American Journal of Botany

RESEARCH ARTICLE

Phylogenetic structure in the *Sphagnum recurvum* complex (Bryophyta) in relation to taxonomy and geography

Aaron M. Duffy[1], Blanka Aguero[1], Hans K. Stenøien[2], Kjell Ivar Flatberg[2], Michael S. Ignatov[3], Kristian Hassel[2], and A. Jonathan Shaw[1,4]

Manuscript received 19 February 2002; revision accepted 28 May 2020.

[1] Lewis E. Anderson Bryophyte Herbarium, Department of Biology, Duke University, Durham, North Carolina 27708, USA

[2] Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway

[3] Tsitsin Main Botanical Garden of Russian Academy of Sciences, Moscow, Russia

[4] Author for correspondence (e-mail: shaw@duke.edu)

Citation: Duffy, A. M., B., Aguero, H. K., Stenøien, K. I., Flatberg, M. S., Ignatov, K., Hassel, and A. J., Shaw. 2020. Phylogenetic structure in the *Sphagnum recurvum* complex (Bryophyta) in relation to taxonomy and geography. *American Journal of Botany* 107(9): 1–13.

doi:10.1002/ajb2.1525

PREMISE: The *Sphagnum recurvum* complex comprises a group of closely related peat mosses that are dominant components of many northern wetland ecosystems. Taxonomic hypotheses for the group range from interpreting the whole complex as one polymorphic species to distinguishing 6–10 species. The complex occurs throughout the Northern Hemisphere, and some of the putative species have intercontinental ranges. Our goals were to delimit the complex and assess its phylogenetic structure in relation to morphologically defined species and intercontinental geography.

METHODS: RADseq analyses were applied to a sample of 384 collections from Europe, North America, and Asia. The data were subjected to maximum likelihood phylogenetic analyses and analyses of genetic structure using the software STRUCTURE and multivariate ordination approaches.

RESULTS: The *S. recurvum* complex includes *S. angustifolium*, *S. fallax*, *S. flexuosum*, *S. pacificum*, and *S. recurvum* as clades with little evidence of admixture. We also resolved an unnamed clade that is referred to here as *S.* "pseudopacificum." We confirm that *S. balticum* and *S. obtusum* are nested within the complex. Species with bluntly acute to obtuse stem leaf apices are sister to those with acute to apiculate leaves. Most of the species exhibit some differentiation between intraspecific population systems disjunct on different continents.

CONCLUSIONS: We recognize seven species in the amended *S. recurvum* complex, including *S. balticum* and *S. obtusum*, in addition to the informal clade *S.* "pseudopacificum." Although we detected some geographically correlated phylogenetic structure within widespread morphospecies, our RADseq data support the interpretation that these species have intercontinental geographic ranges.

KEY WORDS peat moss; Sphagnaceae; Sphagnum; Sphagnum angustifolium; Sphagnum balticum; Sphagnum fallax; Sphagnum flexuosum; Sphagnum obtusum; Sphagnum pacificum; Sphagnum recurvum.

Plants that reproduce with spores rather than seeds, including the bryophytes, lycophytes, and monilophytes, are generally thought to have broad ranges that often span multiple continents (Schofield and Crum, 1972). It has been estimated that ~70% of the mosses found in Europe also occur in North America (Frahm and Vitt, 1993). Indeed, a perusal of the bryophyte volumes in the *Flora of North America* indicate that as presently understood, most temperate and boreal bryophyte species are recorded from multiple continents. Moreover, a substantial number of Neotropical bryophytes are also reported from Africa and/or other tropical continental areas (Gradstein et al., 1983). Consistent with the general pattern of bryophytes having broad, often intercontinental ranges is that many south-temperate bryophyte species are thought to occur disjunctively between Australia/New Zealand and South America, with low rates of endemism in any one area (such as New Zealand) (Muñoz et al., 2004). By contrast, most seed-plant species are restricted to a single continent; Qian (1999) estimated that only ~6% of vascular plants are shared between North America and Europe. Notwithstanding issues such as how to define what constitutes a species, heterogeneity among plant groups in genetic/phylogenetic structure, and differences in the approaches of different taxonomists, the general pattern that spore plants have broader ranges than seed plants has been uncontroversial (but see Vigalondo et al., 2019).

Dated molecular phylogenies have consistently suggested divergence times between intercontinentally disjunct bryophyte populations as being far too recent to be explained by continental drift. Thus, attention has focused on the efficacy of spore dispersal in minimizing or eliminating divergence among distinct populations of bryophytes. At the same time, recent systematic analyses have shown that some species previously thought to have extremely broad intercontinental ranges consist of genetically and sometimes morphologically divergent units that can be interpreted as separate species (Heinrichs et al., 2010; Medina et al., 2012, 2013; Renner et al., 2013, 2017; Hedenäs et al., 2014; Hassel et al., 2018, Vigalondo et al., 2019).

Here, we describe phylogenetic structure in a small clade of closely related plants in the moss genus *Sphagnum* (Sphagnaceae, peat moss). *Sphagnum*, with some 200–400 species, is the largest of four genera in the moss class Sphagnopsida (Shaw et al., 2010, 2016). The genus is especially abundant and diverse in north-temperate and boreal regions of the Northern Hemisphere, where it grows in and actually creates peatlands—bogs and fens—in many wetland habitats (Rydin and Jeglum, 2013). *Sphagnum*-dominated peatlands have long served as a model for research in community assembly and niche differentiation among closely related sympatric species. Moreover, because some 25% of Earth's terrestrial carbon pool is stored in *Sphagnum*-dominated peatlands (Yu, 2012), the genus has recently become a model for linking the plants and their traits, ecosystem function, and global climate (Weston et al., 2018).

There has arguably been more molecular work on interspecific and intraspecific genetic/phylogenetic structure on *Sphagnum* than on any other genus of bryophytes. Most Northern Hemisphere species of *Sphagnum*, like other bryophytes, are thought to have geographic ranges that span multiple continents (McQueen and Andrus, 2007). Genetic analyses have supported these interpretations that most species are widespread and that endemism, even at the continental scale, is low. Most of the North American species are found in Europe; some occur in both eastern and western North America, although some intercontinental species have amphi-Atlantic or amphi-Pacific ranges (Shaw et al., 2004, 2014; Kyrkjeeide et al., 2015, 2016a, b; Yousefi et al., 2017).

In their revision of *Sphagnum* for the *Flora of North America*, McQueen and Andrus (2007) recognized 91 species. The genus comprises five major clades, each recognized as a subgenus (Shaw et al., 2016). The focus of this study, the so-called *S. recurvum* complex (Flatberg, 1992a), falls within the subgenus *Cuspidata*. Taxonomy of the *S. recurvum* complex has been highly variable; Crum (1984) considered it a single species, *S. recurvum* P. Beauv., but McQueen and Andrus (2007) recognized five to seven distinct species (depending on precisely how the complex is delimited). Flatberg (1992a) recognized five "core" species. Most of the putative species are widespread in both North America and Europe, excluding *S. rubroflexuosum* Andrus, which is endemic to a few sites in eastern North America, and *S. recurvum* s. str., which is restricted to the New World other than one confirmed report from the Azores. While most of the species are common and widespread across North America and Europe, some are not strictly circumboreal. *Sphagnum fallax*, for example, is common in Europe and eastern North America but appears to be absent from western North America. A related species, *S. pacificum*, is limited in North America to the Pacific coast and is not known from eastern North America or Europe. *Sphagnum angustifolium*, perhaps the most common species in the complex, occurs in Europe, Asia, and both eastern and western North America.

Our goals in this research were to assess the phylogenetic structure of this complex and address the following questions: (1) How many phylogenetically distinguishable species are there in the complex? (2) Is there genetic differentiation between plants on different continents such that additional allopatric species are resolved, contrary to the interpretation that these species have intercontinental ranges? (3) If the species do have intercontinental ranges, is there detectable differentiation among metapopulation systems on different continents (or between eastern and western North American systems)? This group is especially appropriate for addressing these questions because the traditionally defined species are difficult to distinguish and have been variously interpreted at the taxonomic level. They occupy wet microsites near the water table within peatland communities, possibly facilitating interspecific hybridization, given that sperm are released into the water and the taxa frequently co-occur in close proximity. Most of the putative species are currently thought to have intercontinental ranges.

MATERIALS AND METHODS

Taxon sampling

A total of 384 plants were sampled for the genomic analyses. These included plants generally considered part of the complex, two other taxa not generally included within the complex but sometimes considered close (*S. balticum*, *S. obtusum*), four other species from outside the complex but within subg. *Cuspidata* (*S. annulatum*, *S. majus*, *S. pulchrum*, *S. riparium*), and one other, more distant, outgroup from subg. *Subsecunda* (*S. missouricum*). Of these, 90 were excluded because of poor data quality. The remaining 294 samples that were included in the final analyses comprised 113 samples from Europe (of which 89 were from Norway, one from Portugal [Azores], and 23 from European Russia), 134 from eastern North America, and 47 from western North America.

Most of the species included in this study have broad distributions that span multiple continents, and we tried to sample across their ranges. Our success was limited by the availability of recent specimens that could yield DNA of sufficient quality for the RADseq procedure. As a consequence, our western North American samples were largely from Alaska, where we were able to conduct fieldwork, supplemented by two samples representing two species from Vancouver Island, British Columbia. Our European samples were collected from central Norway, and from western Russia near Moscow. Sampling from eastern North America, where the group is most diverse, was more extensive. The group is poorly known in eastern Asia (e.g., Japan and China) and we were not able to include samples from those regions. Overall, the sampling was sufficient to address our main questions regarding species delimitation and differentiation within species between North America and Europe.

Voucher specimens are archived in the Duke University herbarium (DUKE). Specimen information and voucher data (including geocoordinates for all samples) are included in Appendix S1. The central portion of a single capitulum was sampled for the molecular work, and the remaining tissue from that stem was placed in a small packet and returned to the herbarium specimen.

DNA isolation, library preparation, and sequencing

Genomic DNA was extracted from dried samples using tissue from the capitulum of the gametophytes, each with a mass of ~100 mg. Extractions followed the CTAB protocol outlined in Shaw et al. (2003). DNA concentrations were measured using a Qubit 2.0 Fluorometer (Life Technologies, Waltham, Massachusetts, USA) and standardized to 20 ng/μL. Genomic libraries were made following the double digestion restriction site-associated DNA sequencing (ddRADseq) protocol of Parchman et al. (2012) with modifications described here. Restriction digest of 10 μL of the genomic DNA sample was performed in a 25 μL reaction containing 0.5 μL restriction enzyme EcoRI, 1 μL restriction enzyme MseI, and 2.5 μL Cutsmart buffer (New England BioLabs, Ipswich, Massachusetts) over 3 h at 37°C, followed by 10 min at 65° C for permanent inactivation of the enzymes. Digested fragments of each sample were ligated to uniquely barcoded oligonucleotide adapters in 12 μL reactions containing 9 μL digested DNA, 1 μL barcoded EcoRI adapter, 1 μL MseI adapter, 0.2 μL T4 DNA ligase enzyme, and 0.12 μL 10X ligase buffer (New England BioLabs) at 23°C for 1 h. Amplification of ligated DNA fragments containing both ligated adapters was performed in 20 μL reactions containing 2 μL ligated DNA, 4 μL 1 mM dNTP, 4 μL 5X buffer, 1.3 μL 5 μM premixed polymerase chain reaction (PCR) primers, 0.4 μL $MgCl_2$, 0.15 μL DMSO, and 0.2 μL iProof *Taq* polymerase (Bio-Rad, Brossard, Quebec, Canada). PCR product concentrations were measured using a Qubit 2.0 Fluorometer and pooled into four libraries, each containing 10 ng of 96 PCR products. Each library was cleaned and size-selected for fragments around 350 bp using AMPur XPbeads (Beckman Coulter, Jersey City, New Jersey, USA), checked for quality on a BioAnalyzer (Agilent Technologies, Wilmington, Delaware, USA) and sequenced on a single lane of Illumina HiSeq 2000 with 100 bp single-end reads or a single lane of NextSeq 500 with 150 bp single-ended reads at the Genome Sequencing Shared resource operated by the Duke Center for Genomic and Computational Biology (https://genome.duke.edu/).

RADseq data pipeline

Raw Illumina reads were checked for quality with FastQC (Andrews, 2010), and reads from the NextSeq 500 runs were trimmed to match the length of reads from the HiSeq 2000 runs. Single nucleotide polymorphism (SNP) discovery was performed with ipyrad version 0.7.29 (Eaton, 2014) using default parameters except as noted here. Reads were processed as datatype "ddrad" to match the library preparation method and samples were treated as haploid, since all except six samples of one outgroup species were expected to have haploid gametophytes. A maximum of two mismatched bases were allowed in the barcode during demultiplexing; Illumina adapter sequences and low-quality bases were trimmed from the reads; and trimmed reads <42 bases long or with more than five low-quality bases were discarded. Multiple ipyrad runs were performed using a range of read clustering thresholds to identify the clustering threshold (0.90) that maximized the number of variable and phylogenetically informative loci and to verify that the results of downstream analyses are not sensitive to clustering threshold. Low-read samples and samples with low numbers of loci identified in these exploratory analyses were removed. Only loci present in ≥80% of the remaining samples were kept for the final analyses, and additional runs using different minimum sample coverage values were performed to ensure that inferences are not sensitive to the number of loci and level of missing data. Ipyrad uses Muscle (https://www.ebi.ac.uk/Tools/msa/muscle/) to align sequences in the matrix.

Phylogenetic analyses

RAxML version 8.2.12 (Stamatakis, 2014) was used to estimate phylogenetic relationships among sequences under maximum likelihood (ML) using concatenated loci identified by ipyrad. The ML tree was estimated using random starting trees, the rapid bootstrap analysis and best-scoring ML tree search, and the GTRGAMMA nucleotide substitution model. The rapid hill-climbing search algorithm was used to estimate the best ML tree. Multiple searches did not detect any variation in optimal topology. One hundred bootstrap replicates were performed to determine support for branches.

Phylogenetic relationships among species were also estimated under the multispecies coalescent model using singular value decomposition scores for species quartets (SVDquartets) as implemented in PAUP* version 4.0a, build 165 (Swofford, 2003; Chifman and Kubatko, 2014; Chifman and Kubatko, 2015). Ten million random quartets were sampled (22.28% of total distinct quartets) with 200 bootstrap replicates to determine branch support.

Cluster analyses

Each locus identified by ipyrad may contain multiple SNPs, so to avoid using tightly linked SNPs, one randomly selected SNP per locus was used for clustering analyses. Genetic structure within the *S. recurvum* complex was explored using Bayesian model-based cluster analysis with STRUCTURE version 2.3.2.1 (Pritchard et al., 2000). The most likely number of clusters (K) was evaluated using the method of Evanno et al. (2005) based on 10 independent runs using an admixture model with correlated allele frequencies for each K from 1 to 10 with 50,000 steps of burn-in and 500,000 steps per run. Regardless of how this method evaluated the "optimal" K, we explored higher levels of K to assess the possibility of additional structure in the data. Matrices of membership coefficients across the independent runs were used to search for the optimal alignment with CLUMPP version 1.1.2 (Jakobsson and Rosenberg, 2007). In addition to the entire *S. recurvum* complex, STRUCTURE was also used on subsets of the samples to explore finer-scale genetic structure within major clades identified in the phylogenetic analyses and within individual species when sufficient samples were available.

Principal component analysis (PCA) of scaled allele frequencies using the R package "adagenet" (Jombart, 2011) was used to further explore genetic structure in the entire complex, in major clades, and within individual species.

Genetic diversity analyses

Genetic diversity patterns were explored by calculating diversity statistics and estimating pairwise Nei's unbiased genetic distance with GenAlEx version 6.5 (Peakall and Smouse, 2006, 2012) and estimating pairwise F_{ST} with the R package "hierfstat" (Goudet, 2005). Genetic diversity statistics and distances were calculated for the entire complex, for major clades, and for individual species.

RESULTS

Data characterization

Four lanes of Illumina sequencing yielded 652 million reads. After trimming, removing barcodes and adapter sequences, filtering for quality, and removing samples with low readcounts, 448 million reads of 42 to 92 bp were retained across 294 individual plant samples, with the number of reads per individual ranging from 375,249 to 2,878,373 (median ± SD = 1,518,724 ± 531,416). The assembly pipeline produced 6170 loci shared among ≥80% of the individuals; the final matrix included 484,401 bp, and 6148 of those loci contained one or more SNPs and 6100 contained one or more phylogenetically informative SNPs (i.e., those shared by two or more individuals). The mean locus coverage per individual was 88.2%.

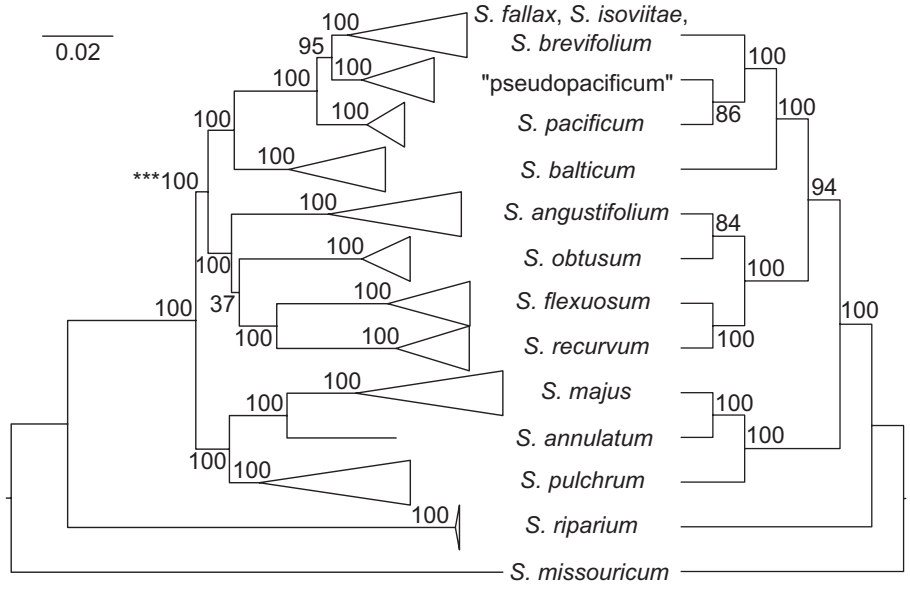


FIGURE 1. Summary of phylogenetic relationships among *Sphagnum recurvum* complex species and related species based on RADseq loci. Relationships were estimated using maximum likelihood (left) and singular value decomposition scores for species quartets (right). Nodes are labeled with bootstrap support values. Asterisks indicate circumscription of the *S. recurvum* complex.

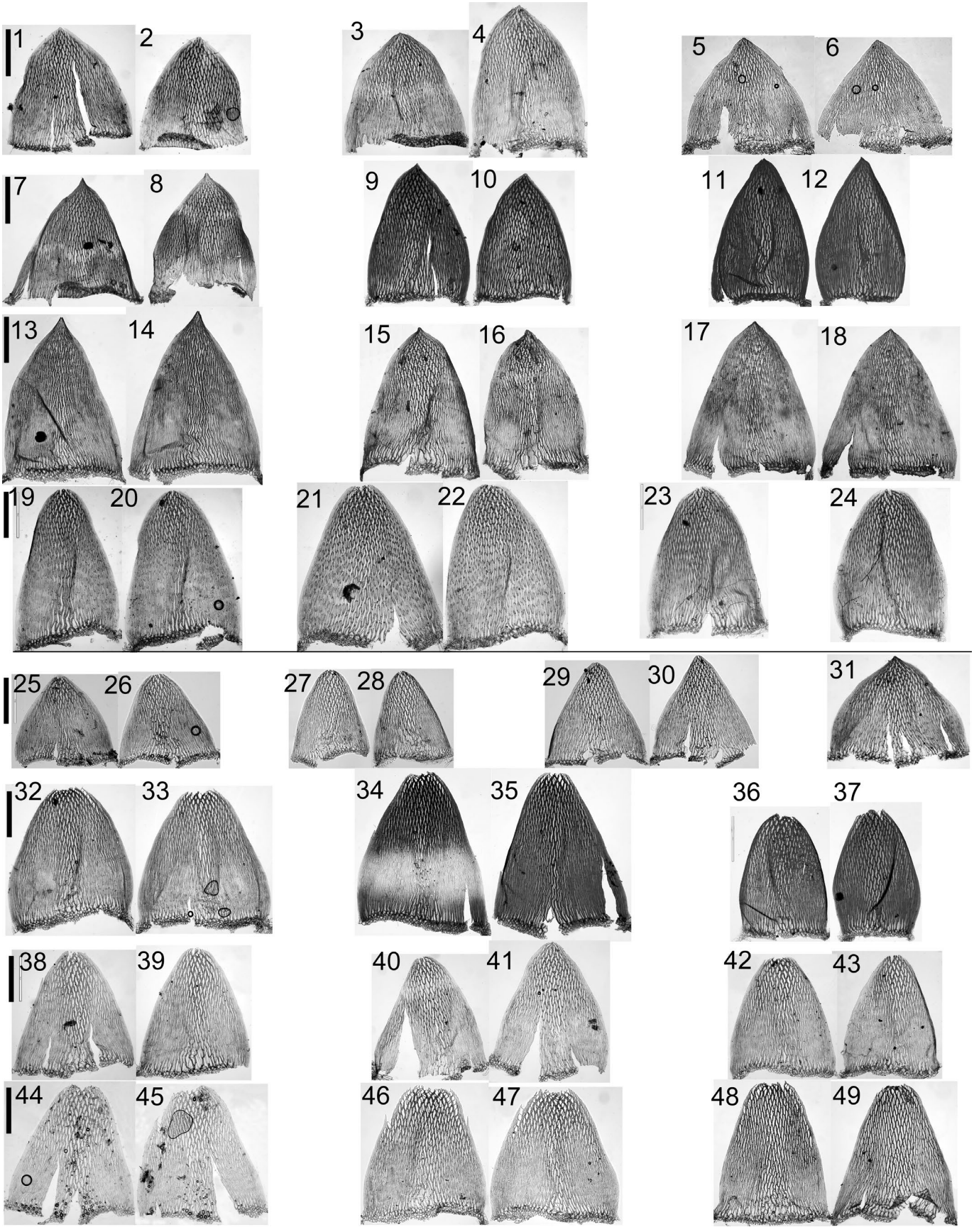
Phylogenetic analyses

Phylogenetic relationships among all individuals are provided in Appendix S2; relationships among species are summarized in Fig. 1. Rooted by a series of increasingly distant *Sphagnum* species, the *S. recurvum* complex is resolved as monophyletic with 100% bootstrap (BS) support (identified by asterisks in Fig. 1 and Appendix S2). The complex, defined phylogenetically, includes two species, *S. balticum* and *S. obtusum*, that have not generally been identified as closely related to the core species. The complex is sister to a clade that includes *S. majus*, *S. annulatum*, and *S. pulchrum*. *Sphagnum majus* has been shown to be an allopolyploid species (i.e., diploid gametophytes, tetraploid sporophytes) derived from a cross between *S. cuspidatum* (also in subg. *Cuspidata*) and *S. annulatum*. It was not possible to distinguish homologous and homeologous loci in allopolyploid *S. majus*, but despite this uncertainty, samples were resolved as a monophyletic group sister to one of its established parental species, *S. annulatum*.

Two reciprocally monophyletic groups are resolved (each with 100% BS) within the *S. recurvum* complex, hereafter referred to as the "Pointed Leaf" and "Rounded Leaf" groups (clades). These labels refer to the stem leaves, which tend to be acute to apiculate in the Pointed Leaf clade and are obtuse to broadly acute in the Rounded Leaf clade (Fig. 2). These informal labels are not perfectly descriptive (for example, *S. balticum*, phylogenetically part of the Pointed Leaf group, has stem leaves that are barely pointed), but they apply in general. Stem leaf shapes and their apices are generally used for distinguishing species in this complex. *Sphagnum fallax* (Fig. 2: 1–6), *S.* "pseudopacificum" (see below) (Fig. 2: 7–12), and *S. pacificum* (Fig. 2: 13–18) have acute to apiculate leaves whereas *S. balticum*, as noted, has broadly acute to more or less obtuse leaves. Species in the Rounded Leaf group, *S. angustifolium* (Fig. 2: 25–31), *S. obtusum* (Fig. 2: 32–37), and *S. flexuosum* (Fig. 2: 38–43) have broadly obtuse leaves that are often resorbed across the apex. Occasional stem leaves of *S. angustifolium* are more acute (e.g., Fig. 2: 31), causing confusion with *S. fallax*, with which it often co-occurs.

Support for species relationships within the complex differ between maximum likelihood (ML) analyses of the concatenated data set and the quartet analyses (Fig. 1). Based on ML, four taxa are resolved, all with 100% BS, within the Pointed Leaf clade. These include *S. fallax*, *S. pacificum*, and *S. balticum*, plus a currently

FIGURE 2. Stem leaf variation in the *Sphagnum recurvum* complex. All photos are at same magnification; scale bar (left side of each row) = 200 µm. Each row represents a species. Within each species, paired leaves were derived from a single stem. Note the generally greater similarity between leaves from individual stems than between stems of a species. Images 23, 24, and 31 are single leaves from those stems. (**1**–**6**) *S. fallax*, 1–2 SB5051 (*BS19013*, PE), 3–4 SB5083 (*Ignatov 2015-14*, Russia), 5–6 SB5112 (*Garrett A149*, PE same site as 1–2). (**7**–**12**) *S.* "pseudopacificum," 7–8 SB5225 (*Garrett AG541*, AK-Matanuska-Susitna), 9–10 SB5212 (*Garrett AG500*, AK-Alyeska), 11–12 SB5220 (*Garrett AG525*, AK-Kenai). (**13**–**18**) *S. pacificum*, 13–14 SB5152 (*JS 2016-26A*, AK-Juneau), 15–16 SB5154 (*JS 2016-30*, AK-Whittier), 17–18 SB5179 (*Piatkowski 2017-60*, AK-Yakutat). (**19**–**24**) *S. balticum*, 19–20 AG237 (*Garrett A225*, Norway), 21–22 AG249 (*Garrett A248*, Norway), 23–24 AG235 (*Garrett A220*, Norway). (**25**–**31**) *S. angustifolium*, 25–26 SB5250 (*Piatkowski BP2018_145B*, WI), 27–28 SB5077 (*Ignatov 2015-6*, Russia), 29–30 SB5230 (*Garrett AG569*, AK-Fairbanks), 31 SB5252 (*Garrett AG586*, AK-Fairbanks). Note the acute-apiculate apex in leaf 31; similar to those in *S. fallax*. (**32**–**37**) *S. obtusum*, 32–33 SB5229 (*Garrett AG579*, AK-Fairbanks), 34–35 SB5189 (*Piatkowski BP2017_264*, AK-Anchorage), 36–37 SB5215 (*Garrett AG513*, AK-Kenai). (**38**–**43**) *S. flexuosum*, 38–39 SB4985 (*BS18906*, MD), 40–41 SB4976 (*Garrett A033*, MD), 42–43 SB5009 (*Garrett A075*, PA). (**44**–**49**) *S. recurvum*, 44–45 SB4995 (*Garrett A064*, MD), 46–47 SB5109 (*Garrett A142*, PA), 48–49 SB5234 (*BA19605*, NC).


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

unnamed taxon that we refer to as *S.* "pseudopacificum." All samples identified morphologically as *S. brevifolium* or *S. isoviitae*, recognized as species by McQueen and Andrus (2007), Flatberg (2002, 2013), and Laine et al. (2018), are scattered within the *S. fallax* clade with no hint that either species forms a monophyletic group (Appendix S2). In the Rounded Leaf clade, *S. angustifolium* is sister to *S. obtusum*, *S. flexuosum*, and *S. recurvum* in the ML analyses, but in the quartet analysis, *S. obtusum* plus *S. angustifolium* are resolved as a clade sister to *S. flexuosum* plus *S. recurvum*. The sister group relationship between *S. flexuosum* and *S. recurvum* is consistent and strongly supported in both (Fig. 1). ML analyses of relationships among all accessions suggest geographically correlated genetic structure within species that have intercontinental ranges. Reciprocally monophyletic groups of European vs. eastern North American plants are resolved within *S. fallax* (Appendix S2). The European clade is supported at 100% BS whereas the North American clade is weakly supported, although there is a perfect geographic segregation of plants between the clades. *Sphagnum fallax* does not occur in western North America.

Geographic structure within *S. angustifolium* is complex (Appendix S2). Most Alaskan samples are resolved as a separate (weakly supported) clade sister to all the other samples. But a well-supported, smaller second group of samples from Alaska (and British Columbia) appears to be more closely related to plants from northern Europe (Norway and Russia) (Appendix S2). Eastern North American plants also form a single clade that spans at least from Wisconsin and Pennsylvania to subarctic Quebec. All European plants (including those from western Russia) form a clade nested within the overall North American paraphyletic group. Similarly, all European (Norwegian) samples of *S. flexuosum* form a clade that is nested within North American samples of that species. *Sphagnum recurvum* is restricted to eastern North America with the exception of one recorded occurrence in the Azores (Dias et al., 2009). A plant from that single European population is resolved as closely related to samples of *S. recurvum* from the southeastern United States (Appendix S2). Among outgroup taxa, samples of *S. majus* from the eastern United States are nested within two European samples, but this pattern, opposite to

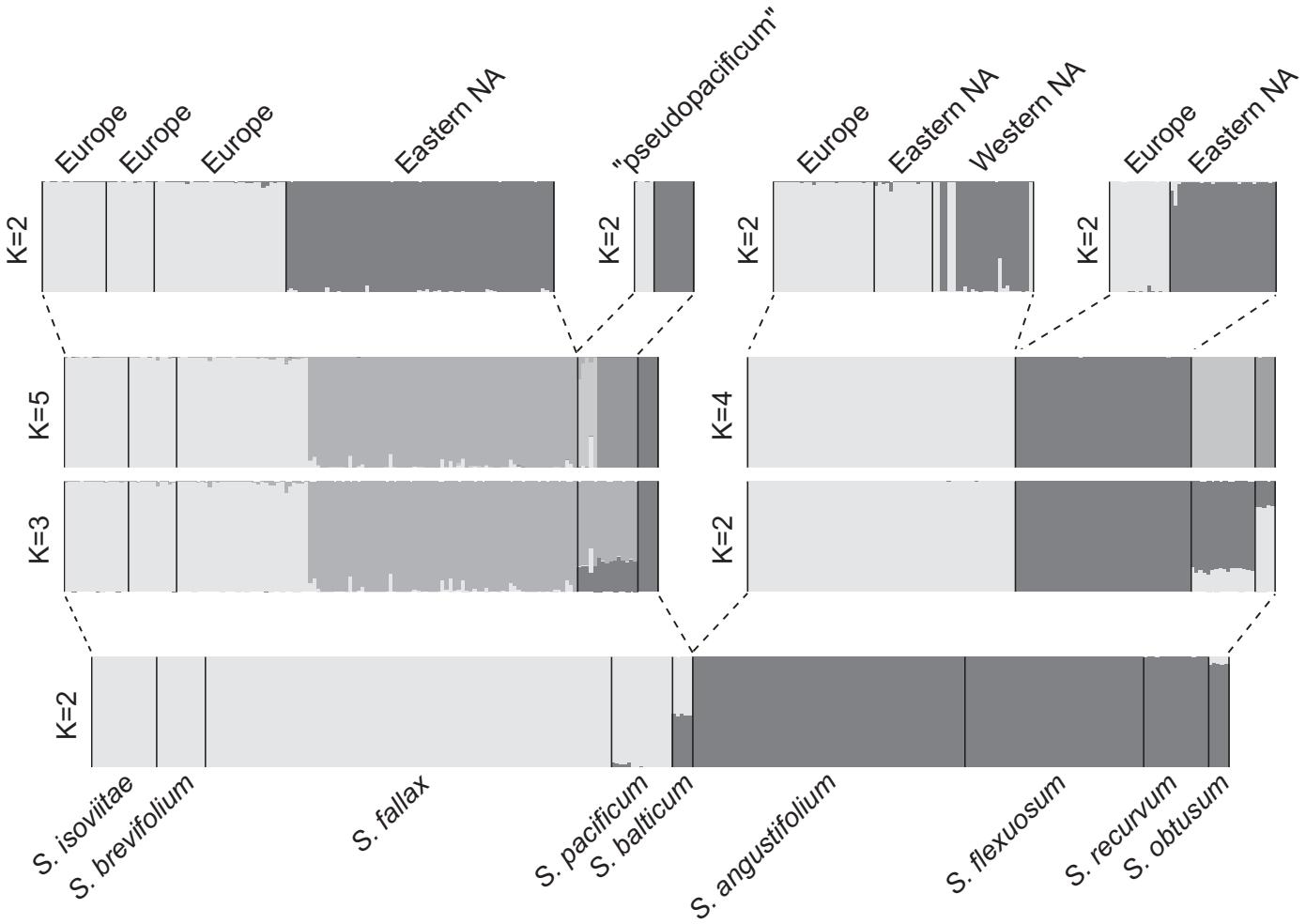


FIGURE 3. Results of STRUCTURE analyses of RADseq loci for all *Sphagnum recurvum* complex species, for the Rounded Leaf and Pointed Leaf species separately, and for individual species. For each analysis, the optimal *K*-value is presented along with a higher *K*-value when it provides additional clustering information. *S. balticum* and *S. obtusum* were not analyzed separately due to small sample sizes, and a separate analysis of *S. recurvum* samples did not provide additional clustering information.

that resolved within *S. angustifolium* and *S. flexuosum*, is based on more limited sampling.

Sampling within continents was too limited to thoroughly assess geographic structure on more local scales, but several species were sampled sufficiently from within eastern North America to suggest that some geographic structure is likely. Within *S. fallax*, for example, samples collected from the same or proximate sites within states generally group together (Appendix S2). As previously noted, Alaskan samples of *S. angustifolium* fall into two clades, both resolved (although weakly supported) as distinct from samples collected in the temperate eastern United States. Some grouping of geographically proximate samples is also evident within *S. recurvum* and *S. flexuosum* (Appendix S2).

Cluster analyses

Results of cluster analyses based on STRUCTURE parallel those from the phylogenetic analyses (Fig. 3). When all samples were analyzed as a group, at $K = 2$ (evaluated as "optimal") the Pointed Leaf and Rounded Leaf groups were resolved, with *S. balticum* showing substantial admixture between the two. *Sphagnum pacificum* and *S. obtusum* also showed admixture, but to a very limited extent (Fig. 2). As with the phylogenetic analyses, no distinction is evident between *S. fallax*, *S. brevifolium*, and *S. isoviitae*.

Separate analyses of the Pointed and Rounded Leaf groups (left and right, respectively, in Fig. 3) reveal additional genetic structure. For the Pointed Leaf group, at $K = 3$ (optimal), two genotype groups are resolved within the phylogenetic species *S. fallax*. Norwegian samples identified as *S. brevifolium* and *S. isoviitae* based on morphology all belong to one of those two groups (Fig. 2), but the group also includes plants that are morphologically unambiguous *S. fallax* s. str. North American samples identified as *S. brevifolium* or *S. isoviitae* are scattered across the same *S. fallax* genotype groups. At $K = 5$, *S. pacificum* and *S.* "pseudopacificum," by contrast, are genetically divergent from both of the two *S. fallax* genotype groups. In an analysis of *S. fallax* alone (including *S. brevifolium* and *S. isoviitae*), $K = 2$ was considered optimal, and this resolves European and North American samples of this species but does not resolve *S. fallax*, *S. brevifolium*, and *S. isoviitae* (which all occur on both continents; Fig. 2). Even at $K = 3$ or $K = 4$, we found no additional genetic structure to support separating *S. brevifolium* or *S. isoviitae* from *S. fallax* (results not shown).

A separate analysis of the Rounded Leaf group found that $K = 2$ was optimal. One of the genotype groups were assigned to different clusters with no admixture within *S. angustifolium* and the other in *S. flexuosum*, with *S. recurvum* and *S. obtusum* showing different patterns of admixture for the two genotype groups. At $K = 4$, each of the four morphospecies, *S. angustifolium*, *S. flexuosum*, *S. recurvum*, and *S. obtusum*, are resolved as different clusters. Within *S. angustifolium*, at $K = 2$, eastern North American and European samples belong to the same genetic group (light gray in Fig. 3) and the western North American (mainly Alaskan) plants mostly belong to a different genetic group but with some admixture. Moreover, five Alaskan plants belong to the light gray genotype group, otherwise restricted to eastern North American and European plants. At increasing levels of *K*, additional genetic structure is suggested within *S. angustifolium*, but also with increasing evidence of admixture (Appendix S3). At $K = 4$, the two phylogenetically resolved groups of Alaskan plants belong to separate clusters. Within *S. flexuosum*, European and eastern North American plants belong to different genotype groups, with a limited amount of admixture (Fig. 2).

TABLE 1. Pairwise estimates of Nei's unbiased distance (*D*, above the diagonal) and F_{ST} (below the diagonal) between species and leaf types in the *Sphagnum recurvum* complex.

	S. fallax	*S.* "pseudopacificum"	*S. pacificum*	*S. balticum*	*S. angustifolium*	*S. flexuosum*	*S. recurvum*	*S. obtusum*
S. fallax	0.000	0.017	0.024	0.084	0.103	0.119	0.123	0.137
S. "pseudopacificum"	0.041	0.000	0.035	0.080	0.105	0.121	0.129	0.135
S. pacificum	0.060	0.248	0.000	0.083	0.117	0.135	0.142	0.149
S. balticum	0.069	0.146	0.194	0.000	0.120	0.124	0.133	0.145
S. angustifolium	0.492	0.237	0.307	0.152	0.000	0.114	0.116	0.131
S. flexuosum	0.511	0.365	0.456	0.237	0.523	0.000	0.088	0.156
S. recurvum	0.250	0.157	0.283	−0.010	0.276	0.238	0.000	0.164
S. obtusum	0.115	0.318	0.381	0.045	0.128	0.220	0.010	0.000
LEAF TYPE:								
	Pointed	Rounded						
Pointed	0	0.085						
Rounded	0.336	0						

TABLE 2. Fixed differences among species in the *Sphagnum recurvum* complex. Note that these fixed differences are between pairs of species and do not necessarily represent diagnostic alleles for individual species in relation to all other species in the complex. Comparisons were limited to loci for which data were available for >50% of the samples representing both groups.

	S. "pseudopacificum"	*S. pacificum*	*S. balticum*	*S. angustifolium*	*S. flexuosum*	*S. recurvum*	*S. obtusum*
S. fallax	22	34	194	227	350	358	363
S. "pseudopacificum"		87	255	308	436	439	434
S. pacificum			288	316	436	443	444
S. balticum				291	424	419	425
S. angustifolium					273	291	249
S. flexuosum						273	403
S. recurvum							419

Genetic patterns resolved by phylogenetic and STRUCTURE analyses are corroborated by PCA (Appendix S4). Differentiation between the Rounded and Pointed Leaf groups is strong (Appendix S4A), as are morphospecies within those groups (Appendix S4B, C). Differentiation between European and North American plants within *S. fallax* (Appendix S4D) and *S. flexuosum* (Appendix S4G) is also evident. Moreover, the greater genetic similarity between *S. angustifolium* plants from eastern North America and Europe compared to either with western North American plants is clear, as is the occurrence of two genetically divergent groups of *S. angustifolium* plants from Alaska (Appendix S4E). The PCA also corroborates differentiation of *S.* "pseudopacificum" and *S. pacificum* (Appendix S4F).

Genetic differentiation and diversity

Genetic differentiation (estimated by Nei's *D* and F_{ST}) among species in the *S. recurvum* complex is generally low, corroborating the view that this is a group of very closely related species (Table 1). In fact, differentiation is also low ($F_{ST} < 0.4$) between the Rounded and Pointed groups of species within the complex, and some species within those two groups are more differentiated than are the groups.

Despite the low values of F_{ST}, we detected a large number of fixed differences among species in pairwise comparisons (Table 2). The greatest number of fixed differences (444) occurred between *S. obtusum* and *S. pacificum* and the least (22) between *S.* "pseudopacificum" and *S. fallax*. In general, a lower number of fixed differences occurred among three morphologically similar species in the Pointed Leaf group: *S. pacificum*, *S.* "pseudopacificum," and *S. fallax* (Table 2). It should be noted that these are not necessarily "diagnostic alleles," since some fixed differences between specific pairs of taxa may not be universally diagnostic for an individual taxon/clade. Those numbers would be smaller. Forty-eight fixed differences occurred between the more inclusive Rounded Leaf and Pointed Leaf clades. No fixed differences were detected between *S. fallax*, *S. isoviitae*, and *S. brevifolium*, nor between North American and European plants of *S. fallax*.

For all estimated statistics, the Rounded Leaf group of species contains higher genetic diversity, and more private alleles, than the Pointed Leaf group, although the sample size was somewhat larger for the latter than for the former (Table 3). Within the Pointed Leaf group, the most common species (and most sampled), *S. fallax*, contains the highest levels of genetic diversity; similarly, *S. angustifolium*, which is the most common species (and most abundantly sampled), is more genetically diverse than any other Rounded Leaf species (Table 3).

DISCUSSION

Systematic/taxonomic implications

Both circumscription of the *S. recurvum* complex and the numbers of species within it have been disputed, and our results inform both issues. The core species comprising the complex (Flatberg, 1989, 1992a, b; McQueen and Andrus, 2007; Laine et al., 2018) are *S. brevifolium*, *S. fallax*, *S. flexuosum*, *S. isoviitae*, *S. pacificum*, and *S. recurvum*. It is referred to as the *S. recurvum* complex because that species was the first described. Members of the complex typically have two side-by-side branch buds in the lower parts of the capitula, whereas most *Sphagnum* have them one above the other or have a single branch bud. These species are further characterized (though not unique to these within subg. *Cuspidata*) by the arrangement of pores on the branch leaves: few other than a single conspicuous pore near the distal end of each hyaline cell on the outer (convex or abaxial) surface, and more numerous, larger round pores on the inner (convex, adaxial) surface. Finally, compared to other taxa in subg. *Cuspidata*, species in the *S. recurvum* group are generally characterized by relatively short stem leaves, sometimes barely longer than wide, although this trait is somewhat variable (as shown in Fig. 2).

Flatberg (1992a) noted that *S. balticum* and *S. pulchrum* appear to be closely related to the *S. recurvum* complex and that the decision to exclude them from his taxonomic studies on this group was largely arbitrary. Based on phylogenetic analyses of organellar (plastid and mitochondrial) genome sequences, *S. balticum* is nested within the *S. recurvum* complex (Shaw et al., 2016), and our results corroborate that conclusion. *Sphagnum balticum* lacks the paired branch buds generally characteristic of the core species, and the stem leaves are somewhat longer than in the other species (Fig. 2). They also spread widely from the stem, unlike the more pendent-spreading stem leaves of most *S. recurvum* complex species. The branch leaf pore pattern, typically with one prominent distal pore on the outer surface of each hyaline cell and more numerous, round, larger pores inside, is similar to that of the core species. *Sphagnum obtusum*, also shown here to be part of the *S. recurvum* complex, has a somewhat different branch leaf pore structure than

TABLE 3. Genetic variability in species and leaf-types in the *Sphagnum recurvum* complex.

	N	PPL	$N_a \pm$ SE	$N_e \pm$ SE	PPr	$I \pm$ SE
Total	280	100.00	1.558 ± 0.015	1.122 ± 0.007		0.131 ± 0.006
Pointed Leaf	148	41.24	1.418 ± 0.021	1.082 ± 0.009	23.76	0.091 ± 0.007
S. fallax	128	29.51	1.300 ± 0.023	1.070 ± 0.010	13.33	0.073 ± 0.008
S. "pseudopacificum"	5	7.73	1.077 ± 0.013	1.049 ± 0.009	1.09	0.044 ± 0.007
S. pacificum	10	5.62	1.056 ± 0.011	1.031 ± 0.007	2.00	0.029 ± 0.006
S. balticum	5	5.39	1.054 ± 0.011	1.037 ± 0.008	3.78	0.032 ± 0.006
Rounded Leaf	132	68.38	1.699 ± 0.020	1.163 ± 0.012	36.40	0.171 ± 0.009
S. angustifolium	67	27.40	1.274 ± 0.022	1.076 ± 0.010	14.89	0.079 ± 0.008
S. flexuosum	44	16.63	1.169 ± 0.018	1.056 ± 0.009	10.42	0.058 ± 0.008
S. recurvum	16	14.75	1.148 ± 0.017	1.046 ± 0.007	11.43	0.053 ± 0.007
S. obtusum	5	7.73	1.077 ± 0.013	1.052 ± 0.009	8.70	0.045 ± 0.008

Notes: N = number of individuals for each species or genetic cluster, excluding clones and individuals showing >10% admixture between clusters; PPL = percent polymorphic loci; N_a = mean alleles per locus; N_e = mean effective number of alleles per locus; PPr = percent private alleles; *I* = Shannon's information index.

the others. Outer pores are more numerous, sometimes in commissural rows, and are often faint and poorly defined.

Sphagnum pulchrum, by contrast, falls outside the *S. recurvum* complex, in a clade that includes the gametophytically haploid *S. annulatum*. That clade also includes two allopolyploid species (i.e., diploid gametophytes and tetraploid sporophytes) that share *S. annulatum* as one of their parents. *Sphagnum majus* is derived from a cross between *S. annulatum* and *S. cuspidatum* (Såstad et al., 2000), and *S. jensenii* derives from a cross between *S. annulatum* and *S. balticum* (Såstad et al., 1999a). *Sphagnum annulatum*, *S. majus*, and *S. jensenii* differ morphologically by numerous pores on the convex surface of branch leaves, stem leaves much longer than broad, and differentiated stem cortex two or three cells wide (except in *S. annulatum*). Flatberg (1992a) noted that *S. pulchrum* shares morphological characteristics with species in both the *S. recurvum* and *S. annulatum* complexes; our results indicate that *S. pulchrum* shares a more recent common ancestor with *S. annulatum* than with any species in the *S. recurvum* complex as we have circumscribed it here.

Several species in the *S. recurvum* complex are reported from South America and/or other tropical areas, and genetic analyses are needed to determine if in fact those plants are conspecific with the temperate and boreal (to subarctic) samples included in this study. Some northern *Sphagnum* species definitely do occur at low latitudes, but some samples from South America identified as *S. recurvum*, for example, are allopolyploids (unlike northern *S. recurvum*) and are clearly not conspecific (A. M. Duffy et al., unpublished data). Flatberg (1992a) speculated that *S. recurvum* could be conspecific with two tropical species, *S. pulchricoma* and *S. cuspidatulum*, which would imply that *S. recurvum* has a pantropical range that includes South America, Africa, and Asia. This view is not supported by genetic information from microsatellites, however, which indicate that both *S. pulchricoma* and *S. cuspidatulum* are distinct from *S. recurvum* (Karlin et al., 2014). We can only confirm the occurrence of *S. recurvum* in eastern North America, with a single known European locality in the Azores (Dias et al., 2009). That collection from the Azores falls within the *S. recurvum* clade in our analyses (Appendix S2), closely related to collections from the southeastern United States. Nevertheless, in addition to cases of northern species possibly occurring in tropical regions, there could be other, distinct, tropical species that would fall within the *S. recurvum* complex. The current circumscription of the complex, limited to the northern species included in this study, is necessarily a work-in-progress.

Our analyses resolve eight clades within the *S. recurvum* complex, seven corresponding to widely recognized species, plus *S.* "pseudopacificum." Crum (1984) considered plants that we attribute to *S. angustifolium*, *S. fallax*, *S. flexuosum*, and *S. recurvum* as conspecific (*S. recurvum* s. lat.). *Sphagnum pacificum* had not been described at the time, and Crum did not consider *S. balticum* or *S. obtusum* as part of the *S. recurvum* complex. McQueen and Andrus (2007) recognized all of the taxa we resolve here as distinct species and added *S. rubruflexuosum*, describing this new species from a few sites in Pennsylvania and Maryland (Andrus, 1988). Plants that we included in the present analyses from the type locality for *S. rubroflexuosum* were not resolved as distinct from *S. flexuosum* based on phylogenetic or cluster (STRUCTURE) analyses.

The two major clades within the *S. recurvum* complex, referred to here as the Rounded and Pointed Leaf groups, correspond more or less to groups recognized previously (Flatberg, 1992a). The Pointed Leaf group contains *S. fallax* (including *S. brevifolium* and *S. isoviitae*) and *S. pacificum*, plus the clade resolved as *S.* "pseudopacificum" in our analyses. These species are characterized by acute to apiculate stem leaves (Fig. 2) and also share yellow spores and somewhat differentiated stem cortical cells. Our results show that *S. balticum* is sister to this clade as well; it has broadly and bluntly acute stems leaves, but it does have yellow spores and a more or less differentiated cortex (Flatberg, 1992a). Species in the Rounded Leaf group, including *S. angustifolium*, *S. flexuosum*, *S. obtusum*, and *S. recurvum*, have brown spores, stem cortex little or not differentiated, and rounded stem leaf apices that are obviously and characteristically resorbed. That apical resorption is minimal in *S. angustifolium* compared to the other species in this subgroup (Fig. 2: 25–49). Our results show that these morphological traits

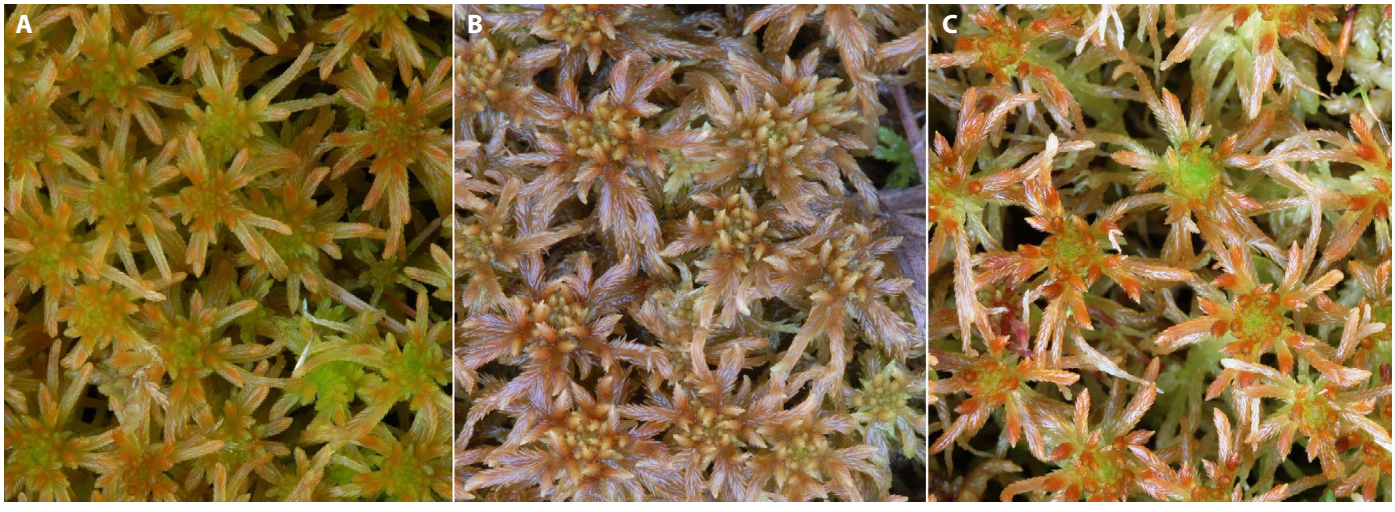


FIGURE 4. Field-derived photographs of three morphologically distinguishable morphs within the phylogenetic species *Sphagnum fallax*: (A) fallax morph, (B) brevifolium morph, and (C) isoviitae morph. These morphs are not distinguished by our molecular results but may warrant further study because of subtle morphological and ecological differences. Photos: Kjell Ivar Flatberg. License: CC BY 4.0 (Norwegian Environmental Specimen Bank).

generally track phylogenetic relationships within the complex with regard to the grouping of species into more inclusive clades.

In terms of species delineation, the most controversial issue in the complex pertains to the delineation of *S. fallax*, *S. isoviitae*, and *S. brevifolium*. Both *S. fallax* and *S. brevifolium* were described in the 19th century (*S. cuspidatum* var. *fallax* Klinggräff and *S. cuspidatum* var. *brevifolium* Röll, respectively). Flatberg (1992a) described *S. isoviitae* from central Norway as part of his taxonomic studies on the *S. recurvum* complex. These three species are closely related morphotypes within the Pointed Leaf group and frequently grow intimately mixed (Fig. 4). Flatberg and collaborators (Flatberg, 1992a, b; Såstad and Flatberg, 1994; Stenøien et al., 1997; Såstad et al., 1999b) have conducted a series of morphometric, experimental, and genetic studies to test the occurrence and degree of discontinuity among them and concluded that the three can be distinguished morphologically (although weakly so, at best, for *S. fallax* vs. *S. isoviitae*). They are frequently distinguishable in the field and/or as dried specimens, and differ in color, capitulum shape, and branch leaf shape and arrangement (e.g., the degree of ranking [running in rows]), and the extent to which the branch leaves of the inner part of the capitula recurve when dry. They can also differ subtly in microscopic characters, including the shape of the chlorophyllose cells in transverse section. Såstad et al. (1999a) showed that whereas isozyme and RAPD markers evidenced genetic differentiation among *S. recurvum*, *S. angustifolium*, and *S. flexuosum* in the Rounded Leaf group, no such differentiation was detected among *S. fallax*, *S. brevifolium*, and *S. isoviitae* in the Pointed Leaf group. Using microsatellites, Szurdoki et al. (2014) showed that *S. angustifolium*, *S. fallax*, and *S. flexuosum* are genetically distinct, but they did not address the issue of *S. fallax* vs. *S. isoviitae* or *S. brevifolium*.

Neither North American nor Norwegian samples in our data set that had been identified morphologically as *S. brevifolium* or *S. isoviitae* were distinguishable from *S. fallax* based on the RADseq data. Our data provide no hint of genetic divergence within *S. fallax* that could be construed as evidence in favor of separating *S. brevifolium*, *S. isoviitae*, or *S. rubroflexuosum*. Analyses using STRUCTURE identified two genotype groups within *S. fallax*, but these groups do not correspond to these morphotypes. Our results strongly suggest that neither *S. brevifolium* nor *S. isoviitae* represents a distinct species phylogenetically, but rather both are morphotypes nested within *S. fallax*.

The absence of differentiation using the molecular markers employed here does not bear on the question of whether the morphological characters used to distinguish them as species are genetically based. Indeed, the fact that morphs corresponding to *S. brevifolium* and *S. isoviitae* are often distinguishable in both Scandinavia and eastern North America suggest that they may well represent genetic variants. It could be that a limited number of genes control those morphological traits, and that they are polymorphic within *S. fallax*. Nevertheless, our results suggest that the characters used to distinguish these taxa, plant color and leaf arrangement (ranked or not), do not resolve genealogical species. By contrast, most of the species in the complex can be distinguished morphologically, especially by stem leaf shape and the degree of apical resorption. Capitulum shape (flat vs. rounded) is sometimes useful (e.g., *S. fallax* vs. *S. angustifolium*), but differences among the morphs (brevifolium and isoviitae) within *S. fallax* show that this character can exhibit intraspecific variation as well.

Szurdoki et al. (2014) found that *S. angustifolium*, *S. fallax*, and *S. flexuosum* could be distinguished by molecular data but suggested that their genetic delineation was sometimes incongruent with morphological patterns. We also first found that some of our plant identifications for these taxa conflicted with subsequent molecular phylogenetic results, but reexamination of all specimens included in the project indicated that essentially every case of morphological-genetic incongruence involved initial misidentifications (by us). One result of our reexaminations was that *S. fallax* turned out to be more common in eastern North America than *S. angustifolium*, and it occupies a broader niche range than we (or others; Johnson et al., 2014) had realized. *Sphagnum angustifolium* is generally thought to form hummocks, whereas *S. fallax* typically grows in lawns and carpets closer to the water table. Our results suggest that not only is *S. fallax* more common than *S. angustifolium* in eastern North America, but it forms low hummocks rather commonly.

Genetic admixture

STRUCTURE software identified genotypic groups within the complex, and by conducting hierarchical analyses at different phylogenetic levels (within and between clades) we found some evidence of genotypic admixture within individual plants. This admixture can reflect retention of ancestral polymorphism and/or introgression. *Sphagnum balticum* is the only species that appeared to have a substantially admixed structure combining genetic attributes of the Rounded and Pointed Leaf clades (Fig. 3). This species is especially variable morphologically, combines stem leaf structure of the two groups (i.e., bluntly acute to rounded stem leaves in the Pointed Leaf clade), and was involved as the paternal parent in hybridization (with another species of subg. *Cuspidata*, *S. tenellum*) that yielded the allopolyploid species *S. troendelagicum* (Stenøien et al., 2010). As such, *S. balticum* is a species that warrants additional study of genetic structure and the possibility of interspecific introgression across its broad intercontinental range. It may facilitate genetic exchange between members of the *S. recurvum* complex, and beyond, in subg. *Cuspidata*. Despite the fact that other species in the complex grow mixed in wet microsites that could facilitate interspecific hybridization, we found surprisingly little evidence of interspecific admixture. Additional analyses are needed, however, since STRUCTURE may not reveal introgression if it occurred long enough in the past for subsequent coalescence within species that are largely reproductively isolated.

Geographic patterns

Low but nonzero levels of migration/gene flow have been detected between North American and European plants of several *Sphagnum* species (Szövényi et al., 2008; Stenøien et al., 2011), and similarly between western North American and eastern Asian plants (Shaw et al., 2014, 2015). Dating of divergences between European and North American conspecifics have generally inferred Pleistocene time scales (Szövényi et al., 2008; Stenøien et al., 2011).

Both the Rounded Leaf and Pointed Leaf groups within the *S. recurvum* complex have intercontinental ranges that include Europe, Asia, and North America. We detected geographic structure within the three species that have the broadest geographic ranges: *S. fallax* in the Pointed Leaf group; *S. angustifolium* and *S. flexuosum* in the Rounded Leaf group. In *S. fallax*, eastern North American and European clades are reciprocally monophyletic. There is no evidence of morphological differentiation between European and North American plants. This species does not occur in western

North America. *Sphagnum angustifolium* does occur in all three regions, and its phylogenetic structure is complex. There are multiple, geographically correlated clades. Alaska alone harbors plants representing genetically and phylogenetically divergent *S.angustifolium* plants. In *S. flexuosum*, European plants form a clade that is nested within a paraphyletic group of North American samples.

Vigalondo et al. (2019) found that a species generally interpreted as having a broad intercontinental distribution in the moss family Orthotrichaceae consists of more or less allopatric clades that also differ in multivariate patterns of morphological variation. Citing other recent studies that have found similar phylogenetic structure within supposedly widespread species, Vigalondo et al. (2019) suggested that species diversity in mosses and liverworts may be significantly underestimated. Evidence to support this broad conclusion is currently limited to fewer than a half dozen bryophyte species/complexes (with a total of some 20,000 species in the combined phyla), and it is noteworthy that all are tropical to temperate groups. The situation in *Sphagnum*, as an example of common and widespread temperate to boreal bryophytes, may be different. As data accumulate, we see a pattern in *Sphagnum* of detectable but minimal divergence among metapopulation systems on different continents. This pattern may be generally true among species that are ecologically abundant community dominants around the Northern Hemisphere. Life history correlates of differing phylogenetic structure need to be investigated. For example, the species of Orthotrichaceae studied by Vigalondo et al. (2019) may be less clonal and more short-lived than are *Sphagnum* species.

Key to species in the *S. recurvum* complex

Because our species delineations differ from those in other recent treatments of the group, we provide the key below to distinguish the seven species that we resolve with RADseq data. The clade we identify as *S.* "pseudopacificum" requires further work, especially additional collections. It is not included in the key and would likely key out by collectors as *S. pacificum*. Because the three morphs of *S. fallax* are often distinguishable, we also provide a key to distinguish them in order to encourage further research into their genetics and ecology. The brevifolium and isoviitae morphs are formally recognized by Lönnell and Hassel (2018) as varieties of *S. fallax*: namely, *S. fallax* var. *brevifolium* (Lindb. ex Braithw.) Lönnell & Hassel and *S. fallax* var. *isoviitae* (Flatberg) Lönnell & Hassel.

Key to species in the *Sphagnum recurvum* complex

1. Stem leaves bluntly acute, obtuse-truncate or truncate 2
1. Stem leaves acute or apiculate 6
2. Stem leaves rounded-obtuse, ± narrowly eroded at apex 3
2. Stem leaves obtuse-truncate to truncate, narrowly to widely eroded/fimbriate at apex 5
3. Stem leaves wide spreading; upper stem leaf cells fibrillose; branch fascicles usually with one pendent branch ..... *S. balticum*
3. Stem leaves appressed to slightly spreading; stem leaf cells efibrillose (very rarely with rudimentary fibrils); branch fascicles with 2(–3) pendent branches 4
4. Capitula green to brownish, convex, with straight branches; stem leaves not or little longer than wide, scarcely or not eroded apically ..... *S. angustifolium*
4. Capitula usually greenish, rather flat with a knoblike inner part of short concentrically crowded branches; stem leaves slightly but definitely longer than wide; eroded apically ..... *S. flexuosum*
5. Capitula flat; branch leaves non-recurved when dry, unranked, branch leaf cells on outer surface with numerous small pores often in two rows; chlorophyllose cells of branch leaves in transverse section isosceles-triangular, slightly enclosed on inner surface ..... *S. obtusum*
5. Capitula +/– convex; branch leaves sharply recurved when dry, often conspicuously ranked; branch leaf cells on outer surface usually with one apical end pore; chlorophyllose cells of branch leaves in transverse section equilateral-triangular and broadly enclosed on inner surface ..... *S. recurvum*
6. Stem leaves acute-apiculate to apiculate; branch leaf tips strongly subulate-involute above; chlorophyllose cells of branch leaves in transverse section equilateral-triangular, broadly enclosed on inner surface ..... *S. pacificum*
6. Stem leaves varyingly acute-obtuse, acute to acute-apiculate; branch leaf tips weakly subulate-involute above; chlorophyllose cells of branch leaves in transverse section varyingly isosceles-triangular and narrowly enclosed on inner surface to equilateral-triangular and broadly enclosed on inner surface ..... *S. fallax*

Key to *Sphagnum fallax* morphs

1. Capitula green to yellow-brown; central capitulum branches nearly straight to slightly curved laterally, inner capitulum branches with markedly recurved leaf tips (45–90°, often ˃90°) when dry; stem leaves obtuse-acute; outer stem cortical cells indistinctly enlarged; chlorophyllose cells of branch leaves in transverse section isosceles-triangular and narrowly enclosed on inner surface ..... fallax morph
1. Capitula yellow-green, yellow-brown to brown; central capitulum branches markedly curved laterally, inner capitulum branches with little to moderately recurved leaf tips (˂45°) when dry; stem leaves acute-apiculate to apiculate; outer layer stem cortical cells enlarged; chlorophyllose cells of branch leaves in transverse section isosceles triangular to ovate-triangular and broadly enclosed on the inner surface 2
2. Capitula rather flat and clearly 5-radiate, central curved branches more or less sharply differentiated from outer branches and rather straight; branch leaves markedly 5-ranked ..... isoviitae morph
2. Capitula somewhat convex and indistinctly 5-radiate, central curved branches not clearly differentiated from outer branches; branch leaves indistinctly 5-ranked ..... brevifolium morph

ACKNOWLEDGMENTS

This research was supported by National Science Foundation grants DEB-1737899 and DEB-1928514. We thank two anonymous reviewers for constructive suggestions that improved the manuscript.

AUTHOR CONTRIBUTIONS

A.J.S. and B.A. developed the project, conducted most of the fieldwork, examined all plants microscopically, and participated in the lab work and data analysis. A.D. conducted the analyses and prepared the paper. H.S., K.H., K.I.F, and M.S.I. participated in fieldwork and preparation of the paper.

DATA AVAILABILITY

The sequences, phylogenetic alignment, and STRUCTURE file analyzed in this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.1g1jwsts7 (Duffy et al., 2020).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Voucher information for accessions included in the RADseq analyses.

APPENDIX S2. Phylogenetic relationships among samples of *Sphagnum recurvum* complex species and related species based on RADseq loci. This is the optimal ML tree with nodes having bootstrap support ≥50% labeled.

APPENDIX S3. Genetic structure of *S. angustifolium* revealed by STRUCTURE analyses at increasing levels of K.

APPENDIX S4. Principal components analysis (PCA) of variation in RADseq loci among samples of (A) all *Sphagnum recurvum* complex species; (B) Pointed Leaf species; (C) Rounded Leaf species; (D) *S. fallax*, *S. isoviitae*, and *S. brevifolium*; (E) *S. angustifolium*; (F) *S. pacificum*;(G) *S. flexuosum*.

LITERATURE CITED

Andrews, S.2010. FastQC: A quality control tool for high throughput sequence data. Website: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Andrus, R. E. 1988. Two new taxa of *Sphagnum* in section *Cuspidata*. *The Bryologist* 91: 364–366.

Chifman, J., and L. Kubatko. 2014. Quartet inference from SNP data under the coalescent. *Bioinformatics* 30: 3317–3324.

Chifman, J., and L. Kubatko. 2015. Identifiability of the unrooted species tree topology under the coalescent model with time-reversible substitution processes, site-specific rate variation, and invariable sites. *Journal of Theoretical Biology* 374: 35–47.

Crum, H. A. 1984. Sphagnopsida, Sphagnaceae. *North American Flora, ser. 2, part* 11: 1–180.

Dias, E., C. Mendes, and A. J. Shaw. 2009. *Sphagnum recurvum* P. Beauv. on Terceira, Azores, new to Macaronesia-Europe. *Journal of Bryology* 31: 199–201.

Duffy, A., M. B. Aguero, H. K. Stenøien, K. I. Flatberg, M. S. Ignatov, K. Hassel, and A. J. Shaw. 2020. Data from: Phylogenetic structure in the *Sphagnum recurvum* complex (Bryophyta) in relation to taxonomy and geography. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.1g1jwsts7.

Eaton, D. A. R. 2014. PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* 30: 1844–1849.

Evanno, G., S. Regnaut, and J. Gaudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* 14: 611–620.

Flatberg, K. I. 1989. *Sphagnum* (*Cuspidata*) *pacificum*, sp. nov. *The Bryologist* 92: 116–119.

Flatberg, K. I. 1992a. The European taxa in the *Sphagnum recurvum* complex. 1. *Sphagnum isoviitae* sp. nov. *Journal of Bryology* 17: 1–13.

Flatberg, K. I. 1992b. The European taxa in the *Sphagnum recurvum* complex. 2. Amended descriptions of *Sphagnum brevifolium* and *S. fallax*. *Oikos* 17: 96–110.

Flatberg, K. I. 2002. The Norwegian Sphagna: A field colour guide. *Universitetet i Trondheim, Vitenskapsmuseet Rapport Botanisk Serie* 1994: 1–42.

Flatberg, K. I. 2013. Norges torvmoser. Akademika forlag, Trondheim.

Frahm, J.-P., and D. H. Vitt. 1993. Comparisons between the mossfloras of North American and Europe. *Nova Hedwigia* 56: 307–333.

Goudet, J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* 5: 184–186.

Gradstein, S. R., T. Pocs, and J. Vana. 1983. Disjunct Hepaticae in tropical America and Africa. *Acta Botanica Hungarica* 29: 127–171.

Hassel, K., M. O. Kyrkjeeide, N. Yousefi, T. Prestø, H. K. Stenøien, A. J. Shaw, and K. I. Flatberg. 2018. *Sphagnum divinum (*sp. nov.) and *S. medium* Limpr. and their relationship to *S. magellanicum* Brid. *Journal of Bryology* 40: 197–222.

Hedenäs, L., A. Desámoré, B. Laenen, B. Papp, D. Quandt, J. M. González-Mancebo, J. Patiño, et al. 2014. Three species for the price of one within the moss *Homalothecium sericeum* sl. *Taxon* 63: 249–257.

Heinrichs, J., J. Hentschel, A. Bombosch, A. Fiebig, J. Reise, M. Edelmann, H.-P. Kreier, et al. 2010. One species or at least eight? Delimitation and distribution of *Frullania tamarisci* (L.) Dumort. s. l. (Jungermanniopsida, Porellales) inferred from nuclear and chloroplast DNA markers. *Molecular Phylogenetics and Evolution* 56: 1105–1114.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801–1806.

Johnson, M. J., G. Granath, T. Teemu, R. Pouliot, H. K. Sten.ien, L. Rochefort, H. Rydin, and A. J. Shaw. 2014. Evolution of niche preference in *Sphagnum* peat mosses. *Evolution* 69: 90–103.

Jombart, T., and I. Ahmed. 2011. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070–3071.

Karlin, E. F., E. M. Temsch, E. Bizuru, J. Marino, S. B. Boles, N. Devos, and A. J. Shaw. 2014. Invisible in plain sight: recurrent double allopolyploidy in the African *Sphagnum* x *planifolium* (Sphagnaceae). *The Bryologist* 117: 187–201.

Kyrkjeeide, M. O., K. Hassel, K. I. Flatberg, A. J. Shaw, C. Brochmann, and H. K. Stenøien. 2016a. Long-distance dispersal and barriers shape genetic structure of peatmosses (*Sphagnum*) across the Northern Hemisphere. *Journal of Biogeography* 43: 1215–1226.

Kyrkjeeide, M. O., K. Hassel, K. I. Flatberg, A. J. Shaw, N. Yousefi, and H. K. Stenøien. 2016b. Spatial genetic structure of the abundant and widespread peatmoss *Sphagnum magellanicum* Brid. *PLoS One* 1111: e0148447.

Kyrkjeeide, M. O., K. Hassel, H. K. Stenøien, T. Prestø, E. Boström, A. J. Shaw, and K. I. Flatberg. 2015. The dark morph of *Sphagnum fuscum* (Schimp.) H.Klinggr. in Europe is conspecific with the North American *S. Beothuk*. *Journal of Bryology* 37: 251–266.

Laine, J., K. I. Flatberg, P. Harju, T. Timonen, K. Minkklinen, A. Laine, E.-S. Tuittila, and H. Vasander. 2018. *Sphagnum* Mosses. The Stars of European Mires. University of Helsinki, Department of Forest Sciences, Sphagna Ky, Helsinki.

Lönnell, N., and K. Hassel (2018) New combinations within the genus Sphagnum (Sphagnaceae, Bryophyta). *Biotaxa* 369: 57–58.

McQueen, C. B., and R. E. Andrus. 2007. Sphagnaceae Dumortier. In: Committee FoNAE, ed. Bryophytes: mosses, part 1. Flora of North America, Vol. 27, 45–101. New York, NY: Oxford University Press.

Medina, R., F. Lara, B. Goffinet, R. Garilleti, and V. Mazimpaka. 2012. Integrative taxonomy successfully resolves the pseudo-cryptic complex of the disjunct epiphytic moss *Orthotrichum consimile* s.l. (Orthotrichaceae). *Taxon* 61: 1180–1198.

Medina, R., F. Lara, B. Goffinet, R. Garilleti, and V. Mazimpaka. 2013. Unnoticed diversity within the disjunct moss *Orthotrichum tenellum* s.l. validated by morphological and molecular approaches. *Taxon* 62: 1133–1152.

Muñoz, J., A. M. FeIicíslmo, F. Cabezas, A. R. Burgaz, and I. Martinez. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science* 304: 1144–1147.

Parchman, T. L., Z. Gompert, J. Mudge, F. D. Schilkey, C. W. Benkman, and C. A. Buerkle. 2012. Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology* 21: 2991–3005.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537–2539.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Qian, H. 1999. Spatial pattern of vascular plant diversity in North America North of Mexico and its floristic relationships with Eurasia. *Annals of Botany* 83: 271–283.

Renner, M. A. M., N. Devos, J. Patiño, E. A. Brown, A. Orme, M. Elgey, T. C. Wilson, et al. 2013. Integrative taxonomy resolves the cryptic and pseudo-cryptic *Radula buccinifera* complex (Porellales, Jungermanniopsida), including two reinstated and five new species. *PhytoKeys* 27: 1–113.

Renner, M. A. M., M. M. Heslewood, S. D. F. Patzak, A. Schäfer-Verwimp, and J. Heinrichs. 2017. By how much do we underestimate species diversity of liverworts using morphological evidence? An example from Australasian *Plagiochila* (Plagiochilaceae: Jungermanniopsida). *Molecular Phylogenetics and Evolution* 107: 576–593.

Rydin, H., and J. Jeglum. 2013. The Biology of Peatlands. Ed. 2. Oxford University Press, New York, NY, USA.

Såstad, S. M. 1999. Genetic and environmental sources of variation in leaf morphology of *Sphagnum fallax* and *Sphagnum isoviitae* (Bryopsida): comparison of experiments conducted in the field and laboratory. *Canadian Journal of Botany* 77: 1–10.

Såstad, S. M., and K. I. Flatberg. 1994. Leaf size and shape in the *Sphagnum recurvum* complex: taxonomic significance and habitat variation. *Journal of Bryology* 18: 261–275.

Såstad, S. M., K. I. Flatberg, and N. Cronberg. 1999a. Electrophoretic evidence supporting a theory of allopolyploid origin of the peatmoss *Sphagnum jensenii*. *Nordic Journal of Botany* 19: 355–362.

Såstad, S. M., K. I. Flatberg, and L. Hanssen. 2000. Origin, taxonomy and population structure of the allopolyploid peat moss *Sphagnum majus*. *Plant Systematics and Evolution* 225: 73–84.

Såstad, S. M., H. K. Stenoien, and K. I. Flatberg. 1999b. Species delimitation and relationships of the *Sphagnum recurvum* complex (Bryophyta)—as revealed by isozyme and RAPD markers. *Systematic Botany* 24: 95–107.

Schofield, W. B., and H. A. Crum. 1972. Disjunctions in bryophytes. *Annals of the Missouri Botanical Garden* 59: 174–202.

Shaw, A. J., C. J. Cox, and S. B. Boles. 2003. Polarity of peatmoss (*Sphagnum*) evolution: who says bryophytes have no roots? *American Journal of Botany* 90: 1777–1787.

Shaw, A. J., C. J. Cox, and S. B. Boles. 2004. Phylogenetic relationships among *Sphagnum* sections: *Hemitheca*, *Isocladus*, and *Subsecunda*. *The Bryologist* 107: 189–196.

Shaw, A. J., C. J. Cox, W. R. Buck, N. Devos, A. M. Buchanan, L. Cave, R. Seppelt, et al. 2010. Newly resolved relationships in an early land plant lineage: Bryophyta class Sphagnopsida (peat mosses). *American Journal of Botany* 97: 1511–1531.

Shaw, A. J., N. Devos, C. J. Cox, and B. Shaw. 2016. Organellar phylogenomics of an emerging model system: *Sphagnum* (peatmoss). *Annals of Botany* 118: 185–196.

Shaw, A. J., G. K. Golinski, E. G. Clark, B. Shaw, H. K. Stenøien, and K. I. Flatberg. 2014. Intercontinental genetic structure in the amphi-Pacific peatmoss *Sphagnum miyabeanum* (Bryophyta: Sphagnaceae). *Biological Journal of the Linnean Society* 111: 17–37.

Shaw, A. J., B. Shaw, M. G. Johnson, N. Devos, and B. Carter. 2015. Genetic and phylogenetic structure of the Pacific Rim clade of *Sphagnum* subg. *Subsecunda*: haploid and allodiploid taxa. *Biological Journal of the Linnaean Society* 116: 295–311.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Stenøien, H. T., S. Bakken, and K. I. Flatberg. 1997. Phenotypic variation in the *Sphagnum recurvum* complex: a cultivation experiment. *Journal of Bryology* 19: 731–750.

Stenøien, H. K., A. J. Shaw, B. Shaw, K. Hassel, and U. Gunnarsson. 2011. North American origin and recent European establishment of the amphi-Atlantic peat moss: *Sphagnum angermannicum*. *Evolution* 65: 1181–1194.

Stenøien, H. K., A. J. Shaw, K. Stengrundet, and K. I. Flatberg. 2010. The narrow endemic Norwegian peat moss *Sphagnum troendelagicum* originated before the last glacial maximum. *Heredity* 2010: 1–13.

Szurdoki, E., O. Márton, and P. Szövényi (2014) Genetic and morphological diversity of Sphagnum angustifolium, S. flexuosum and S. fallax in Europe. *Taxon* 63: 237–248.

Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Szövényi, P., S. Terracciano, M. Ricca, and A. J. Shaw. 2008. Recent divergence, intercontinental dispersal and shared polymorphisms are shaping the genetic structure of amphi-Atlantic peatmoss populations. *Molecular Ecology* 17: 5364–5377.

Vigalondo, B., R. Garilleti, A. Vanderpoorten, J. Patiño, I. Draper, J. A. Calleja, V. Mazimpaka, and F. Lara. 2019. Do mosses really exhibit so large distribution ranges? Insights from the integrative taxonomic study of the *Lewinskya affinis* complex (Orthotrichaceae, Bryopsida). *Molecular Phylogenetics and Evolution* 140: 106598.

Weston, D. J., M. R. Turetsky, M. G. Johnson, G. Granath, Z. Lindo, L. R. Belyea, S. K. Rice, et al. 2018. The Sphagnome Project: enabling ecological and evolutionary insights through a genus-level sequencing project. *New Phytologist* 217: 16–25.

Yousefi, N., K. Hassel, K. I. Flatberg, P. Kemppainen, E. Trucchi, A. J. Shaw, M. O. Kyrkjeeide, et al. 2017. Divergent evolution and niche differentiation within the common peatmoss *Sphagnum magellanicum*. *American Journal of Botany* 104: 1060–1072.

Yu, Z. C. 2012. Northern peatland carbon stocks and dynamics: a review. *Biogeosciences* 9: 4071–4085.

Zanten, B. O. van. 1978. Experimental studies on transoceanic long-range dispersal of moss spores in the Southern Hemisphere. *Journal of the Hattori Botanical Laboratory* 44: 458–482.