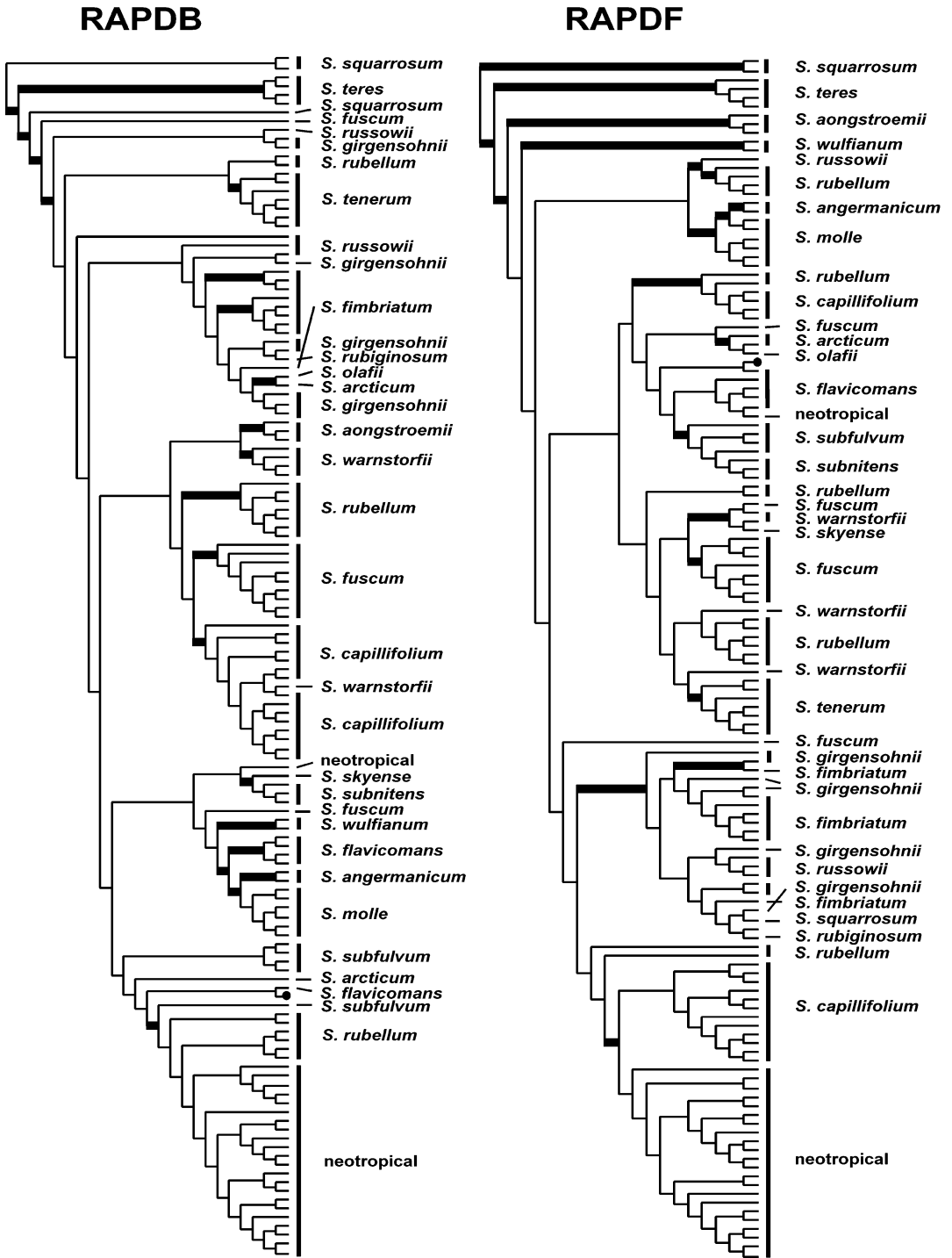


FIG. 3. Phylogenetic reconstruction for *Sphagnum* section *Acutifolia*, based on heterogeneous Bayesian analyses of anonymous RAPDB and RAPDF sequences. See text for details concerning the amplification and sequencing of the anonymous region. Thick branches are supported by $\geq 95\%$ Bayesian joint posterior probability. The accession of *S. subtile* (no. 1281, identified by J. Shaw and L. E. Anderson) indicated by a solid black circle does not group with other accessions of the morphospecies.

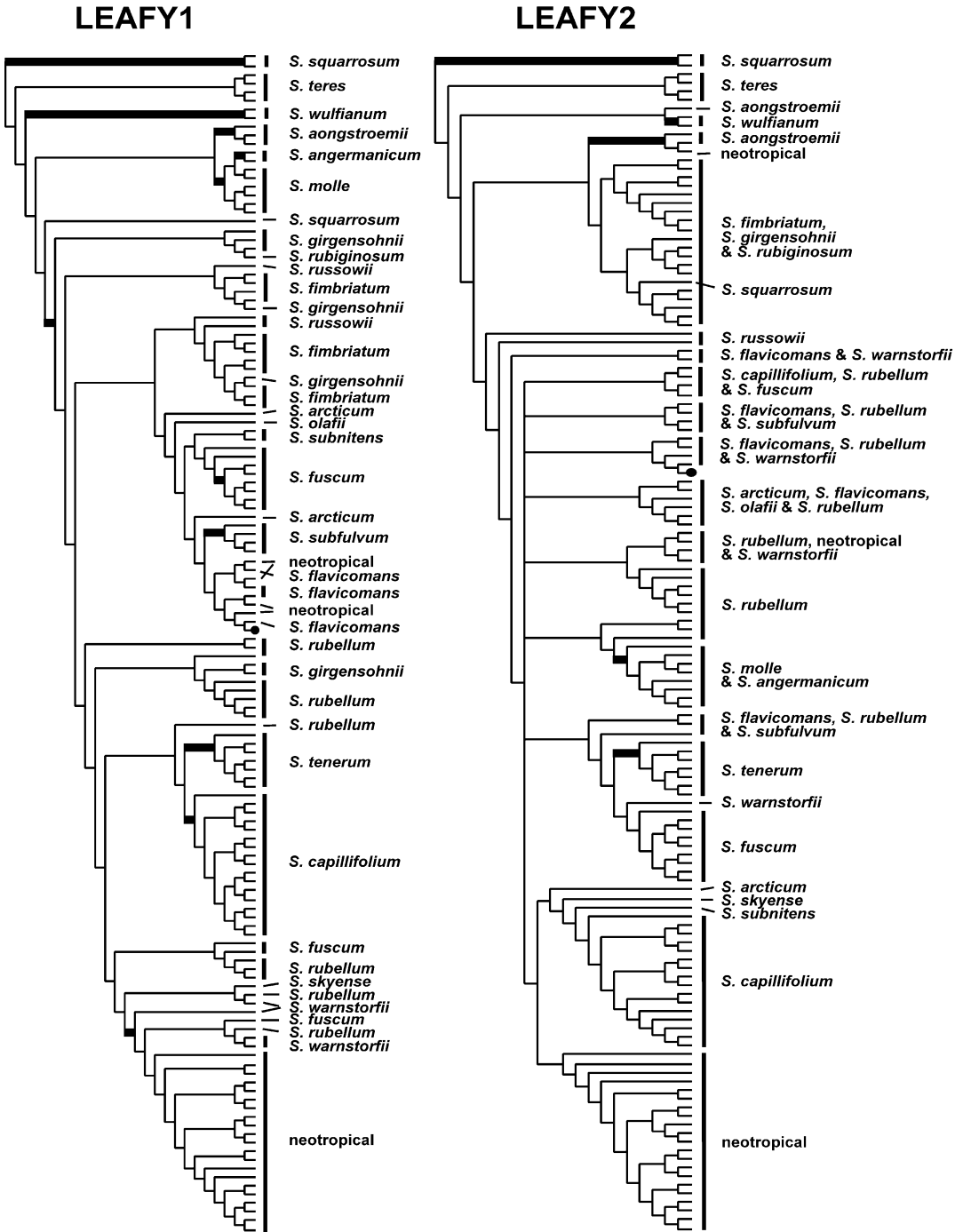


FIG. 4. Phylogenetic reconstruction for *Sphagnum* sect. *Acutifolia*, based on heterogeneous Bayesian analyses of *LEAFY1* and *LEAFY2* (intron) sequences. Thick branches are supported at $\geq 95\%$ Bayesian joint posterior probability. The accession of *S. subtile* (no. 1281, identified by J. Shaw and L. E. Anderson) indicated by a solid black circle does not group with other accessions of the morphospecies.

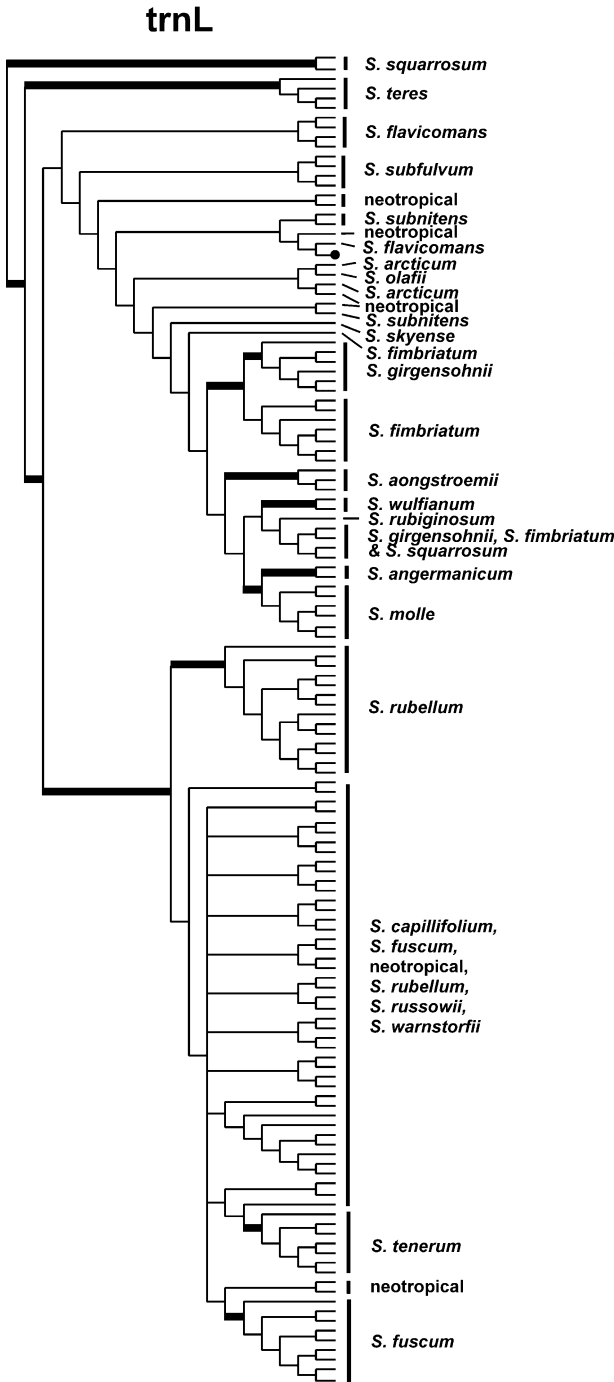


FIG. 5. Phylogenetic reconstruction for *Sphagnum* section *Acutifolia*, based on heterogeneous Bayesian analyses of *trnL* (cpDNA) sequences. Thick branches are supported at ≥ 95% Bayesian joint posterior probability. The accession of *S. subtile* (no. 1281, identified by J. Shaw and L. E. Anderson) indicated by a solid black circle does not group with other accessions of the morphospecies.

well-supported clade includes *S. capillifolium*, *S. rubellum*, *S. tenerum*, *S. fuscum*, and a group of Neotropical populations, but many relationships within this clade are unresolved.

Single Locus Relationships of *S. squarrosom*. Two of the three populations of *S. squarrosom* consistently formed a clade, but a third population (No. 1204 from India) appears to have a genealogically heterogeneous composition. Its ITS and RAPDB sequences, while not providing evidence for a monophyletic relationship with the other two populations, place it outside section *Acutifolia*, with other populations of section *Squarrosa*. The placement of this population relative to other *Squarrosa* and *Acutifolia* species depends on how the phylogeny is rooted, but sequence data indicate that population 1204 is divergent from other *S. squarrosom* accessions. Sequences from RAPDA, RAPDF, and *trnL* provide strong support for a relationship with populations of *S. girgensohnii* and *S. rubiginosum*. A similar relationship is suggested by *LEAFY1* and *LEAFY2* sequences, but without support. *LEAFY2* suggests that *S. squarrosom* 1204 is nested within the *S. girgensohnii*-*S. fimbriatum* clade whereas *LEAFY1* sequences place it as sister to a larger clade that includes these taxa. The heterogeneous *S. squarrosom* plant lacks an 18 base deletion in RAPDA that is shared by the other two populations of *S. squarrosom* (Table 2). The two populations of *S. girgensohnii* to which *S. squarrosom* No. 1204 is related are from Japan. It is noteworthy that No. 1204 is from India, although the population also shows a relationship to *S. rubiginosum* from Norway.

Single Locus Relationships of *S. russowii*. Different loci suggest different relationships for *S. russowii*, suggesting an allopolyploid origin for the species. These patterns are summarized in Table 2. ITS sequences of all *S. russowii* populations show strongly supported relationships to *S. rubellum* and/or *S. capillifolium*. RAPDA sequences from three of the four *S. russowii* accessions, in contrast, provide strong support for a relationship to the *S. girgensohnii*-*S. fimbriatum* clade, while RAPDA sequences from one population are allied with *S. rubellum* (Table 2). Other loci provide evidence of relationships to these same divergent clades, but with the patterns varying among accessions and loci (Table 2). *trnL* sequences from *S. russowii* No. 588 suggest a relationship to *S. warnstorffii*. RAPDA sequences support a relationship between *S. russowii*, No. 577 from Norway, and a clade that includes both *S. girgensohnii* and *S. fimbriatum*. This *S. russowii* population shares a 6-base deletion in RAPDA with two populations of *S. girgensohnii*, one from Quebec (No. 1372) and one from Minnesota (No. 98).

Single Locus Relationships of *S. fuscum*. Most populations of *S. fuscum* are relatively similar, but one, no. 1238 from Japan, is an outlier for several loci. No. 1238 is excluded from a well-supported clade of *S. fus-*

cum populations based on the combined multilocus data set sequences. Its position is unresolved for several loci, but it is in a strongly supported clade with populations of *S. warnstorffii* and *S. skyense* for RAPDF. Other populations of *S. fuscum* also have divergent relationships as evidenced by RAPDF sequences (Fig. 3). *LEAFY1* sequences place No. 1238, along with another population of *S. fuscum* (No. 1185), in an unsupported clade with *S. rubellum*. *LEAFY2* sequences also place No. 1238 with populations of *S. rubellum* (and an *S. capillifolium* accession).

Single locus relationships of *S. skyense*. ITS sequences resolve *S. skyense*, known only from the type locality in Scotland, in a well-supported clade with four populations of *S. warnstorffii* (from Alaska, Finland, and Russia [x2]), but RAPDA and RAPDB place *S. skyense*, with strong support, in a clade with populations of *S. subnitens* from Alaska and Norway. RAPDF sequences indicate a relationship, also strongly supported, with two populations of *S. warnstorffii* and one of *S. fuscum*. The *S. warnstorffii* populations, from Alaska and Finland, are the same as those to which ITS sequences indicate a relationship. The population of *S. fuscum* included in this clade is another accession (no. 1238) that appears to have heterogeneous relationships based on different loci.

Single Locus Relationships of *S. arcticum* and *S. olafii*. These two species were described from the arctic island of Spitzbergen—*S. arcticum* was also recorded from Canada and Alaska (Flatberg and Frisvoll 1984). One of the two populations of *S. arcticum* included in these analyses was collected in the Northwest Territories of Canada (No. 1191) while the other was collected at or near the type locality in Spitzbergen (No. 1178). The collection of *S. olafii* is from Spitzbergen. ITS sequences place all three accessions in unsupported relationships within the (well-supported) *S. fimbriatum*-*S. girgensohnii* clade. An insertion of one nucleotide in ITS2 is shared between the Spitzbergen population of *S. arcticum*, *S. olafii*, five populations of *S. girgensohnii* (all from North America) and seven populations of *S. fimbriatum* (from North America, Europe, and Russia). Only two populations of *S. girgensohnii* lack the insertion (Nos. 1236 and 1242, both from Japan) and two populations of *S. fimbriatum* also lack the insertion (No. 1367 from Alaska and No. 1369 from Alberta). The insertion is also lacking in the second (Canadian) population of *S. arcticum*. RAPDA sequences resolve the three populations of *S. arcticum* and *S. olafii* as monophyletic with strong (99%) support, sister to two populations of *S. subnitens* from Norway, plus *S. skyense* with 93% posterior probability. RAPDB provides strong support for monophyly of the Spitzbergen populations of *S. olafii* and *S. arcticum* (100% posterior probability), but this clade does not include the Canadian population of *S. arcticum* that bears relation-

ships to *S. flavicomans*, *S. subfulvum*, *S. rubellum*, and Neotropical populations. RAPDF provides strong support for monophyly of all three populations, but, in contrast to RAPDB, yields a sister group relationship between the Canadian population of *S. arcticum* and *S. olafii*. The other three loci did not permit strong inferences about relationships, but for *LEAFY1* sequences, neither *S. arcticum* nor *S. olafii* are resolved as members of the well-supported *S. fimbriatum*-*S. girgensohnii* clades.

DISCUSSION

Two patterns are evident from the analyses of multilocus sequence data from *Acutifolia*: many of the commonly recognized morphospecies correspond to monophyletic groups of populations, but individuals that are admixtures of different species are rather frequent. The analysis of genetic admixture in the ancestry of individual organisms has been largely restricted to human populations (e.g., Chikhi 2001; Cerda-Flores et al. 2002), but such mosaic individuals have been documented in some animals and plants and may have implications for conservation efforts (Whittemore and Schaal 1991; Ciofi et al. 1999; Estroup et al. 2001; Pierpaoli et al. 2003). Statistical theory underlying admixture analysis has been developed mainly for microsatellite data. Interspecific genetic admixture is here inferred from phylogenetic data. It is always difficult to distinguish shared nucleotide sequences that reflect retained polymorphism from those resulting from more recent interbreeding. In the case of sect. *Acutifolia*, many well-supported relationships are in conflict between loci.

It is hard to escape the conclusion from multilocus molecular data presented here that interspecific hybridization is common in *Sphagnum* sect. *Acutifolia*. This conclusion is consistent with the impression one gains from studying collections of *Sphagnum* morphologically. Many of the species are commonly recognizable based on diagnostic morphological traits, but intermediate collections are not rare. Indeed, it is remarkable that the species maintain their coherence, in terms of both morphology and molecular structure, in the face of interspecific gene flow. Varying taxonomic treatments among investigators reflect perceptions about which is more common—the “rules” (i.e., species) or the exceptions (intermediates). There is no correct taxonomic answer.

Previous isozyme studies provided evidence that *S. russowii* originated through allopolyploidization (Cronberg 1993, 1996). Only two chromosome numbers are known in *Sphagnum*: $n = 19$ and $n = 38$ (Fritsch 1982), with *Sphagnum russowii* having $n = 38$. Cronberg (1996) showed that *S. russowii* exhibits fixed heterozygosity at five isozyme loci. Based on allelic profiles of other *Acutifolia* species, Cronberg (1996) concluded that

S. girgensohnii is likely one parent of *S. russowii*, and identified the second putative parent as either *S. rubellum* or *S. quinquefarium*. Unfortunately, *S. quinquefarium* was not included in the present analyses, but the DNA sequence data are consistent with *S. rubellum* being one parental species, and *S. girgensohnii* is almost certainly the other. Cronberg (1996) argued on the basis of morphological similarities that *S. rubellum* is a more likely parent than is *S. quinquefarium*. In addition to nucleotide substitution data, one population of *S. russowii* has a deletion in the RAPDB locus that is otherwise found only in two populations of *S. girgensohnii*. Although Cronberg (1996) thought that multiple independent origins of allopolyploid *S. russowii* are unlikely, the DNA data support multiple origins, even among the four samples included in these analyses. One sample, No. 588 from Washington, even seems to have some *S. warnstorffii* in its genetic makeup.

The chromosome numbers of *S. arcticum* and *S. olafii* have not been reported, but Greilhuber et al. (2002) described flow cytometry measurements that clearly indicate gametophytic diploidy for both species. Flatberg and Frisvoll (1984) hypothesized that *S. arcticum* may have originated through hybridization between *S. fimbriatum* and *S. subfulvum*. It is similar to *S. olafii* in morphology (Flatberg 1993), but differs in color and growth form, stem leaf shape, and the shape of the branch leaf hyaline cells. Flatberg (1993) suggested that *S. olafii* rather than *S. subfulvum* could be involved in the ancestry of *S. arcticum*. Flatberg (pers. comm.) has also suggested that *S. subnitens* may be involved in the ancestry of *S. olafii*.

Molecular data presented here corroborate a close relationship between *S. olafii* and *S. arcticum*. RAPDA and RAPDF sequences, and the combined data set, indicate that the three populations representing *S. arcticum* and *S. olafii* form a monophyletic group (with 99 and 100% Bayesian support, respectively). The data do not permit resolution of their ancestry with regard to *S. girgensohnii* versus *S. fimbriatum*: ITS places them in an unresolved clade that includes both species. RAPDA sequences support a relationship of both *S. arcticum* and *S. olafii* with *S. subnitens* (and *S. skyense*). *Sphagnum olafii* and one of the two *S. arcticum* populations (No. 1178 from Spitzbergen) are placed by RAPDB sequences in a clade that is sister to three populations of *S. girgensohnii* (with 91% Bayesian probability). The other population of *S. arcticum* is not included in that clade. RAPDF sequences, on the other hand, indicate that the *S. arcticum* population from Canada is sister to *S. olafii* (within the clade that includes all three samples). An insertion in the ITS2 region is shared by the Spitzbergen populations of *S. arcticum* and *S. olafii*, but not the Canadian population of *S. arcticum*.

The DNA data support a single origin of *S. olafii* plus *S. arcticum*, although the molecular patterns could

also be explained by separate allopolyploid origins and subsequent hybridization. Neither species is known to reproduce sexually on Spitzbergen (Flatberg and Frisvoll 1984; Flatberg 1993, 2002b), but sexual reproduction may have occurred in the past, and/or currently without discovery. Sequence data further indicate that *S. subnitens* is a possible ancestor; the other ancestor is clearly *S. fimbriatum* or *S. girgensohnii*, with the evidence weakly supporting *S. girgensohnii* as the more likely of the two. Sequence data do not provide evidence of a relationship to *S. subfulvum*, although limited sampling could constrain this conclusion.

Hybrid ancestry has also been proposed for *S. skyense*. The species was described from northern Scotland (Isle of Skye) by Flatberg (1988), who noted morphological similarities to *S. quinquefarium* and *S. subnitens* (or possibly *S. flavicomans*). Sequence data support a hybrid origin for *S. skyense*, involving *S. warnstorffii* (supported by ITS and RAPDF) and *S. subnitens* (supported by RAPDA and RAPDB). Because *S. quinquefarium* was not included in the present analyses, involvement of that species cannot be excluded. All of the populations of *S. warnstorffii* that appear to be related to *S. skyense* (Nos. 371, Alaska; 580, Finland; 886 & 881, Russia), as well as those of *S. subnitens* (Nos. 570, Norway; 1297, Alaska) are from arctic or subarctic regions, not unlike the type locality for *S. skyense* in Scotland.

Population sampling clearly supports the hypothesis that at least occasional interbreeding occurs among *Sphagnum* species. Allopolyploid taxa obviously require interspecific hybridization, and individual collections—for example, *S. squarrosus* No. 1204 and *S. fuscum* No. 1238 are likely admixtures of different taxa. *Sphagnum fuscum* and *S. warnstorffii* appear to be especially heterogeneous among loci and populations. In addition, the molecular data provide evidence for reticulation deeper in the phylogeny of section *Acutifolia*. As a consequence, relationships among species and groups of species are not resolved with strong support in the combined analysis. ITS sequences, for example, resolve the *S. molle* plus *S. angermanicum* clade as sister to *S. flavicomans* + *S. subfulvum* + *S. subnitens*, whereas RAPDA resolves *S. molle* + *S. angermanicum* as an early-diverging lineage of *Acutifolia*, outside a well-supported clade that includes *S. subnitens* + *S. subfulvum* + *S. flavicomans*. RAPDF sequences place *S. girgensohnii* and *S. fimbriatum* in a clade with *S. capillifolium*, whereas ITS resolves the two species, and *S. capillifolium*, in two separate well-supported clades.

Of particular interest is the relationship of the Neotropical populations to other *Acutifolia*. The combined analysis resolves all but one of them as members of a monophyletic group, although without support. The number of species in our sample cannot be specified at present, pending revisionary work, but the level of

molecular diversity is not high in the 16 samples (unpublished). The weight of the evidence from the seven loci supports a single origin of the *Acutifolia* Neotropical clade from a boreal ancestor. The sister group to this clade, however, remains unresolved. ITS sequences lend strong support to a sister group relationship between the Neotropical group and *S. fuscum*, RAPDB supports a relationship to *S. rubellum*, and RAPDF provides strong support for a sister group relationship with *S. capillifolium*. One Neotropical population is even part of a well-supported clade that includes two populations of *S. aongstroemii* based on *LEAFY2* sequences.

Incongruent phylogenetic patterns involving individual collections that are admixtures of related taxa strongly implicate ongoing gene flow among species, but gene flow between Neotropical and boreal populations is highly unlikely. Phylogenetic conflicts concerning ancestry of the Neotropical clade may reflect ancient reticulation, or possibly more recent gene flow between *S. rubellum*, *S. fuscum*, and *S. capillifolium*, such that a clear phylogenetic signal resolving the sister group to Neotropical taxa has been obscured.

One of the most controversial issues in *Acutifolia* systematics concerns the relationship between *S. rubellum* and *S. capillifolium*. They have been treated as distinct species (Isoviita 1966; Andrus 1980; Flatberg 2002a), varieties of one species (e.g., Andrews 1913; Dixon 1924; Crum 1984; Daniels and Eddy 1990), or considered not distinct at all (Hill 1976). The two differ in stem leaf shape, branch leaf arrangement (ranked or spiral), branch leaf pore size, and other features, but no one argues that the variation in morphology is discontinuous. Cronberg (1996) found that although there were no fixed allelic differences characterizing the two taxa, differences in allele frequencies at several isozyme loci support their separation. DNA sequence data presented here unambiguously support their separation. Indeed, only one or two samples may be genetic admixtures of the two species. The results indicate that *S. rubellum* and *S. capillifolium* are different phylogenetic entities, even if they cannot always be distinguished morphologically.

Crum (1984) included *S. rubellum*, *S. subtile*, and *S. andersonianum* in *S. capillifolium* var. *tenellum* (Schimp.) Crum. Nucleotide sequence data do not provide support for separating *S. subtile* from *S. capillifolium*, even as a variety. The combined data resolved two clades within *S. capillifolium*, but plants having the morphology of *S. subtile* occurred in both clades. Crum (1984) and Natcheva and Cronberg (2002) associated the "subtile" phenotype with *S. rubellum* rather than *S. capillifolium*. This could reflect incongruent morphological versus molecular evidence, or possibly differences in the criteria used to define the "subtile" phenotype. Criteria used in this study agree with those discussed by

Crum (1984). Crum (1984) also included *S. andersonianum* in *S. capillifolium* var. *tenellum*, but the DNA data indicate that unlike *S. subtile*, *S. andersonianum* is indistinguishable from *S. rubellum*. *Sphagnum rubellum* and *S. andersonianum* are said to differ in branch leaf arrangement (ranked or spiral), the number of pendent branches per fascicle, and branch leaf pore size and shape, but in a broader survey we have been unable to consistently distinguish the two morphologically. There is no indication of any separation based on sequence data. Specimens identified in this study as *S. andersonianum* had at least some branch fascicles with two pendent branches (diagnostic for *S. andersonianum*). However, some of these had clearly ranked leaves (more characteristic of *S. rubellum*).

Molecular data do not support the separation of *S. bartlettianum* from *S. rubellum*. Crum (1984) and Andrus (1980) both distinguish *S. bartlettianum* as a distinct species, and there has been no published discussion of intermediates between the two, nor any suggestion that they should be combined. Nevertheless, *S. bartlettianum* and *S. rubellum* can be difficult to distinguish morphologically, especially in the mountains of the southeastern United States. *Sphagnum rubellum* is a common circumboreal species of ombrotrophic bogs, whereas *S. bartlettianum* is the most common member of section *Acutifolia* in the southeastern U.S. Coastal Plain. Coastal plain populations appear distinct from *S. rubellum*, but in the Appalachian mountains where the two are sympatric, the species can be nearly indistinguishable. Coastal plain *S. bartlettianum* differs from northern *S. rubellum* in having less (or not at all) secund leaves, more flat-tipped capitula, and longer stem leaves. However, the two species share more or less ranked leaves, and both have relatively small branch leaf pores. Sequence data do not provide any evidence of phylogenetic discontinuity between them. Significantly, three populations each of *S. bartlettianum* and *S. rubellum* uniquely share a 3-base deletion in the RAPDF locus. Assuming this deletion is homologous in the six specimens, this synapomorphy provides strong evidence that *S. rubellum* and *S. bartlettianum* belong to a single interbreeding species.

Crum (1984) recognized *S. tenerum* as a variety of *S. capillifolium*, but the molecular data not only provide support for *S. tenerum* as a separate species, but demonstrate that this species is one of the most distinct in section *Acutifolia*. *Sphagnum tenerum* is resolved as monophyletic by every one of the seven genes individually, as well as by the combined data. Crum described the geographic distribution of *S. tenerum* (at the varietal level) as including eastern North America west to Kansas, Missouri, and Minnesota, and also Europe west to the Caucasus, and Japan. Andrews (1947) and Crum (1990a) also include *S. tenerum* in the flora of South America. All of the samples of *S. tenerum* included in

the present analyses were from eastern North America and we cannot confirm its distribution outside this region. Recent treatments of European sphagna do not include *S. tenerum* in the flora (Daniels and Eddy 1990; Flatberg 2002a). A plant identified by Crum as *S. tenerum* from South America (Churchill 18330 from Colombia: MICH) unambiguously grouped with other Neotropical samples in the present analyses and is clearly not conspecific with North American *S. tenerum*.

Taxonomic Implications. Because of conflicting patterns from different loci, this study was largely unsuccessful in resolving phylogenetic relationships among species of *Acutifolia* sufficiently to propose a well-supported infra-sectional classification.

It is clear that *S. girgensohnii* and *S. fimbriatum* are closely related, and that they are more closely related to *S. subnitens*, *S. subfulvum*, and *S. flavicomans* than they are to the *S. capillifolium* group, including *S. tenerum* and *S. rubellum*. ITS sequences resolve these as the two major lineages within the *Acutifolia*, but this resolution is not supported by other loci. The general pattern of relationships obtained from sequence data are broadly consistent with those resolved by multivariate analyses of stem leaf shapes (Gerdol 1987) and isozyme variation (Cronberg 1996). If ancient hybridization is the reason for the non-resolution of deep relationships within the *Acutifolia*, interspecific relationships may never be fully resolved.

The molecular results do not support the suggestion made by Eddy (1979) that *S. fimbriatum* and *S. girgensohnii* should be excluded from section *Acutifolia*. Cronberg (1996) found that *S. girgensohnii* and *S. fimbriatum* are more similar at isozyme loci to *S. teres* and *S. squarrosus* in section *Squarrosa* than to other species of section *Acutifolia*. Phylogenetic analyses of sequence data indicate that *S. girgensohnii* and *S. fimbriatum* share a more recent common ancestor with other species of section *Acutifolia* than with either *S. teres* or *S. squarrosus*. Isozyme data also suggest that *S. molle* is more similar genetically to *S. fimbriatum*, *S. girgensohnii*, and section *Squarrosa*, than to other *Acutifolia* (Cronberg 1996), but sequence data consistently and unambiguously show that *S. molle* is nested within *Acutifolia*, although its precise relationship remains uncertain. Its placement as an early-diverging lineage within *Acutifolia*, as shown in the combined tree, may result from a compromise between conflicting signals provided by the different loci. ITS sequences alone, for example, suggest a sister group relationship between *S. molle* + *S. angermanicum* and *S. subfulvum* + *S. subnitens* + *S. flavicomans*. Molecular data do not support the separation of *S. wulfianum* and *S. aongstroemii* as separate monospecific sections.

It is possible to define objective criteria for delimiting species, and some argue that morphological diag-

noses should be replaced by genetic data, almost like "DNA barcodes" (Hebert et al. 2002). The level at which population systems attain the status of species could be based on numbers of mutations or some other measure of genetic distinctiveness. However, in *Sphagnum*, as in most genera, some "species" are closely related to other species, while others are highly isolated. Some are more distinct at the morphological or ecological than molecular levels; others are genetically isolated but are morphologically indistinguishable from closely related congeners. The degree to which a species is genetically isolated could reflect age, effective population size, ecological distinctiveness, and/or mode of speciation (e.g., involving a population bottleneck). This study adopts a pragmatic approach to species; taxonomic implications are discussed in the context of common definitions of morphospecies.

Our results support the separation of *S. rubellum*, *S. capillifolium*, and *S. tenerum*, but not *S. subtile*, which should be merged with *S. capillifolium*. Moreover, the molecular results strongly support merging *S. rubellum*, *S. andersonianum*, and *S. bartlettianum*. Merging *S. bartlettianum* is likely to be the most controversial conclusion, and it can always be argued that additional data will resolve it as a separate clade. This is unlikely, however, since there is no hint of separation in the seven-locus data set, which provides sufficient information to resolve other closely related taxa. We consider the RAPDF deletion shared by some populations of *S. bartlettianum* and *S. rubellum* to be especially significant, and it supports evidence from nucleotide substitutions alone.

Sequence data support the separation of *S. subnitens*, *S. subfulvum*, and *S. flavicomans*, corroborating arguments made by Flatberg (1985b), Crum (1984), and Andrus (1980) that these are distinct species. Sjörs (1944) suggested *S. subfulvum* could have been derived by introgressive hybridization between *S. fuscum* and *S. subnitens*, and although both of the latter species do seem to be involved in the ancestry of some of the genetically heterogeneous samples encountered in this study, there is no strong evidence that samples of *S. subfulvum*, *S. subnitens*, or *S. flavicomans* are genetic admixtures. The three are morphologically similar and overlap in stem and branch leaf shapes (Björbäck and Norling 1984), but nucleotide sequence data indicate that at least those samples included in the present study are mutually monophyletic. Nevertheless, taxonomic confusion surrounding them persists, as Crum (1984) interpreted both *S. subnitens* and *S. subfulvum* as being present in eastern and western North America, while Andrus (1980) limits *S. subnitens* to the western part of the continent. The prospect of molecular data clarifying this issue is promising, but larger sample sizes with extensive geographic sampling are needed. *Sphagnum subnitens* is the only species of section *Acu-*

tifolia known from New Zealand and may have been introduced recently (Fife 1996). This hypothesis may also be testable using nucleotide sequence variation.

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APPENDIX 1

Accession data for plants included in the molecular analyses of *Sphagnum* sect. *Acutifolia*. GenBank accession numbers are in the following sequence: ITS, RAPDa, RAPDb, RAPDf, LEAFY-1, LEAFY-2, trnL.

S. andersonianum, 361, *Andrus 8466* (DUKE), USA: Alaska, AY298366, AY346522, AY346660, AY346794, AY347105, AY346935, AY298000; *S. andersonianum*, 363, *Allen 9438* (DUKE), USA: Maine, AY298367, AY346523, AY346661, AY346795, AY347106, AY346936, AY298001; *S. andersonianum*, 379, *Andrus 8164* (DUKE), Ireland, AY298370, AY346524, AY346662, AY346796, AY347109, AY346939, AY298004; *S. angermanicum*, 401, *Town 2253* (DUKE), USA: New York, AF193747, AY346526, AY346664, AY346798, AY347112, AY346943, AY298006; *S. angermanicum*, 1374, *Andrus & Karlin 6995* (DUKE), USA: New Jersey, AY298372, AY346525, AY346663, AY346797, AY347110, AY346940, AF192618; (DUKE), USA: *S. aongstroemii*, 412, *Andrus & Flatberg 7531* (DUKE), Norway, AF193748, AY309636, AY309655, AY309674, AY309513, AY309739, AF192619; *S. aongstroemii*, 882, *Cronberg* s.n. (DUKE), Russia: Siberia, AY298376, AY346527, AY346665, AY346799, AY347113, AY346944, AY298010; *S. aongstroemii*, 889, *Cronberg* s.n. (DUKE), Russia: Siberia, AY298377, AY346528, AY346666, AY346800, AY347114, AY346945, AY298011; *S. arcticum*, 1178, *Flatberg et al. 270–00* (DUKE), Norway,

- AY298378, AY346529, AY346667, AY346801, AY347115, AY346946, AY298012; *S. arcticum*, 1191, *Scotter 76467* (DUKE), Canada: NWT, AY298379, AY346530, AY346668, AY346802, AY347116, AY346947, AY298013; *S. austro-americanum*, 615, *Churchill & Betancur 18752* (MO), Colombia, AF193695, AY346531, AY346669, AY346803, AY347117, AY346948, AF192625; *S. azuayense*, 875, *Laegaard 53594E* (NY), Ecuador, AY298385, AY346532, AY346670, AY346804, AY347118, AY346949, AY298019
- S. bartlettianum*, 336, *Shaw 9280* (DUKE), USA: South Carolina, AY298390, AY346536, AY346674, AY346808, AY347122, AY346953, AY298021; *S. bartlettianum*, 338, *Shaw 9279* (DUKE), USA: South Carolina, AY298391, AY346537, AY346675, AY346809, AY347123, AY346954, AY298022; *S. bartlettianum*, 341, *Shaw 9282* (DUKE), USA: South Carolina, AY298392, AY346538, AY346676, AY346810, AY347124, AY346955, AY298023; *S. bartlettianum*, 1315, *Anderson 27686* (DUKE), USA: South Carolina, AY298387, AY346533, AY346671, AY346805, AY347119, AY346950, AY298024; *S. bartlettianum*, 1316, *Sheridan & Underwood 3* (DUKE), USA: Maryland, AY298388, AY346534, AY346672, AY346806, AY347120, AY346951, AF192600; *S. bartlettianum*, 1319, *Shaw 9950* (DUKE), USA: Alabama, AY298389, AY346535, AY346673, AY346807, AY347121, AY346952, AY298025
- S. capillifolium*, 368, *Talbot 228* (DUKE), USA: Alaska, AY298403, AY346542, AY346680, AY346814, AY347128, AY346959, AY298033; *S. capillifolium*, 562, *Shaw 9739* (DUKE), Finland, AY298404, AY346543, AY346681, AY346815, AY347129, AY346960, AY298034; *S. capillifolium*, 568, *Shaw 9648* (DUKE), Norway, AY298405, AY346544, AY346682, AY346816, AY347130, AY346961, AY298031; *S. capillifolium*, 569, *Shaw 9650* (DUKE), Norway, AY298406, AY346545, AY346683, AY346817, AY347131, AY346962, AF192621; *S. capillifolium*, 581, *Shaw 9739* (DUKE), Finland, AY298408, AY346546, AY346684, AY346818, AY347132, AY346963, AY298036; *S. capillifolium*, 1038, *Redfearn et al. 33655* (NY), USA: Missouri, AY298400, AY346540, AY346678, AY346812, AY347126, AY346957, AY298037; *S. capillifolium*, 1040, *Town s.n.* (NY), USA: New York, AY298401, AY346541, AY346679, AY346813, AY347127, AY346958, AY298038; *S. capillifolium*, 1253, *Vanderpoorten 691* (DUKE), England, AY298398, AY346539, AY346677, AY346811, AY347125, AY346956, AY298040; *S. capillifolium*, 1313, *Shaw 10173* (DUKE), Canada: Alberta, AY298714, AY346652, AY346784, AY346924, AY347246, AY347081, AY298089
- S. fimbriatum*, 883, *Cronberg s.n.* (DUKE), Russia: Siberia, AY298459, AY346557, AY346695, AY346829, AY347146, AY346977, AY298081; *S. fimbriatum*, 892, *Cronberg s.n.* (DUKE), Russia: Siberia, AY298458, AY346556, AY346694, AY346828, AY347144, AY346975, AY298338; *S. fimbriatum*, 1152, *Forbes 91–10C* (MICH), Russia: Siberia, AY298457, AY346555, AY346693, AY346827, AY347142, AY346973, AY298082; *S. fimbriatum*, 1213, *Heras & Infanti VII 21165* (DUKE), Hungary, AY298449, AY346548, AY346686, AY346820, AY347135, AY346966, AY298084; *S. fimbriatum*, 1243, *Uchida 2004* (DUKE), Japan, AY298450, AY346549, AY346687, AY346821, AY347136, AY346967, AY298085; *S. fimbriatum*, 1364, *Shaw 11554* (DUKE), USA: New York, AY298452, AY346550, AY346688, AY346822, AY347137, AY346968, AY298086; *S. fimbriatum*, 1367, *Schofield 105892* (DUKE), USA: Alaska, AY298453, AY346551, AY346689, AY346823, AY347138, AY346969, AY298087; *S. fimbriatum*, 1368, *Schofield 98564* (DUKE), Canada: BC, AY298454, AY346552, AY346690, AY346824, AY347139, AY346970, AY298088; *S. fimbriatum*, 1370, *Nelson 19541* (DUKE), USA: West Virginia, AY298456, AY346554, AY346692, AY346826, AY347141, AY346972, AY298091; *S. flavicomans*, 100, *Schofield 101051* (DUKE), Canada: NFLD, AF193690, AY346558, AY346696, AY346830, AY347147, AY346978, AY298090; *S. flavicomans*, 1289, *Town O2241* (DUKE), USA: New York, AY298460, AY346697, AY346971, AY346831, AY347148, AY346979, AF192571; *S. flavicomans*, 1290, *Allen 16775* (DUKE), USA: Maine, AY298461, AY346560, AY346698, AY346832, AY347149, AY346980, AY298093; *S. flavicomans*, 1293, *Hedderston 8702* (DUKE), Canada: Quebec, AY298462, AY346561, AY346699, AY346833, AY347150, AY346981, AY298094; *S. flavicomans*, 1295, *Belland & Schofield 17174* (DUKE), Canada: NS, AY298463, AY346562, AY346700, AY346834, AY347151, AY346982, AY298095; *S. fuscum*, 419, *Risk 1322* (DUKE), USA: New York, AF193736, AY346569, AY346707, AY346841, AY347159, AY346990, AY298096; *S. fuscum*, 420, *Bowers 24013* (DUKE), USA: Minnesota, AY298473, AY346570, AY346708, AY346842, AY347160, AY346991, AY298098; *S. fuscum*, 571, *Shaw 9678* (DUKE), Norway, AY309503, AY309641, AY309660, AY309679, AY309518, AY309743, AY298099; *S. fuscum*, 574, *Shaw 9680* (DUKE), Norway, AY298474, AY346571, AY346709, AY346843, AY347161, AY346992, AY298100; *S. fuscum*, 1184, *Belland & Schofield 17956* (DUKE), Canada: NB, AY298465, AY346563, AY346701, AY346835, AY347152, AY346983, AY298101; *S. fuscum*, 1185, *Schofield 101020* (DUKE), Canada: NFLD, AY298466, AY346564, AY346702, AY346836, AY347153, AY346984, AY298102; *S. fuscum*, 1187, *Risk et al. 6591* (DUKE), USA: Kentucky, AY298467, AY346565, AY346703, AY346837, AY347154, AY346985, AY298103; *S. fuscum*, 1238, *Yamaguchi 18783* (DUKE), Japan, AY298468, AY346566, AY346704, AY346838, AY347155, AY346986, AY298106; *S. fuscum*, 1240, *Yamaguchi 18775* (DUKE), Japan, AY298469, AY346567, AY346705, AY346839, AY347156, AY346987, AF192601; *S. fuscum*, 1246, *Higuchi 40992* (DUKE), Japan, AY298470, AY346568, AY346706, AY346840, AY347157, AY346988, AY347095
- S. girgensohnii*, 590, *Andreas s.n.* (DUKE), USA: Ohio, AY298485, AY346579, AY346716, AY346851, AY347171, AY347002, AF192603; *S. girgensohnii*, 1236, *Tsukamoto MT-582* (DUKE), Japan, AY298478, AY346573, AY346710, AY346845, AY347164, AY346996, AY298110; *S. girgensohnii*, 1242, *Uchida 2009* (DUKE), Japan, AY298480, AY346574, AY346711, AY346846, AY347165, AY346997, AY298112; *S. girgensohnii*, 1365, *Shaw 11553* (DUKE), USA: New York, AY298481, AY346575, AY346712, AY346847, AY347167, AY346998, AY298113; *S. girgensohnii*, 1371, *Shaw 9392* (DUKE), USA: North Carolina, AY298482, AY346576, AY346713, AY346848, AY347168, AY346999, AY298114; *S. girgensohnii*, 1372, *Price 694* (DUKE), Canada: QB, AY298483, AY346577, AY346714, AY346849, AY347169, AY347000, AY298115; *S. girgensohnii*, 1373, *Schofield 97076* (DUKE), Canada: NS, AY298484, AY346578, AY346715, AY346850, AY347170, AY347001, AY298116
- S. laxirameum*, 638, *Linares & Churchill 3740* (MO), Colombia, AF193703, AY346584, AY346717, AY346856, AY347176, AY347007, AF192604; *S. limbatum*, 625, *Dauphin 2065* (MO), Costa Rica, AF193693, AY346586, AY346719, AY346858, AY347178, AY347009, AY298359
- S. meridense*, 107, *Allen 11978* (DUKE), Honduras, AF193692, AY346588, AY346720, AY346860, AY347181, AY347013, AF192632
- S. meridense*, 612, *Lewis 38738d-1* (MO), Bolivia, AY298541, AY346590, AY346722, AY346862, AY347183, AY347015, AF192628; *S. meridense*, 825, *LaFarge s.n.* (DUKE), Mexico, AY298542, AY346591, AY346723, AY346863, AY347184, AY347016, AY298173; *S. meridense*, 1189, *Price et al. 1254* (DUKE), Bolivia, AY298540, AY346589, AY346721, AY346861, AY347182, AY347014, AY298174; *S. molle*, 391, *Andrus 8178* (DUKE), England, AY346499, AY346592, AY346724, AY346864, AY347186, AY347019, AF192624; *S. molle*, 392, *Andrus 8113* (DUKE), Ireland, AY298545, AY346593, AY346725, AY346865, AY347187, AY347020, AY298175; *S. molle*, 394, *Anderson 27323* (DUKE), USA: New Jersey, AY298546, AY346594, AY346726, AY346866, AY347188, AY347021, AY347099; *S. molle*, 395, *Risk & Kiser 7025* (DUKE), USA: Mississippi, AY298547, AY346595, AY346727, AY346867, AY347189, AY347022, AY298179; *S. molle*, 396, *Risk 6629* (DUKE), USA: Kentucky, AY298548, AY346596, AY346728, AY346868, AY347190, AY347023, AY298180; *S. molle*, 399, *MacDonald 3923* (DUKE), USA: Alabama, AY298549, AY346597, AY346729, AY346869, AY347191, AY347024, AY298181
- S. olafii*, 1179, *Flatberg et al. 288–00* (DUKE), Norway, AY298567, AY346608, AY346740, AY346880, AY347202, AY347035, AF192599; *S. oxyphyllum*, 611, *Lewis 89–953d-3* (MO), Bolivia, AF193685, AY346609, AY346741, AY346881, AY347203, AY347036, AY298182
- S. rubellum*, 306, *Shaw 95–27–6a* (DUKE), USA: New York,

- AF193730, AY347104, AY346934, AY346521, AY346659, AY346793, AY297999; *S. rubellum*, 563, *Shaw* 9842 (DUKE), Finland, AY298619, AY346610, AY346742, AY346882, AY347204, AY347037, AY298200; *S. rubellum*, 564, *Shaw* 9643 (DUKE), Norway, AY298620, AY346611, AY346743, AY346883, AY347205, AY347038, AF192623; *S. rubellum*, 565, *Shaw* 9646 (DUKE), Norway, AY298621, AY346612, AY346744, AY346884, AY347206, AY347039, AF192614; *S. rubellum*, 566, *Shaw* 9646 (DUKE), Norway, AY298622, AY346613, AY346745, AY346885, AY347207, AY347040, AY298253; *S. rubellum*, 572, *Shaw* 9679 (DUKE), Norway, AY346508, AY346614, AY346746, AY346886, AY347208, AY347041, AY298254; *S. rubellum*, 579, *Shaw* 9842 (DUKE), Finland, AY298624, AY346615, AY346747, AY346887, AY347209, AY347042, AY298255; *S. rubellum*, 886, *Cronberg* s.n. (DUKE), Russia: Siberia, AY298409, AY346547, AY346685, AY346819, AY347133, AY346964, AY347100; *S. rubellum*, 891, *Cronberg* s.n. (DUKE), Russia: Siberia, AY298718, AY346656, AY346788, AY346928, AY347253, AY347088, AY298041; *S. rubellum*, 1281, *Muselman* 99157 (DUKE), USA: Michigan, AY298684, AY346636, AY346769, AY346909, AY347231, AY347066, AY298353; *S. rubellum*, 1322, *Nelson* 17459 (DUKE), USA: South Carolina, AY298715, AY346653, AY346785, AY346925, AY347247, AY347082, AF192609; *S. rubiginosum*, 583, *Shaw* 9630 (DUKE), Norway, AF193742, AY346616, AY346748, AY346888, AY347210, AY347043, AY298260; *S. russowii*, 322, *Shaw* 9266 (DUKE), USA: New York, AF193731, AY346617, AY346749, AY346889, AY347211, AY347044, AY298261; *S. russowii*, 577, *Shaw* 9681 (DUKE), Norway, AY298626, AY346618, AY346750, AY346890, AY347212, AY347045, AF192610; *S. russowii*, 588, *Anderson* 27411 (DUKE), USA: Washington, AY298627, AY346619, AY346751, AY346891, AY347213, AY347046, AY298262; *S. russowii*, 885, *Cronberg* s.n. (DUKE), Russia: Siberia, AY298628, AY346620, AY346752, AY346892, AY347214, AY347047, AF192611
- S. skyense*, 466, *Flatberg* s.n. H, Scotland, AF193737, AY346621, AY346753, AY346893, AY347215, AY347049, AY298185
- S. sp.*, 1320, *Shaw* 11015 (DUKE), Ecuador, AY298552, AY346598, AY346730, AY346870, AY347192, AY347025, AY298187; *S. sp.*, 1321, *Shaw* 10990 (DUKE), Ecuador, AY298553, AY346599, AY346731, AY346871, AY347193, AY347026, AY298188; *S. sp.*, 1359, *Shaw* 11215 (DUKE), Ecuador, AY298554, AY346600, AY346732, AY346872, AY347194, AY347027, AY298189; *S. sp.*, 1360, *Shaw* 11235 (DUKE), Ecuador, AY298555, AY346601, AY346733, AY346873, AY347195, AY347028, AY298190; *S. sp.*, 1361, *Shaw* 11468 (DUKE), Ecuador, AY298556, AY346602, AY346734, AY346874, AY347196, AY347029, AY298191; *S. sp.*, 1362, *Shaw* 11195 (DUKE), Ecuador, AY298557, AY346603, AY346735, AY346875, AY347197, AY347030, AY298192; *S. sp.*, 1363, *Shaw* 11267 (DUKE), Ecuador, AY298558, AY346604, AY346736, AY346876, AY347198, AY347031, AY298193; *S. sp.*, 1377, *Shaw* 11365 (DUKE), Ecuador, AY298559, AY346605, AY346737, AY346877, AY347199, AY347032, AY298283; *S. sp.*, 1378, *Shaw* 11390 (DUKE), Ecuador, AY298560, AY346606, AY346738, AY346878, AY347200, AY347033, AF192592; *S. sp.*, 1379, *Shaw* 11313 (DUKE), Ecuador, AY298561, AY347201, AY347034, AY346607, AY346739, AY346879, AY298284
- S. sparsum*, 128, *Linares & Churchill* 3957 (NY), Colombia, AY346509, AY346623, AY346755, AY346895, AY347217, AY347051, AF192592
- S. sparsum*, 613, *McQueen* 7172 (MO), Costa Rica, AF193694, AY346624, AY346756, AY346896, AY347218, AY347052, AF192581
- S. sparsum*, 633, *Linares & Churchill* 3957 (MO), Colombia, AY298648, AY346625, AY346757, AY346897, AY347219, AY347053, AY298285; *S. sparsum*, 1175, *Price et al.* 1457 (DUKE), Bolivia, AY298647, AY346622, AY346754, AY346894, AY347216, AY347050, AY298287; *S. squarrosom*, 103, *Belland & Schofield* 17919 (DUKE), Canada: NB, AF193708, AY346627, AY346759, AY346899, AY347221, AY347056, AY298295; *S. squarrosom*, 1204, *Long* 26424 (DUKE), India, AY298649, AY346626, AY346758, AY346898, AY347220, AY347054, AY298296; *S. squarrosom*, 1248, *Higuchi* 40888 (DUKE), Japan, AY298651, AY309647, AY309666, AY309684, AY309525, AY309749, AY298297; *S. subfulvum*, 545, *Shaw* 9824 (DUKE), Finland, AY298661, AY346631, AY346763, AY346903, AY347225, AY347060, AF192612; *S. subfulvum*, 1245, *Higuchi* 40898 (DUKE), Japan, AY298658, AY346628, AY346760, AY346900, AY347222, AY347057, AY298300; *S. subfulvum*, 1303, *Andrus* 8878 (DUKE), USA: Alaska, AY298659, AY346629, AY346761, AY346901, AY347223, AY347058, AY298303; *S. subfulvum*, 1304, *Shaw* 10236 (DUKE), USA: New Jersey, AY298660, AY346630, AY346762, AY346902, AY347224, AY347059, AF192613; *S. subnitens*, 538, *Shaw* 9723 (DUKE), Norway, AY298667, AY309649, AY309668, AY309685, AY309527, AY309751, AY298318; *S. subnitens*, 570, *Shaw* 9658 (DUKE), Norway, AF193741, AY346633, AY346766, AY346906, AY347228, AY347063, AY298319; *S. subnitens*, 1297, *Schofield* 109532 (DUKE), USA: Alaska, AY298664, AY346632, AY346764, AY346904, AY347227, AY347062, AY298320; *S. subtile*, 1279, *Shaw* 10266 (DUKE), USA: New Jersey, AY298682, AY346634, AY346767, AY346907, AY347229, AY347064, AY298321; *S. subtile*, 1280, *Summers* 7913 (DUKE), USA: Missouri, AY298683, AY346635, AY346768, AY346908, AY347230, AY347065, AY298322; *S. subtile*, 1282, *Allen* 22485 (DUKE), USA: Maine, AY298685, AY346637, AY346770, AY346910, AY347232, AY347067, AY298323; *S. subtile*, 1283, *Pedano* 476 (DUKE), USA: Maine, AY298686, AY346638, AY346771, AY346911, AY347233, AY347068, AY298324; *S. subtile*, 1284, *Risk et al.* 6875 (DUKE), USA: Kentucky, AY298687, AY346639, AY346772, AY346912, AY347234, AY347069, AY298325; *S. subtile*, 1285, *Price* 665 (DUKE), Canada: Quebec, AY298688, AY346640, AY346773, AY346913, AY347235, AY347070, AY298328; *S. subtile*, 1294, *Karlin* 9510-0723 (DUKE), USA: Virginia, AY298689, AY346641, AY346774, AY346914, AY347236, AY347071, AY298329
- S. tenerum*, 75, *Shaw* 9335 (DUKE), USA: North Carolina, AF193672, AY309650, AY309669, AY309686, AY309528, AY309752, AY298330; *S. tenerum*, 335, *Shaw* 9271 (DUKE), USA: North Carolina, AY298695, AY346646, AY346779, AY346919, AY347241, AY347076, AY298331; *S. tenerum*, 337, *Shaw* 9283 (DUKE), USA: South Carolina, AY298696, AY346647, AY346780, AY346920, AY347242, AY347077, AY298332; *S. tenerum*, 1039, *Andrus & Karlin* 7080 (NY), USA: New Jersey, AY298691, AY346642, AY346775, AY346915, AY347237, AY347072, AY298333; *S. tenerum*, 1181, *Buck* 30560 (DUKE), USA: Georgia, AY298692, AY346643, AY346776, AY346916, AY347238, AY347073, AF192588; *S. tenerum*, 1192, *Shaw* 10217 (DUKE), USA: New Jersey, AY298693, AY346644, AY346777, AY346917, AY347239, AY347074, AY298337; *S. tenerum*, 1197, *Clampitt* 1111 (DUKE), USA: Virginia, AY298694, AY346645, AY346778, AY346918, AY347240, AY347075, AF192596; *S. teres*, 366, *Hedderon* 7928 (DUKE), Canada: British Columbia, AF193720, AY309651, AY309670, AY309687, AY309529, AY309753, AY298349; *S. teres*, 1099, *Flatberg* 161833 (DUKE), Norway, AY298701, AY361686, AY361844, AY361263, AY361120, AY361362, AY298350; *S. teres*, 1232, *Flatberg et al.* 309-00 (DUKE), Norway, AY298702, AY346650, AY346783, AY346923, AY347245, AY347080, AY298351; *S. teres*, 1369, *Shaw* 10189 (DUKE), Canada: Alberta, AY298455, AY346553, AY346691, AY346825, AY347140, AY346971, AY347102
- S. warnstorffii*, 371, *Schofield* 99947 (DUKE), USA: Alaska, AY298716, AY346654, AY346786, AY346926, AY347249, AY347084, AY298357; *S. warnstorffii*, 580, *Shaw* 9818 (DUKE), Finland, AY346519, AY346655, AY346787, AY346927, AY347251, AY347086, AY298358; *S. wulfianum*, 98, *Bowers* 22980 (DUKE), USA: Minnesota, AF193712, AY346658, AY346792, AY346932, AY347257, AY347093, AY298041; *S. wulfianum*, 1288, *Shaw* 9855 (DUKE), Finland, AY298722, AY309652, AY309671, AY309688, AY309530, AY309754, AY298194; *S. wulfianum*, 1382, *Ireland* 23753 (DUKE), Canada: Ontario, AY298723, AY346657, AY346791, AY346931, AY347256, AY347092, AY298353