

## Divergent and Reticulate Evolution in Closely Related Species of *Sphagnum* Section *Subsecunda*

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**Abstract.** *The Sphagnum subsecundum complex includes a group of closely related, morphologically intergrading species in section Subsecunda. Nucleotide sequences from six genes (four nuclear and two chloroplast) were obtained from 74 populations representing all the putative species in this complex (S. denticulatum, S. inundatum, S. lescurii, S. subsecundum) to determine if the morphologically-defined taxa represent genetically distinct units. Sampling included populations from North America, Europe, and Asia. Parsimony analyses resolved two major groups of populations, one containing only North American plants (plus one from northern Russia) and the other containing all but two of the European samples, a few from North America, and one from Japan. Two of the four morphospecies occurred in both groups. Shimodaira-Hasegawa (SH) tests indicate that monophyly of S. inundatum, S. subsecundum, and S. lescurii can be rejected, whereas monophyly of S. denticulatum cannot be rejected with our data. Intragenic recombination was detected in both groups of populations, but was substantially higher in the “American” group. Because recombination calls into question the applicability of character-based phylogenetic methods, including parsimony, molecular similarity among populations was estimated using neighbor-joining. Neighbor-joining also resolved geographically correlated groups and corroborated the conclusion that morphologically defined species do not form genetically coherent groups. Groups of populations more closely reflect geographic than morphological patterns.*

**Keywords.** Allopolyploidy, ITS, moss phylogeny, polyploidy, recombination, *Sphagnum inundatum*, *Sphagnum lescurii*, *Sphagnum subsecundum* complex, *trnG*, *trnL-trnF*.

With some 200 species (Crum 2001), *Sphagnum* L. is one of the world's largest genera of mosses. However, the total number of species is unknown, and the species-level taxonomy of *Sphagnum* is arguably one of the most contentious among moss genera. Much of the taxonomic disagreement centers around the status of taxa within complexes of closely related species; where one taxonomist “sees” a single species, another might see six or eight. Most *Sphagnum* species fall within one of four large monophyletic sections (or subgenera): *Sphagnum*, *Subsecunda*, *Cuspidata*, and *Acutifolia* (Shaw 2000; Shaw et al. 2003). Complexes of closely related and morphologically similar taxa surround *S. imbricatum* (section *Sphagnum*; Flat-

berg 1986), *S. recurvum* (section *Cuspidata*; S astad 1998), *S. capillifolium* (section *Acutifolia*; McQueen 1989; Cronberg 1994) and *S. subsecundum* (section *Subsecunda*; Krzakowa & Melosik 2000), and each of these groups remains taxonomically controversial.

Relationships within the section *Subsecunda* are especially problematic because the section is speciose worldwide, and because gametophyte morphology appears to be highly plastic in response to habitat conditions (Flatberg 1985; Goosens & De Sloover 1981). Taxa within the *S. subsecundum* complex are distinguished by size and shape of stem and branch leaves, and by the extent to which stem leaves are fibrillose and porose (Crum 1984; Eddy 1977; Hill 1975; Melosik 2000b). Lindberg (1882), for example, distinguished four species of

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section *Subsecunda* in Europe and North America, but Warnstorf (1911) recognized 17 species in Europe and 36 species in North America. Isoviita (1966) reduced this number to six for Europe while Åberg (1937) recognized only three European species. Taking the most conservative approach, Andrews (1913) recognized a single North American species in the group: *S. subsecundum* Nees. However, Andrews later (1959) acknowledged that “*Sphagnum subsecundum* Nees, taken in a broad sense, has by universal agreement long been considered the most variable species of the genus.” Crum (1984) also recognized just *S. subsecundum* at the specific level, but with nine varieties. Most authors have separated at least those species having a multistratose stem cortex; i.e., *S. contortum* Schultz, *S. platyphyllum* (Lindb. ex Braithw.) Sull. ex Warnst. and *S. carolinianum* Andrus, but the taxonomic status of taxa having a unistratose cortex remains controversial. The latter group is referred to here as the “*S. subsecundum* complex.”

The *S. subsecundum* complex includes taxa that have been distinguished at the specific (Flatberg 1985; Melosik 2000b; Russow 1894), subspecific (Daniels & Eddy 1985; Eddy 1977), or varietal level (Crum 1984; Hill 1975; Rahman 1972). The separation of *S. lescurii*, *S. denticulatum* and *S. inundatum* as distinct species is not easy, in spite of the fact that all these taxa seem to have their own morphological (but mainly quantitative), ecological, and distributional characteristics. *Sphagnum inundatum* Russow poses particular problems. The taxon was described by Russow (1894) as a species, but Rahman (1972) considered it a variety of *S. subsecundum*. Hill (1975), on the other hand, recognized it as a variety of *S. auriculatum*. Similarly, the taxonomic position of the North American *S. lescurii* Sull. remains unclear, as it has been related both to European *S. auriculatum* (Andrus 1980; Andrus & Vitt 1977; Crum & Anderson 1981; Crum 1984) or *S. inundatum* (Flatberg 1985).

Taxonomic difficulties among species of sect. *Subsecunda* have also led to nomenclatural confusion. Some bryologists use the name *S. auriculatum* for what others call *S. denticulatum*. Dirkse and Isoviita (1986) argued that *S. auriculatum* and *S. denticulatum* are synonyms and that the earlier name, *S. denticulatum*, should be used. In contrast, others (e.g., Flatberg 2002) thought that the type specimen of *S. denticulatum* is in such poor shape that it cannot be reliably identified, and the name should therefore be rejected (Flatberg, pers. comm.).

A number of authors have discussed putative ecological differences between taxa in the *S. subsecundum* complex (e.g., Brzeg et al. 2000; Eddy 1977; Goosens & DeSloover 1981). *Sphagnum sub-*

*secundum* occurs across northern North America, Europe, and Asia, and is also reported from South America (Crum, pers. comm.) and Australasia, south to New Guinea (Eddy 1977). The range of *Sphagnum inundatum* is much the same as *S. subsecundum*, but according to Daniels and Eddy (1985), it does not extend as far to the north and east and is more common than *S. subsecundum* in the British Isles. The geographic distributions of *S. denticulatum* and *S. lescurii* are unclear because of the taxonomic confusion surrounding them. Crum (1984) considered those taxa [as *S. subsecundum* var. *rufescens* (Nees & Hornsch.) Huebener] to be widespread in eastern North America, south through the West Indies and into northern South America. He also attributed them to Japan, Australia, New Zealand, and northern Africa. Recent work suggests that at least some tropical and Southern Hemisphere records belong to endemic taxa related to, but not conspecific with, the Northern Hemisphere species (e.g., Fife 1996). Eddy (1985) reported *S. auriculatum* (= *S. denticulatum*) but not *S. subsecundum* from northern Africa. Most European authors have considered *S. denticulatum* as a species with predominantly oceanic tendencies in its distribution (Eddy 1977; Hill et al. 1992; Isoviita 1966; Melosik 2000a; Ochyra 1995).

The purpose of this study is to assess genetic and phylogenetic relationships among members of the *S. subsecundum* complex using nucleotide sequences from six nuclear and chloroplast genes. Our samples include collections that conform to morphological concepts of *S. subsecundum*, *S. inundatum*, *S. denticulatum*, and *S. lescurii*. By utilizing a relatively narrow species concept in which we distinguish all four species, we were able to test whether these names correspond to distinguishable groups of multilocus haplotypes, and how these haplotypes are related.

#### MATERIALS AND METHODS

*Taxon sampling.*—The molecular data set included sixty-nine accessions, each identified as one of the four species in the *S. subsecundum* complex. Five additional populations representing other species of section *Subsecunda* were included as outgroups, namely, *S. capense* Hornsch. and *S. cymbifolioides* Breutel from Africa, and *S. contortum* Schultz from North America. Of the 69 collections of “the complex,” 31 originated in Europe, 36 in North America, and two from Asia (Japan and northern Russia). Voucher specimens are deposited in DUKE unless otherwise noted. In most cases, the six genomic regions (see below) were sequenced directly, but when unambiguous results were not obtained from direct sequencing, the regions were cloned as in Shaw et al. (2003). Generally, only a single clone was sequenced for each individual, and when more than one clone was sequenced, the first was arbitrarily chosen for inclusion in the phylogenetic analysis.

Inferences about how the molecular results correspond

to morphologically defined species depend, of course, on how the species are identified. Three systematists who specialize on *Sphagnum* examined the collections: L. E. Anderson–Durham, North Carolina; and two of the authors—I. Melosik and J. Shaw. Species identifications were accomplished both subjectively using characters identified as diagnostic by Crum and Anderson (1981), Flatberg (2002), and Melosik (2000b), and by multivariate statistical analyses of a larger sample of collections within which most of these accessions were included (Melosik, unpubl.). There was disagreement among the specialists on the identity of some samples. In most cases the consensus taxonomic interpretation was used, or one of us (AJS) made the final determination. These disagreements highlight taxonomic difficulties in the group and reflect differing views about which morphological characters are diagnostic when they provide contradictory evidence. Our major conclusions are robust to these disagreements. Specimen data, along with GenBank accession numbers, are provided in Table 1.

**Genomic sequencing.**—Nucleotide sequences were obtained from six loci. From the chloroplast genome, we sequenced transfer RNA<sup>Gly</sup> (UCC) (hereafter, *trnG*), and the *trnL* (UAA) 5' exon—*trnF*(GAA) intergenic spacer (*trnL*). Nuclear sequences were obtained from the ITS1–5.8S–ITS2 region (ITS). Three anonymous regions, assumed to be nuclear, were sequenced using primers designed for loci identified from an analysis of RAPDs (random amplified polymorphic DNA) in *Sphagnum*. Primer sequences for amplifying and sequencing *trnG* are provided in Pacak and Szweykowska (2003). Amplification and sequencing protocols, and the other primers utilized in this study, are described in Shaw et al. (2003).

**Phylogenetic analyses.**—A first round of analyses was conducted under maximum parsimony (MP) and Bayesian statistical inference. Equally-weighted parsimony analyses were conducted with 300 random taxon-addition replicates and TBR branch swapping, saving as many as 50 trees per replicate. Branch swapping was then conducted on these trees, saving as many as 20,000 total trees. Support for nodes was assessed by 300 non-parametric bootstrap replicates each with ten random taxon-addition replicates, saving 500 trees per replicate.

The most appropriate substitution model for the data was determined by hierarchical likelihood ratio tests with the aid of MrModeltest 1.1b (Nylander 2002). The model was utilized for a homogeneous Bayesian analysis using MrBayes3 (Huelsenbeck & Ronquist 2002). We also undertook a heterogeneous Bayesian analysis of the data in which the optimal substitution model was determined independently for each of the six regions. Consensus trees reconstructed from the collection of trees contained in the 95% posterior probability space of the Bayesian analyses were largely unresolved, reflecting conflicts among (and within) genomic regions. These trees are not presented. We describe our attempt to accomplish Bayesian analyses of the data set, even though presentable results were not obtained, because our failure may well reflect recombination in the study taxa. The Bayesian reconstructions will not be discussed further.

Hypotheses of monophyly for the four morphospecies were evaluated using Shimodaira–Hasegawa (SH) tests, implemented in PAUP (Swofford 2001). Ten randomly selected trees obtained from the unconstrained maximum parsimony analysis were compared to 100 trees recovered from analyses in which each species was constrained (sequentially) as monophyletic. Significance of the SH tests were assessed using Rell optimization with 1,000 bootstrap replicates.

Because recombination was detected in all four nuclear loci (see below), molecular similarities among the samples were also assessed using neighbor joining, implemented in PAUP. Uncorrected distances were used to cluster the samples, and support for groups was assessed by 5,000 bootstrap replicates.

Parsimony and Bayesian analyses were undertaken for each locus separately, and for various two and three locus combinations. Trees derived from these analyses were unresolved and uninformative, and are not presented.

**Population genetics and recombination.**—Descriptive genetic statistics (including Tajima's D; Tajima 1989) and analysis of molecular variance (AMOVA) were implemented using ARLEQUIN (Schneider et al. 2000). Outgroup samples (four) were excluded from the genetic analyses. The AMOVAs estimated partitioning of molecular variation within and among two geographically correlated clades resolved by the parsimony analysis, and also within and among four clusters of genetically differentiated populations identified by neighbor-joining (Table 3). For the AMOVA that partitioned molecular variation within and between the two groups identified under parsimony, two ingroup populations that were not included in either of the two major clades were excluded so that the AMOVA was based on 67 populations. The second AMOVA, which partitioned variation within and among the four groups identified by neighbor-joining, included all 69 ingroup populations.

Population-level intragenic recombination was estimated separately for the groups identified by parsimony ( $N = 2$ ) and neighbor-joining ( $N = 4$ ) using SITES (Hey & Wakeley 1997) on a locus by locus basis. The test for recombination implemented in SITES is based on the four-gamete test of Hudson and Kaplan (1985). Recombination is implied when two sites are incongruent; i.e., when all four possible gametic combinations exist in the data. At least one recombination event must have occurred within an interval bounded by two incongruent sites. Gamma ( $\gamma$ ) (Hey & Wakeley 1997) provides an estimate of the population recombination rate,  $2Nc$  (for a population of haploids), where  $c$  is the crossing over rate for the sequenced region per generation. Also calculated is the ratio of number of recombination events per mutation ( $c/\mu$ ), derived by dividing gamma ( $Nc$ ) by theta ( $\theta = 2Nc\mu$  for haploids). This ratio gives an estimate of the relative contribution of recombination and mutation to molecular diversity on a per site basis.

An important assumption of this approach is that the data conform to the infinite sites model; that is, that mutations have occurred no more than once at each site. Otherwise, incongruence could result from parallel evolution because of multiple hits, and SITES does not give an accurate estimate of recombination in such cases (Hey & Wakeley 1997). The number of sites segregating more than two nucleotides provides an indication of the extent to which multiple hits may have occurred. These numbers are presented, along with recombination estimates, in Tables 4 and 5.

## RESULTS

**Parsimony analyses.**—The aligned nucleotide matrix included 74 taxa and 4,379 characters, of which 3,886 were monomorphic, 248 were autapomorphic, and 245 were parsimony informative. The breakdown of nucleotide variation among loci is provided in Table 2. The nuclear ribosomal ITS region included more than twice as many singletons

TABLE 1. Collection information and GenBank accession numbers for samples included in the phylogenetic and genetic analyses.

Isolate	Species	Collector	Collection	Herb	ITS	RAPDa	RAPDb	RAPDf	tmL	tmG
5	<i>S. inundatum</i>	Melosik	14-922	DUKE	AY298502	AY361599	AY361745	AY361176	AY298134	AY361419
13	<i>S. inundatum</i>	Melosik	1-13a	AMU	AY298489	AY361586	AY361731	AY361163	AY298121	AY361406
15	<i>S. inundatum</i>	Stebel	3	DUKE	AY298491	AY361588	AY361733	AY361165	AY298123	AY361408
59	<i>S. denticulatum</i>	Melosik	43-963	DUKE	AY298444	AY361578	AY361722	AY361155	AY298075	AY361397
63	<i>S. denticulatum</i>	Melosik	36-956	DUKE	AY298445	AY361579	AY361723	AY361156	AY298076	AY361398
64	<i>S. denticulatum</i>	Stebel	2-2	DUKE	AY298446	AY361580	AY361724	AY361157	AY298077	AY361399
67	<i>S. inundatum</i>	Melosik	46(67)-968	DUKE	AY298503	AY361600	AY361746	AY361177	AY298135	AY361420
70	<i>S. subsecundum</i>	Melosik	5-5	DUKE	AY298641	AY361673	AY361825	AY361248	AY298275	AY361498
73	<i>S. subsecundum</i>	Melosik	18(231)-685	DUKE	AY298642	AY361674	AY361826	AY361249	AY298276	AY361499
95	<i>S. inundatum</i>	Stachnowicz	4	DUKE	AY298504	AY361601	AY361747	AY361178	AY298136	AY361421
127	<i>S. cristatum</i>	Streichmann	47192	NY	AF193713	AY309640	AY309659	AY309678	AF192584	AY309762
129	<i>S. denticulatum</i>	Brzeg	12(111)-12	DUKE	AY298434	AY361566	AY361708	AY361141	AY298065	AY361384
132	<i>S. subsecundum</i>	Melosik	21(B-933)	DUKE	AY298629	AY361651	AY361801	AY361224	AY298263	AY361473
141	<i>S. inundatum</i>	Melosik	58(249)-1001	DUKE	AY298490	AY361587	AY361732	AY361164	AY298122	AY361407
142	<i>S. capense</i>	Snook	7352	NY	AF193664	AY361560	AY361702	AY361134	AF192563	AY361377
162	<i>S. denticulatum</i>	Melosik	9(PZ-1038)	DUKE	AY361006	AY361567	AY361709	AY361142	AY361522	AY361385
174	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY361007	AY361568	AY361710	AY361143	AY361523	AY361386
177	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298435	AY361569	AY361711	AY361144	AY298066	AY361387
180	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298436	AY361570	AY361712	AY361145	AY298067	AY361388
189	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298437	AY361571	AY361713	AY361146	AY298068	AY361389
195	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298438	AY361572	AY361714	AY361147	AY298069	AY361390
196	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298439	AY361573	AY361715	AY361148	AY298070	AY361391
198	<i>S. denticulatum</i>	Melosik	live-no voucher	AMU	AY298440	AY361574	AY361716	AY361149	AY298071	AY361392
201	<i>S. lescurii</i>	Risk	7457	DUKE	AY298492	AY361589	AY361734	AY361166	AY298124	AY361409
202	<i>S. lescurii</i>	Alford	236	DUKE	AY298493	AY361590	AY361735	AY361167	AY298125	AY361410
203	<i>S. lescurii</i>	Anderson	25199	DUKE	AY298515	AY361607	AY361754	AY361182	AY298147	AY361427
206	<i>S. lescurii</i>	Alford & Alford	1665	DUKE	AY298516	AY361608	AY361755	AY361183	AY298148	AY361428
207	<i>S. lescurii</i>	Summers	8174	DUKE	AY298494	AY361591	AY361736	AY361168	AY298126	AY361411
208	<i>S. lescurii</i>	Allen & Pursell	13160	DUKE	AY298495	AY361592	AY361737	AY361169	AY298127	AY361412
210	<i>S. lescurii</i>	Nelson	20863	DUKE	AY298517	AY361609	AY361756	AY361184	AY298149	AY361429
213	<i>S. lescurii</i>	Bachmann	245	DUKE	AY298518	AY361610	AY361757	AY361185	AY298150	AY361430
215	<i>S. inundatum</i>	Andrus	8319	DUKE	AY298496	AY361594	AY361739	AY361171	AY298128	AY361414
216	<i>S. lescurii</i>	Allen	8400	DUKE	AY361011	AY361611	AY361758	AY361186	AY361527	AY361431
217	<i>S. lescurii</i>	Shaw	10275	DUKE	AY298631	AY361652	AY361802	AY361225	AY298265	AY361474
218	<i>S. subsecundum</i>	Ireland	22827	DUKE	AY361016	AY361653	AY361803	AY361226	AY361531	AY361475
219	<i>S. inundatum</i>	Schofield & Belland	96929	DUKE	AY361017	AY361654	AY361804	AY361227	AY361532	AY361476
220	<i>S. subsecundum</i>	Schofield	109317	DUKE	AY361018	AY361655	AY361805	AY361228	AY361533	AY361477
221	<i>S. subsecundum</i>	Schofield et al.	101508	DUKE	AY361019	AY361656	AY361806	AY361229	AY361534	AY361478
222	<i>S. subsecundum</i>	Schofield et al.	84276	DUKE	AY361020	AY361657	AY361807	AY361230	AY361535	AY361479
224	<i>S. subsecundum</i>	Schofield et al.	102425	DUKE	AY361021	AY361659	AY361809	AY361232	AY361537	AY361481
225	<i>S. subsecundum</i>	Shevock & York	18317	DUKE	AY298632	AY361660	AY361810	AY361233	AY298266	AY361482
228	<i>S. inundatum</i>	Schofield et al.	97577	DUKE	AY298497	AY361595	AY361740	AY361172	AY298129	AY361415
231	<i>S. lescurii</i>	Price	752	DUKE	AY361023	AY361661	AY361811	AY361234	AY361539	AY361484

TABLE 1. Continued.

Isolate	Species	Collector	Collection	Herb	ITS	RAPDa	RAPDb	RAPDf	trnL	trnG
232	<i>S. lescurii</i>	Sanford	B-26a	DUKE	AY361012	AY361612	AY361759	AY361187	AY361528	AY361432
233	<i>S. lescurii</i>	Risk	11003	DUKE	AY361013	AY361613	AY361760	AY361188	AY361529	AY361433
235	<i>S. lescurii</i>	Allen	20268	DUKE	AY298519	AY361614	AY361761	AY361189	AY298151	AY361434
236	<i>S. inundatum</i>	Shaw	10301	DUKE	AY298498	AY361596	AY361741	AY361173	AY298130	AY361416
237	<i>S. inundatum</i>	Allen	21783	DUKE	AY298633	AY361662	AY361812	AY361235	AY298267	AY361485
239	<i>S. subsecundum</i>	Shevock	21002	DUKE	AY298634	AY361663	AY361813	AY361236	AY298268	AY361486
242	<i>S. inundatum</i>	Kutschka	s.n.	DUKE	AY298499	AY361597	AY361742	AY361174	AY298131	AY361417
244	<i>S. inundatum</i>	Musselman	99115	DUKE	AY361024	AY361665	AY361238	AY361154	AY361541	AY361488
245	<i>S. lescurii</i>	Nelson & Robinson	18744	DUKE	AY298520	AY361615	AY361762	AY361190	AY298152	AY361435
247	<i>S. lescurii</i>	Anderson	24873	DUKE	AY298442	AY361575	AY361718	AY361151	AY298073	AY361394
250	<i>S. lescurii</i>	Allen	22474	DUKE	AY298635	AY361666	AY361816	AY361239	AY298269	AY361489
251	<i>S. subsecundum</i>	Musselman	99155	DUKE	AY361025	AY361667	AY361817	AY361240	AY298270	AY361490
253	<i>S. subsecundum</i>	Shaw	10341	DUKE	AY361026	AY361668	AY361818	AY361241	AY361543	AY361491
255	<i>S. subsecundum</i>	Anderson	24276	DUKE	AY298636	AY361669	AY361820	AY361243	AY298271	AY361493
257	<i>S. subsecundum</i>	Kartolinen & Hyvönen	9809	DUKE	AY298638	AY361670	AY361822	AY361245	AY298272	AY361495
259	<i>S. subsecundum</i>	Andrus & Flatberg	7514	DUKE	AY298640	AY361671	AY361823	AY361246	AY298274	AY361496
261	<i>S. subsecundum</i>	Granzow de la Cerda	GC-2045	DUKE	AY361027	AY361672	AY361824	AY361247	AY361545	AY361497
265	<i>S. inundatum</i>	Andrus & Flatberg	7515	DUKE	AY298501	AY361598	AY361744	AY361175	AY298133	AY361418
266	<i>S. deniculatum</i>	Andrus	8172	DUKE	AY361009	AY361577	AY361721	AY361154	AY361525	AY361396
647	<i>S. subsecundum</i>	Andrus & Talbot	8573	DUKE	AF193668	AY361637	AY361786	AY361210	AF192635	AY361458
648	<i>S. contortum</i>	Anderson	25410	DUKE	AF193669	AY361563	AY361705	AY361138	AF192636	AY361381
881	<i>S. sp.</i>	Buck	6817	NY	AY298681	AY361685	AY361842	AY361261	AY298317	AY361514
1059	<i>S. lescurii</i>	Schofield	101087	DUKE	AY298670	AY361679	AY361834	AY361254	AY298306	AY361506
1092	<i>S. deniculatum</i>	Heras	VIT 19275	DUKE	AY361003	AY361551	AY361692	AY361127	AY361520	AY361369
1093	<i>S. deniculatum</i>	Infanti & Heras	VIT 16670	DUKE	AY298380	AY361552	AY361693	AY361128	AY298014	AY361370
1140	<i>S. cymbifoloides</i>	Miehe & Miehe	U71-10970	DUKE	AY298672	AY361680	AY361836	AY361256	AY298308	AY361508
1171	<i>S. inundatum</i>	Andrus	9277	DUKE	AY298507	AY361604	AY361749	AY361180	AY298139	AY361422
1218	<i>S. deniculatum</i>	Infanti & Heras	VIT 21068	DUKE	AY298381	AY361553	AY361694	AY361129	AY298015	AY361371
1391	<i>S. subsecundum</i>	Ignatov	00/1011	DUKE	AY298677	AY361682	AY361838	AY361257	AY298313	AY361510
1393	<i>S. inundatum</i>	van Tienhoven	20116	DUKE	AY298679	AY361683	AY361840	AY361259	AY298315	AY361512
1394	<i>S. deniculatum</i>	Heidestein	201032	DUKE	AY298680	AY361684	AY361841	AY361260	AY298316	AY361513

TABLE 2. Gene regions included in phylogenetic analyses of the *S. subsecundum* complex, their lengths, numbers of autapomorphic and informative sites.

Locus	Length	Autapomorphic sites	Informative sites
ITS	773	70	30
RapdA	1,099	54	84
RapdB	605	64	64
RapdF	544	37	47
<i>trnG</i>	722	11	11
<i>trnL</i>	636	12	12

(autapomorphic sites) than polymorphisms that were shared among two or more accessions (parsimony informative sites). The RAPD loci, which were also variable, contained as many or more informative than autapomorphic sites. As expected, the two chloroplast loci (*trnG*, *trnL*) contained less nucleotide variation than the nuclear loci (Table 2).

20,000 equally most-parsimonious trees of 931 steps were recovered from the phylogenetic analyses. The trees had a CI of 0.570, an RI of 0.794, and an RC of 0.453. The strict consensus of these trees is shown as Figure 1. The *S. subsecundum* complex plus *S. contortum* form a monophyletic group with 100% bootstrap support, but populations of the four species that comprise the complex, identified morphologically, are scattered and do not

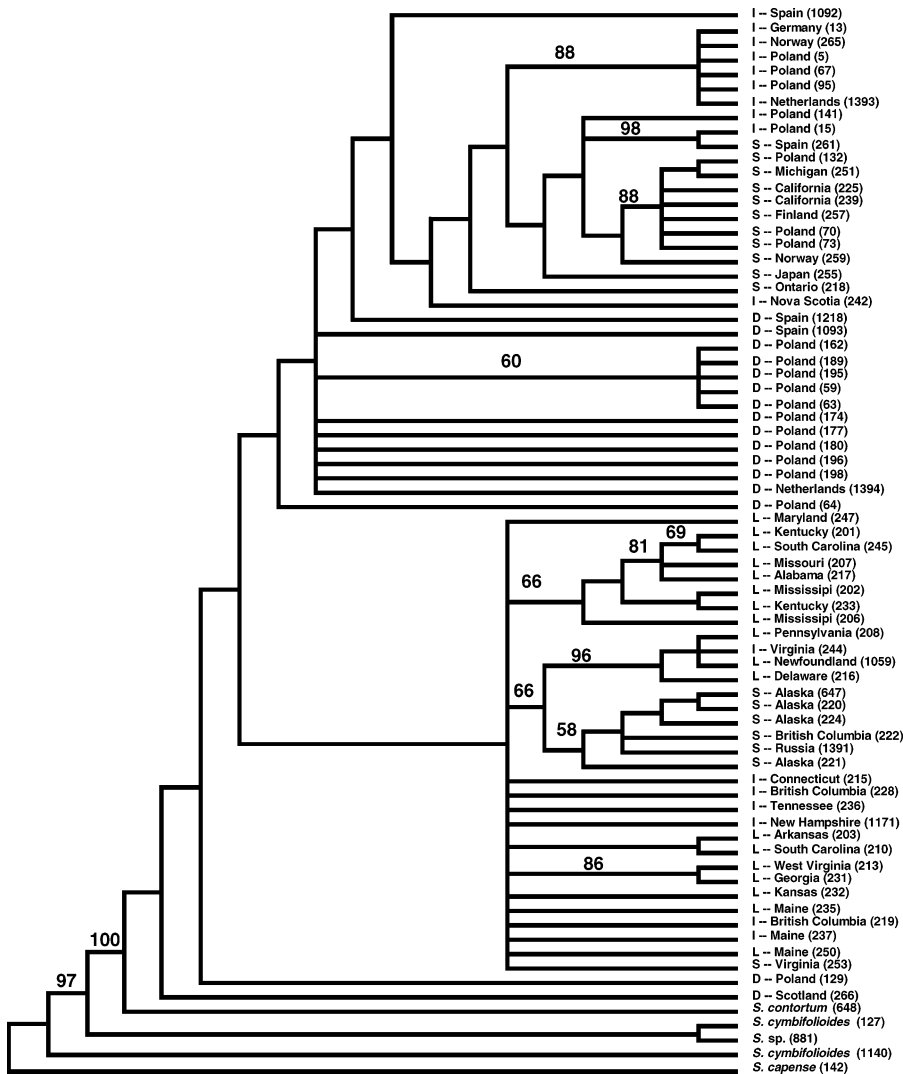


FIGURE 1. Maximum parsimony strict consensus tree showing genealogical relationships among populations in the *Sphagnum subsecundum* complex. Nonparametric bootstrap values of >50% are shown above branches. D = *S. deniculatum*, I = *S. inundatum*, L = *S. lescurii*, S = *S. subsecundum*.

group together as monophyletic units. Few groups of populations were supported with high bootstrap values, but two major groups were resolved within the complex. Only two populations of *S. denticulatum*, from Scotland and Poland, fell outside these two groups (#266 and #129; Fig. 1). With those exceptions, all the European populations fell into one group, which also included North American populations from California, Michigan, Nova Scotia, and Ontario; hereafter, referred to as the "European group." A population from Japan was also included in this group. The other group of populations included all the remaining North American populations; hereafter, the "North American group." A population from Russia grouped with the American populations.

Populations of *S. subsecundum* and *S. inundatum* occurred in both geographic groups (Fig. 1). Within the North American and European groups, a few smaller well-supported subgroups were resolved, and in some cases these are related to geography. One subgroup within the European group includes populations from Poland, Norway, and the Netherlands, and one within the North American group includes populations from the eastern part of the continent: Newfoundland, Pennsylvania, Delaware, and Virginia. A weakly supported group includes populations from northwestern North America (Alaska, British Columbia) and Russia. On the other hand, a strongly supported cluster of populations in the European group includes plants from Poland, Finland, California, and Michigan (Fig. 1). In some cases, collections from widely separated localities share a well-supported sister group relationship [e.g., *S. subsecundum* (#261) from Spain and *S. inundatum* (#15) from Poland]. What little grouping of morphospecies exists in the tree is confounded with geography.

SH tests rejected monophyly for *S. subsecundum* ( $p < 0.001$ ), *S. inundatum* ( $p < 0.001$ ), and *S. lescurii* ( $p < 0.05$ ), but monophyly of *S. denticulatum* could not be rejected. The first two species are represented in both the North American and European groups whereas *S. lescurii* and *S. denticulatum* are restricted (in our sample) to one or the other (North America and Europe, respectively).

*Population genetics of clades resolved by parsimony.*—An analysis of molecular variance (AMOVA) conducted on the 67 populations that were included by parsimony analyses in either the North American or European groups indicates that approximately 13% of the total sequence variation can be attributed to differentiation between the two geographically correlated groups (Table 3). This is (statistically) significant differentiation, although 87% of the total molecular variation occurred among samples within the groups.

TABLE 3. Analysis of molecular variation (AMOVA) between the two major clades in the *S. subsecundum* complex resolved by parsimony analysis, and among the four clusters identified by neighbor-joining. The first analysis includes only those 67 populations that were grouped within the two clades shown in FIG. 1; seven populations (including outgroups) that are outside those clades are not included. In the second analysis, all 69 ingroup samples were included.

Source	df	S.S.	V.C.	% of variation
<i>Parsimony analysis</i>				
Between clades	1	320.943	8.008	13.08
Within clades	65	3,459.446	53.222	86.92
Total	66	3,780.388	61.230	
<i>Neighbor-joining</i>				
Among clusters	3	554.083	10.214	36.69
Within clusters	65	1,145.642	17.625	63.31
Total	68	1,699.725	27.839	

On a locus-by-locus basis, there appears to be no consistent difference in nucleotide diversity (estimated as  $\theta$ ) between the North American and European groups (Table 4). There is, however, a substantial difference in estimated levels of intragenic recombination. Recombination was detected within each of the four nuclear genes, but no recombination was found in the chloroplast loci. The latter was expected, although the lack of evidence for recombination could also be the result of relatively low nucleotide polymorphism at these loci. A total of 19 recombination intervals were detected in the North American group, versus seven for the European group. Similarly, estimates of gamma ( $\gamma$ ) for the North American populations were consistently higher than for the European populations, in some cases by 700% or more (Table 4). Based on combined data from the six genes, Tajima's D was significantly negative for the North American clade ( $D = -1.480$ ,  $p < 0.05$ ) but did not differ from zero for the European clade ( $D = -0.175$ ,  $p > 0.05$ ). The ratio of gamma ( $\gamma$ ) to theta ( $\theta$ ) was less than 1 for all loci in both groups, indicating that recombination contributes less to nucleotide diversity on a per site basis than does mutation. Only a few of the 493 variable nucleotide sites segregated more than two nucleotides (Table 4), suggesting that while multiple hits have occurred, this does not appear to be a substantial problem for estimating recombination within these sequences.

*Neighbor-joining analyses.*—The neighbor-joining tree also resolved geographically correlated groups. Most European populations clustered in two groups: Europe I and Europe II (Fig. 2). The former includes only European populations while the latter includes, in addition to European populations, one sample each from Japan and Ontario,

TABLE 4. Molecular diversity ( $\theta$ ), the number of detectable recombination intervals, population-level recombination ( $\gamma$ ), the ratio of recombinational to mutational variability on a per site basis, and the number of nucleotides segregating  $>3$  nucleotides (nts), in two clades within the *Sphagnum subsecundum* complex.

		“Europe”	“North America”
ITS	$\theta$	0.01000	0.00928
	# intervals	2	4
	$\gamma$	0.000607	0.001205
	$\gamma/\theta$	0.0492	0.0618
	$>3$ nts	0	1
RapidA	$\theta$	0.01242	0.01590
	# intervals	3	6
	$\gamma$	0.000782	0.005445
	$\gamma/\theta$	0.0502	0.2618
	$>3$ nts	4	3
RapidB	$\theta$	0.02113	0.01500
	# intervals	1	4
	$\gamma$	0.001150	0.004228
	$\gamma/\theta$	0.0363	0.1541
	$>3$ nts	2	3
RapidF	$\theta$	0.01088	0.01062
	# intervals	1	5
	$\gamma$	0.003792	0.016962
	$\gamma/\theta$	0.1391	0.6289
	$>3$ nts	1	2
<i>trnL</i>	$\theta$	0.00392	0.00329
	# intervals	0	0
	$\gamma$	0.0000	0.0000
	$\gamma/\theta$	0.0000	0.0000
	$>3$ nts	0	0
<i>trnG</i>	$\theta$	0.00293	0.00290
	# intervals	0	0
	$\gamma$	0.0000	0.0000
	$\gamma/\theta$	0.0000	0.0000
	$>3$ nts	0	0

Canada. The America I cluster consists of populations from the southeastern United States (with a western outlier from Kansas); one Polish population is genetically similar to these populations (Fig. 2). The America II cluster is more geographically heterogeneous, with accessions from across North America. The America II cluster also includes four accessions from Poland and Finland. A well-supported subgroup contains only populations from northwestern North America (British Columbia, Alaska) and adjacent Russia.

Compared to parsimony, neighbor-joining revealed somewhat greater genetic similarity among accessions that were identified as the same morphospecies. For example, North American and European plants that were identified as *S. subsecundum* grouped mainly in one cluster, albeit with populations from the two continents forming two separate subgroups. Nevertheless, plants that conform to *S. subsecundum* morphology, from Spain (#261), Japan (#255), and Norway (#259), grouped outside these clusters, and are more genetically similar to

accessions identified as *S. inundatum* and *S. denticulatum*. In addition, the Ontario accession (#218), which was also identified as *S. subsecundum*, grouped with European plants identified as *S. inundatum*. A similar picture emerges with regard to the other morphologically defined species in the *S. subsecundum* complex; some of the populations of a particular morphotype grouped together, but none formed exclusive groups (Fig. 2). Thus, a group of North American populations identified as *S. inundatum* form a cluster, but this group is distinct from *S. inundatum* in Europe. Similarly, plants identified as *S. lescurii* group mainly in one cluster, but other accessions (#s 213, 231, 235, 235) are more genetically similar to plants identified as other species.

*Population genetics of groups resolved by neighbor-joining.*—An AMOVA, in which molecular variation was partitioned within and among the four geographically correlated groups revealed by neighbor-joining, indicates that differentiation among the groups accounts for approximately 37% of the total variation (Table 3). Although this level of molecular differentiation is highly significant, 67% of the molecular variation in the complex occurs within clusters.

A general pattern across loci is that the Europe I cluster contains less nucleotide variation than do the other three groups (Table 5). This cluster consists mainly of a group of genetically uniform Polish populations, with four additional samples from Spain and the Netherlands. The America II cluster, in contrast, contains a geographically disparate group of populations that are also genetically heterogeneous. Within that group, two subgroups are resolved by neighbor-joining, and these appear to differ in levels of genetic diversity (Fig. 2). The two other clusters of populations, Europe II and America I, tend to have intermediate levels of genetic diversity.

The America II group appears to be characterized by higher levels of intragenic recombination than do the other groups (Table 5). This is evidenced both by the numbers of recombination intervals inferred from the four gamete tests, and by estimates of gamma, the population recombination rate. However, because nucleotide diversity is generally higher within the America II group than in the other groups, recombination appears to contribute less than mutation to molecular diversity on a per site basis. Indeed, for no locus does recombination appear to contribute more than mutation in the America II group, whereas the ratio,  $\gamma/\theta$ , is greater than one for several loci in the other populations (Table 5).

#### DISCUSSION

Genealogical relationships among plants of the *S. subsecundum* complex based on multilocus DNA



TABLE 5. Molecular diversity ( $\theta$ ), the number of detectable recombination intervals, population-level recombination ( $\gamma$ ), the ratio of recombinational to mutational variability on a per site basis, and the number of nucleotides segregating  $>3$  nucleotides (nts), in four clusters of populations identified within the *Sphagnum subsecundum* complex using neighbor-joining analysis. NA means that the data were insufficient for estimation.

		Europe-1	Europe-2	N.America-1	America-2
ITS	$\theta$	0.00079	0.00834	0.00626	0.01255
	# intervals	0	0	0	4
	$\gamma$	NA	NA	NA	0.001964
	$\gamma/\theta$	NA	NA	NA	0.1088
	$>3$ nts	0	1	0	1
RapdA	$\theta$	0.00187	0.00446	0.00713	0.01750
	# intervals	2	1	3	5
	$\gamma$	0.009564	NA	0.006928	0.007733
	$\gamma/\theta$	1.8114	NA	0.7444	0.3302
	$>3$ nts	0	0	0	6
RapdB	$\theta$	0.00297	0.02494	0.01467	0.01820
	# intervals	0	1	0	3
	$\gamma$	NA	0.001666	0.000000	0.007841
	$\gamma/\theta$	NA	0.0634	0.0000	0.2638
	$>3$ nts	0	0	0	4
RapdF	$\theta$	0.00626	0.00728	0.00744	0.01318
	# intervals	0	2	1	2
	$\gamma$	NA	0.018250	0.018954	0.011104
	$\gamma/\theta$	NA	1.7765	2.2384	0.4247
	$>3$ nts	0	0	0	2
<i>trnL</i>	$\theta$	0.00000	0.00260	0.00254	0.00264
	# intervals	0	0	0	0
	$\gamma$	NA	NA	0.00000	0.00000
	$\gamma/\theta$	NA	NA	0.00000	0.00000
	$>3$ nts	0	0	0	0
<i>trnG</i>	$\theta$	0.00000	0.00208	0.00345	0.00453
	# intervals	0	0	0	0
	$\gamma$	NA	0.00000	0.00000	0.00000
	$\gamma/\theta$	NA	0.00000	0.00000	0.00000
	$>3$ nts	0	0	0	0

Results of the neighbor joining analyses, in general, support the major inferences gained from parsimony: populations that conform to the same morphospecies do not form strictly exclusive groups, and genetic similarity among populations is more closely related to geographic proximity (at least on a continental scale) than to morphology. Geographically-correlated subgroups resolved by parsimony are also revealed by neighbor-joining. For example, neighbor-joining identified a group of *S. subsecundum* populations from British Columbia, Alaska, northern Russia, and a group of *S. lescurii* populations from the eastern United States. A group of genetically similar plants from Virginia, Delaware, Pennsylvania, and Newfoundland includes plants that were identified morphologically as *S. inundatum* and *S. lescurii*. On the other hand, a well-supported group of *S. subsecundum* populations, assigned to that species on the basis of their morphology, included plants from California, Michigan, Finland, and Poland.

The major difference between the parsimony and neighbor-joining trees is that neighbor-joining does not resolve the North American and European groups as two exclusive assemblages, as did the

parsimony analysis. If both trees are viewed in a genealogical framework, parsimony resolved the European and North American populations as mutually monophyletic sister groups whereas under neighbor-joining, the North American and European assemblages are both para- or polyphyletic.

Analyses of recombination suggest geographically-correlated differences in reproductive biology within the *S. subsecundum* complex. The two major groups of populations identified by parsimony indicate that recombination is substantially higher in the North American than in the European group. A total of 19 recombination intervals were detected among sequences in the American group, versus seven for the European group. Differences in estimated values for gamma ( $\gamma$ ) parallel these results. Comparable, geographically-correlated differences in recombination are indicated by genetic analyses of the groups of populations identified by neighbor-joining, and suggest that the biggest difference pertains to the Europe I and North America II groups.

The frequency of sexual reproduction, evidenced by sporophyte formation, is variable across the genus *Sphagnum* (Cronberg 1993). Sporophytes are rare in many species and it generally assumed that

asexual propagation must be important. Sporophytes are not common in any of the species in the *S. subsecundum* complex and are unknown in some of the taxa in some areas of Europe (Cronberg 1993). Evidence of intragenic recombination even among European plants indicates at least some sexuality, but sexual reproduction may be more prevalent in North America than in Europe. Field studies are needed to assess the relative frequency of sporophyte formation in North American versus European populations, and more intensive genetic analyses may reveal further differences in population genetic structure. These taxa are not among the major peat-forming *Sphagna* of boreal regions but are common components of more mineral-rich fens throughout North America, Europe, and northern Asia.

The disconnect between groups of populations identified from molecular data, and species identified by morphological characters, could have several explanations. If the morphospecies are of recent origin, there may have been insufficient time for intraspecific coalescence of nucleotide sequences. If the sequenced loci are strictly neutral, expected coalescence times depend on population size and structure in the ancestral species, metapopulation processes, and other changes in population size since the taxa were isolated (Edwards & Beeli 2000; Hudson & Coyne 2002; Pannell 2003; Rannala & Yang 2003; Rosenberg 2003; Vogl et al. 2003). Diversifying and directional selection increase and decrease, respectively, expected coalescence times for particular loci. At  $5.298N$  generations after divergence, 99% of the loci in newly isolated species are expected to attain intraspecific monophyly (Rosenberg 2003). If the effective population size ( $N$ ) is large and the generations are long-lived, this can amount to many thousands of years. Stenøien and Sæstad (1999) attributed the lack of divergence between American and European populations of *Sphagnum angustifolium* (Russov) C. Jens. to large population sizes and consequent low rates of genetic drift.

Because of high levels of variation and rapid coalescence times, animal mitochondrial DNA has been utilized extensively to investigate phylogenetic and phylogeographic relationships within and between closely related species, yet almost one quarter of investigated species appear to be nonmonophyletic (Funk and Omland 2003). It is perhaps therefore not surprising that closely related morphospecies in the *S. subsecundum* complex appear to be poly- and/or paraphyletic. Nevertheless, the results of this study can be compared to those from several other empirical studies of *Sphagnum* species. Shaw et al. (2005), using the same set of loci employed for the present study, found that most

morphospecies in *Sphagnum* sect. *Acutifolia* are demonstrably monophyletic. Some individual samples appeared to be genetic admixtures, yet despite these mixed samples almost all the traditionally recognized species contained a core of populations that were resolved (and well supported) as monophyletic. Indeed, some controversial species that are barely distinct morphologically were surprisingly distinct in terms of DNA sequences. Shaw et al. (2004) found that three other species of *Sphagnum* sect. *Subsecunda* are demonstrably monophyletic at each of the loci utilized in the present study, as well as in combined analyses. An additional informative study of closely related species of sect. *Subsecunda* (Zhou, Menzel & Shaw, unpubl.) compared two morphotypes that have generally been considered conspecific (*S. macrophyllum* var. *macrophyllum* Bernh. ex Brid. and *S. macrophyllum* var. *floridanum* Austin) and found that these two morphologically similar taxa are mutually monophyletic using a subset of the loci included in the present study. These studies raise the expectation that if morphospecies in the *S. subsecundum* complex have divergent genealogical histories, the present data set is likely to provide supportive evidence. That is, judging from other groups within *Sphagnum*, these loci provide sufficient signal to resolve closely related taxa. Although it can (always) be argued that the species may be resolved with additional sequence data, it is clear that these taxa are not comparable in a phylogenetic sense to many other species of *Sphagnum*.

One possibility is that species in the *S. subsecundum* complex may be of more recent origin compared to other *Sphagnum* species. Alternatively, there may be too much "interspecific" interbreeding for the morphospecies to attain mutual monophyly. There is also the possibility that morphological differences between the taxa represent environmental modifications without a genetic basis, or that genetic differences underlying the morphotypes have arisen in parallel. In either case, it would appear that the species have been "oversplit" and do not represent phylogenetically distinct entities.

In contrast to the true mosses (class Bryopsida), chromosome numbers are relatively invariant in *Sphagnum*. Only two gametophytic numbers are known:  $n = 19 + m$  and  $n = 38 + m$  (Newton 1993). Both numbers occur in the *S. subsecundum* complex and polyploidy may at least partly underlie incongruence between morphology and DNA-based genealogical relationships. Although counts are relatively few, and multiple populations of each taxon still need to be investigated, a haploid ( $n = 19$ ) chromosome complement has been reported for a Finnish collection and six Polish samples of *S.*

*subsecundum* (Sliwinska et al. 2000; Sorsa 1956) and two North Carolina populations of *S. lescurii* [as *S. subsecundum*; Bryan 1955 (specimens in DUKE)]. However, more recent photometric analyses (Melosik, unpublished) of genome size in *S. lescurii* indicate that this species is gametophytically diploid (i.e.,  $n = 38$ ). *Sphagnum inundatum* and *S. denticulatum* have diploid gametophytes ( $n = 38$ ) (Holmen 1955 and Smith & Newton 1968, respectively). Temsch et al. (1998) used photometry to assess ploidy levels in 30 *Sphagna* based on DNA content and reported that *S. denticulatum* is haploid. However, subsequent analyses of seven samples indicate that *S. denticulatum* is diploid (Temsch, pers. comm.). Sliwinska et al. (2000) also inferred from photometric analyses that six samples of *S. denticulatum* from Poland had  $n = 38$ .

Various hypotheses of allopolyploid origins have been advanced for the diploids, *S. denticulatum* (e.g., Hill 1975) and *S. inundatum* (Maass & Harvey 1973). *Sphagnum subsecundum* is thought to be one likely parent, but a putative second parent has not been identified and little concrete evidence exists to support allopolyploid origins for either species (although possibly correct). Eddy (1977) suggested that *S. inundatum* evolved from *S. subsecundum*, probably through autodiploidy. If the diploids (*S. denticulatum*, *S. inundatum*, *S. lescurii*) are allopolyploids, this could explain the lack of differentiation between them, and between them and the haploids, at the molecular level. Moreover, the allopolyploids could have arisen more than once, explaining the apparent polyphyly based on DNA sequences. The allopolyploids in Europe appear to have a separate origin(s) from those in North America. In addition, the allopolyploid hypothesis does not explain the manner in which even the haploid species (*S. subsecundum*) is non-monophyletic in our analyses. Nevertheless, chromosome numbers and additional genetic data may clarify the systematic and evolutionary status of morphospecies in the *S. subsecundum* complex.

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#### LITERATURE CITED

- ÅBERG, G. 1937. Untersuchungen über die *Sphagnum*-Arten der Gruppe *Subsecunda* in Europa. *Arkiv för Botanik* 29A 1: 1–77.
- ANDREWS, A. L. 1913. Order Sphagnales. *North American Flora* 15: 1–31.
- . 1959. Notes on North American *Sphagnum*. XI. *Sphagnum subsecundum*. *THE BRYOLOGIST* 62: 87–96.
- ANDRUS, R. E. 1980. Sphagnaceae (peat moss family) of New York State.—Contributions to a flora of New York State III. *New York State Museum Bulletin* 422: 1–89.
- & D. H. VITT. 1977. *Sphagnotheca Boreali-americana*: fascicle II. *THE BRYOLOGIST* 80: 645–647.
- BRYAN, V. S. 1955. Chromosome studies in the genus *Sphagnum*. *THE BRYOLOGIST* 58: 16–39.
- BRZEG, A., I. MELOSİK, W. STACHNOWICZ & A. STEBEL. 2000. Outline phytosociological scale and ecology of three related species of peatmosses—*Sphagnum subsecundum* s.l., in the light of chosen data from Poland. In M. Krzakowa & I. Melosik (eds.), *The variability in Polish populations of Sphagnum taxa (Subsecunda section)*, according to morphological, anatomical and biochemical traits, 49–59. *Bogucki Wydawnictwo Naukowe S.C.: Poznan, Poland.*
- CRONBERG, N. 1993. Reproductive biology of *Sphagnum*. *Lindbergia* 17: 69–82.
- . 1994. Genetic diversity and reproduction in *Sphagnum* (Bryophyta): isozyme studies in *S. capillifolium* and related species. Ph.D. dissertation, University of Lund, Lund, Sweden.
- CRUM, H. A. 1984. Sphagnopsida, Sphagnaceae. *North American Flora, Series 2*, 11: 1–180.
- . 2001. *Structural Diversity of Bryophytes*. University of Michigan Herbarium, Ann Arbor.
- & L. E. ANDERSON. 1981. *Mosses of Eastern North America*. 2 Vols. Columbia University Press, New York.
- DANIELS, R. E. & A. EDDY. 1985. *Handbook of European Sphagna*. Institute of Terrestrial Ecology. HMSO, London.
- DIRKSE, G. M. & P. ISOVIITA. 1986. *Sphagnum denticulatum*, an older name for *S. auriculatum*. *Journal of Bryology* 14: 388–389.
- EDDY, A. 1977. *Sphagnum subsecundum* agg. in Britain. *Journal of Bryology* 9: 309–319.
- . 1985. A revision of African Sphagnales. *Bulletin of the British Museum, Natural History (Botany)* 12: 77–172.
- EDWARDS, S. V. & P. BEERLI. 2000. Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* 54: 1839–1854.
- FIFE, A. J. 1996. A synopsis of New Zealand *Sphagna*, with a description of *S. simplex* sp. nov. *New Zealand Journal of Botany* 34: 309–328.
- FLATBERG, K. I. 1985. Taxonomy of crispate morphotypes in *Sphagnum* sect. *Subsecunda*. *Lindbergia* 11: 99–113.
- . 1986. Taxonomy, morphovariation, distribution and ecology of the *Sphagnum imbricatum* complex with main reference to Norway. *Gunneria* 54: 1–118.
- . 2002. *The Norwegian Sphagna: a field colour guide*. Norges Tehnisk-naturvitenskapelige Universitet Vitenskapsmuset, Rapport Botanisk, Serie 2002-1: 1–44.
- FRITSCH, R. 1982. Index to plant chromosome numbers—Bryophyta. Scheltema and Holkema, The Hague & Boston.
- FUNK, D. J. & K. E. OMLAND. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics* 34: 397–423.
- GOOSSENS, M. & J. DE SLOOVER. 1981. Étude taxono-

- mique et synécologique des espèces du genre *Sphagnum* section *Subsecunda* dans une tourbière de Haute Ardenne. Bulletin de la Société Royale de Botanique de Belgique 114: 89–105.
- HEY, J. & J. WAKELEY. 1997. A coalescent estimator of the population recombination rate. *Genetics* 145: 833–846.
- HILL, M. O. 1975. *Sphagnum subsecundum* Nees and *S. auriculatum* Schimp. in Britain. *Journal of Bryology* 8: 435–441.
- , C. D. PRESTON & A. J. E. SMITH. 1992. Atlas of the Bryophytes of Britain and Ireland Vol. 2 Mosses (except Diplolepidaceae). Horley Book, London.
- HOLMEN, K. 1955. Chromosome numbers of some species of *Sphagnum*. *Botanisk Tidsskrift* 52: 37–42.
- HUDSON, R. R. & J. A. COYNE. 2002. Mathematical consequences of the genealogical species concept. *Evolution* 56: 1557–1565.
- & N. L. KAPLAN. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics* 111: 147–164.
- HUELSENBECK, J. P. & F. RONQUIST. 2002. MrBayes version 3.0B. [www document] URL <http://morphbank.ebc.uu.se/mrbayes3/info.php>
- ISOVIITA, P. 1966. Studies on *Sphagnum* L. I. Nomenclatural revisions of the European taxa. *Annales Botanici Fennici* 7: 157–162.
- KRZAKOWA, M. & I. MELOSİK (Eds.). 2000. The variability in Polish populations of *Sphagnum* taxa (*Subsecunda* section), according to morphological, anatomical and biochemical traits. Bogucki Wydawnictwo Naukowe S.C., Poznan, Poland.
- LINDBERG, S. O. 1882. Europas och Nord Amerikas Hvitmossor (*Sphagna*). Promotionsprogram. J.C. Frenckell & Son, Helsinki.
- MAASS, W. S. G. & M. J. HARVEY. 1973. Studies on the taxonomy and distribution of *Sphagnum* VII. Chromosome numbers in *Sphagnum*. *Nova Hedwigia* 24: 193–205.
- MCQUEEN, C. B. 1989. A biosystematic study of *Sphagnum capillifolium* sensu lato. *THE BRYOLOGIST* 92: 1–24.
- MELOSİK, I. 2000a. Distribution of species of the *Subsecunda* section of *Sphagnum* genus in Poland. In M. Krzakowa & I. Melosik (eds.), The variability in Polish populations of *Sphagnum* taxa (*Subsecunda* section), according to morphological, anatomical and biochemical traits, 27–47. Bogucki Wydawnictwo Naukowe S.C., Poznan, Poland.
- . 2000b. Morphological and anatomical variation of taxa belonging to the *Subsecunda* section of the *Sphagnum* genus, as presented by materials collected in Poland. In M. Krzakowa & I. Melosik (eds.), The variability in Polish populations of *Sphagnum* taxa (*Subsecunda* section), according to morphological, anatomical and biochemical traits, 61–109. Bogucki Wydawnictwo Naukowe S.C., Poznan, Poland.
- NEWTON, M. E. 1993. Cytogenetics of *Sphagnum*. *Advances in Bryology* 5: 61–78.
- NYLANDER, J. A. A. 2002. MrModeltest—version 1.1b. [www document] URL <http://www.ebc.uu.se/systzoo.staff/nylander.html>
- OCHYRA, R. 1995. *Sphagnum auriculatum* Schimp.: a genus new to the bryophyte flora of Crete. *Journal of Bryology* 18: 827–828.
- PACAK, A. & Z. SZWEJKOWSKA. 2003. Organellar inheritance in liverworts: an example of *Pellia borealis*. *Journal of Molecular Evolution* 56: 11–17.
- PANNELL, J. R. 2003. Coalescence in a metapopulation with recurrent local extinction and recolonization. *Evolution* 57: 949–961.
- RAHMAN, S. M. A. 1972. Taxonomic investigations on some British *Sphagna*. *Sphagnum subsecundum* sensu lato. *Journal of Bryology* 7: 169–179.
- RANNALA, B. & Z. YANG. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164: 1645–1656.
- ROSENBERG, N. A. 2003. The shapes of neutral gene phylogenies in two species: probabilities of monophyly, paraphyly, and polyphyly in a coalescent model. *Evolution* 57: 1465–1477.
- RUSSOW, E. A. F. 1894. Zur Kenntnis der *Subsecundum*- und *Cymbifolium* Gruppe europäischer Torfmoose, nebst einem Anhang, enthaltend eine Aufzählung der bisher im Ostbalticum beobachteten *Sphagnum*-Arten und einen Schlüssel zur Bestimmung dieser Arten. *Archiv für die Naturkunde Liv- Ehst- und Kurlands, Série 2*, 10: 361–527.
- SÅSTAD, S. M. 1998. Species delimitation and phylogenetic relationships within the *Sphagnum recurvum* complex (Bryophyta): genetic variation and phenotypic plasticity. Ph.D. dissertation, Norwegian University of Science and Technology, Trondheim: Norway.
- SCHNEIDER, S., D. ROESSLI & L. EXCOFFIER. 2000. ARLEQUIN ver. 2.000. [www document] URL <http://anthro.unige.ch/arlequin>
- SHAW, A. J. 2000. Phylogeny of the Sphagnopsida based on nuclear and chloroplast DNA sequences. *THE BRYOLOGIST* 103: 277–306.
- , C. J. COX & S. B. BOLES. 2003. Polarity of peatmoss (*Sphagnum*) evolution: who says mosses have no roots? *American Journal Botany* 90: 1777–1787.
- , ——— & ———. 2004. Phylogenetic relationships among *Sphagnum* sections: *Hemitheca*, *Isocladus*, and *Subsecunda*. *THE BRYOLOGIST* 107: 189–196.
- , ——— & ———. 2005. Phylogeny, species delimitation, and interspecific recombination in *Sphagnum* section *Acutifolia*. *Systematic Botany* 30: 16–33.
- SLIWINSKA, E., M. KRZAKOWA & I. MELOSİK. 2000. Estimation of ploidy level in four *Sphagnum* species (*Subsecunda* section) by flow cytometry. In M. Krzakowa & I. Melosik (eds.), The variability in Polish populations of *Sphagnum* taxa (*Subsecunda* section), according to morphological, anatomical and biochemical traits, 137–150. Bogucki Wydawnictwo Naukowe S.C., Poznan, Poland.
- SMEDMARK, J. E. E., T. ERIKSSON, R. C. EVANS & C. S. CAMPBELL. 2003. Ancient allopolyploid speciation in Geinae (Rosaceae): evidence from nuclear granule-bound starch synthase (BGSSI) gene sequences. *Systematic Biology* 52: 374–385.
- SMITH, A. J. E. & M. E. NEWTON. 1968. Chromosome studies on some British and Irish mosses. III. *Transactions of the British Bryological Society* 5: 463–522.
- SORSA, V. 1956. The quadripolar spindle and the change in orientation of the chromosomes in meiosis of *Sphagnum*. *Annales Academiae Scientiarum Fennicae, Series A, IV, Biologica* 33: 1–64.
- STENØJEN, H. K. & S. M. SÅSTAD. 1999. Genetic structure in three haploid peatmosses (*Sphagnum*). *Heredity* 82: 391–400.
- SWOFFORD, D. L. 2001. PAUP\*: phylogenetic analysis using parsimony (\*and other methods). Vers. 4.0b8. Sinauer Associates, Sunderland, Mass.
- TAJIMA, D. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

- TEMSCH, E. M., J. GREILHUBER & R. KRISAI. 1998. Genome size in *Sphagnum* (peat moss). *Botanica Acta* 111: 325–330.
- VOGL, C., A. DAS, M. BEAUMONT, S. MOHANTY & W. STEPHAN. 2003. Population subdivision and molecular sequence variation: theory and analysis of *Drosophila ananassae* data. *Genetics* 165: 1385–1395.
- WARNSTORF, C. 1911. Sphagnales-Sphagnaceae (Sphagnologia Universalis). In H. G. A. Engler (ed.), *Das Pflanzenreich* 51: 1–546. Wilhelm Englemann, Leipzig.
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