

VARIATION IN “BIODIVERSITY VALUE” OF PEATMOSS SPECIES IN *SPHAGNUM* SECTION *ACUTIFOLIA* (SPHAGNACEAE)¹

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Species are the most common currency by which biodiversity is measured, but species are not equivalent in “biodiversity value” because of differences in phylogenetic history and current population processes. Morphologically defined species in *Sphagnum* section *Acutifolia* (Bryophyta) were compared with regard to how phylogenetically distinct each is from its sister species and how much nucleotide variation each encompasses. Comparisons were based on sequence variation at seven nuclear and chloroplast loci. Assignment of collections to morphospecies accounted for about 57–75% of the overall nucleotide variation at the seven loci, but morphospecies differed greatly in how much nucleotide diversity they encompass. In addition, morphospecies varied widely in their genetic distinctiveness, estimated as the length of the stem branch from the most recent common ancestor and numbers of differentially fixed and shared polymorphic nucleotides among taxa. Levels of molecular diversity within morphospecies were not correlated with their degree of isolation. Factors that affected the biodiversity value of species include age, mode of origin, demographic history, and reproductive biology.

Key words: *Acutifolia*; biodiversity value; molecular diversity; nucleotide diversity; peat mosses; *Sphagnum*.

Systematics at the species level is central to conservation biology (Humphries et al., 1995; Soltis and Gitzendanner, 1999; Crandall et al., 2000; Amos and Balmford, 2001; Barber, 2002; Funk et al., 2002; Funk and Richardson, 2002; Moritz, 2002; Sechrest et al., 2002; Wayne and Morin, 2004). Among other things, systematic studies can identify monophyletic groups that are in many cases the most appropriate units on which to focus conservation efforts (Moritz, 1994, but see also Crandall et al., 2000).

Biodiversity studies typically focus on species as the units for comparison and global analyses often use community and regional endemism as measures of “uniqueness” and “irreplaceability” of the biodiversity in an area (Meyers et al., 2000; Hobohm, 2003). There has been extensive debate about how to define and recognize species (e.g., Mishler, 1985; Baum and Donoghue, 1995; Davis, 1997; Wheeler and Meier, 2000). Some argue that species are constructs (e.g., Hendry et al., 2000) whereas others cite empirical data to support an objective reality to species as discrete units of evolution and biodiversity (Wilson, 1992; Avise and Walker, 1999; Coyne and Orr, 2004). Nevertheless, there does seem to be universal agreement that species are not phylogenetically equivalent units of biodiversity (Faith, 1992, 1994). Researchers over the past 30 years have amply demonstrated that species differ in genetic structure because of demographic and evolutionary processes such as gene flow, changes in population size, natural selection, and genetic drift (Hamrick and Godt, 1990; Buckler and Thornsberry, 2002). Species also differ in age and the inherited genetic background that results from their deeper phylogenetic history.

Correlations between biodiversity and ecosystem function have been documented, but the patterns are complex (Grime,

1997; Tilman et al., 1997; Loreau et al., 2001), and the cause and effect relationship between biodiversity and ecosystem processes may be bidirectional (Worm and Duffy, 2003). One contentious issue is the extent to which species are equivalent and hence mutually replaceable; are ecosystem functions more closely related to biodiversity levels per se than to community composition? Population-level biodiversity may be as or more critical to ecosystem function than species-level patterns of biodiversity (Luck et al., 2003). Genetic variation is an important component of any population-level estimate of biodiversity.

The potential non-equivalence of species as evolutionary units complicates the interpretation of biodiversity patterns. Simply counting species as the units of biodiversity is crude at best and may be positively misleading if non-equivalent evolutionary units are also non-equivalent in terms of their contributions to ecosystem function. Recognition that units assigned the rank of species are often non-equivalent has led to alternative metrics for quantifying levels of biodiversity (e.g., Faith, 1992, 1994).

Sphagnum is one of the largest genera of mosses and dominates many habitats in which it occurs. *Sphagnum*-dominated peatlands are widespread throughout the boreal zone of the Northern Hemisphere. These peatlands, constituting a large and important pool of global carbon, are involved in the transfer of other atmospheric gases that affect global climate and affect regional patterns of hydrology (Gorham, 1991). Boreal peatlands provide habitat for diverse organisms including many endemics (Crum, 1988; Hingley, 1993). However, *Sphagnum* is arguably one of the most taxonomically difficult genera of mosses; what constitutes a species of *Sphagnum* is often controversial. In North America, for example, Andrews (1913) recognized 39 species, Crum (1984) recognized 51 species and 21 varieties, and Anderson (1990) included 72 species in his North American checklist. McQueen and Andrus (2003) included 90 species in their recent treatment of *Sphagnum* for the Flora of North America project.

Global patterns of *Sphagnum* biodiversity based on nucle-

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7 genes

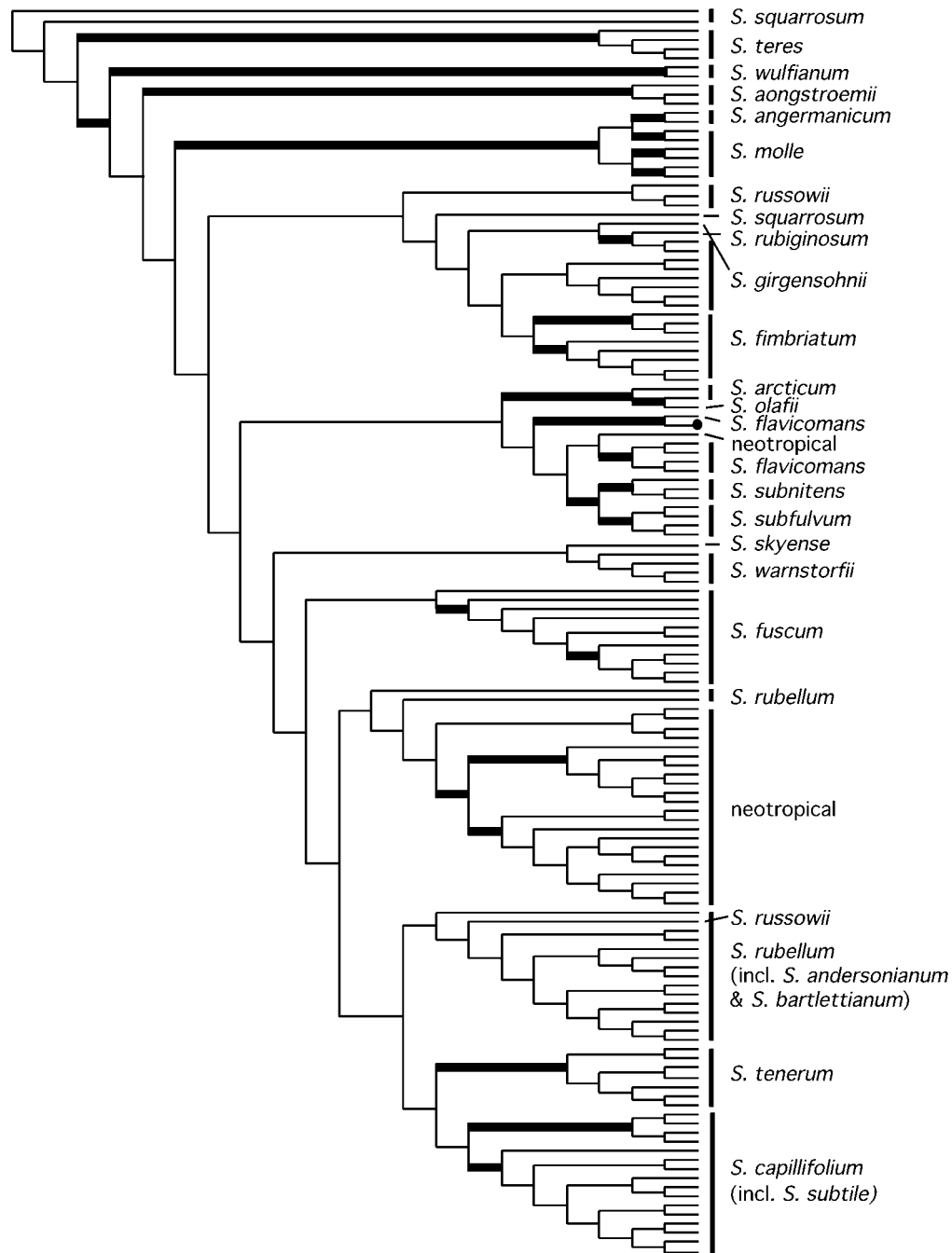


Fig. 1. Phylogenetic reconstruction for *Sphagnum* sect. *Acutifolia* based on heterogeneous Bayesian analyses of seven genomic regions, from Shaw et al. (2005). Several of the species included in this tree (*S. arcticum*, *S. olafii*, *S. russowii*, and *S. skyense*) were not included in some or all of the present genetic analyses (see Materials and Methods for additional details). Thick branches are supported at $\geq 95\%$ Bayesian joint posterior probability.

otide sequence data were analyzed by Shaw et al. (2003b). The present study is an analysis of biodiversity patterns in *Sphagnum* section *Acutifolia*, focusing on genetic data so that inferences are not constrained by particular species concepts. Section *Acutifolia* is one of four large monophyletic groups within *Sphagnum* (Shaw, 2000; Shaw et al., 2003a). Species of sect. *Acutifolia* are widespread and abundant throughout the boreal zone of North America, Europe, and Asia, and include

major peat formers such as *S. fuscum*. Species of sect. *Acutifolia* are also fairly abundant at high altitudes of the South American Andes mountains (Andrews, 1947; Crum, 1990), but the section is absent from other non-boreal regions or is represented by one or two disjunct northern species. Phylogenetic relationships within section *Acutifolia* were described by Shaw et al. (2005) and are reproduced here as Fig. 1.

The goals of this research were to compare morphologically

defined species of *Sphagnum* sect. *Acutifolia* with regard to their “biodiversity value.” Species differ along two important axes of variation: the degree of phylogenetic isolation from their nearest relatives and the amount of genetic and/or phenotypic (including ecological) diversity they encompass. Genetic distance from congeners has been used as a proxy for time since divergence in studies of both plants (Moyle et al., 2004) and animals (Coyne and Orr, 1997; reviewed in Coyne and Orr, 2004). However, genetic and phylogenetic architecture, including interspecific distances, reflect both time per se and demographic/reproductive processes since divergence. Likewise, the degree of infraspecific genetic variation is determined by age, reproductive processes, and demographic factors. This study describes variation in these two axes of species architecture in *Sphagnum* sect. *Acutifolia*, and relates the results to information about relative taxon ages and other features of their biology, including morphological distinctiveness and breeding system. Species are indeed heterogeneous, but it is clear that age alone is insufficient to fully explain variation in either parameter of species biodiversity value.

MATERIALS AND METHODS

Population sampling—The majority of data for the analyses presented here were previously reported by Shaw et al. (2005) in a phylogenetic analysis of *Sphagnum* sect. *Acutifolia*. These data include 136 populations of sect. *Acutifolia* ($N = 129$) and its sister group, sect. *Squarrosa* ($N = 7$). Of the 136 samples, 66 (49%) are from North America, 30 (22%) from Europe, 23 (17%) from South or Central America (including Mexico), eight (6%) from northern Asia (Russia), eight (6%) from Japan, and one from India. Samples include almost all the northern hemisphere species of sect. *Acutifolia*; species-level systematic knowledge about the South American taxa is too crude to reliably specify how many of the putative species are included. Collection localities, specimen information, and GenBank accession numbers were provided in Shaw et al. (2005). Each population was represented by nucleotide sequences from seven loci; the data set described by Shaw et al. (2005) was complete (i.e., no locus was missing for any population). Additional populations not included in the previous study (because some loci were unavailable for some populations) were added for the present analyses of infraspecific nucleotide diversity to maximize population sampling. Voucher information, collection localities, and GenBank accession numbers for these additional populations are provided in the Appendix.

All specimens were assigned to morphospecies by J. Shaw and L. E. Anderson (Duke University), following the nomenclature of Anderson (1990). *Sphagnum* collections frequently contain interspecific mixtures; hence, the individual stem from which DNA was extracted was placed in a separate small packet within the larger herbarium specimen (preserved in DUKE) so that all identifications can be verified.

A description of cladistic structure within sect. *Acutifolia*, based on analyses of the seven separate genes and on the combined data set, was provided by Shaw et al. (2005). The all-compatible splits tree from the posterior probability distribution identified by Bayesian analyses of the combined data is reproduced here as Fig. 1. Some but not all of the morphospecies are well supported as monophyletic. In several species, a core of populations is demonstrably monophyletic, but one or more populations appear to be admixtures of two or more species. One population was such an extreme genetic admixture that it could not be unambiguously assigned to any single species (no. 1204 in Shaw et al., 2005); hence, it was deleted from the present analyses. Other plants that appear to be genetic admixtures are generally aberrant for only one or two loci and are included in the morphospecies to which they best fit genetically and morphologically. In some cases, analyses are reported for more inclusive clades that include two or three closely related species—*S. girgensohnii* plus *S. fimbriatum*, and *S. flavicomans*, *S. subnitens*, and *S. subfulvum*. These clades represent well-supported monophyletic groups (based

on results described in Shaw et al., 2005), and the morphospecies included within them are closely related in terms of both morphology and molecules.

For purposes of the molecular biodiversity analyses, *S. olafii* and *S. arcticum*, both restricted to arctic regions (Flatberg and Frisvoll, 1984; Flatberg, 1993a), were combined as one species because phylogenetic analyses indicate that the two cannot be distinguished by the seven loci used in this study (Shaw et al., 2005).

Sphagnum rubiginosum, a rare species known from scattered arctic and subarctic localities in North America and Europe, is morphologically similar to *S. girgensohnii* (Flatberg, 1993b). It is phylogenetically nested within *S. girgensohnii* and was deleted from the current analyses. *Sphagnum skyense* Flatb., known only from a single locality in Scotland (Flatberg, 1988), appears to be of hybrid ancestry (Shaw et al., 2005) and was also excluded from these analyses. *Sphagnum russowii*, a widespread and common circumboreal allopolyploid species (Cronberg, 1996), was included in the analyses of molecular diversity, but not in the phylogenetically based estimates of stem clade lengths. Populations from the neotropics (Mexico to South America) presented special problems with regard to assigning plants to morphospecies. Three taxa, *S. limbatum*, *S. meridense*, and *S. sparsum*, are relatively well known (e.g., Allen, 1994; Sharp et al., 1994), whereas the taxonomy of *Sphagnum* sect. *Acutifolia* in South America is much in need of revision (Andrews, 1947; Crum, 1990). For purposes of molecular diversity analyses, all the neotropical accessions were combined and are referred to as the “neotropical” taxon. Previous phylogenetic analyses weakly support the neotropical populations as monophyletic but with little well-supported cladistic structure within the group (Shaw et al., 2005). One population was an outlier and may contain genetic material from other boreal species of sect. *Acutifolia* (Shaw et al., 2005); it is retained in the current analyses as a member of the neotropical taxon. For the analyses of genetic distinctiveness, *S. limbatum*, *S. meridense*, *S. sparsum*, and the remaining neotropical accessions were distinguished as four separate taxa. Results are also reported for all neotropical accessions combined as a single group.

Molecular methods—Analyses are based on nucleotide sequences from seven genomic regions. Chloroplast DNA sequences were obtained from 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*). It is not clear whether these latter introns are from one gene or if two different *LEAFY* paralogues are amplified. Three anonymous regions were identified from RAPDs (random amplified polymorphic DNA) as described in Shaw et al. (2003a) and are assumed to be nuclear because they do not BLAST (Basic Local Alignment Search Tool) to known chloroplast or mitochondrial genes. These regions are referred to as *RapdA*, *RapdB*, and *RapdF*. The occurrence of numerous indels and the absence of an open-reading frame (ORF) in the three regions indicate that they are noncoding sequences. Primer sequences and sequencing protocols for the seven regions are described in Shaw et al. (2003a).

Phylogenetic analyses—Shaw et al. (2005) described the analytical methods used to reconstruct phylogenetic relationships in sect. *Acutifolia*. 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) and heterogeneous Bayesian analyses were conducted with each genomic region allowed to evolve according to its own model of substitution.

Genetic analyses—The degree to which the classification of samples into morphospecies “captures” information about patterns of molecular biodiversity was tested using analysis of molecular variance (AMOVA), implemented in ARLEQUIN (Schneider et al., 2000). Molecular diversity was partitioned separately for each locus among morphospecies and among population samples within morphospecies. Molecular variation within morphospecies was summarized by theta (θ_s) using ARLEQUIN (Schneider et al., 2000).

The degree to which each morphospecies is phylogenetically isolated was

TABLE 1. Partitioning of nucleotide variation among and within morphospecies of *Sphagnum* section *Acutifolia* revealed by separate single-gene AMOVAs.

Genomic region	Species variation (%)	
	Among	Within
ITS	66.59*	33.41
<i>Leafy 1</i>	66.20*	33.80
<i>Leafy 2</i>	63.99*	36.01
<i>RapdA</i>	74.69*	25.31
<i>RapdB</i>	64.60*	35.40
<i>RapdF</i>	56.54*	43.46
<i>trnL</i>	70.28*	29.72

* = $F_{ST} > 0$ at $P < 0.00001$.

estimated in two ways. The length of the branch leading to the species from its most recent common ancestor was estimated, and these values are hereafter referred to as stem clade lengths. Branch lengths were optimized on trees derived from Bayesian phylogenetic analyses of data sets in which each species was represented by a single accession. Phylogenetic uncertainty was taken into account by conducting 20 separate Bayesian analyses, each time selecting one population at random to represent each species. Some species are represented in the full data set by only one or two populations, whereas others are represented by 10–16. Thus, some samples were included in more than one analysis. Nevertheless, each of the 20 replicate analyses was based on a unique combination of samples. Stem clade lengths for each morphospecies included in the analyses was summarized by a mean (\pm SD, that reflects phylogenetic uncertainty).

As another measure of distinctiveness, the numbers of nucleotide sites with shared polymorphic nucleotides were estimated for all pairwise combinations of species using SITES (Hey and Wakeley, 1997). The numbers of fixed nucleotide differences among species were similarly estimated.

Sphagnum arcticum, *S. olafii*, *S. skyense*, and *S. russowii*, shown previously to be genetic admixtures of likely hybrid origin, were excluded from the analyses of stem clade lengths. *Sphagnum russowii*, a common and widely recognized species, was included in the summaries of shared polymorphisms and fixed differences.

RESULTS

Molecular diversity within and among morphospecies—

Locus by locus analyses of molecular variation (AMOVAs) indicate that the percentage of total variation attributable to molecular differentiation among morphospecies ranges from 57% for *RapdF* to 75% for *RapdA* (Table 1). Differences among morphospecies account for 60–70% of the total variation in the other five loci, with the remaining variation attributable to differences among intraspecific populations.

Averaged across the seven loci, levels of nucleotide diversity (θ_{π}) within species of sections *Acutifolia* and *Squarrosa* ranged from 0.0016 (*S. teres*) to 0.0362 (*S. girgensohnii*) (Table 2). Both species of sect. *Squarrosa*, namely *S. squarrosum* and *S. teres*, are characterized by relatively low diversity. Data for a third recently described arctic species of sect. *Squarrosa*, *S. tundrae*, are only available for ITS and *trnL*. Two *S. tundrae* populations from Svalbard had identical ITS haplotypes, although they differed at six sites in *trnL*.

In some cases, putative sister species (Fig. 1) differ substantially in molecular diversity (Table 2). *Sphagnum girgensohnii*, for example, contains higher nucleotide diversity than *S. fimbriatum* at five of the seven loci (data for individual loci not shown). *Sphagnum tenerum*, an eastern North American endemic, is sister to either *S. rubellum* or *S. capillifolium*, both of which are circumboreal, and is notably low in genetic di-

TABLE 2. Nucleotide variation (as θ_{π} ; mean number of pairwise nucleotide differences) within sect. *Acutifolia*, summarized across seven loci; total aligned sequence lengths = 5176 nucleotides. These estimates are based on the data set that consists of 129 accessions for which complete data are available for all seven loci.

Species	N	θ_{π}
<i>S. angermanicum</i>	2	0.0119 \pm 0.0120
<i>S. aongstroemii</i>	3	0.0086 \pm 0.0065
<i>S. arcticum</i>	3	0.0044 \pm 0.0034
<i>S. capillifolium</i>	16	0.0104 \pm 0.0053
<i>S. fimbriatum</i>	10	0.0219 \pm 0.0117
<i>S. flavicomans</i>	5	0.0058 \pm 0.0036
<i>S. fuscum</i>	10	0.0111 \pm 0.0060
<i>S. girgensohnii</i>	8	0.0362 \pm 0.0198
<i>S. molle</i>	6	0.0142 \pm 0.0083
“neotropical”	21	0.0146 \pm 0.0073
<i>S. rubellum</i>	16	0.0119 \pm 0.0061
<i>S. russowii</i>	4	0.0114 \pm 0.0075
<i>S. squarrosum</i>	2	0.0031 \pm 0.0032
<i>S. subfulvum</i>	4	0.0042 \pm 0.0280
<i>S. subnitens</i>	3	0.0034 \pm 0.0026
<i>S. tenerum</i>	7	0.0035 \pm 0.0020
<i>S. teres</i>	3	0.0016 \pm 0.0013
<i>S. warnstorffii</i>	6	0.0180 \pm 0.0105
<i>S. wulfianum</i>	2	0.0049 \pm 0.0050

versity relative to either of these putative sister species. *Sphagnum capillifolium* is closely related to *S. rubellum* although they are probably not sister species (because of *S. tenerum*). Levels of molecular diversity are higher at six of the seven loci in *S. rubellum*, by several hundred percent in some cases. Only in the chloroplast locus, *trnL*, is *S. capillifolium* more variable than *S. rubellum*.

Phylogenetic distance among species—The mean number of fixed nucleotide differences between species, averaged across the whole section, is 46.7. Some species, for example *S. angermanicum*, *S. aongstroemii*, and *S. molle*, have a higher than average number of fixed differences in relation to other species of sect. *Acutifolia* (Table 3). *Sphagnum fuscum*, on the other hand, has relatively few fixed differences relative to other species in the section. Surprisingly, the 21 neotropical accessions also have relatively few fixed differences relative to the boreal species. In particular, the neotropical group is distinguished from *S. fuscum* and *S. warnstorffii* by only one fixed difference each, and from *S. rubellum* by two fixed differences.

Patterns of shared polymorphic nucleotides among species largely parallel numbers of fixed differences (Table 3). *Sphagnum angermanicum*, *S. aongstroemii*, and *S. molle* share few segregating nucleotides with other species. The mean number of sites with shared polymorphic nucleotides among all pairwise comparisons of species in sect. *Acutifolia* species is 6.5. Many pairs of species have fewer than five sites with shared polymorphic nucleotides, but some pairs of morphospecies share high numbers (e.g., >15 sites) of segregating nucleotides (Table 3).

The neotropical group of accessions shares a high number of polymorphic nucleotides with some of the boreal species; in particular, *S. fuscum* (20 sites), *S. rubellum* (34), and *S. warnstorffii* (27), and to a lesser extent, *S. capillifolium* (13) and *S. wulfianum* (16) (Table 3). In general, *S. fuscum*, *S. rubellum*, and *S. warnstorffii* share a high number of segregating bases with each other, as well as with the neotropical group. *Sphagnum russowii* shares relatively high numbers of bases at

TABLE 3. Number of nucleotide sites with fixed differences (above the diagonal) and shared polymorphisms (below the diagonal) among morphospecies of *Sphagnum* section *Acutifolia*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. <i>S. angermanicum</i>	—	109	93	93	97	92	87	27	82	91	112	101	95	132	86	66	97
2. <i>S. aongstroemii</i>	0	—	72	69	93	66	64	82	53	68	88	87	79	111	62	41	74
3. <i>S. arcticum/olafii</i>	0	0	—	34	34	22	31	65	10	22	41	28	22	59	14	21	32
4. <i>S. fimbriatum</i>	2	0	2	—	61	39	0	62	24	38	59	58	47	81	30	2	30
5. <i>S. flavicomans</i>	0	0	0	0	—	35	60	68	22	37	60	34	33	78	32	42	51
6. <i>S. fuscum</i>	0	0	1	5	0	—	38	64	1	5	20	34	28	34	3	21	12
7. <i>S. girgensohnii</i>	7	0	5	27	0	4	—	60	23	37	55	53	43	76	29	0	31
8. <i>S. molle</i>	0	3	1	4	0	6	5	—	55	64	85	74	67	104	59	40	68
9. "neotropical"	0	6	3	4	1	20	5	6	—	2	17	17	12	34	1	14	10
10. <i>S. rubellum</i>	0	0	3	3	0	24	10	4	34	—	0	33	24	24	0	22	6
11. <i>S. capillifolium</i>	0	0	0	1	0	7	3	1	13	17	—	55	41	45	0	39	47
12. <i>S. subfulvum</i>	0	0	3	1	0	3	1	3	3	2	0	—	23	73	29	38	47
13. <i>S. subnitens</i>	0	0	0	1	0	3	1	0	3	5	0	0	—	59	21	28	40
14. <i>S. tenerum</i>	0	1	0	1	0	1	1	1	2	2	0	0	0	—	26	57	46
15. <i>S. warnstorffii</i>	0	0	1	1	1	23	8	3	27	40	14	0	4	0	—	18	7
16. <i>S. wulfianum</i>	1	2	2	14	0	10	31	6	16	15	5	2	2	1	13	—	21
17. <i>S. russowii</i>	0	0	3	4	2	6	23	4	6	13	4	1	0	1	10	19	—

polymorphic sites with *S. girgensohnii* (23) and *S. rubellum* (13) and also with *S. wulfianum* (19). *Sphagnum russowii* is likely an allopolyploid (Cronberg, 1996) and both *S. girgensohnii* and *S. rubellum* have been implicated as possible parents. *Sphagnum wulfianum*, a species sometimes classified in its own section, *Polyclada*, shares high numbers of segregating bases with *S. fimbriatum* (14), *S. girgensohnii* (31), *S. rubellum* (15), *S. warnstorffii* (13), and the neotropical group (16) (Table 3).

Clade stem branch lengths leading to morphospecies are highly variable, confirming the impression from fixed differences and numbers of shared polymorphic sites that species of sect. *Acutifolia* are heterogeneous in the degree of genetic and phylogenetic isolation. The most phylogenetically distinct species are *S. aongstroemii* and *S. wulfianum* (Fig. 2). The clade that includes accessions of *S. molle* and *S. angermanicum* is also highly distinct, although the two morphospecies themselves are not mutually monophyletic relative to one another. The two species are easy to distinguish morphologically, but phylogenetic analyses indicate that *S. angermanicum* may be nested within *S. molle* (Fig. 1).

Three species from tropical America that can be separated on morphological grounds from the remaining neotropical accessions, *S. limbatum*, *S. meridense*, and *S. sparsum*, are very closely related to their nearest relatives, as indicated by very short stem clade branch lengths (Fig. 2). *Sphagnum flavicomans*, *S. subnitens*, and *S. subfulvum* are morphologically similar species but are resolved as mutually monophyletic (Fig. 1). The inclusive clade that includes these taxa is also on a short branch relative to other species of sect. *Acutifolia*.

Phylogenetic distinctiveness of morphospecies, quantified as the length of the stem branch leading to morphospecies, is not correlated with nucleotide diversity within species (Fig. 3).

DISCUSSION

Morphologically defined species in *Sphagnum* sect. *Acutifolia* are not equivalent with regard to molecular biodiversity. Morphospecies differ in levels of nucleotide variation that they encompass and their degree of genetic/phylogenetic separation from closely related species. These two aspects of molecular biodiversity are themselves uncorrelated and result in hetero-

geneous units of molecular diversity being described as species. Some morphospecies are highly polymorphic but closely related to congeners, some are less diverse but more isolated, and yet others are both diverse and isolated. Comparisons between the numbers of species in different peatland communities do not incorporate these meaningful differences at the genetic level. Measures of biodiversity within *Sphagnum*, based on species richness where each species is considered equivalent, yield different inferences about global geographic patterns in peatmoss biodiversity than those based on molecular diversity without regard to traditional definitions of taxonomic species (Shaw et al., 2003b).

Species are also heterogeneous in terms of morphological isolation. Some are very distinctive, whereas others intergrade within complexes of closely related forms. Such complexes in *Sphagnum* have engendered taxonomic disputes about the delimitation of species for more than a century (Lindberg, 1882; Warnstorff, 1911; Sjörs, 1944; Hill, 1976; Andrus, 1980; Crum, 1984; Flatberg, 1985, 1992; McQueen, 1989; Daniels and Eddy, 1990; Cronberg, 1996). Similar systematic patterns occur in each of the other three large sections of *Sphagnum*. In section *Subsecundum*, species delimitation is especially difficult in the so-called *S. subsecundum* complex, which includes one (Crum, 1984) to four or more species (McQueen and Andrus, 2003); in section *Sphagnum*, the *S. imbricatum* complex is controversial (Flatberg, 1986), and in section *Cuspidata*, there is much disagreement about how to define species in the *S. recurvum* complex (Såstad, 1998). In each section there are also species with numerous autapomorphies.

Complexes of closely related species occur in many other groups of plants. Sometimes clusters of genetically close, morphologically similar species are associated with agamous modes of reproduction, interspecific hybridization, or both (Stebbins, 1950; Grant, 1981). Especially notorious complexes of microspecies occur in the angiosperm genera, *Alchemilla*, *Crataegus*, *Rubus*, and *Taraxacum*, (Richards, 1973, 1997; Grant, 1981). Mayr (1992) estimated that almost 10% of 838 species making up a local Massachusetts flora were difficult to distinguish morphologically from related congeners, while the majority of species were more distinct. Moss species in general tend to be more distinct, at least in terms of isozyme

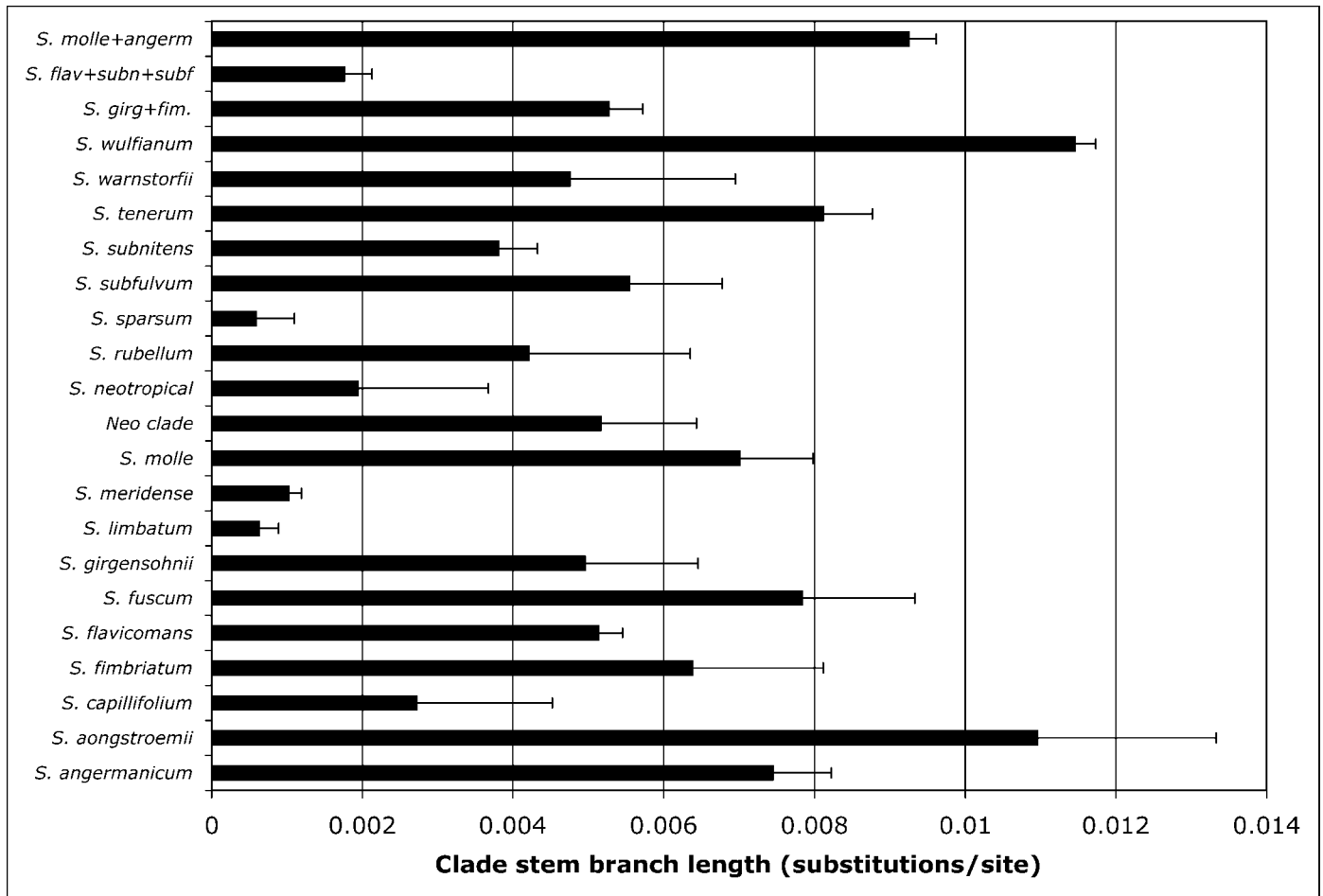


Fig. 2. Stem branch lengths (substitutions/site) for morphospecies in *Sphagnum* section *Acutifolia*. Mean branch lengths (+SD) for each species based on separate phylogenetic analyses with random sampling of accessions to include one sample of each morphospecies in each data set.

loci, than are congeneric angiosperm species (Wyatt et al., 1989). Hedrén (2004) explored different species delimitations, from narrow (taxonomic “splitting”) to broad (taxonomic “lumping”) in the difficult *Carex flava* complex relative to the partitioning of genetic variation at isozyme loci. Alternative taxonomic approaches affected not only the total amount of variation encompassed by “species,” but also the partitioning of variation within and among populations. Under the best of circumstances, species are non-equivalent in biodiversity value. Different taxonomic practices make them non-equivalent in different ways.

Everything else being equal, old species that diverged early might have had time to accumulate high levels of genetic diversity and autapomorphic mutations that distinguish them from congeners. Genetic/phylogenetic isolation would therefore be positively correlated with age, at least until mutational saturation destroys any detectable pattern.

Sphagnum aongstroemii and *S. wulfianum* are the most phylogenetically isolated among species of sect. *Acutifolia*. These species have each been separated from the *Acutifolia* as monospecific sections of *Sphagnum* based on morphological differences (Warnstorff, 1911; Crum, 1984; Flatberg, 2002; McQueen and Andrus, 2003). They do in fact appear to represent early diverging lineages within the section *Acutifolia*, and their genetic isolation probably reflects their relatively great age.

Nevertheless, phylogenetic analyses suggest that age is not the primary factor underlying differences in genetic distance among *Sphagnum* taxa.

An especially clear example of factors other than age as determinants of phylogenetic distinctiveness (as measured by stem clade lengths and fixed nucleotide differences) comes from *S. tenerum*. *Sphagnum tenerum* is a relatively recent species and is morphologically very close to *S. capillifolium*, within which Crum (1984) considered it a variety. However, *S. tenerum* is one of the most genetically distinct in sect. *Acutifolia*. Reconstructions are ambiguous with regard to whether *S. tenerum* is sister to *S. capillifolium* or *S. rubellum* (Shaw et al., 2005). However, the latter two have broad, circumboreal distributions, whereas *S. tenerum* is restricted to eastern North America and is common in the southeastern U.S. Low levels of genetic diversity, low numbers of sites with shared polymorphic nucleotides, and high levels of fixed differences relative to both *S. capillifolium* and *S. rubellum* are consistent with an origin of *S. tenerum* through peripheral isolation. Demographic factors, rather than age, must underlie the phylogenetic isolation of *S. tenerum*. A population bottleneck could have occurred either during or subsequent to speciation.

Sphagnum subfulvum, *S. subnitens*, and *S. flavicomans* are very similar morphologically and are closely related phylogenetically, but the species are each surprisingly distinctive in

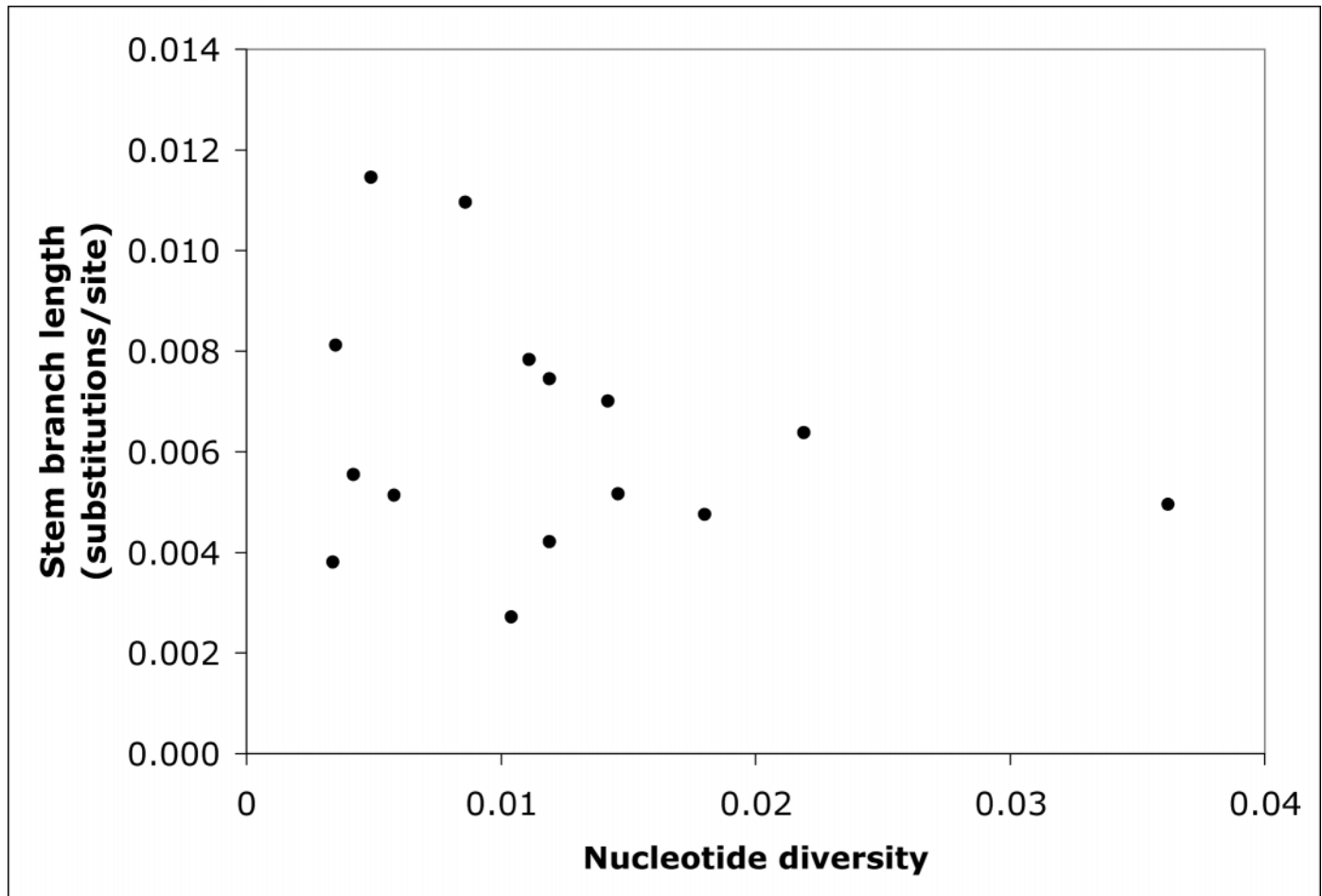


Fig. 3. Relationship between nucleotide diversity (θ_w) within species and stem branch length (substitutions/site) leading to the species.

terms of DNA sequences. Allopatric speciation involving cleavage of an ancestral geographic range into two more or less equal parts would, at least for some period of time, lead to high numbers of shared polymorphism between the daughter species. In contrast, a founder event, or speciation through peripheral isolation, could result in higher numbers of fixed differences and fewer shared polymorphisms, even in recently diverged species. Overall, demographic factors (including reproductive biology since mating patterns determine effective population sizes) appear to be more important determinants of species distinctiveness than age.

In addition to differences in the degree of genetic isolation, species of sect. *Acutifolia* are heterogeneous in levels of infraspecific nucleotide sequence variation, corroborating inferences from isozymes (Cronberg, 1996). Many species in sect. *Acutifolia* appear to be variable in gametophyte sexual expression (Crum, 1984), so it is difficult to relate levels of nucleotide diversity to mating system. There does not appear to be a clear correlation between nucleotide diversity and uni- vs. bisexual gametophytes. Correlations between life history traits and genetic structure in vascular plants have been extensively reviewed (Hamrick et al., 1979; Hamrick, 1989; Hamrick and Godt, 1990, 1996).

Levels of variation within species were not correlated with the degree of phylogenetic isolation among species. Given the number of different factors that can affect genetic diversity

within species and the degree of differentiation between them, it is not surprising that species are nonequivalent in biodiversity value. Nevertheless, it is interesting that assignment of plants to morphospecies accounts for roughly equivalent percentages of the total molecular diversity in each of the seven loci included in the present analyses. Roughly two thirds of the molecular diversity in each locus is partitioned among morphospecies, with the remaining third among conspecific populations. Morphospecies designations clearly do provide significant information about the architecture of molecular variation in sect. *Acutifolia*.

A close relationship between boreal and neotropical species of sect. *Acutifolia* inferred from the phylogenetic topology (Fig. 1; Shaw et al., 2005) is corroborated by shared polymorphism between pairwise combinations of species. Among the boreal taxa, *S. fuscum*, *S. rubellum*, and *S. warnstorffii* share especially high numbers of polymorphic nucleotides with neotropical populations, as do *S. capillifolium* and *S. wulfianum* to a lesser extent. These same taxa are implicated in the ancestry of neotropical *Acutifolia* in phylogenetic analyses, but different loci provide significantly incongruent inferences about relationships (Shaw et al., 2005). *Sphagnum fuscum* and *S. warnstorffii* also share significant numbers of polymorphic nucleotides, as well as high levels of isozyme similarity (Cronberg, 1996), with several other boreal species in addition to the neotropical taxa. It may be that these taxa provide conduits

through which genetic information is shared among multiple generally distinct species of sect. *Acutifolia*. Phylogenetic analyses suggest that while many morphospecies of sect. *Acutifolia* are monophyletic, interspecific hybridization is not rare and plants that appear to be genetic admixtures are not uncommon (Shaw et al., 2005). The ability to hybridize may not be uniform across sect. *Acutifolia* species and genetic data suggest that *S. fuscum*, *S. rubellum*, and *S. warnstorffii* might be especially promiscuous.

Morphological and molecular patterns in sect. *Acutifolia* suggest somewhat fussy boundaries among the species. There is much research yet to be done on two important classes of questions: why are the species as distinct as they are given some interbreeding, and why do the species not interbreed more frequently? Studies of mating patterns and reproductive isolation are fundamental to these questions as well as to understanding patterns of gene coalescence and the phylogenetic delimitation of species.

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APPENDIX. Accession information for specimens included in the molecular analyses. Information for previously published collections (marked as NA, below) are reported in Shaw et al. (in press).

Taxon; DNA isolate number; locality; *voucher specimen*; GenBank accession numbers: ITS; RAPDa; RAPDb; RAPDf; LEAFY-1; LEAFY-2; *trnL*.

Sphagnum aciphyllum Müll. Hat.; **1100**; Colombia; *Churchill & Betancur 18687* (MICH); AY346468; NA; NA; NA; NA; AY346933; NA.

Sphagnum andersonianum R.E. Andrus; **284**; USA: New York; *Shaw 95–27–7a* (DUKE); AY346469; NA; NA; NA; NA; NA. *S. andersonianum*; **285**; USA: New York; *Shaw 8007* (DUKE); AY346470; NA; NA; NA; NA; NA. *S. andersonianum*; **287**; USA: New York; *Shaw 8014* (DUKE); AY346471; NA; NA; NA; NA; NA. *S. andersonianum*; **307**; USA: New York; *Shaw 95–27–8a* (DUKE); AY346472; NA; NA; NA; NA; NA. *S. andersonianum*; **362**; USA: Maine; *Crum s.n.* (DUKE); AY346473; NA; NA; NA; NA; NA. *S. andersonianum*; **365**; USA: New York; *Andrus 8281* (DUKE); AY346474; NA; NA; NA; NA; NA. *S. andersonianum*; **377**; Ireland; *Andrus 8111b* (DUKE); AY298368; NA; NA; NA; AY347107; AY346937; AY298002. *S. andersonianum*; **378**; United Kingdom; *Andrus 8182* (DUKE); AY298369; NA; NA; NA; AY347108; AY346938; AY298003.

Sphagnum angermanicum Melin; **358**; USA: New Jersey; *Anderson 27360* (DUKE); AF193727; NA; NA; NA; NA; AY298026. *S. angermanicum*; **1375**; Canada: Nova Scotia; *Andrus 6703* (DUKE); NA; NA; NA; NA; AY347111; AY346941; NA. *S. angermanicum*; **1376**; Canada: New Brunswick; *Belland 14176* (DUKE); NA; NA; NA; NA; NA; AY346942; NA.

Sphagnum aongstroemii C. Hartum; **1098**; Norway; *Flatberg 161818* (DUKE); NA; NA; NA; NA; NA; NA; AY347094.

Sphagnum aureum C. B. McQueen; **1004**; Costa Rica; *McQueen 7165* (NY); AY298524; NA; NA; NA; AY347179; AY347010; AY298156.

Sphagnum bartlettianum Warnst.; **308**; USA: New York; *Shaw 8011* (DUKE); AY346475; NA; NA; NA; NA; NA. *S. bartlettianum*; **310**; USA: New York; *Shaw 8010* (DUKE); AY346476; NA; NA; NA; NA; NA. *S. bartlettianum*; **311**; USA: New York; *Shaw 8013* (DUKE); AY346477; NA; NA; NA; NA; NA. *S. bartlettianum*; **334**; USA: North Carolina; *Shaw 9285* (DUKE); AY346478; NA; NA; NA; NA; NA. *S. bartlettianum*; **340**; USA: South Carolina; *Shaw 9281* (DUKE); AY346479; NA; NA; NA; NA; NA. *S. bartlettianum*; **342**; USA: South Carolina; *Shaw 9284* (DUKE); AY346480; NA; NA; NA; NA; NA. *S. bartlettianum*; **345**; USA: North Carolina; *Shaw 9286* (DUKE); AY346481; NA; NA; NA; NA; NA. *S. bartlettianum*; **1314**; USA: Washington; *Andrus 7970* (DUKE); AY298386; NA; NA; NA; NA; NA; AY298020.

Sphagnum capillifolium (F. W. Weiss) Schrank; **283**; USA: New York; *Shaw 8045* (DUKE); AY346482; NA; NA; NA; NA; NA. *S. capillifolium*; **323**; USA: New York; *Shaw 9233* (DUKE); AY346483; NA; NA; NA; NA; NA. *S. capillifolium*; **324**; USA: New York; *Shaw 9231* (DUKE); AY346484; NA; NA; NA; NA; NA. *S. capillifolium*; **325**; USA: New York; *Shaw 9230* (DUKE); AY346485; NA; NA; NA; NA; NA. *S. capillifolium*; **326**; USA: New York; *Shaw 9232* (DUKE); AY346486; NA; NA; NA; NA; NA. *S. capillifolium*; **329**; USA: New York; *Shaw*

- 9258 (DUKE); AY346487; NA; NA; NA; NA; NA; NA. *S. capillifolium*; **332**; USA: New York; *Shaw 9256* (DUKE); AY346488; NA; NA; NA; NA; NA; NA. *S. capillifolium*; **333**; USA: New York; *Shaw 9255* (DUKE); AY346489; NA; NA; NA; NA; NA; NA. *S. capillifolium*; **372**; Canada: New Brunswick; *Belland 17944* (DUKE); AF193724; NA; NA; NA; NA; NA; NA. *S. capillifolium*; **578**; Norway; *Shaw 9683* (DUKE); AY298407; NA; NA; NA; NA; NA; NA. *S. capillifolium*; **607**; China; *Vitt 34803* (MO); AY346490; NA; NA; NA; NA; NA; NA.
- Sphagnum ecuadorensense* Warnst.; **866**; Ecuador; *Luteyn et al. 6554* (NY); AY298447; NA; NA; NA; AY347134; AY346965; AY298078.
- Sphagnum fimbriatum* Wilson; **96**; USA: Alaska; *Andrus & Talbot 8554* (DUKE); AF193673; NA; NA; NA; AY347145; AY346976; AF192591. *S. fimbriatum*; **888**; Russia: Siberia; *Cronberg s.n.* (DUKE); AY346492; NA; NA; NA; NA; NA; NA. *S. fimbriatum*; **890**; Russia: Siberia; *Cronberg s.n.* (DUKE); AY346491; NA; NA; NA; AY347143; AY346974; NA. *S. fimbriatum*; **1300**; USA: California; *Shevock 18330* (DUKE); AY298451; NA; NA; NA; NA; NA; AY298083.
- Sphagnum fuscum* Seooekt & H. A. Crum; **573**; Norway; *Shaw 9680* (DUKE); AY346493; NA; NA; NA; NA; NA; NA. *S. fuscum*; **575**; Norway; *Shaw 9680* (DUKE); AY298475; NA; NA; NA; NA; NA; AY346993; AY298107. *S. fuscum*; **584**; Ireland; *Andrus et al. 8088* (DUKE); AY346494; NA; NA; NA; NA; NA; NA. *S. fuscum*; **1251**; Norway; *Vanderpoorten 717* (DUKE); AY298471; NA; NA; NA; AY347158; AY346989; AY298104. *S. fuscum*; **1301**; USA: California; *Shevock 18333* (DUKE); AY298472; NA; NA; NA; NA; NA; AY298105.
- Sphagnum girgensohnii* H. A. Crum; **101**; USA: Alaska; *Schofield et al. 101946* (DUKE); AF193675; NA; NA; NA; AY347162; AY346994; AF192604. *S. girgensohnii*; **1177**; Norway; *Flatberg et al. 312–00* (DUKE); AY298477; AY346572; NA; AY346844; AY347163; AY346995; AY298109. *S. girgensohnii*; **1237**; Japan; *Tsukamoto MT-473* (DUKE); AY298479; NA; NA; NA; NA; NA; AY298111. *S. girgensohnii*; **1250**; Belgium; *Vanderpoorten 146* (DUKE); AY346495; NA; NA; NA; NA; AY347166; NA; NA.
- Sphagnum junghuhnianum* Dozy & Molck.; **628**; China; *Shevock 14370* (MO); AF193728; AY346583; NA; AY346855; AY347175; AY347006; AF192630. *S. junghuhnianum*; **453**; Papua New Guinea; *De Sloover 42750* (H); AY298511; AY346580; NA; AY346852; AY347172; AY347003; AY298143. *S. junghuhnianum*; **468**; Papua New Guinea; *Is-erentant B-22* (H); AY298512; AY346581; NA; AY346853; AY347173; AY347004; AY298144. *S. junghuhnianum*; **860**; Philippines; *Schwartz 3768* (DUKE); AY298513; AY346582; NA; AY346854; AY347174; AY347005; AY298145. *S. junghuhnianum*; **1174**; India; *Long 22368* (DUKE); NA; NA; NA; NA; NA; NA; AY347096.
- Sphagnum lewisii*; **635**; Colombia; *Lewis 88–1446* (MO); AF193701; AY346585; AY346718; AY346857; AY347177; AY347008; NA.
- Sphagnum limbatum* Mitt.; **99**; Mexico; *Norris 77524* (DUKE); AY346497; AY346587; NA; AY346859; AY347180; AY347012; AY347097. *S. limbatum*; **634**; Mexico; *Norris 77524* (MO); AY346496; NA; NA; NA; NA; AY347011; NA.
- Sphagnum mendocinum*; **376**; USA: California; *Mishler 3760* (DUKE); AY346518; NA; NA; NA; AY347250; AY347085; NA.
- Sphagnum molle* Sull.; **303**; USA: South Carolina; *Shaw 8729* (DUKE); AF193723; NA; NA; NA; NA; AY347017; AY298178. *S. molle*; **390**; Norway; *Andrus 7630* (DUKE); AY346498; NA; NA; NA; NA; AY347185; AY347018; AY347098.
- Sphagnum quinquefarium* (Lindb.) Warnst.; **370**; USA: Alaska; *Schofield 104094* (DUKE); AF193722; NA; NA; NA; AY347248; AY347083; AF192590.
- Sphagnum rubellum* Wilson; **312**; USA: New York; *Shaw 8015* (DUKE); AY346500; NA; NA; NA; NA; NA; NA. *S. rubellum*; **315**; USA: New York; *Shaw 8003* (DUKE); AY346501; NA; NA; NA; NA; NA; NA. *S. rubellum*; **316**; USA: New York; *Shaw 8001* (DUKE); AY346502; NA; NA; NA; NA; NA; NA. *S. rubellum*; **317**; USA: New York; *Shaw 8043* (DUKE); AY346503; NA; NA; NA; NA; NA; NA. *S. rubellum*; **318**; USA: New York; *Shaw 8017* (DUKE); AY346504; NA; NA; NA; NA; NA; NA. *S. rubellum*; **319**; USA: New York; *Shaw 8018* (DUKE); AY346505; NA; NA; NA; NA; NA; NA. *S. rubellum*; **321**; USA: New York; *Shaw 8016* (DUKE); AY346506; NA; NA; NA; NA; NA; NA. *S. rubellum*; **331**; USA: New York; *Shaw 9234* (DUKE); AY346507; NA; NA; NA; NA; NA; NA. *S. rubellum*; **567**; Norway; *Shaw 9647* (DUKE); AY298623; NA; NA; NA; NA; NA; AY298256. *S. rubellum*; **582**; Norway; *Shaw 9733* (DUKE); AF193742; NA; NA; NA; NA; NA; NA; AY298258.
- Sphagnum russowii* Warnst.; **330**; USA: New York; *Shaw 9259* (DUKE); AF193732; NA; NA; NA; NA; NA; NA.
- Sphagnum schofieldii* H. A. Crum; **373**; Canada: British Columbia; *Schofield 64344* (DUKE); AF193733; NA; NA; NA; AY347048; AY298279.
- Sphagnum* sp. (neotropical); **632**; Colombia; *Churchill 18330* (MO); AY298697; AY346648; AY346781; AY346921; AY347243; AY347078; AY298333.
- Sphagnum squarrosum* Dixon & Sherrin; **1235**; Japan; *Tsukamoto MT-467* (DUKE); AY298650; NA; NA; NA; NA; AY347055; AY298286.
- Sphagnum subfulvum* Sjors; **546**; Finland; *Shaw 9800* (DUKE); AY346511; NA; NA; NA; NA; NA; NA. *S. subfulvum*; **1298**; Canada: New Brunswick; *Belland 13652* (DUKE); AY346510; NA; NA; NA; NA; NA; NA.
- Sphagnum subnitens* Russi & Warnst.; **200**; Poland; *Lisowski & Tobolski B-12324* (POZG); ; NA; AY346765; AY346905; NA; NA; NA. *S. subnitens*; **369**; USA: Alaska; *Belland 13659* (DUKE); AY346512; NA; NA; NA; NA; NA; NA. *S. subnitens*; **589**; Canada: Nova Scotia; *Schofield 97180* (DUKE); AY346513; NA; NA; NA; NA; NA; NA. *S. subnitens*; **1296**; USA: Alaska; *Schofield 106106* (DUKE); AY298663; NA; NA; NA; AY347226; AY347061; AY298299. *S. subnitens*; **1305**; Canada: New Brunswick; *Belland 14186* (DUKE); AY298665; NA; NA; NA; NA; NA; AY298301. *S. subnitens*; **1306**; Belgium; *Vanderpoorten 49* (DUKE); AY298666; NA; NA; NA; NA; NA; AY298302.
- Sphagnum tenerum* Sull & Lesq.; **339**; USA: South Carolina; *Shaw 9274* (DUKE); AY346514; NA; NA; NA; NA; NA; NA. *S. tenerum*; **343**; USA: North Carolina; *Shaw 9272* (DUKE); AY346515; NA; NA; NA; NA; NA; NA.
- Sphagnum triporosum* H. A. Crum; **864**; Brazil; *Frahm 1834* (NY); AY298704; NA; NA; NA; NA; NA; AY298340.
- S. triporosum*; **1073**; Brazil; *Frahm 1834* (MICH); AY346516; NA; NA; NA; NA; NA; NA.
- Sphagnum tundrae* Flatberg; **1041**; Norway; *Flatberg s.n.* (NY); AY298707; NA; NA; NA; NA; NA; AY298343. *S. tundrae*; **1097**; Norway; *Flatberg 161883* (DUKE); AY298708; NA; NA; NA; NA; NA; AY298344.
- Sphagnum warnstorffii* Russow; **360**; USA: Alaska; *Andrus 8358* (DUKE); AY298365; NA; NA; NA; NA; NA; AF192589. *S. warnstorffii*; **631**; China; *Vitt 34808* (MO); AY298717; NA; NA; NA; NA; AY347252; AY347087; AY298352. *S. warnstorffii*; **1310**; Luxembourg; *Vanderpoorten 43* (DUKE); AY346517; NA; NA; NA; NA; NA; AY347101. *S. warnstorffii*; **1311**; USA: Michigan; *Schofield 98518* (DUKE); AY298712; AY346651; NA; NA; NA; NA; NA; AY298347. *S. warnstorffii*; **1312**; USA: Maine; *Spaulding 79* (DUKE); AY298713; NA; NA; NA; NA; NA; AY298348.
- Sphagnum wilfii* H. A. Crum; **374**; Canada: British Columbia; *Schofield 83645* (DUKE); AF193744; NA; NA; NA; NA; NA; AY298355.
- Sphagnum wulfianum* Girg.; **124**; USA: Minnesota; *Bowers 22665* (NY); AY298720; NA; NA; NA; NA; NA; AY347089; AF192587. *S. wulfianum*; **1287**; USA: Michigan; *Taub 96* (DUKE); AY298721; NA; AY346789; AY346929; AY347254; AY347090; AY298356. *S. wulfianum*; **1380**; USA: New York; *Town 1813* (DUKE); AY346520; NA; AY346790; AY346930; AY347255; AY347091; NA. *S. wulfianum*; **1381**; Canada: Ontario; *Vitt 35150* (DUKE); NA; NA; NA; NA; NA; NA; AY347103.