Biological Journal of the Linnean Society, 2023, **XX**, 1–27
https://doi.org/10.1093/biolinnean/blad111
Advance access publication 14 September 2023
Original Article

Original Article

The *Sphagnum cuspidatum* complex: phylogeny, species delimitation, and morphology

Sean C. Robinson[1,*], Marta Nieto-Lugilde[2], Aaron M. Duffy[2], Katherine Martinez Muñoz[2], Blanka Aguero[2], Amelia Merced[3], Kristian Hassel[4], Kjell Ivar Flatberg[4] and A. Jonathan Shaw[2]

[1]Department of Biology, SUNY Oneonta, Oneonta, NY 13820, United States
[2]Department of Biology & L. E. Anderson Bryophyte Herbarium, Duke University, Durham, NC 27708, United States
[3]USDA Forest Service, International Institute of Tropical Forestry, San Juan, PR 00926, United States
[4]Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

*Corresponding author. Biology Department, SUNY Oneonta, 108 Ravine Parkway, Oneonta, NY 13820, United States. E-mail: sean.robinson@onoenta.edu

ABSTRACT

The use of species as a concept is an important metric for assessing biological diversity and ecosystem function. However, delimiting species based on morphological characters can be difficult, especially in aquatic plants that exhibit high levels of variation and overlap. The *Sphagnum cuspidatum* complex, which includes plants that dominate peatland hollows, provides an example of challenges in species delimitation. Microscopic characters that have been used to define taxa and the possibility that these characters may simply be phenoplastic responses to variation in water availability make species delimitation in this group especially difficult. In particular, the use of leaf shape and serration, which have been used to separate species in the complex, have resulted in divergent taxonomic treatments. Using a combination of high-resolution population genomic data (RADseq) and a robust morphological assessment of plants representing the focal species, we provide evidence to evaluate putative species in this complex. Our data support the recognition of *S. cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, and *S. trinitense* as genetically distinct species that can be separated morphologically. These results indicate that *S. viride* does not differ genetically from *S. cuspidatum*. Our results are broadly relevant to other aquatic groups where leaf shape and marginal teeth are used to distinguish species.

Keywords: peat moss; *Sphagnum*; *Sphagnum fitzgeraldii*; *Sphagnum trinitense*; *Sphagnum mississippiense*; integrative taxonomy; species delimitation; morphological variation; RADseq

INTRODUCTION

Biological diversity is an important component of ecosystem function from local to global scales (Naeem *et al.* 1994, 2012, Tilman *et al.* 1996). While there are numerous ways by which biological diversity can be defined, species richness is still a standard and important metric (Gaston 1996, Bengtsson 1998). With over 34 different species concepts, however, defining what constitutes a species can be difficult (Zachos 2018). The use of morphological characters can be problematic and differentiating between what characters are diagnostic regarding species boundaries and which ones constitute intraspecific variability is not easy. This problem can be especially acute in aquatic taxa (Shaw 1986, Vanderpoorten and Jacquemart 2004, Li *et al.* 2019).

Within the bryophyte genus *Sphagnum* L., phenotypic plasticity is very common, making species delimitation challenging. Intraspecific variation in morphological characters in response to differences in water availability, nutrient levels, and/or pH is well documented (Flatberg 1988, Baker & Boatman 1990, Anderson *et al.* 1992, Li *et al.* 1992, Såstad and Flatberg 1993, 1994, Stenøien *et al.* 1997, 2014, Såstad *et al.* 1999, Oke *et al.*, 2020, Rastogi *et al.* 2020, Nieto-Lugilde *et al.* 2022). Having a good understanding of species boundaries within *Sphagnum* impacts not only the field of systematics, but also ecological studies that focus on the role *Sphagnum* communities play as ecosystem engineers in peatlands (Rydin and Jeglum 2013, Johnson *et al.* 2014, Jassey and Signarbieux 2019, Bengtsson *et al.* 2020). For example, Bengtsson *et al.* (2020) used two globally distributed *Sphagnum* species [*Sphagnum magellanicum* Brid. and *S. fuscum* (Schimp.) H. Klinggr.] as models to assess the importance of abiotic and biotic variables to carbon sequestration in northern peatlands by analysing variation in the growth of these two species across 99 Holarctic peatlands. However,

Received 11 May 2023; revised 2 August 2023; accepted 4 August 2023

Hassel *et al.* (2018) provided evidence that plants previously referred to as *S. magellanicum* in the Northern Hemisphere are in fact two different species (*S. divinum* Flatberg & K.Hassel and *S. medium* Limpr.) and that they differ in morphology and ecology. Bengtsson *et al.* (2020) recognized this taxonomic change and acknowledged that their results may have been affected by the ecological differences between these taxa. More recently, Shaw *et al.* (2022, 2023) provided evidence that the *S. magellanicum* complex contains two additional North American species, *S. diabolicum* A.J.Shaw, Aguero & Nieto-Lugilde and *S. magniae* A.J.Shaw, Aguero & Nieto-Lugilde, that are distinct in ecology and geographical distribution.

The challenge of species delimitation in *Sphagnum* has resulted in several complexes within the genus in which a handful of related species are difficult to differentiate due to high levels of morphological variation within species, and morphological similarity between them (Flatberg 1988, Såstad and Flatberg 1993, 1994, Stenøien *et al.* 1997, Duffy *et al.* 2020, Shaw *et al.* 2023). The *Sphagnum cuspidatum* complex within subgenus *Cuspidata*, which comprises aquatic species that dominate peatland hollows, is one such complex. McQueen and Andrus (2007) recognized five haploid species in this group, including *Sphagnum cuspidatum* Ehrh. Ex Hoffm., *Sphagnum fitzgeraldii* Renauld & Cardot, *Sphagnum mississippiense* R.E. Andrus, *Sphagnum trinitense* Müll.Hal., and *Sphagnum viride* Flatberg (Fig. 1). Two of these, *S. cuspidatum* and *S. viride*, are amphiatlantic, occurring in Europe and eastern North America. Karlin *et al.* (2011) suggested that *S. cuspidatum* occurs on every continent except Antarctica, based on microsatellite data. The other three are restricted to the Western Hemisphere, primarily along parts of the Atlantic and Gulf coastal plains. *Sphagnum fitzgeraldii* has been reported from Virginia to Florida and west to Louisiana (McQueen and Andrus, 2007) whereas *S. mississippiense* was originally described from just four sites in Louisiana and Mississippi and later reported from one site in New Jersey (Andrus 1987, McQueen and Andrus 2007). *Sphagnum trinitense* is known from southern New York to Florida, west to Louisiana and up the Mississippi drainage, as well as from the West Indies and South America (McQueen and Andrus 2007). These three taxa are unique within subgenus *Cuspidata* for having prominent teeth along branch leaf margins.

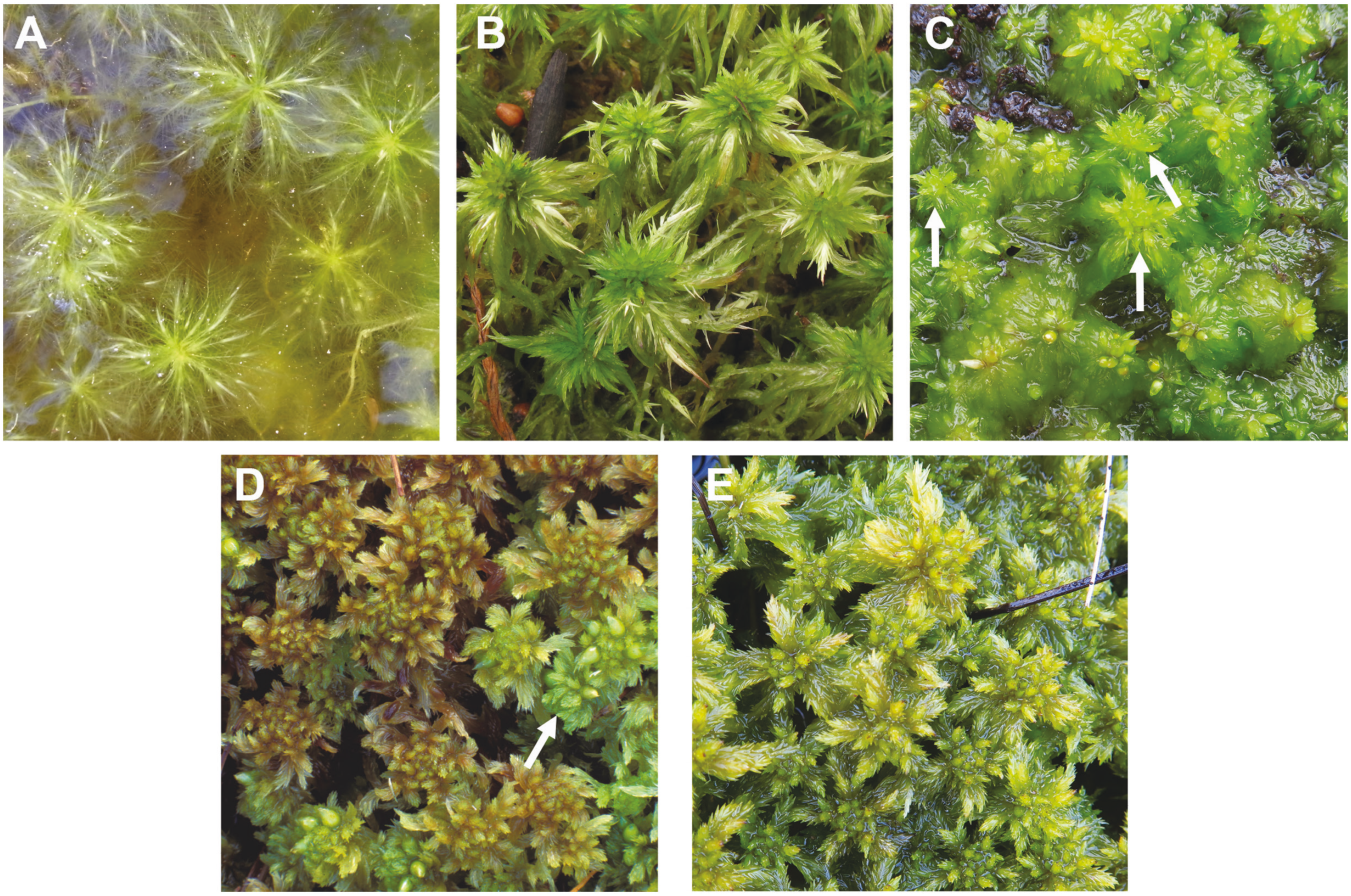

Figure 1. A, *Sphagnum trinitense*: USA, SOUTH CAROLINA: Sumter Co., Woods Mill Pond. Photo by B. Aguero, 23 January 2010. B, *Sphagnum mississippiense*: USA, MISSISSIPPI: Greene Co., Along MS-57, 1.19 miles ESE from Chickasawhay River, W side of rd. Photo by B. Aguero, November 2021. C, *Sphagnum fitzgeraldii* (with *S. cuspidatum* mixed in): USA, NORTH CAROLINA: Brunswick Co., Green Swamp. Photo by A. J. Shaw, March 2020. Arrows point to plants of *S. cuspidatum*. D, *Sphagnum viride* (with *S. cuspidatum* mixed in): NORWAY, Hordaland Co., Tysnes, Photo by K. I. Flatberg, August 1978. *Sphagnum viride* plants are green with young sporophytes, while *S. cuspidatum* plants are green-brownish without sporophytes. Arrow points to one of the *S. viride* plants. E, *Sphagnum cuspidatum*: USA, MAINE, Hancock Co., Winter Harbor Fen. Photo by B. Aguero, 17 June 2021.

Sphagnum fitzgeraldii is noticeably smaller than *S. mississippiense* or *S. trinitense*, with a prominent terminal bud and has broad oblong–ovate branch leaves with rounded truncate apices that make it easily distinguishable from other members of the *S. cuspidatum* complex. Furthermore, it can be separated on the basis of its ecology, occurring primarily in seasonal pools of coastal plain pine flatwoods and savannas, rarely occurring with *S. cuspidatum* in more permanently wet habitats (Crum 1997, McQueen and Andrus 2007, Anderson *et al.* 2009). For these reasons, the taxonomic status of *S. fitzgeraldii* is not under debate; however, its phylogenetic position within subgenus *Cuspidata* is unresolved. In contrast to *S. fitzgeraldii*, taxonomic treatments of *S. mississippiense*, *S. trinitense*, and *S. viride* have been inconsistent.

Known to grow alongside *S. cuspidatum* in weakly minerotrophic pools, *S. trinitense* can be separated from *S. cuspidatum* by having larger stem leaves with many septate hyaline cells, an undifferentiated stem cortex, and strongly to weakly serrate branch leaves (Andrus 1980, Crum 1997, McQueen and Andrus 2007). However, given variability in the size and extent of branch leaf teeth in *S. trinitense*, and the overall variability seen in plants of *S. cuspidatum*, including the occasional presence of teeth on branch leaves, the legitimacy of *S. trinitense* has been questioned, and in some cases it is considered a synonym of *S. cuspidatum* or treated as a variety of that species [*S. cuspidatum* var. *serrulatum* (Schlieph.) Schlieph.] (Blomquist 1937, 1938, Crum and Anderson 1981, Lane 1981, Allen 2005, Anderson *et al.* 2009).

Sphagnum mississippiense is morphologically similar to *S. trinitense* in having serrulate branch leaves. In addition, it has obtuse stem leaves with septate hyaline cells and undifferentiated stem cortical cells. Compared to *S. trinitense* and *S. cuspidatum*, *S. mississippiense* has shorter, less tapered branches, and ovate to broadly ovate branch leaves at branch bases. *Sphagnum cuspidatum* has ovate–lanceolate leaves and *S. trinitense* has ovate–lanceolate to lanceolate leaves (Andrus 1987, McQueen and Andrus 2007). Considering the obvious overlap in characters, Anderson *et al.* (2009) listed *S. mississippiense* as a synonym of *S. cuspidatum*. Crum (1997) stated that 'an isotype of *S. mississippiense* belongs to *S. cuspidatum* and several of the paratypes belong to *S. trinitense*'.

Of the species being examined in this study, *S. viride* is the most difficult to differentiate from *S. cuspidatum* because of its morphological similarity and the overlap in geographical and ecological ranges. Originally described by Flatberg (1988), *S. viride* can be separated from *S. cuspidatum* in the field by having less curved spreading branches and a lack of brown to red colour often seen at the base of branch leaves within the capitulum of female plants of *S. cuspidatum* (Flatberg 1988, McQueen and Andrus 2007). Microscopically, *S. viride* has branch leaves that are broader, straighter, and less tubular at their apices than those of *S. cuspidatum*, which also has shorter and broader hyaline and chlorophyllose cells. In addition, *S. viride* typically has more pores along the commissures of hyaline cells on the concave surface of branch leaves, and narrower hyaline cells in the upper portion of stem leaves. Nevertheless, these two characters are said to vary with water availability (Flatberg 1988). Ecologically, *S. viride* is described as preferring poor fens, while *S. cuspidatum* has a broader ecological range occurring in ombrotrophic to weakly minerotrophic sites (Flatberg 1988, McQueen and Andrus 2007). Unfortunately, as explained in Flatberg (1988), both *S. cuspidatum* and *S. viride* express a wide range of phenotypic plasticity 'along the wet–dry ecogradient'. Furthermore, Hanssen *et al.* (2000) was unable to separate plants of *S. cuspidatum* from *S. viride* based on isozyme patterns.

The aim of the present study was to use a combination of high-resolution population genomic data (RADseq) and a robust morphological assessment of plants from sites representing the known distributions of the focal species to: (i) establish if *S. mississippiense*, *S. trinitense*, and *S. viride* should be recognized at the species level; (ii) resolve phylogenetic relationships among species in the complex; (iii) determine which morphological characters best differentiate phylogenetically delimited species in the complex; and (iv) evaluate the taxonomic merit of leaf shape and leaf serration in these aquatic bryophytes.

MATERIALS AND METHODS

Taxon sampling

Material from 65 collections was used for the molecular analyses covering the known distributions of *S. cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, and *S. trinitense*, in eastern North America. These included 25 *S. cuspidatum*, seven *S. fitzgeraldii*, four *S. mississippiense* collected in the same area as the paratypes of this species, and 29 *S. trinitense* collections, based on preliminary morphological determinations (Fig. 2; Supporting Information, Table S1). In addition, one collection of *S. trinitense* from Puerto Rico and three collections identified as *S. viride* from Europe were included. The majority of collections were from one of three locations in North Carolina: Jessups Pond (20 collections), a site where *S. cuspidatum* and *S. trinitense* appear to occur sympatrically, Duke Forest (10 collections), and Green Swamp (16 collections), a site where *S. cuspidatum* and *S. fitzgeraldii* co-occur. Other collections were from sites in Florida ($N = 2$), Massachusetts ($N = 4$), Mississippi ($N = 6$), New York ($N = 1$), South Carolina ($N = 2$), and Virginia ($N = 3$). Thirteen samples of *S. cuspidatum* from Europe and Asia were included for a broader sampling of the genetic diversity in this species (Table S1). Data from *S. riparium* Ångström, *S. obtusum* Warnst., and *S. torreyanum* Sull. were included as outgroup data in the phylogenetic analyses.

For the morphometric analyses comparing *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, 50 of the collections included in the molecular analyses were examined, including 23 *S. cuspidatum*, seven *S. mississippiense*, and 20 *S. trinitense*. The seven *S. mississippiense* collections included the four collections originally identified as *S. mississippiense* and three collections that were originally identified as *S. cuspidatum* or *S. trinitense* but were later determined to be in the *S. mississippiense* clade based on molecular results. Two type specimens of *S. mississippiense* (DUKE 48; DUKE 49) were also examined. However, given the age of these collections, DNA extracted from them was too poor to include in the genetic analyses.

In our morphological comparisons of *S. cuspidatum* and *S. viride*, in addition to the three European collections of *S. viride* used in the genetic analyses, 17 collections of *S. cuspidatum* and 18 collections of *S. viride* from sites where these two putative species occur sympatrically were examined (Supporting

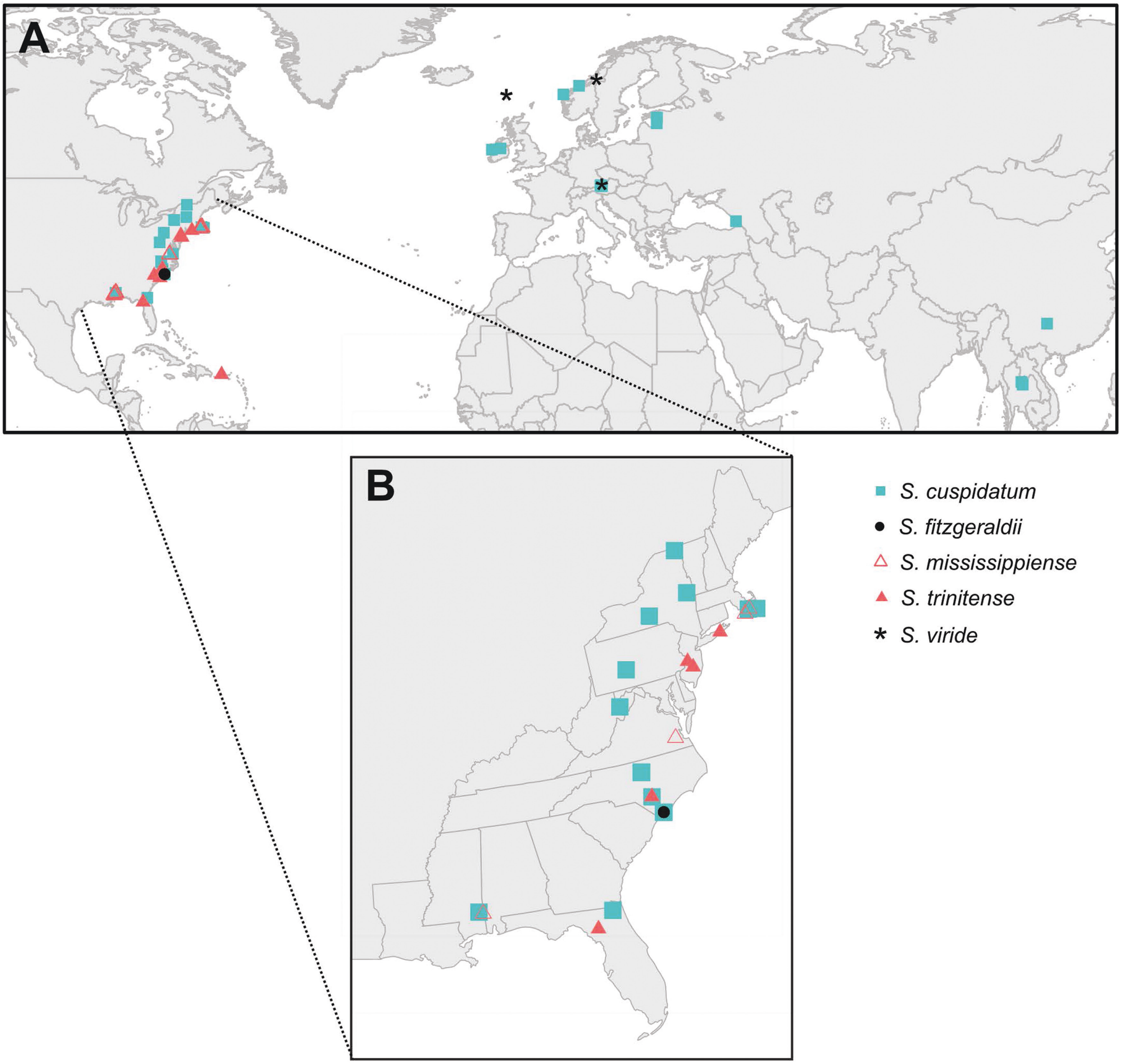

Figure 2. Geographical locations for collections of *Sphagnum cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, *S. trinitense*, and *S. viride* included in this study.

Information, Table S1). These additional collections were not included in the genetic analyses due to a lack of sufficient DNA.

Sphagnum fitzgeraldii was not included in the morphometric analyses because its taxonomic status was not in question.

DNA extraction, ddRADseq library preparation and sequencing

One capitulum of each gametophyte was used to extract genomic DNA from dried samples. Extractions followed the CTAB protocol outlined in Nieto-Lugilde *et al.* (2022). Genomic libraries were made following the double digestion restriction site-associated DNA sequencing (ddRADseq; using *Eco*RI and *Mse*I enzymes) protocol of Parchman *et al.* (2012), with modifications described by Duffy *et al.* (2020). Aliquots of two extractions were included twice in the RADseq library as duplicates to assess sequencing errors. The libraries were cleaned and size-selected for fragments of ~350 bp using AMPur XPbeads (Beckman Coulter), checked for quality on a BioAnalyzer (Agilent), and sequenced on a single lane of an Illumina NovaSeq 6000 with 150-bp single-end reads at the Genome Sequencing Shared resource at the Duke Center for Genomic and Computational Biology (https://oit.duke.edu/comp-print/research/). For 44 of the collections used (20 *S. cuspidatum*, three *S. fitzgeraldii*, four *S. mississippiense*, and 17 *S. trinitense* collections) two plants from each packet were sampled for genetic analysis to assess local-scale variation within populations and the degree to which plants of different species may or may not grow intermixed.

'RADseq-like' sequences for five samples (*S.* sp.: IYSB; *S. fitzgeraldii*: IYRH; *S. cuspidatum*: IYRJ, ISTB; *S. riparium*: IUTX) were generated from *Sphagnum* genome resequencing assemblies using the *in silico* digestion method described in Shaw *et al.* (2022).

RADseq data pipeline

Single nucleotide polymorphism (SNP) discovery was performed with ipyrad v.0.9.50 (Eaton and Overcast 2020) using default parameters except as noted here. Reads were processed as datatype 'ddrad' to match the library preparation method. A maximum of one mismatched base was allowed in the barcode during demultiplexing, Illumina adapter sequences and low-quality bases were trimmed from the reads, and trimmed reads less than 92 bases long or with more than five low-quality bases were discarded. Loci were assembled *de novo* and multiple ipyrad runs were performed using a range of read clustering thresholds to identify the clustering threshold (0.88) that maximized the number of variable and parsimony-informative loci and to verify that the results of downstream analyses were not sensitive to clustering threshold. Only loci present in at least 80% of the samples were kept for final analyses. Exploratory assemblies were done treating all samples as haploid or diploid to identify probable polyploid taxa (see below). Genotype calling was then performed again treating *S. mississippiense* and *S. torreyanum* as diploids and the remaining species (*S. cuspidatum*, *S. fitzgeraldii*, *S. obtusum*, *S. pulchrum*, and *S. trinitense*) as haploids, and the resulting assemblies were merged into a single dataset.

Previous microsatellite work suggests that all species in this analysis except *S. torreyanum* are haploid. Our results suggest that *S. mississippiense* is a polyploid as well, as evidenced by elevated error rates in this species when genotypes are called as haploid. This was also true of *S. torreyanum*, for which independent evidence (microsatellites) previously indicated is a polyploid. All samples were treated as diploid in the ipyrad heterozygosity estimation step. Because most species are expected to be haploid, higher heterozygosity rates seen in *S. torreyanum* and *S. mississippiense* when estimated under these conditions support the inference that these two species are polyploids (Supporting Information, Fig. S1).

Phylogenetic analyses and genomic admixture

IQ-TREE 2.0.3 (Minh *et al.* 2020) was used to infer phylogenetic relationships among sequences by maximum likelihood (ML) using concatenated loci identified by ipyrad. The ML tree was estimated using random starting trees and the ultrafast bootstrap approximation (UFBoot: Minh *et al.* 2013, Hoang *et al.* 2018). The best nucleotide substitution model was estimated using the ModelFinder algorithm (Kalyaanamoorthy *et al.* 2017) as implemented in IQ-TREE, and chosen according to the Bayesian Information Criterion (BIC). The rapid hill-climbing search algorithm was used to estimate the best ML tree using 1000 bootstrap replicates to determine support for branches. A network was constructed using the NeighborNet algorithm implemented in SplitsTree v.4.17 (Huson and Bryant 2006) to visualize potential phylogenetic conflict among species in the *S. cuspidatum* complex.

We assumed that the plastid genome is inherited maternally (Natcheva and Cronberg 2007) and used plastid sequences to further assess relationships and maternal ancestry, especially for the putative polyploid, *S. mississippiense*. For a subset of the samples with the least missing data, RADseq loci were mapped to the *S. fallax* chloroplast genome (GenBank KU725463.1) in bwa v.0.7.17 (Li and Durbin 2009), to identify RADseq loci that are probably from the chloroplast genome. The sample set was then further trimmed to include only samples with no missing data for these loci, and in one sample of *S. obtusum* (SB7057) as an outgroup. The plastid loci were concatenated, and maternal phylogenetic relationships were inferred with IQ-TREE as above. Only samples with no missing data and the one sample of *S. obtusum* (SB7057) were kept.

Population structure and genetic statistics

STRUCTURE 2.3.4 (Pritchard *et al.* 2000) was used to assess genetic structure in the *S. cuspidatum* complex. STRUCTURE assumes a lack of linkage disequilibrium among markers, so samples with low locus coverage and samples with distances similar to the distance between duplicated DNAs (probably clones or very closely related samples) were deleted. Moreover, one SNP per locus was randomly selected to avoid including tightly linked SNPs. STRUCTURE does not permit mixed haploid–diploid models in a single analysis, so all samples were coded as diploid, with the haploids treated as homozygous. The analyses were also conducted with all samples coded as haploid and results were consistent (data not shown). The optimal number of clusters (*K*) was evaluated using the Δ*K* method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER v.0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/, Earl and von Holdt 2012) 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 different levels of *K* to assess the possibility of additional structure in the data. STRUCTURE PLOT v.2.0 (http://omicsspeaks.com/strplot2/; Ramasamy *et al.* 2014) was used to visualize the STRUCTURE results.

Morphological characters

In a comparison of *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, 21 quantitative and five qualitative characters were examined based on descriptions provided by Andrus (1987), McQueen and Andrus (2007) and our own observations (Table 1; Fig. 3). All measurements were made using an Olympus BX43 light microscope with an attached Olympus EP50 camera and associated software. For each of two plants randomly selected from each collection (a total of 104 plants), a portion of the stem and three spreading branches were removed for dissection. Three stem leaves were removed from the stem, and three leaves located at the terminal end of each of the three spreading branches (nine leaves in total), along with three leaves located at the midpoint of each of the spreading branches (nine leaves in total) were removed. The data described in Table 1 and Figure 3 were collected from each branch and leaf. Cross-sections of the stem were made to assess the level of cortical cell differentiation (Table 1; Fig. 3). Measurements were taken at two randomly selected locations on one of the cross-sections.

For the comparison of *S. cuspidatum* and *S. viride*, 12 quantitative characters were examined based on descriptions provided

Table 1. Quantitative and qualitative characters used in morphological evaluation of *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense*. Character ID corresponds to the abbreviations used for each character in data analyses.

	Character	Character ID	Data collected
Quantitative characters			
Stem leaf	Leaf lamina length	StLf Length	Length measured in μm
	Leaf lamina width	StLf Width	Width at widest point of proximal end measured in μm
	Leaf lamina length/width	StLf Ratio	Length measured in μm/width measured in μm
	Area of leaf with fibrillose cells	StLf Fibrils	Percentage area of leaf with fibrillose cells
Basal branch leaf	Leaf lamina length	BrLfBa Length	Length measured in μm
	Leaf lamina width	BrLfBa Width	Width at widest point of proximal end measured in μm
	Leaf lamina length/width	BrLfBa Ratio	Length measured in μm/width measured in μm
Mid-branch leaf	Leaf lamina length	BrLfMi Length	Length measured in μm
	Leaf lamina width	BrLfMi Width	Width at widest point of proximal end measured in μm
	Leaf lamina length/width	BrLfMi Ratio	Length measured in μm/width measured in μm
	Leaf tooth length	BrLfMi Teeth	Length of marginal tooth in upper half of leaf measured in μm (three teeth measured on each leaf)
	Leaf Tubularity	BrLfMi Tub	Width of gap between inrolled margins in μm/width of leaf at the same point in μm (see Fig. 3)
	Extent of leaf tubularity	BrLfMi Tub Dist	Length of tubular region of leaf in μm/length of leaf in μm (see Fig. 3)
Terminal branch leaf	Leaf lamina length	BrLfEn Length	Length measured in μm
	Leaf lamina width	BrLfEn Width	Width at widest point of proximal end measured in μm
	Leaf lamina length/width	BrLfEn Ratio	Length measured in mm/width measured in mm
	Leaf tooth length	BrLfEn Teeth	Length of marginal tooth in upper half of leaf measured in μm (three teeth measured on each leaf)
	Leaf tubularity	BrLfEn Tub	Width of gap between inrolled margins in μm/width of leaf at the same point in μm (see Fig. 3)
	Extent of leaf tubularity	BrLfEn Tub Dist	Length of tubular region of leaf in μm/Length of leaf in μm (see Fig. 3)
Spreading Branches	Length of spreading branches	Branch Length	Length of spreading branch in μm
Cortical cells	Differentiation of cortical cells	Cortical Cells	Mean width of two outer layers of cortical cells/mean width of third and fourth layers of cortical cells measured in μm
Qualitative characters			
Stem leaf	Number of septate cells	StLf Septa	Estimated number of septate hyaline cells scored as a 0 (<5 septate cells), 1 (5–10 septate cells), or 2 (>10 septate cells)
Mid-branch leaf	Shape of terminal end	BrLfMi Tip	Degree to which the tip of the leaf was acute vs. obtuse scored as a 0 (acute) or 1 (obtuse)
	Leaf tubularity	BrLfMi Tub	Degree to which leaf was tubular in upper half scored as a 1 (tubular), 2 (somewhat tubular), or 3 (not tubular) (see Fig. 2). Leaves were later scored as 0 (tubular) or 1 (not tubular) in MCAs
Terminal branch leaf	Shape of terminal end	BrLfEn Tip	Degree to which the tip of the leaf was acute vs. obtuse scored as 0 (acute) or 1 (obtuse)
	Leaf tubularity	BrLfEn Tub	Degree to which leaf was tubular in upper half scored as 1 (tubular), 2 (somewhat tubular), or 3 (not tubular) (see Fig. 2). Leaves were later scored as 0 (tubular) or 1 (not tubular) in MCAs

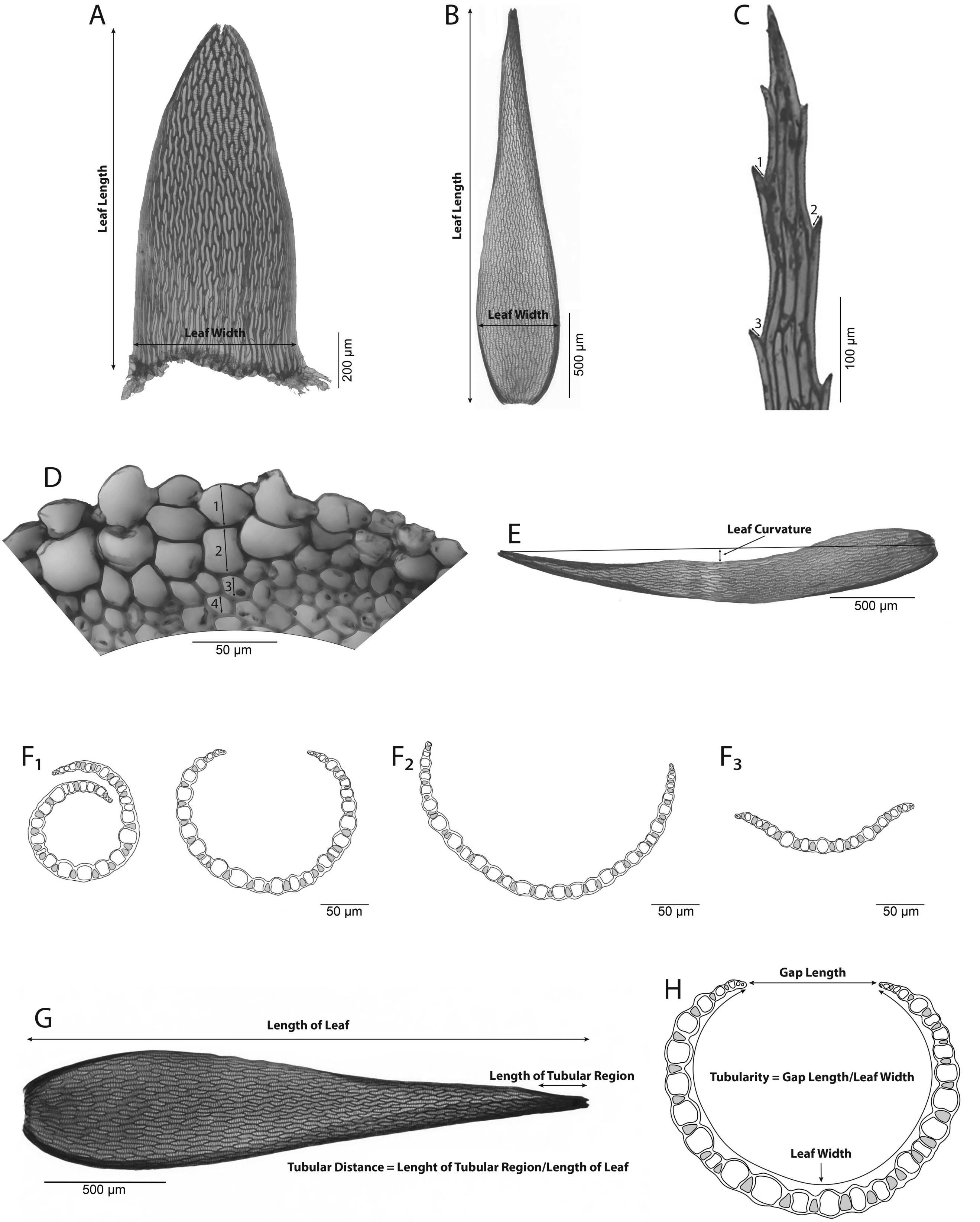

Figure 3. Explanations of how some of the qualitative and quantitative measurements were made. A, stem leaf length and width. B, branch leaf length and width. C, length of branch leaf teeth. D, differentiation of stem cortical cells, which was calculated by dividing the mean width of the outer two cells by the mean width of the third- and fourth-layer cells. E, leaf curvature F, tubularity of branch leaf apices (qualitative assessment): F1 = tubular (scored as 1); F2 = somewhat tubular (scored as 2); F3 = not tubular (scored as 3). G, branch leaf tubular distance. H, branch leaf tubularity (quantitative assessment).

by Flatberg (1988) and McQueen and Andrus (2007) (Table 2). With the exception of three *S. viride* collections, comparisons were only made between plants occurring sympatrically, as mixed stands in some cases, and collected on the same date (Supporting Information, Table S1). This was to ensure that any differences detected were not simply the result of plastic responses to different ecological conditions or sampling dates. For each plant randomly selected from each collection (a total of 38 plants), three spreading branches were removed for dissection. Three leaves located at the midpoint of each of the three dissected spreading branches were removed (nine leaves total). The data described in Table 2 were collected from each branch and leaf. Given the high variability observed in stem leaf morphology in a preliminary examination of characters, and Flatberg's (1988) conclusion that stem leaves were not reliable in separating *S. viride* from *S. cuspidatum*, stem leaf characters were removed from the final morphometric analyses.

Statistical analyses

Plants were grouped by species for morphological comparisons between *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* based on results of the phylogenetic analyses. For comparisons between *S. cuspidatum* and *S. viride*, plants were grouped based on previous identifications (because these taxa were not distinguished by our molecular data). Means from replicate measurements for each of the quantitative characters were calculated. Nonparametric Kruskal–Wallis tests followed by Wilcoxon tests with significance levels subjected to Benjamini–Hochberg corrections were used to evaluate variation in the five qualitative characters (Koutecký 2015). For the quantitative characters, characters for which the data were not normally distributed based on a Shapiro–Wilks test were log transformed. The significance of group differences was evaluated considering all quantitative characters simultaneously using a multivariate analysis of variance (MANOVA). Variation in each character was then evaluated by analysis of variance (ANOVA) followed by a post-hoc Tukey test for multiple comparisons of group means. Principal component analyses (PCAs) and multiple correspondence analyses (MCAs) were performed to examine the distribution of morphological variation in quantitative and qualitative characters, respectively. Comparisons were made between (i) *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* and (ii) *S. cuspidatum* and *S. viride*. PCAs (for quantitative characters) and MCAs (for qualitative characters) were done using (i) all characters examined (data not shown) and (ii) only those characters that were found to be significantly different between the lineages based on the Kruskal–Wallis, Wilcoxon, and ANOVA tests. A Pearson and Spearman's matrix of the correlation coefficients of the characters was calculated to avoid highly corelated characters ($r > |.95|$) (Koutecký 2015). All analyses were conducted using R v.4.1.2 (R Core Team 2022).

To test the extent to which variation in morphological characters within *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* may be due to plasticity in response to habitat variation, plants for which there was sufficient habitat data were coded as 'emergent' (plants found growing above the water table) or 'submergent' (plants found growing at or below the water table). The significance of group (emergent vs. submergent) differences within species was evaluated considering all quantitative characters simultaneously using a MANOVA. Variation in characters was then evaluated by ANOVA and PCA.

RESULTS

Molecular data characterization

After removing barcodes and adapter sequences, trimming and filtering for quality, and removing samples with low read counts, 475 million reads of 32–92 bp were retained across 131 individual samples and four individual DNA replicates. The number of reads per individual ranged from 228 040 to 9 166 863 (median ± SD = 3691 558 ± 2146 240). In a preliminary analysis, the 88% clustering similarity threshold to identify sequences as homologous generated the highest number of loci and parsimony-informative sites and was therefore used to construct the final data sets.

The assembly pipeline produced 8367 loci shared among ≥80% of the individuals. The final matrix included 648 135 bp, and 8326 of those loci contained one or more SNPs and 8254

Table 2. Quantitative characters used in morphological evaluation of *Sphagnum cuspidatum* and *S. viride*. Character ID corresponds to the abbreviations used for each character in data analyses.

	Character	Character ID	Data collected
Quantitative characters			
Mid-branch leaf	Leaf lamina length	BrLfMi Length	Length measured in µm
	Leaf lamina width	BrLfMi Width	Width at widest point of proximal end measured in µm
	Leaf lamina length/width	BrLfMi Ratio	Length measured in µm/width measured in µm
	Leaf curvature	BrLf Curve	Depth of concavity at maximum point of curvature in µm (see Fig. 3)
	Hyaline cell length	BrLf Hyal Length	Length of hyaline cells near apical region of leaves in µm
	Hyaline cell width	BrLf Hyal Width	Width of hyaline cells near apical region of leaves in µm
	Hyaline cell length/width	BrLf Hyal Ratio	Length of hyaline cells in µm/width of hyaline cells in µm
	Green cell length	BrLf Chloro Length	Length of green cells near apical region of leaves in µm
	Green cell width	BrLf Chloro Width	Width of green cells near apical region of leaves in µm
	Green cell length/width	BrLf Chloro Ratio	Length of green cells in µm/width of green cells in µm
	Number of pores	BrLf Pores	Number of pores along commissures of hyaline cells on concave surface
	Extent of leaf tubularity	BrLfEn Tub Dist	Length of tubular region of leaf in µm/Length of leaf in µm (see Fig. 3)

contained one or more phylogenetically informative SNPs (i.e. those shared by two or more individuals). The mean locus coverage per individual was 84.25%.

Phylogenetic reconstructions

Iqtree estimated TVM+F+R3 as the best-fit substitution model according to the BIC. With the exception of *S. viride*, which grouped with *S. cuspidatum*, all individual species included in this study were each resolved as monophyletic (Fig. 4). Rooted with *S. obtusum*, *S. riparium*, and *S. torreyanum*, the complex comprises two clades. One clade includes all *S. cuspidatum* samples. Samples identified morphologically as *S. viride* are scattered within *S. cuspidatum* and do not form a clade. The *S. cuspidatum* clade is sister to a clade that includes *S. fitzgeraldii*, *S. trinitense*, and *S. mississippiense*. Within the latter clade, *S. mississippiense* is resolved as sister to *S. fitzgeraldii* and *S. trinitense*.

SplitsTree network analyses also showed that *S. cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, and *S. trinitense* formed distinct clusters, but did not resolve *S. viride* (Fig. 5). SplitsTree shows conflict in the complex associated with the putative polyploid *S. mississippiense*, which was linked to both *S. fitzgeraldii* and to the outgroup species.

Five loci (of 58–87 bp) mapped to the plastid genome of *S. fallax*, for a total of 403 bp with only 10 variable and two parsimony-informative SNPs. The BIC identified the best-fit substitution model as K2P for these data. The ML tree indicates that *S. trinitense* is separated from *S. fitzgeraldii* and *S. mississippiense*, which share identical plastid sequences. *Sphagnum viride* and *S. cuspidatum*, which also share identical plastid sequences, were placed at the base of the tree (Fig. 6). These results agree with patterns obtained from the nuclear data (Figs 4, 5). *Sphagnum mississippiense* is more closely related to *S. fitzgeraldii* and *S. trinitense* than to *S. cuspidatum* and shares a closer relationship with *S. fitzgeraldii* than with *S. trinitense* (notwithstanding low bootstrap support for this inference).

Population structure

Cluster analyses based on STRUCTURE, which included *S. cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, *S. trinitense*, and *S. viride*, suggested that $K = 2$ genetic groups was optimal (Fig. 7A). Clustering the samples in these two groups, samples of *S. viride* and *S. cuspidatum* belong to the green group, *S. trinitense* corresponds with the red group, and *S. mississippiense* and *S. fitzgeraldii* showed admixture between these two groups. The levels of admixture, however, differed between these last two taxa, which are genetically closer to *S. trinitense* than to *S. cuspidatum*. At $K = 3$, *S. fitzgeraldii* is resolved as separate from the previous groups (yellow group; Fig. 7B), and *S. mississippiense* plants continue to show admixture, but 85% are from the yellow group and the rest belong to the green group (*S. cuspidatum*). Finally, at $K = 4$, all taxa are resolved as separate with the exception of *S. viride*. The latter is never distinguished as a group separate from *S. cuspidatum* at any level of K (Fig. 7C). At increasing levels of K (>4) no additional genetic structure is suggested (data not shown). These results agree with previously performed network analyses in which the two most differentiated species are *S. cuspidatum* and *S. trinitense*. The results also support the existence of genetic conflict in the polyploid *S. mississippiense* between *S. fitzgeraldii* and *S. cuspidatum*, or other species outside the *S. cuspidatum* complex.

Within individual taxa, phylogenetic analyses did not reveal any clear patterns of population structure, with one exception. Within *S. cuspidatum*, plants from European populations formed a clade nested within the North American clade, which was sister to a clade with plants from Asia.

Morphometric analyses comparing *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*

Results of the quantitative and qualitative trait analyses are summarized in Figure 8 and Table 3. Images from representative herbarium collections clearly show the morphological differences between *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, as well as *S. fitzgeraldii* (Figs 9, 10). All three species were discriminated by the MANOVA (Table 4). Numerical values for ANOVA and Kruskal–Wallis tests are presented in Supporting Information Table S2. Eighteen of the 21 quantitative characters used to compare *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* were significantly different between at least two of the three species according to post-hoc tests. These are illustrated by different lower-case letters in the beanplots (Fig. 8). Of these 21 characters, 11 were significantly different between *S. cuspidatum* and *S. trinitense*; 12 and 14 characters separated *S. mississippiense* from *S. trinitense*, and *S. cuspidatum*, respectively. Characters that were not significantly different between any of the species included area of stem leaves with fibrillose cells, length of basal branch leaves, and length of terminal branch leaves. Of the five qualitative characters examined, two were significantly different between all three species (Table S2). These were number of septate hyaline cells in stem leaves and tubularity of terminal branch leaves. *Sphagnum trinitense* plants differed from *S. cuspidatum* in shape of the terminal branch leaf tip, and from both *S. cuspidatum* and *S. mississippiense* in tubularity of mid-branch leaves. Of the 231 pairs of traits evaluated in the Pearson and Spearman's matrix, 123 (53%) were significantly correlated (Fig. S2). The most highly correlated characters (a strong negative correlation) were tubularity and marginal tooth length of branch leaves.

In the PCAs of significantly different quantitative traits comparing *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, the first two principal components (PCs) accounted for 48% (27% PC1, 21% PC2) of the variance (Fig. 11). Length of terminal and mid-branch leaf teeth, tubularity of terminal and mid-branch leaves, extent of tubularity on mid-branch leaves, and stem cortical cell differentiation were the most important variables in the first PC, separating all three species. The most important variables in PC2, separating *S. mississippiense* plants from *S. cuspidatum* and *S. trinitense*, were basal, mid-, and terminal branch leaf widths. In addition, extent of mid-branch leaf tubularity and length of mid-branch leaf teeth were also important in separating *S. mississippiense* from *S. cuspidatum* and *S. trinitense*, respectively, along PC2. The contributions of each quantitative character to the PCA axes are summarized in Figure 11A.

In the MCAs of significant qualitative traits comparing all three lineages, *S. cuspidatum* and *S. mississippiense* were clearly separated from *S. trinitense* plants (Fig. 12). Estimated number of septate stem leaf hyaline cells, tubularity of terminal branch leaves (tubular vs. non-tubular), and tubularity of mid-branch leaves (tubular vs. non-tubular) contributed the most to the first two

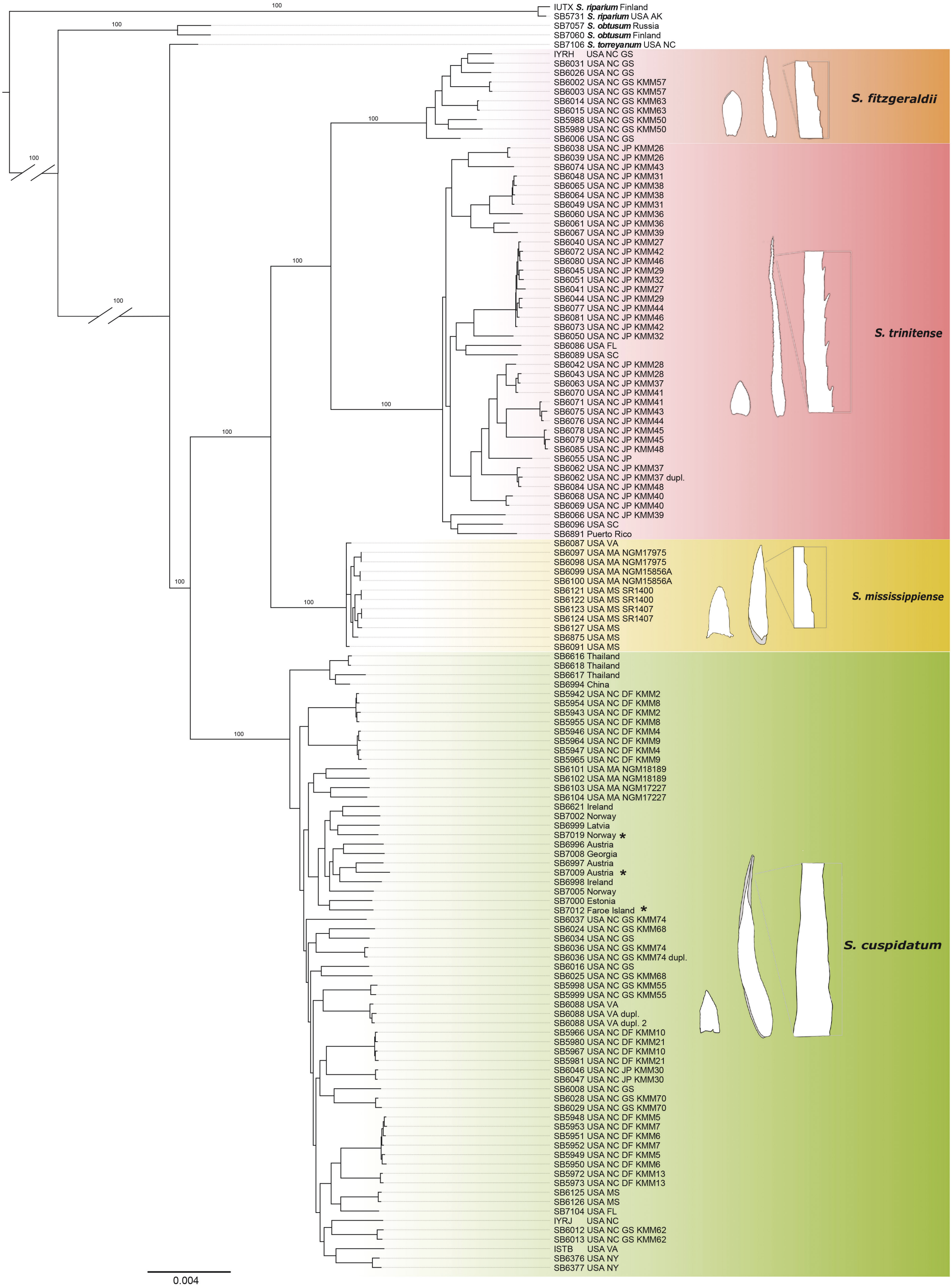

Figure 4. Phylogenetic relationships among species in the *Sphagnum cuspidatum* complex based on RADseq loci. Nodes of the clades supported at >95% are labelled with support values. Extraction numbers are followed by countries, and state abbreviations are provided for samples collected within the eastern United States. Samples from the three most heavily sampled sites in North Carolina are identified using the following abbreviations: DF (Duke Forest), GS (Green Swamp), and JP (Jessups Pond). Codes following these abbreviations represent collector abbreviations and collection numbers (for locality details see Supporting Information, Table S1). This additional information is provided only in those cases where more than one DNA extraction was made from a collection packet. The abbreviation dupl. discriminates sequences from a DNA extraction sequenced more than once. Asterisks designate samples originally identified as *Sphagnum viride*. Slash marks indicate where branches were shortened to better visualize ingroup variation.

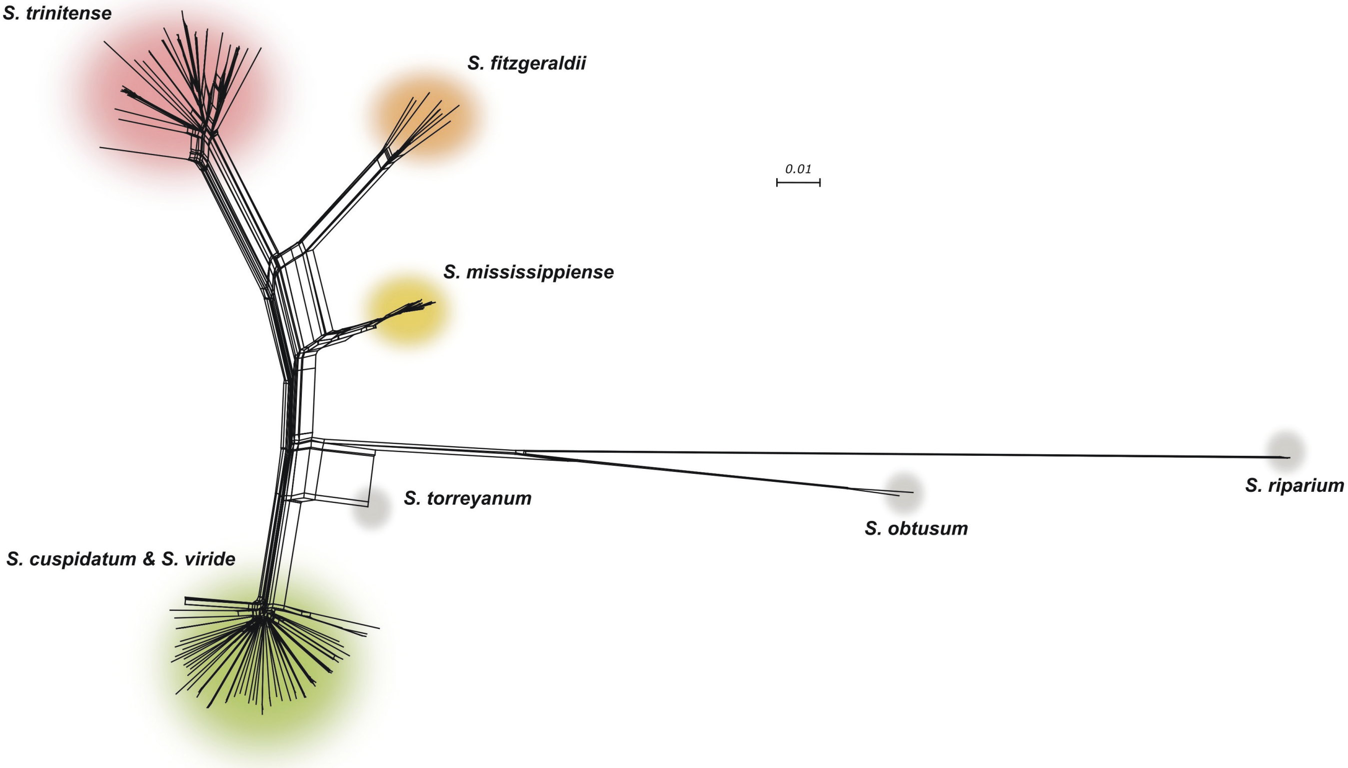

Figure 5. Results of SplitsTree network analyses (Neighbor-Net algorithm) for the *S. cuspidatum* complex.

dimensions, which accounted for 76% of the variability. Shape of terminal branch leaf apices contributed the least and also did not correlate with species because of the high variability in this character within species and a lack of consistent differences between them (Fig. 12B). These results highlight the reduced and/or lack of tubularity in the branch leaves of *S. trinitense* vs. clear tubularity in the branch leaves of *S. cuspidatum* and *S. mississippiense*, as well as the increased number of septate hyaline stem leaf cells in *S. trinitense* (Figs 9, 10). While *S. mississippiense* clearly falls morphologically closer to *S. cuspidatum*, its placement between *S. cuspidatum* and *S. trinitense* reflects the presence of septate stem leaf hyaline cells and a lower degree of tubularity in the terminal branch leaves of at least some of the plants examined.

Emergent vs. submergent plants

In a comparison of *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, emergent and submergent plants were discriminated by the MANOVA in *S. cuspidatum* and *S. mississippiense*, but not in *S. trinitense* (Table 5). Numerical values for ANOVA results are presented in Supporting Information Tables S3–S5. Seven, three, and none of the 20 quantitative characters compared were significantly different between submergent and emergent plants of *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, respectively, according to post-hoc tests. The seven characters that varied within *S. cuspidatum* included stem leaf width, mid-branch leaf length/width, length of mid-branch leaf teeth, terminal branch leaf length, length of terminal branch leaf teeth, and extent of tubular region on terminal branch leaves. The three significantly different characters in *S. mississippiense* included mid-branch leaf width, terminal branch leaf width, and length/width of terminal branch leaves.

In the PCAs of all quantitative characters in emergent vs. submergent plants of *S. cuspidatum*, *S. mississippiense*, and *S. trinitense*, the first two components accounted for 44.4% (27.5% PC1, 16.9% PC2), 53.6% (33.1% PC1, 20.5% PC2), and 43.4% (25.3% PC1, 18.1% PC2) of the variance, respectively (Figs 13–15). For *S. cuspidatum*, the most important variables in the first PC separating emergent and submergent plants were stem leaf width, mid-branch leaf length/width, terminal branch leaf length, and length of terminal branch leaf teeth (Fig. 13). The relationships of each of the quantitative characters to the PCA axes are summarized in Figures 13A, 14A, and 15A.

Morphometric analyses comparing *S. cuspidatum* and *S. viride*

Results of the quantitative trait analyses are summarized in Figure 16 and Table 6. *Sphagnum cuspidatum* and *S. viride* were discriminated by the MANOVA (Table 7). Numerical values for ANOVA results are presented in Supporting Information Table S6. Six of the 12 characters used to compare *S. cuspidatum* and *S. viride* were significantly different between them. These included branch leaf length and length/width, branch leaf hyaline cell length and length/width, number of pores along hyaline cell commissures on concave surface of branch leaves, and extent of tubular region at apices of branch leaves (Fig. 16; Table S6). In addition, while not statistically significant, width and curvature of branch leaves were also noticeably different between the two species (Table 6). Of the 78 pairs of traits evaluated in the Pearson and Spearman's matrix, 32 (41%) were significantly correlated (Fig. S3).

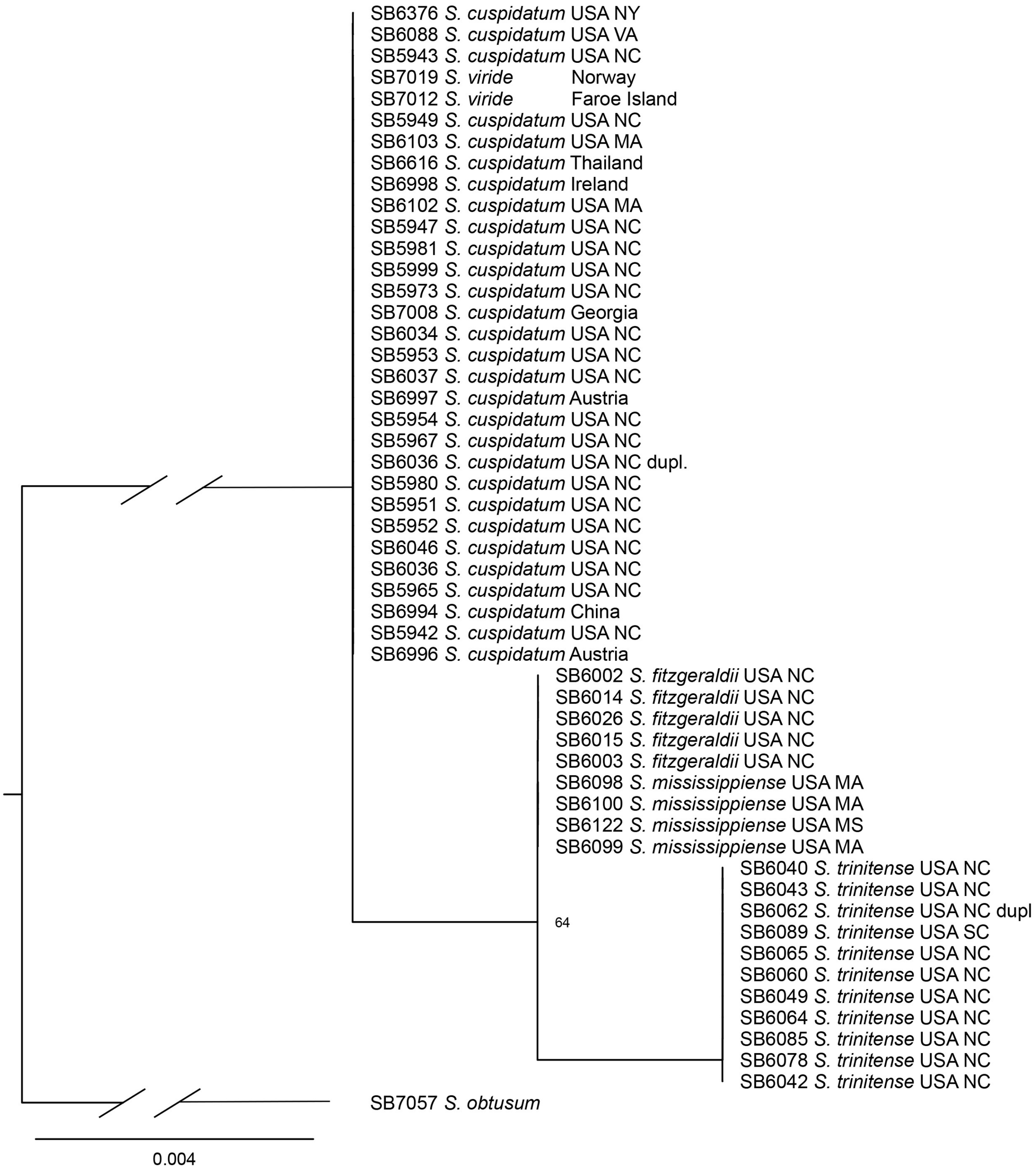

Figure 6. Phylogenetic relationships among RADseq loci probably located in the chloroplast genome from samples of *Sphagnum cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, *S. trinitense*, and *S. viride*.

In the PCAs of the six significantly different characters comparing *S. cuspidatum* and *S. viride*, the first two components accounted for 76% (62% PC1, 14% PC2) of the variance (Fig. 17). Length/width of branch leaves, length/width of branch leaf hyaline cells, and extent of tubular region at branch leaf apices were the most important variables in the first PC, which separates *S. cuspidatum* from *S. viride*. However, there is clear overlap in the morphology of these two species (Fig. 17; Table 6), and PC2 provides little to no separation. The six *S. cuspidatum* specimens that fall within the *S. viride* cluster exhibit no geographical pattern as they were collected from sites in New York, New Hampshire, Michigan, Indiana, and England. The three additional *S. viride*

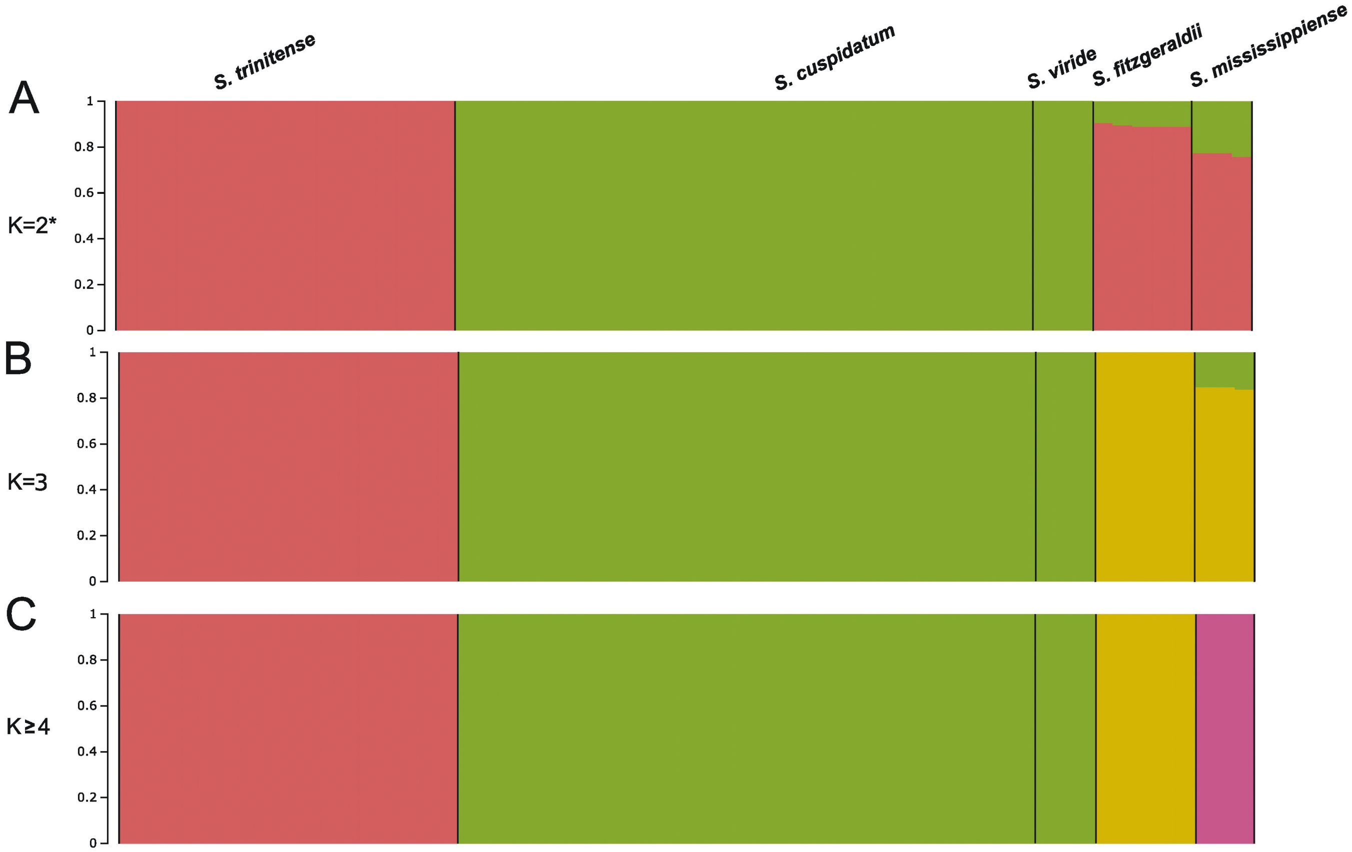

Figure 7. Results of STRUCTURE analyses of RADseq loci. The analysis with the optimal *K*-value is indicated with an asterisk.

specimens included in the phylogenetic analyses occur within the region of overlap between the two species. The relationships of each of the quantitative characters examined to the PCA axes are summarized in Figure 17A.

DISCUSSION

Because of the high levels of morphological diversity found within *Sphagnum* species and substantial overlap in characters between taxa, hypotheses regarding species delimitation within the genus have always been controversial. Physiological and morphological differences among *Sphagnum* species are directly related to the ecological roles they play, so establishing species boundaries and determining what characters represent interspecific vs. intraspecific variation is important beyond the field of systematics (Rice and Schuepp 1995, Johnson *et al.* 2014, Jassey and Signarbieux 2019).

The taxonomic status of species within the *S. cuspidatum* complex has been challenged repeatedly. Four of the five species in this complex are unusual within *Sphagnum* in having serrate to serrulate branch leaves, which is one of the primary characters used to distinguish them. However, variation in the presence/absence and size of branch leaf teeth, and in leaf shape characters (e.g. length, width, length/width, involute leaf apices), have brought into question the taxonomic merit of these characters. The aim of this study was therefore to investigate the phylogenetic, morphological, and taxonomic status of species in the *S. cuspidatum* complex.

Our results support the recognition of *S. cuspidatum*, *S. fitzgeraldii*, *S. mississippiense*, and *S. trinitense* as distinct groups based on phylogenetic and cluster (STRUCTURE) analyses, and they can be separated morphologically. Of the characters examined in this study, those of leaves from the middle of spreading branches are the most reliable. While stem leaf characters such as leaf length and width were significantly different between species, high variability and overlap in these characters make them less reliable. The greater occurrence of septate hyaline cells in stem leaves of *S. trinitense*, however, is a relatively consistent character. In addition, shape of stem leaf apices (acute in *S. cuspidatum* vs. obtuse in *S. mississippiense* and *S. trinitense*) is diagnostic, an important character for separating other taxa in *Sphagnum* subgen. *Cuspidata* (Duffy *et al.* 2020). *Sphagnum trinitense* has narrower branch leaves than *S. mississippiense*, resulting in a higher and lower length/width, respectively. Branch leaf apices in *S. trinitense* are flat. Though at times they may show signs of tubularity, opposite margins do not touch or overlap. In contrast, branch leaf apices of *S. mississippiense* are tubular, as in *S. cuspidatum*. The extent of tubularity in the branch leaves of *S. mississippiense*, however, is significantly less than in plants of *S. cuspidatum*, extending down an average of just 4% of the leaf, as opposed to an average of 20% in *S. cuspidatum.* While teeth occur on the margins of terminal branch leaves in some *S. cuspidatum* plants, the size and number of teeth are significantly lower than those of *S. trinitense* and *S. mississippiense*. Differences in this character are best observed on leaves from the middle of spreading branches. Margins of these leaves are entire in *S.*

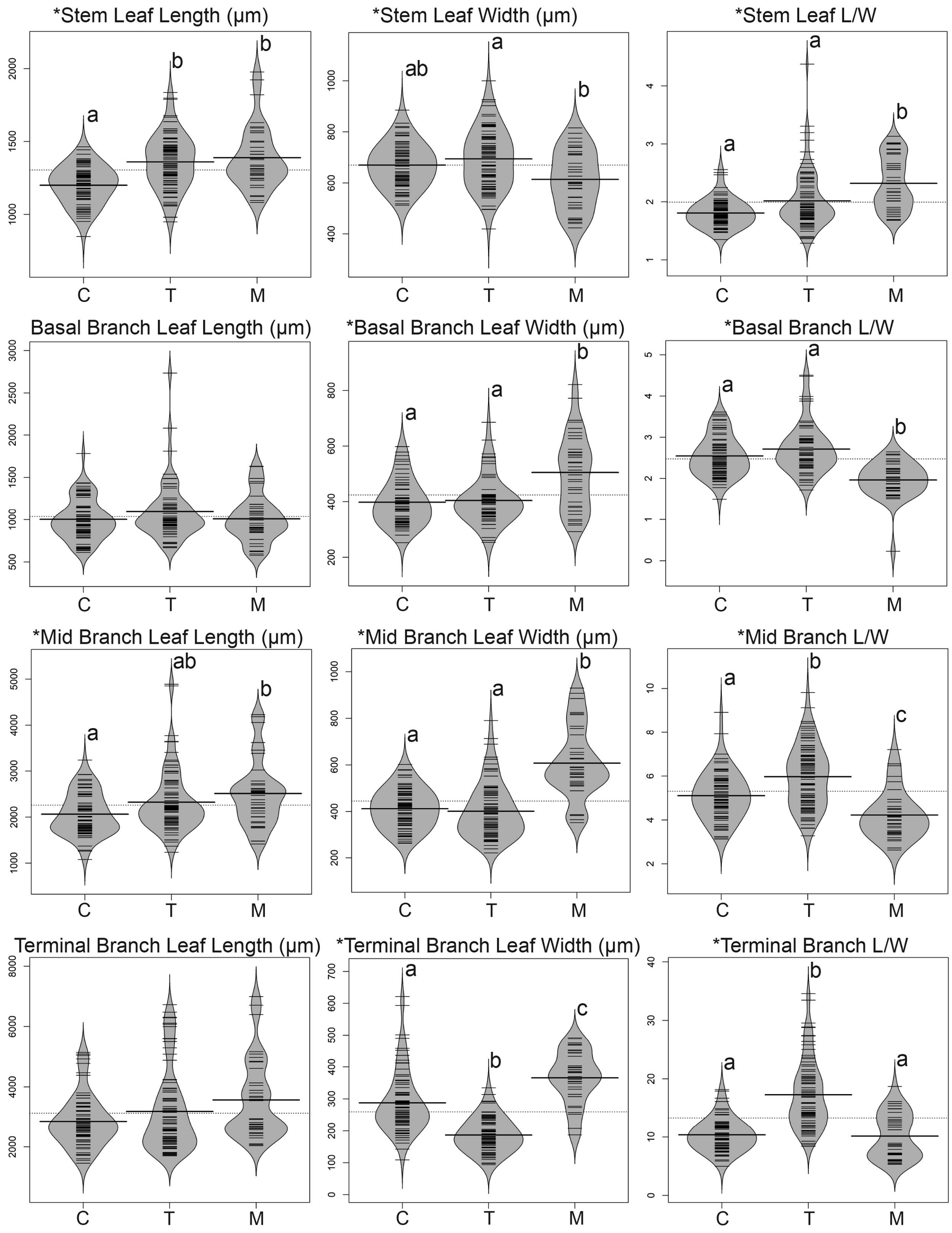

Figure 8. Beanplots of 19 quantitative characters examined in a comparison of plants identified as *S. cuspidatum* (labeled as C), *S. trinitense* (labeled as T), and *S. mississippiense* (labelled as M) by molecular analyses. Individual observations are represented by short horizontal lines and longer, bold horizontal lines represent mean values for each taxon. The dotted line in each plot shows the overall mean value for all observations made across taxa. The shape of each plot is a density trace representing the estimated density of the data distribution (see Kampstra 2008). Characters for which there were statistically significant differences between taxa ($P < .05$) based on ANOVAs are highlighted with an asterisk. Lower-case letters indicate which taxa were significantly different based on Tukey post-hoc tests. The absence of a species from some of the plots is due to a lack of variability in data for that species for that character. Plots for terminal branch leaf tubularity distance and mid-branch leaf tubularity were not included due to a lack of variability in the data for two out of the three species examined.

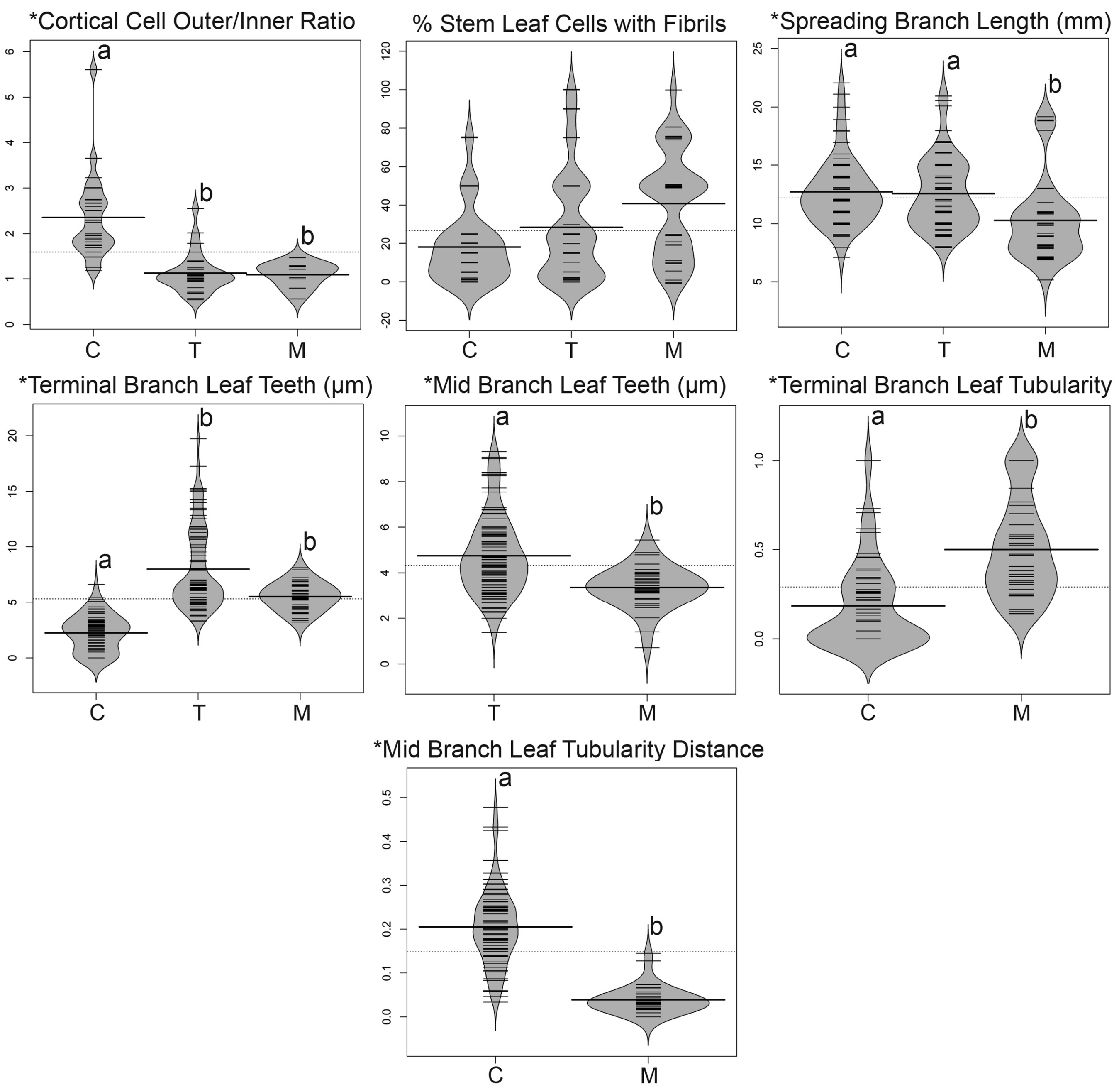

Figure 8. Continued

cuspidatum, although one or two extremely small teeth have been observed on the leaves of a few plants. They are serrate to serrulate in *S. trinitense* and *S. mississippiense*, with *S. trinitense* having significantly larger teeth than *S. mississippiense*. Leaf serration and tubularity were significantly correlated with one another.

Other important differences observed between *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* include the degree of stem leaf cortical cell differentiation and length of spreading branches. *Sphagnum cuspidatum* has two layers of enlarged thin-walled cortical cells, while *S. trinitense* and *S. mississippiense* have one layer, with this outer layer of cells tending to be less differentiated in *S. mississippiense*. Spreading branches tend to be shorter and blunter in *S. mississippiense*, a feature highlighted in its original description that could be used to separate it from *S. trinitense* (Andrus 1987). In our analyses, however, we found spreading branches in some plants of *S. mississippiense* to be similar in length and shape as those in *S. trinitense*.

Originally known from just four sites in Louisiana and Mississippi and later reported from one site in New Jersey (Andrus 1987, McQueen and Andrus 2007), one unexpected finding of this study was the occurrence of *S. mississippiense* in Massachusetts. In addition, we found evidence suggesting that *S. mississippiense* is a polyploid. Andrus (1987) hypothesized that *S. mississippiense* evolved from *S. trinitense*. Our results, however, show a closer relationship with *S. fitzgeraldii*. In fact, assuming *S. mississippiense* is an allopolyploid, the plastid DNA tree suggests that *S. fitzgeraldii* is the maternal parent. This could explain the wider spreading branch leaves in *S. mississippiense*, a feature

Table 3. Descriptions of morphological characters compared across specimens of *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense* plants. Bold text designates characters which were found to be the most diagnostic.

	S. cuspidatum	*S. mississippiense*	*S. trinitense*
Stem leaves			
Leaf shape	Leaves triangular–ovate, apex is typically more acute (or apiculate) than that seen in *S. trinitense* and *S. mississippiense*	Leaves triangular and more elongate than that seen in *S. cuspidatum*, apex typically more obtuse	Leaves triangular–ovate, slightly more elongate than that seen in *S. cuspidatum*, apex ranging from acute to obtuse
Leaf length (mm)	(0.88–)1.17–1.23(–1.46)	(1.08–)1.32–1.46(–1.98)	(0.95–)1.31–1.39(–1.84)
Leaf width (mm)	(0.51–)0.65–0.69(–0.89)	(0.42–)0.58–0.65(–0.82)	(0.41–)0.66–0.72(–1)
Hyaline cell fibrillosity	Hyaline cells often fibrillose in upper portion of leaves	Hyaline cells range from efibrillose to mostly fibrillose throughout leaf	Hyaline cells often fibrillose throughout most of leaf
Leaf length/width	(1.35–)1.75–1.86(–2.56)	(1.68–)2.17–2.47(–3.13)	(1.29–)1.89–2.13(–4.37)
Hyaline cell septa	Stem leaves with some to no septate hyaline cells	Stem leaves range from having no septate hyaline cells to several septate cells in proximal half of leaf	Stem leaves with several septate hyaline cells typically concentrated near the upper half of the leaf, but sometimes extending down to proximal end
Basal branch leaves			
Leaf length (mm)	(0.61–)0.95–1.06(–1.78)	(0.58–)0.92–1.10(–1.63)	(0.67–)0.99–1.22(–2.73)
Leaf width (mm)	(0.25–)0.37–0.42(–0.60)	(0.29–)0.46–0.55(–0.82)	(0.25–)0.38–0.43(–0.68)
Leaf length/width	(1.50–)2.43–2.66(–3.61)	(0.23–)1.82–2.11(–2.64)	(1.72–)2.54–2.90(–4.50)
Mid-branch leaves			
Leaf length (mm)	(1.08–)1.96–2.17(–3.24)	(1.42–)2.27–2.75(–4.23)	(1.23–)2.19–2.52(–4.89)
Leaf width (mm)	(0.26–)0.39–0.43(–0.60)	(0.35–)0.56–0.66(–0.93)	(0.22–)0.37–0.43(–0.79)
Leaf length/width	(3.13–)4.85–5.36(–8.92)	(2.63–)3.86–4.59(–7.20)	(3.63–)5.72–6.37(–9.82)
Leaf margin	Leaf margins entire, but sometimes having a few widely spaced teeth	Leaf margins serrulate with teeth typically smaller than those seen in *S. trinitense*	Leaf margins strongly serrulate to serrate
Marginal teeth (μm)	0–0.24(–2.23)	(0.71–)3.05–3.67(–5.44)	(1.38–)4.35–5.19(–9.31)
Leaf tubularity	Leaves tubular in upper ~20% of leaf, with opposite margins of leaf touching or overlapping	Leaves tubular in upper ~4% of leaf, with opposite margins of leaf touching	Leaves flat throughout. Terminal ends of these leaves can sometimes show signs of tubularity, but opposite margins of the leaf will not be touching or overlapping
Shape of leaf tip	Obtuse with inrolled margins	Obtuse with inrolled margins	Obtuse to somewhat acute and flat
Terminal branch leaves			
Leaf length (mm)	(1.45–)2.65–3.05(–5.14)	(2.04–)3.11–4.02(–7)	(1.70–)2.95–3.61(–6.73)
Leaf width (mm)	(0.11–)0.26–0.31(–0.62)	(0.19–)0.34–0.39(–0.49)	(0.09–)0.18–0.20(–0.33)
Leaf length/width	(4.99–)9.72–11(–18.18)	(5.31–)8.83–11.44(–18.69)	(8.62–)16.19–19(–34.57)
Leaf margin	Leaf margins entire, but sometimes having a few widely spaced teeth	Leaf margins serrulate to serrate with teeth typically smaller than those seen in *S. trinitense*	Leaf margins strongly serrate
Marginal teeth (μm)	(0–)1.86–2.26(–6.63)	(3.21–)5.11–5.95(–8.12)	(3.30–)7.24–9.02(–19.27)
Leaf tubularity	Leaves tubular in upper ~10% of leaf, with opposite margins of leaf almost touching to overlapping	Leaves mostly flat throughout. However, leaves are often tubular at the very tip	Leaves flat throughout. Terminal ends of these leaves can sometimes show signs of tubularity, but opposite margins of the leaf will never be touching or overlapping
Shape of leaf tip	Mostly obtuse, but sometimes acute, with slightly inrolled to fully inrolled margins	Obtuse and ranging from flat to fully inrolled margins	Mostly acute, but sometimes obtuse and flat. Margins can sometimes be slightly inrolled

Table 3. Continued

	S. cuspidatum	*S. mississippiense*	*S. trinitense*
Spreading branches			
Branch length (mm)	(7–)12–13(–22)	(5–)9–11(–19)	(8–)12–13(–21)
Stem cortical cells			
Level of differentiation	Two layers of enlarged thin-walled cells	Outer layer of cells typically thin-walled, but not enlarged or differentiated	Cells undifferentiated or with one layer of slightly enlarged thin-walled cells

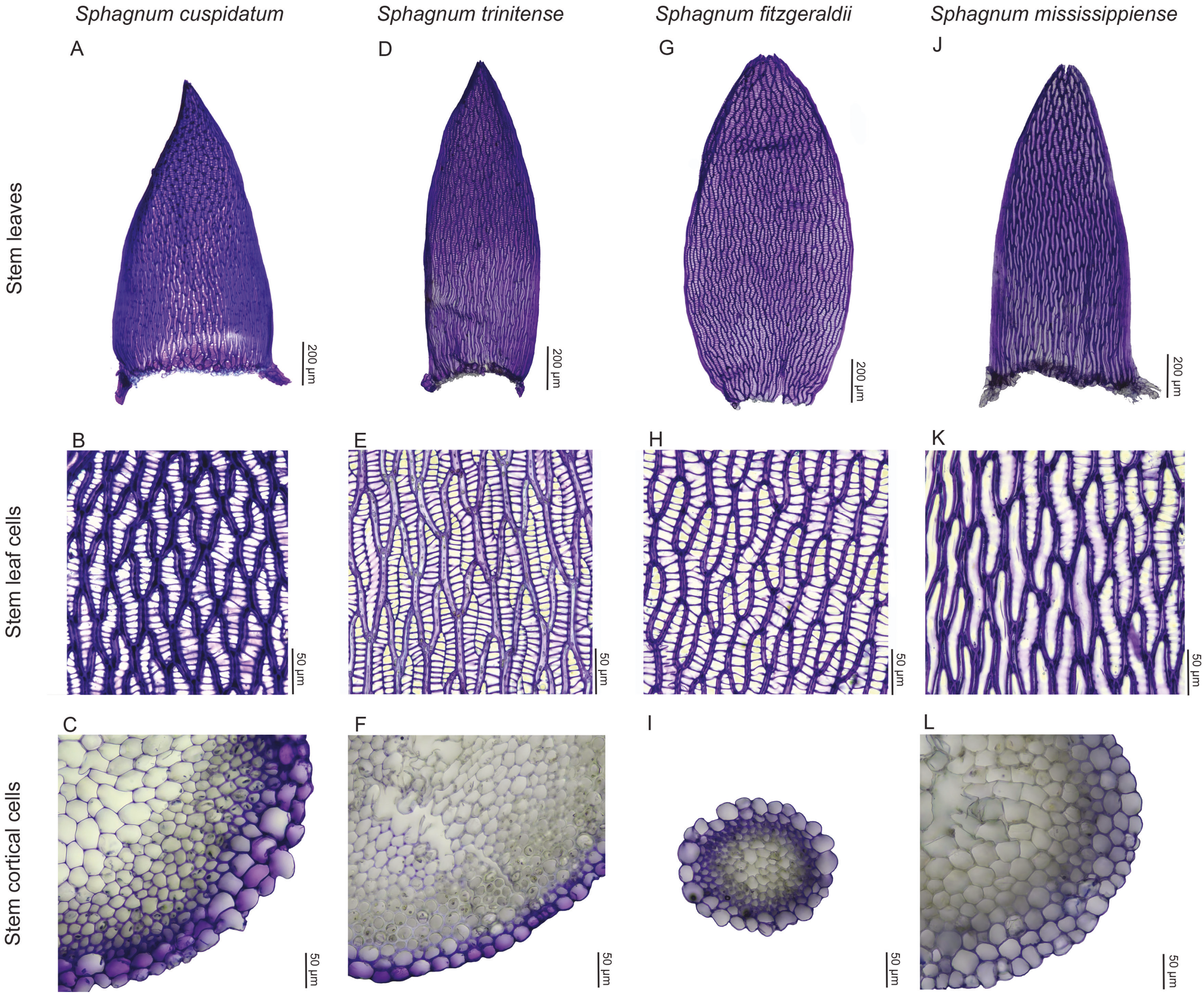

Figure 9. Comparison of stem leaves, stem leaf cells, and stem cortical cells in (A–C) *Sphagnum cuspidatum*, SB5972 (KMM13, Duke Forest, NC); (D–F) *S. trinitense*, SB6043 (KMM28, Jessups Pond, NC); (G–I) *S. fitzgeraldii*, SB6002 (KMM57, Green Swamp, NC); and (I–L) *S. mississippiense*, SB6121 (SR1400, Lucedale, MS).

it shares with *S. fitzgeraldii*. While these inferences should be treated with caution, it is clear that *S. mississippiense* warrants additional study. Furthermore, *S. mississippiense* probably has a broader distribution than current evidence suggests.

Taxonomic merit of leaf shape and marginal teeth

The primary reason why the taxonomic status of *S. trinitense* and *S. mississippiense* have been questioned is because the value of the characters used to define them has been questioned. Species

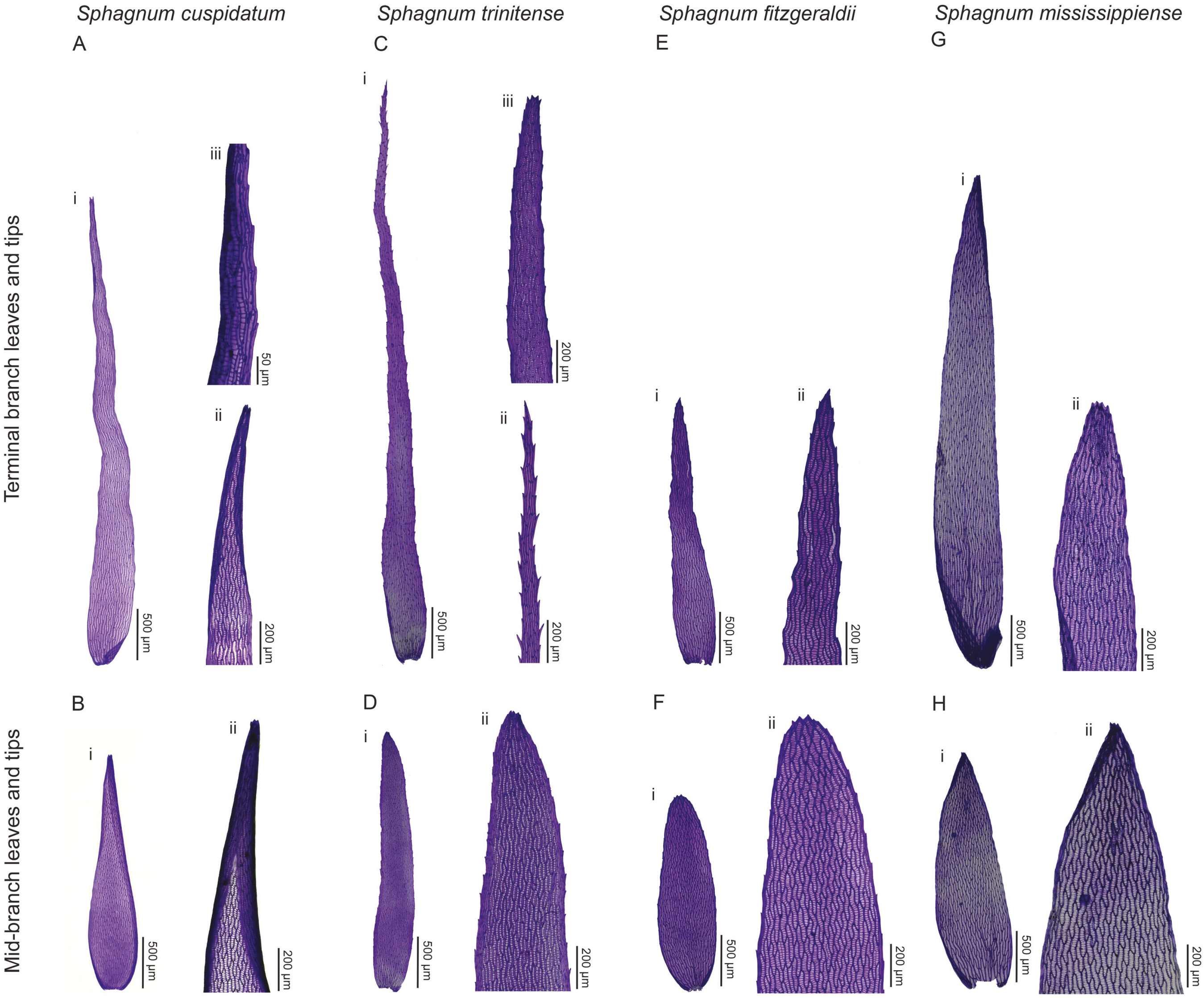

Figure 10. Comparison of leaves and leaf apices from the terminal end of spreading branches and the middle part of spreading branches in (Ai/Aii and Bi/Bii) *Sphagnum cuspidatum*, SB5972 (KMM13, Duke Forest, NC); (Ci/Cii and Di/Dii) *S. trinitense*, SB6043 (KMM28, Jessups Pond, NC); (Ei/Eii and Fi/Fii) *S. fitzgeraldii*, SB6002 (KMM57, Green Swamp, NC); and (Gi/Gii and Hi/Hii) *S. mississippiense*, SB6121 (SR1400, Lucedale, MS). Variation in terminal branch leaf apices seen in (Aiii) *S. cuspidatum*, SB6036 (KMM74, Green Swamp, NC) and (Biii) *S. trinitense* (DUKE005531; Buck31010, Sanderson, FL) are shown.

Table 4. MANOVA of quantitative characters used in morphometric analyses comparing *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense*. Values with statistically significant differences ($\alpha < .05$) are indicated with an asterisk.

	d.f.	**Pillai**	**Approx. *F***	**num d.f.**	**den d.f**	***Pr* (>*F*)**
Species	2	1.8761	54.373	44	159	<2.2e-16 *
Residuals	99					

in the *S. cuspidatum* complex are aquatic and it has been hypothesized that variation in leaf shape and size, and marginal teeth prominence are plastic responses to water and/or nutrient availability (Agnew 1958, Anderson *et al.* 1992, 2009). The seasonal instability of aquatic habitats presents a unique set of physical and physiological challenges (Glime and Vitt 1984). These challenges often result in high variability in morphological characters that can be attributed to either genetically based differences or phenotypic plasticity (Hedenäs 1996, 2008, Wells and Pigliucci 2000, Dorken and Barrett 2004, Spitale and Petraglia 2010, Li *et al.* 2019). This can be an issue, particularly in bryophytes, given that morphological definitions of species are typically based on a small number of microscopic characters that are susceptible to plasticity (Cronberg 1989, Baker and Boatman 1992, Såstad and Flatberg 1993, 1994, Stenøien *et al.* 1997, 2014, Vanderpoorten and Jacquemart 2004, Oke *et al.* 2020).

While our study did not include experiments specifically designed to evaluate levels of plasticity in morphological characters, our results provide evidence supporting the taxonomic merit of some of the characters used to distinguish species within the *S. cuspidatum* complex. Using a mixed stands approach focused on differences in nutrient-richness

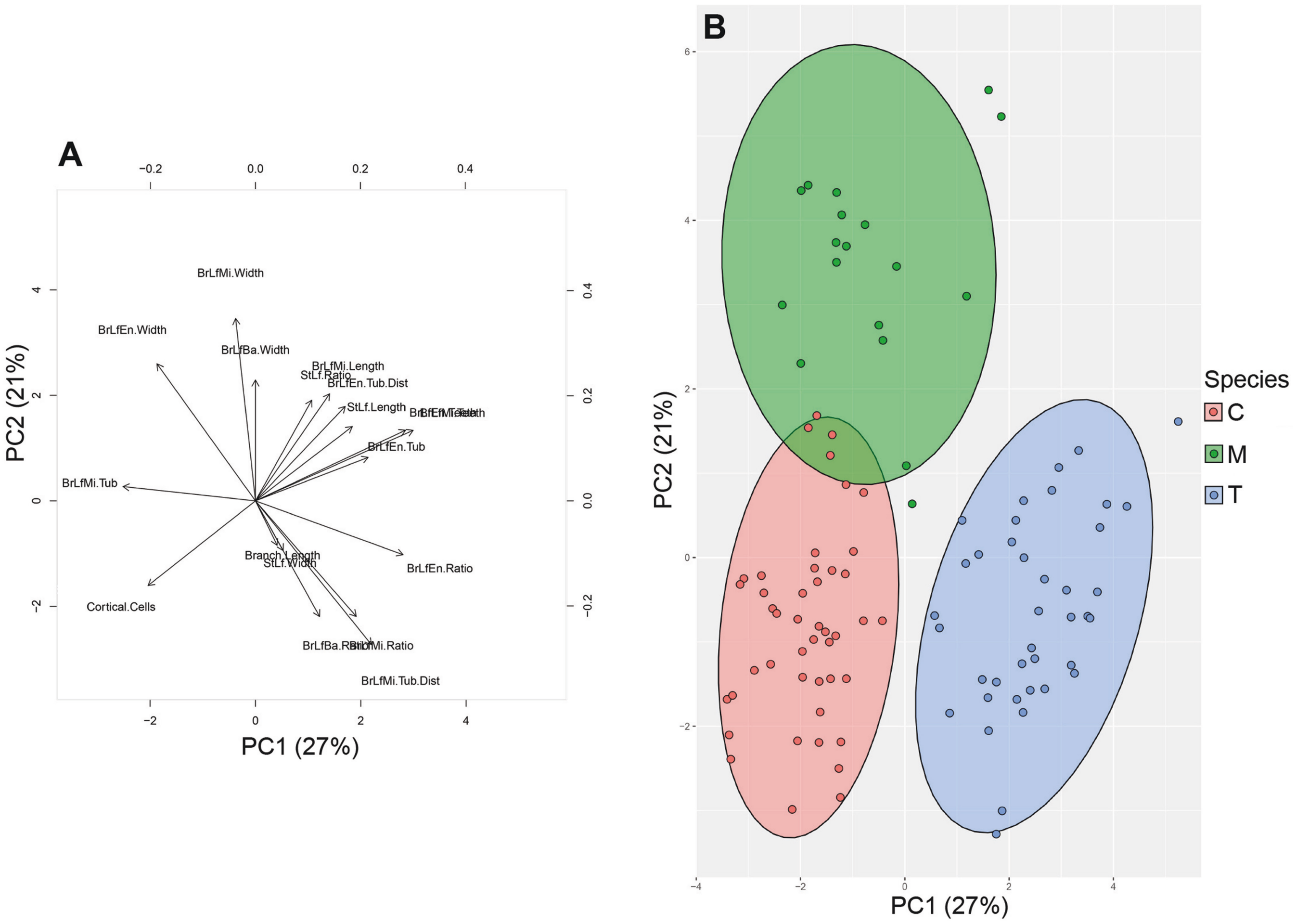

Figure 11. Results of principal components analyses of variation in the 18 statistically significant quantitative characters in *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense*. A, relationships of individual morphological characters to the first two principal components. B, plots of samples in relation to the first two principal components.

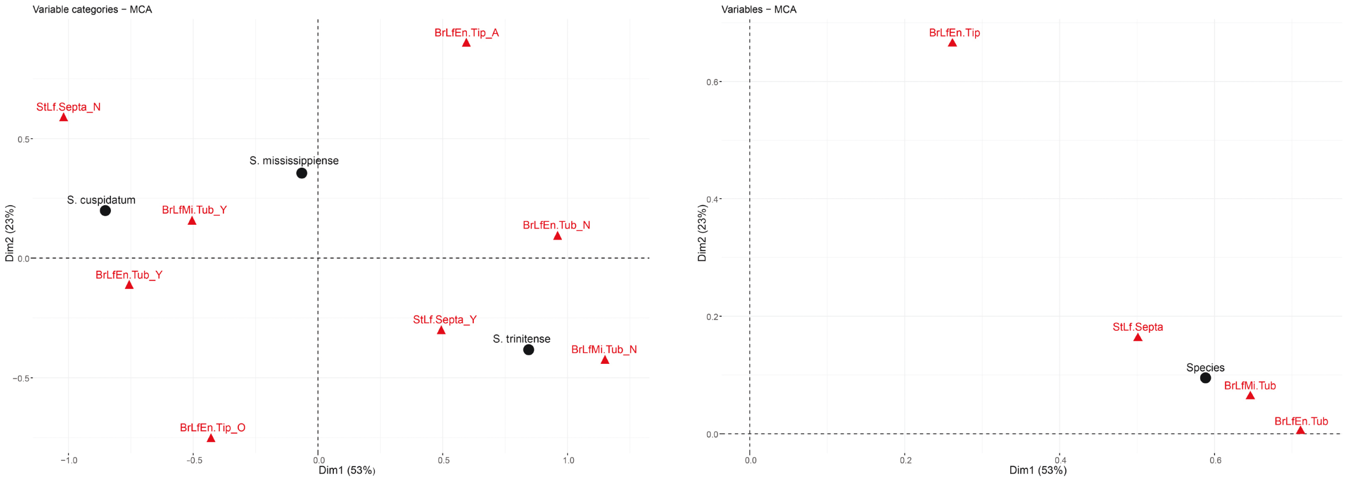

Figure 12. Results of multiple correspondence analysis using the four statistically significant qualitative characters in *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense*. Characters used include tubularity of terminal branch leaves (BrLfEn.Tub; Y = tubular, N = not tubular), tubularity of leaves from middle of branches (BrLfMi.Tub; Y = tubular, N = not tubular), estimated number of septate stem leaf hyaline cells (StLf.Septa; N = not septate, Y = septate), and shape of terminal branch leaf apices (BrLfEn.Tip; A = acute, O = obtuse). A, plot of individual morphological character categories (red triangles) and species categories (black circles) in relation to the first two dimensions. B, plot of morphological characters (red triangles) and species (black circle) in relation to the first two dimensions showing the correlation between each of the morphological characters examined and species as a category.

Table 5. MANOVAs of quantitative characters used in morphological comparisons of emergent and submergent plants in *Sphagnum cuspidatum*, *S. mississippiense*, and *S. trinitense*. Values with statistically significant differences ($\alpha < 0.05$) are indicated with an asterisk.

	d.f.	Pillai	Approx. *F*	num d.f.	den d.f.	*Pr* (>*F*)
Sphagnum cuspidatum						
Habitat	1	0.99473	17.982	21	2	0.04395*
Residuals	22					
Sphagnum mississippiense						
Habitat	1	0.99568	49.417	14	3	0.004096*
Residuals	16					
Sphagnum trinitense						
Habitat	1	0.63994	1.5996	20	18	0.1605
Residuals	37					

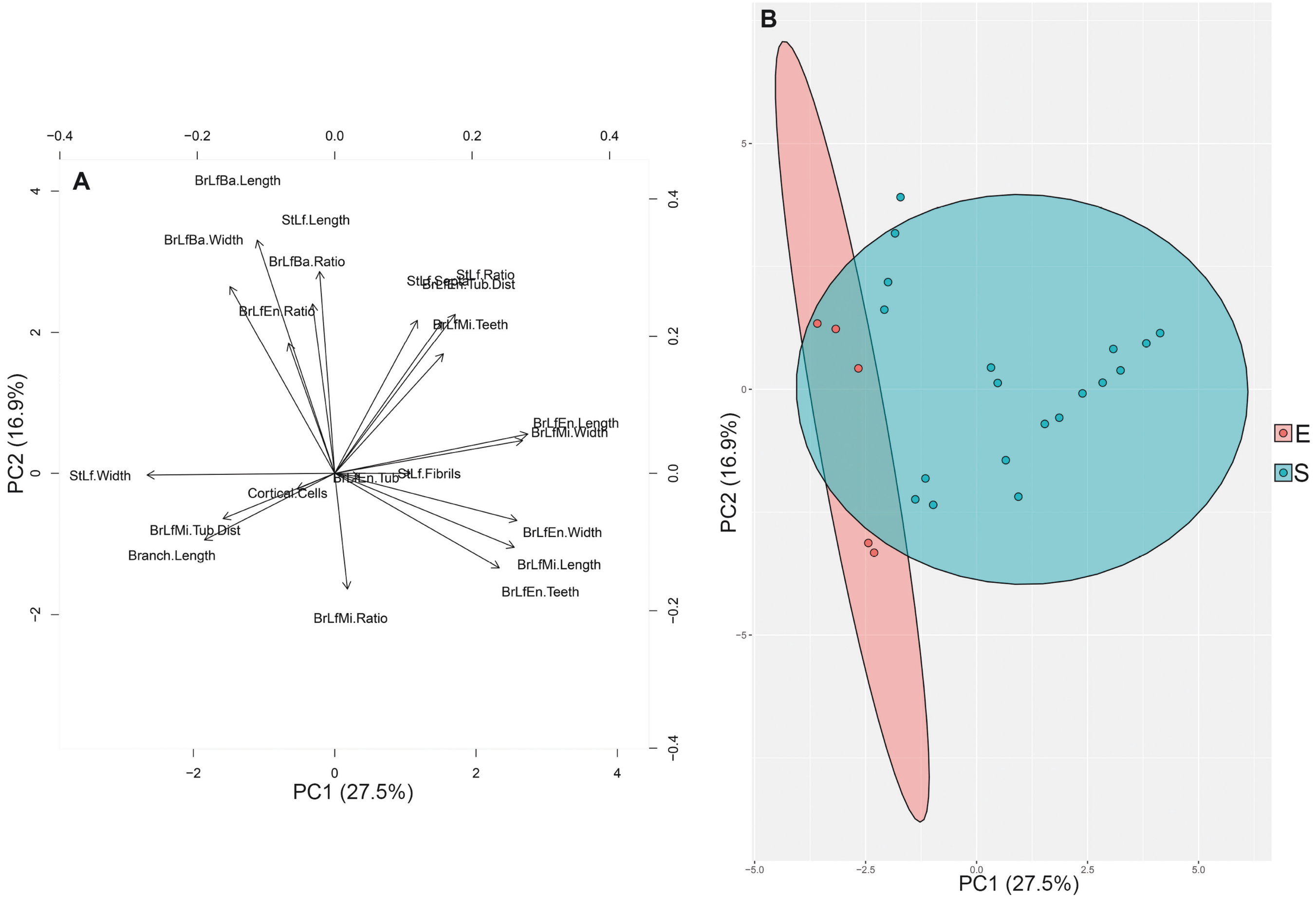

Figure 13. Results of principal components analysis of variation in quantitative characters in emergent and submergent plants of *Sphagnum cuspidatum*. A, relationships of individual morphological characters to the first two principal components. B, plots of samples in relation to the first two principal components.

and water-level conditions between sites, Såstad and Flatberg (1994) found that while breadth and length of branch leaves varied too much in response to wetness to be considered reliable taxonomic characters, breadth/length of branch leaves are useful in the *S. recurvum* complex. Similarly, our results indicate that branch leaf length is an unreliable character, but length/width of the leaves is more reliable, particularly for separating *S. mississippiense* from *S. cuspidatum* and *S. trinitense*. Within species, differences in branch leaf length/width were not significant between submerged and emergent plants, further supporting this as a stable character.

In addition to length/width of mid-branch leaves, our study found the degree of tubularity in mid-branch leaf apices to be an informative character in the *S. cuspidatum* complex. Såstad and Flatberg (1993) found more involute branch leaves to be associated with warmer/drier sites in *S. strictum* (subgen. *Rigida*)

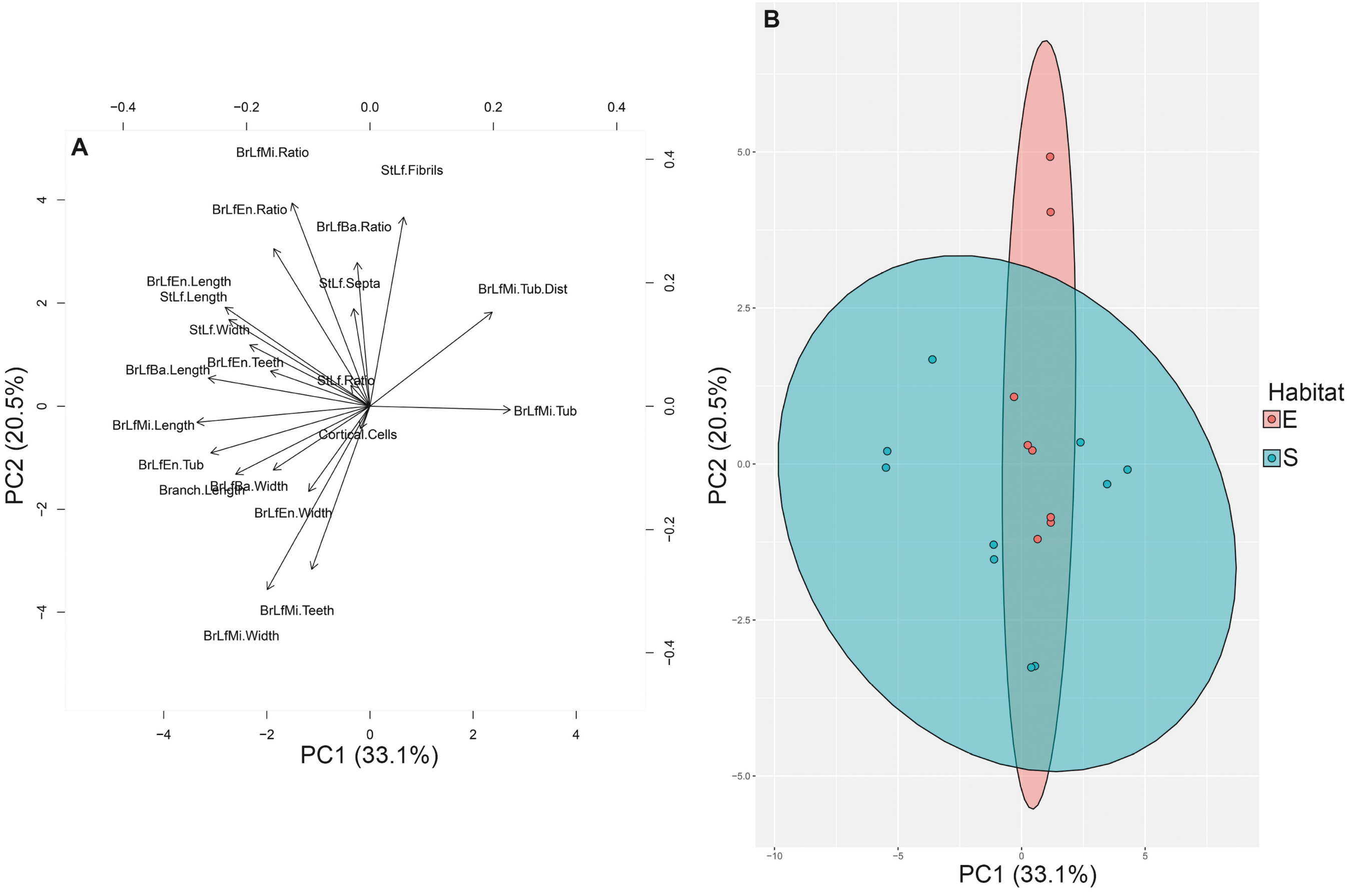

Figure 14. Results of principal components analysis of variation in quantitative characters in emergent and submergent plants of *Sphagnum mississippiense*. A, relationships of individual morphological characters to the first two principal components. B, plots of samples in relation to the first two principal components.

suggesting that this may be an adaptive strategy for retaining water. Our results showed that leaf tubularity is significantly different between species, and there was no difference within species between submergent and emergent plants. This suggests that degree and extent of branch leaf tubularity is a stable character, at least in the *S. cuspidatum* complex.

Leaf serration is strongly correlated with leaf tubularity. Agnew (1958) found that when submerged plants of *S. trinitense* were grown in dry conditions they produced leaves with fewer and smaller teeth, suggesting that leaf serration is a plastic character. Similarly, in a common garden experiment performed by Anderson *et al.* (1992), plants of both *S. cuspidatum* and *S. trinitense* produced 'well-developed' teeth on branch leaf margins, making them indistinguishable. Anderson *et al.* (1992) also stated that they were unable to find a correlation between leaf serration and leaf shape. However, the taxonomic status of the plants used in their experiment were not confirmed genetically, and the authors commented that the *S. cuspidatum* plants used had characters that fit the description of *S. trinitense* in Andrus (1980). Anderson *et al.* (1992) did not quantify variation in the leaf characters they examined, but they found that the leaf serrations in the cultured *S. trinitense* plants were 'more prominent' than those seen in the *S. cuspidatum* plants. Our results show that the size and occurrence of marginal teeth, particularly in leaves from the middle of spreading branches, are reliable characters. It is noteworthy that our phylogenetic analyses indicate that the three 'toothed' taxa form a clade, suggesting that leaf serration is not only genetically based but was conserved through several speciation events. Furthermore, at one of the sites in this study, Jessups Pond, where plants of *S. cuspidatum* and *S. trinitense* occurred sympatrically, no teeth were found on branch leaves of the *S. cuspidatum* plants. Across all plants from all sites included in this study, teeth occurred on branch leaves in a few *S. cuspidatum* plants, but these teeth were not abundant and were significantly smaller than those in *S. trinitense*.

In our comparisons of emergent and submergent plants, *S. cuspidatum* showed the greatest level of plasticity, with significant differences for seven of the 20 characters, as opposed to three in *S. mississippiense* and none in *S. trinitense*. In a study evaluating branch and leaf morphology in aquatic *Sphagnum*, which included *S. trinitense*, Rice and Schuepp (1995) documented a plastic response in leaf characters when plants were grown in submerged vs. emerged conditions. However, regardless of the variation recorded within species, a significant difference between species remained. In addition, Rice and Schuepp (1995) found these differences to be stable in a common garden experiment, and therefore were probably genetically based. Given

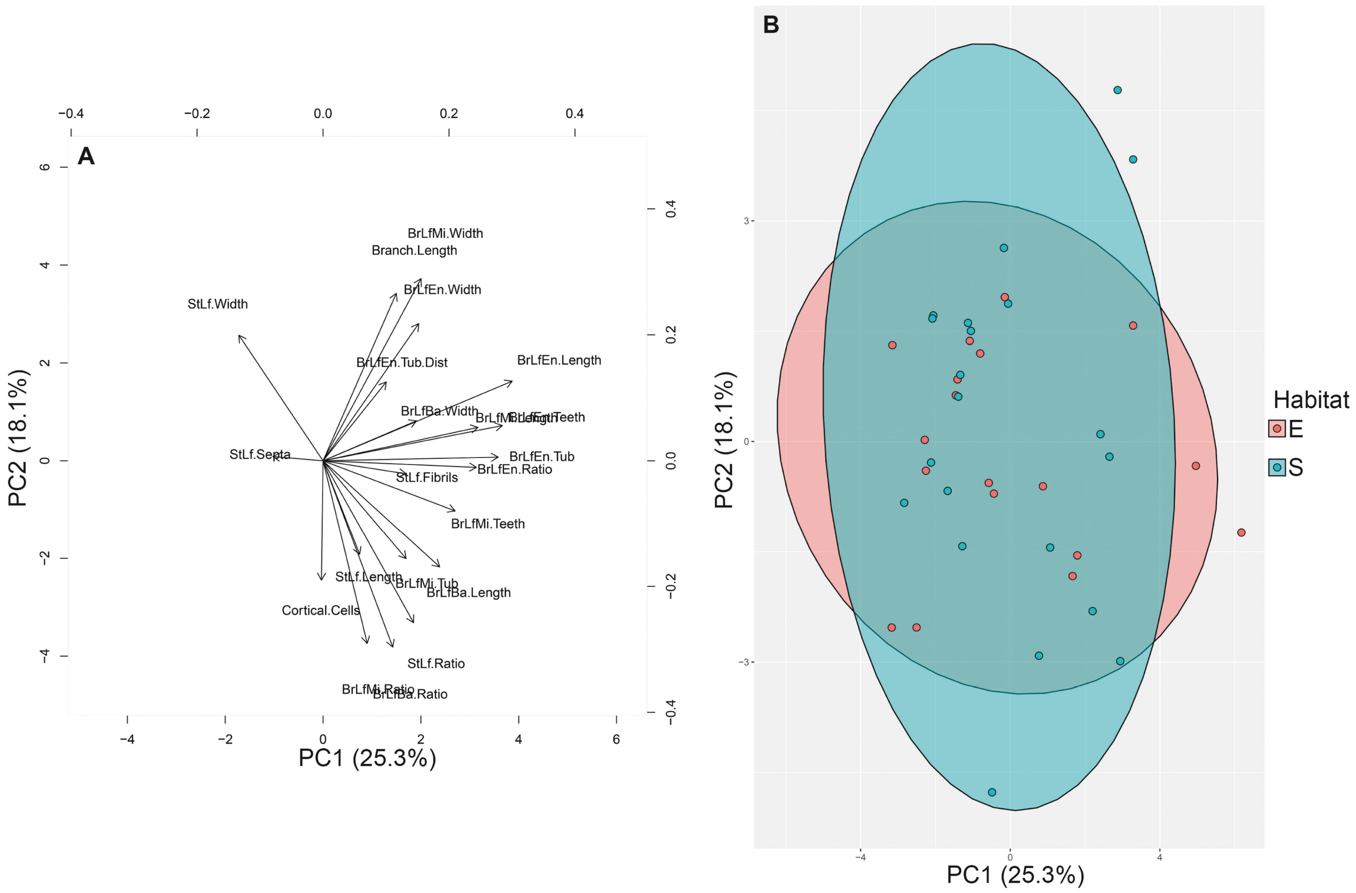

Figure 15. Results of principal components analysis of variation in quantitative characters in emergent and submergent plants of *Sphagnum trinitense*. A, relationships of individual morphological characters to the first two principal components. B, plots of samples in relation to the first two principal components.

the results of our study, the intraspecific variability observed in leaf shape and serration in *S. cuspidatum*, *S. mississippiense*, and *S. trinitense* appears to reflect a combination of intrinsic genetic variability and phenotypic plasticity.

The case of *S. viride*

The case of *S. viride* and its distinction from *S. cuspidatum* is similar to the case of *S. fallax* H.Klinngr. vs. *S. brevifolium* Lindb. ex Braithw. and *S. isoviitae* Flatberg, *S. torreyanum* vs. *S. atlanticum* R.E.Andrus, and *S. majus* subsp. *majus* vs. subsp. *norvegicum* (Flatberg 1988, Såstad and Flatberg 1993, Såstad *et al.* 1999, Såstad, 1999, Andrus 2007, Shaw *et al.* 2009, Duffy *et al.* 2020, Nieto-Lugilde *et al.* 2022). In each case, morphological differences made it possible to separate forms, but later genetic analyses did not support them as phylogenetically distinct taxa (Såstad *et al.* 1999, Shaw *et al.* 2009, Duffy *et al.* 2020, Nieto-Lugilde *et al.* 2022). Nieto-Lugilde *et al.* (2022) did find significant differences in branch leaf characters in *S. majus* subsp. *majus* vs. subsp. *norvegicum* (which were not differentiated by molecular data) and suggested that there may be a genotype × environment interaction causing variability in trait expression across different environments.

In our morphological comparisons of *S. viride* and *S. cuspidatum*, we were careful to only include plants from sites where these two taxa were found growing sympatrically in an effort to reduce the possible effects of habitat and temporal variability. Our results indicate that the two taxa differ morphologically, with differences similar to those described by Flatberg (1988). In particular, plants identified as *S. viride* have wider branch leaves, resulting in a reduced length/width, shorter branch leaf hyaline cells, and a reduced branch leaf hyaline cell length/width, less tubularity in the apical region of branch leaves, and a greater number of pores along commissures of hyaline cells on the concave surface of branch leaves. Nonetheless, our molecular data indicate that *S. viride* does not represent a phylogenetically distinct taxon. This is in line with Hanssen *et al.* (2000), who found that *S. viride* and *S. cuspidatum* did not differ in isozyme patterns. While only three plants of *S. viride* were included in our molecular analyses, we argue that the robust genetic analyses used should have revealed some level of differentiation between *S. viride* and *S. cuspidatum* if it existed. In addition, the three plants of *S. viride* were not sister to one another within the *S. cuspidatum* clade.

Similar to the findings of Nieto-Lugilde *et al.* (2022), while plants of *S. viride* and *S. cuspidatum* were not resolved as distinct on the basis of molecular data, that these plants were growing sympatrically provides evidence that the morphological differences between them may be due to a genotype × environment interaction rather than simply plasticity. Given this, the

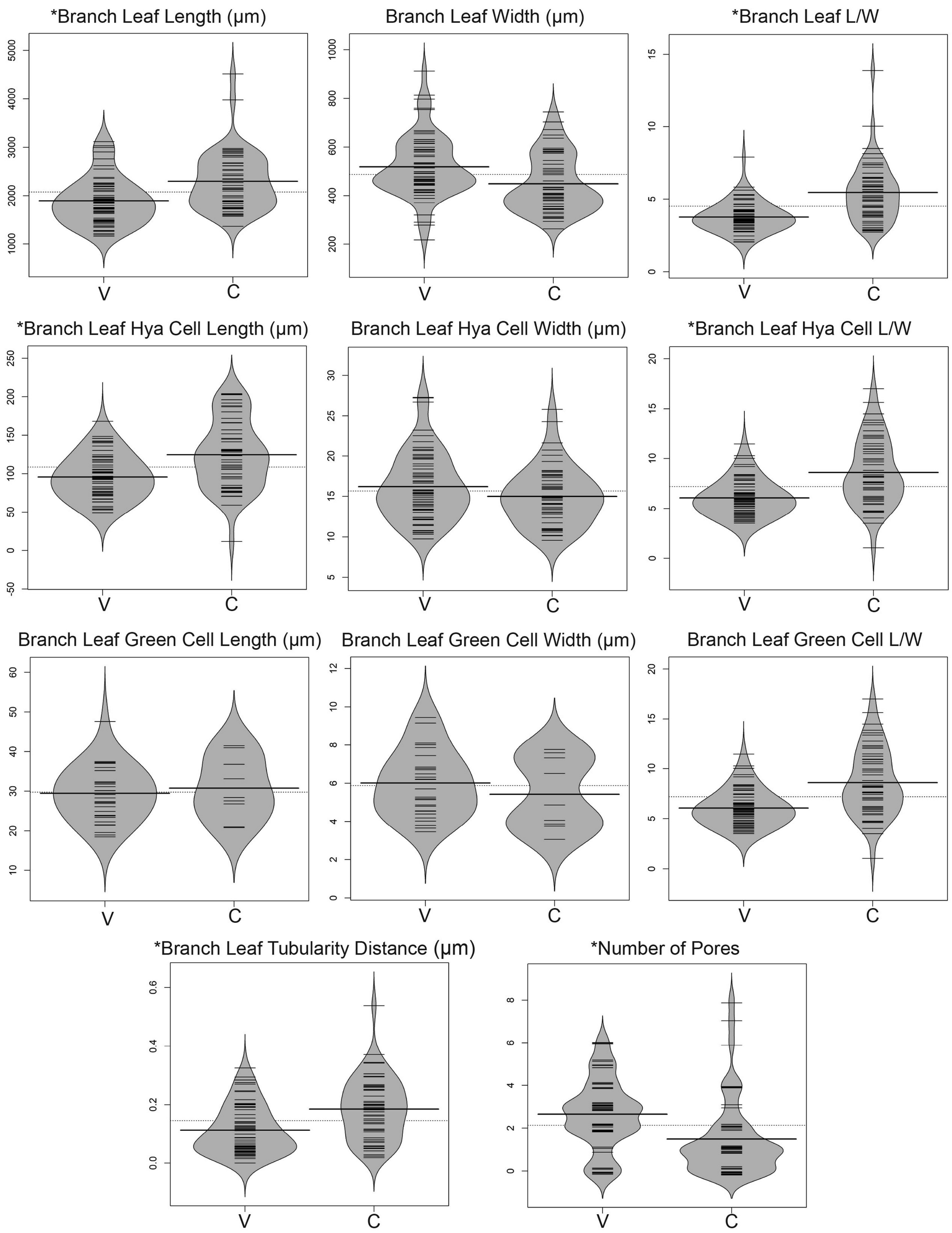

Figure 16. Beanplots of 11 quantitative characters examined in a comparison of plants identified as *S. cuspidatum* (labelled as C) and *S. viride* (labelled as V). Individual observations are represented by short horizontal lines and longer, bold horizontal lines represent mean values for each taxon. The dotted line in each plot shows the overall mean value for all observations made across taxa. The shape of each plot is a density trace representing the estimated density of the data distribution (see Kampstra 2008). Characters for which there were statistically significant differences between taxa ($P < .05$) based on ANOVAs are highlighted with an asterisk. A plot for leaf curvature was not included due to a lack of variability in the data.

Table 6. Descriptions of morphological characters compared between specimens of *Sphagnum cuspidatum* and *S. viride* plants. Bold text designates characters which were found to be the most diagnostic.

	S. cuspidatum	*S. viride*
Mid-branch leaves		
Leaf length (mm)	(1.36–)2.13–2.47(–4.51)	(1.16–)1.77–2(–3.12)
Leaf width (mm)	(0.26–)0.42–0.48(–0.74)	(0.22–)0.49–0.55(–0.91)
Leaf length/width	(2.73–)4.88–6.04(–13.89)	(2.05–)3.52–4.02(–7.9)
Leaf curvature (µm)	(0–)43.61–102.38(–571.42)	(0–)4.92–26.48(–260)
Hyaline cell length (µm)	(115.21–)115.58–142.32(–277)	(49.02–)88.46–102.92(–168.21)
Hyaline cell width (µm)	(6–)13.61–15.71(–25.81)	(9.74–)15.16–17.25(–27.28)
Hyaline cell length/width	(1.05–)8.09–10.74(–30.12)	(3.52–)5.61–6.50(–11.46)
Green cell length (µm)	(20.83–)24.77–36.74(–41.48)	(18.46–)26.94–31.95(–47.58)
Green cell width (µm)	(3.05–)3.99–6.86(–7.77)	(3.45–)5.37–6.66(–9.44)
Green cell length/width	(4.12–)4.96–6.92(–8.13)	(2.76–)4.56–5.96(–10.74)
Number of pores	(0–)1–2(–6)	(0–)2.25–3.06(–6)
Leaf tubularity	Leaves tubular in upper ~20% of leaf, with opposite margins of leaf touching or overlapping	Leaves tubular in upper ~10% of leaf, with opposite margins of leaf touching or overlapping

Table 7. MANOVA of quantitative characters used in morphometric analyses comparing *Sphagnum cuspidatum* and *S. viride*. Values with statistically significant differences ($\alpha < 0.05$) are indicated with an asterisk.

	d.f.	Pillai	Approx. *F*	num d.f.	den d.f.	*Pr* (>*F*)
Species	1	0.56455	2.4849	12	23	0.02931*
Residuals	34					

high genetic variability found in *S. cuspidatum*, and evidence of greater plasticity in comparisons made between emergent and submergent plants, *S. cuspidatum* appears to be an excellent candidate for a future study examining genotype × environment interactions.

Key to species in the *S. cuspidatum* complex

Below is a key that reflects morphological differences between species in the *S. cuspidatum* complex that were resolved in our phylogenetic analyses of RADseq data. While the separation of *S. viride* from *S. cuspidatum* was not supported by genetics, we provide an additional key based in microscopic characters to separate these 'morphs' in an effort to encourage future work investigating the basis for the differences seen in the morphology of these plants. Flatberg (1988) provided additional information about differences between plants growing in dry vs. wet conditions.

Key to species in the *Sphagnum cuspidatum* complex

1. Branch leaves serrate to serrulate 2
1. Branch leaves entire.................................... *Sphagnum cuspidatum*
2. Plants small with a prominent terminal bud; stem leaves ovate to oblong; branch leaves ovate to ovate–lanceolate with a broad, rounded apex.................................... *Sphagnum fitzgeraldii*
2. Plants larger, lacking a prominent terminal bud; stem leaves triangular to triangular–ovate; branch leaves ovate–lanceolate to lanceolate with a pointed apex.................................... 3
3. Stem leaves acute to apiculate with little to no septate hyaline cells; leaves from middle of spreading branches tubular with inrolled margins in upper 15–20% of leaf; marginal teeth few and ≤2 µm in length; outer stem cortex differentiated with two layers of enlarged, thin-walled cells.................................... *Sphagnum cuspidatum*
3. Stem leaves acute to obtuse with some to several one-septate hyaline cells; leaves from middle of spreading branches not tubular or tubular with inrolled margins restricted to the very tip; marginal teeth many and ≥3 µm in length; outer stem cortex not differentiated or differentiation with one layer of enlarged cells 4
4. Branch leaves narrow, ovate–lanceolate to lanceolate; leaves from terminal end of spreading branches with length/width >12:1, not tubular, margins serrate with the majority of teeth ≥7 µm in length; leaves from middle of spreading branches with length/width >5:1, not tubular, margins serrate with the majority of teeth ≥4 µm in length; spreading branches long.................................... *Sphagnum trinitense*
4. Branch leaves broader, ovate to ovate–lanceolate; leaves from terminal end of spreading branches with length/width ≤12:1, ranging from flat to tubular at apex, margins serrulate with the majority of teeth ≤6 µm in length; leaves from middle of spreading branches with length/width ≤5:1, tubular with inrolled margins at apex, margins serrulate with the majority of teeth ≤4 µm in length; spreading branches short.................................... *Sphagnum mississippiense*

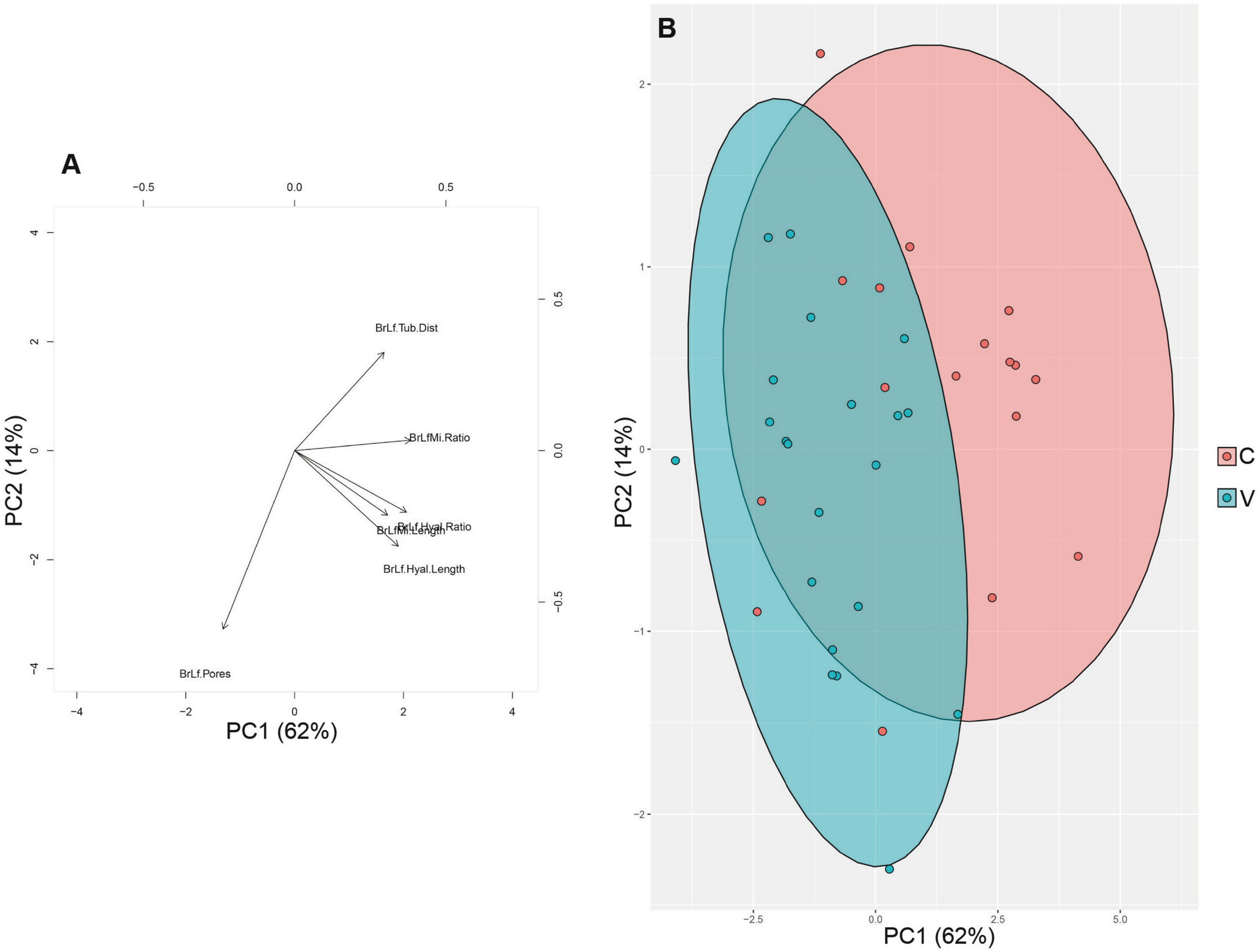

Figure 17. Results of principal components analysis of variation in the six statistically significant quantitative characters in *Sphagnum cuspidatum* and *S. viride*. A, relationships of individual morphological characters to the first two principal components. B, plots of samples in relation to the first two principal components.

Key to cuspidatum and viride morphs of *S. cuspidatum*

1. Leaves from middle part of spreading branches more narrow with a length/width >4:1, a more extensive tubular region at apex extending down ~20% of leaf, longer hyaline cells in the upper portion of leaves with a length/width >8:1, and zero to two commissural pores along hyaline cells on concave surface .... cuspidatum morph
2. Leaves from middle part of spreading branches less narrow with a length/width <4:1, a less extensive tubular region at apex extending down ~10% of leaf, shorter hyaline cells in the upper portion of leaves with a length/width <8:1, and more than two commissural pores along hyaline cells on concave surface .... viride morph

SUPPLEMENTARY DATA

Supplementary data are available at *Biological Journal of the Linnean Society* Journal online.

ACKNOWLEDGEMENTS

This research was supported by the U.S. National Science Foundation Grant DEB-1928514 to AJS.

AUTHOR CONTRIBUTIONS

Sean C. Robinson (contributed to study design, was lead writer of manuscript, conducted field collections of plants used in study, produced and analysed morphometric data); Marta Nieto-Lugilde (contributed to study design, wrote sections of manuscript, conducted field collections of plants used in study, produced and analysed genetic data); Aaron M. Duffy (contributed to study design, assisted with writing of manuscript, assisted with production and analysis of genetic data); Katherine Martinez Munoz (contributed to study design, conducted field collections of plants used in study); Blanka Aguero (contributed to study design, conducted field collections of plants used in study, provided assistance with herbarium collections used in study); Amelia Merced (conducted field collections of plants used in study); Kristian Hassel (conducted field collections of plants used in study, provided assistance with herbarium collections used in study, provided input regarding morphometric analysis of plants); Kjell Ivar Flatberg (conducted field collections of plants used in study, provided assistance with herbarium collections used in study, provided input regarding morphometric analysis of plants); and A. Jonathan Shaw (contributed to study design, assisted with writing of manuscript, conducted field collections of plants used in study). All authors reviewed the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

DATA ACCESSIBILITY AND BENEFIT-SHARING STATEMENT

The sequences, phylogenetic alignments, and STRUCTURE file analysed in this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.crjdfn39t.

REFERENCES

Agnew S. A study in the experimental taxonomy of some British Sphagna (Sect. *Cuspidata*) with observations on their ecology. *D. Phil. Thesis*, University of Wales, 1958.

Allen B. *Maine Mosses: Sphagnaceae–Timmiaceae. Memoirs of the New York Botanical Garden 93*. New York: The New York Botanical Garden Press, 2005.

Anderson LE, Basile MR, Basile DV. Common garden experiments with *Sphagnum* in axenic culture. *Journal of Bryology* 1992;**17**:15–25.

Anderson LE, Shaw AJ, Shaw B. *Peat Mosses of the Southeastern United States*. New York: The New York Botanical Garden Press, 2009.

Andrus RE. Sphagnaceae (peat moss family) of New York State, Bulletin No. 441. In: Mitchell RS (ed.), *Contributions to the Flora of New York State III*. Albany, NY: New York State Museum, 1980.

Andrus RE. *Sphagnum mississippiense* sp. nov. (Sphagnaceae) and related species on the southeastern United States coastal plain. *Memoirs of the New York Botanical Garden* 1987;**45**:237–41.

Andrus RE. *Sphagnum atlanticum*, a new species from eastern North America. *The Bryologist* 2007;**110**:274–8.

Baker RGE, Boatman DJ. Some effects of nitrogen, phosphorus, potassium and carbon dioxide concentration on the morphology and vegetative reproduction of *Sphagnum cuspidatum* Ehrh. *New Phytologist* 1990;**116**:605–11.

Baker RGE, Boatman DJ. The effect of nitrogen, phosphorous and carbon dioxide on cell development in branch leaves of *Sphagnum*. *Journal of Bryology* 1992;**17**:35–46.

Bengtsson F, Rydin H, Baltzer JL *et al.* Environmental drivers of *Sphagnum* growth in peatlands across the Holarctic region. *Journal of Ecology* 2020;**109**:417–31.

Bengtsson J. Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. *Applied Soil Ecology* 1998;**10**:191–9.

Blomquist HL. Mosses of North Carolina, I. Sphagnales. *The Bryologist* 1937;**40**:67–71.

Blomquist HL. Peatmosses of the southeastern states. *The Bryologist* 1938;**54**:1–17.

Cronberg N. Patterns of variation in morphological characters and isoenzymes in populations of *Sphagnum capillifolium* (Ehrh.) Hedw. and *S. rubellum* Wils. from two bogs in southern Sweden. *Journal of Bryology* 1989;**15**:683–96.

Crum H. A seasoned view of the North American Sphagna. *Journal of the Hattori Botanical Laboratory* 1997;**82**:77–98.

Crum H, Anderson LE. *Mosses of Eastern North America*, Vol. **2**. New York: Columbia University Press, 1981.

Dorken ME, Barrett SCH. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology* 2004;**92**:32–44.

Duffy AM, Aguero B, Stenøien HK *et al.* Phylogenetic structure in the *Sphagnum recurvum* complex (Bryophyta) in relation to taxonomy and geography. *American Journal of Botany* 2020;**107**:1283–95.

Earl DA, vonHoldt BM. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 2012;**4**:359–61.

Eaton DAR, Overcast I. ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics* 2020;**36**:2592–4.

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology* 2005;**14**:2611–20.

Flatberg KI. *Sphagnum viridum* sp. nov., and its relation to *S. cuspidatum*. *Kongelige Norske Videnskabers Selskab Skrifter* 1988;**1**:1–64.

Gaston KJ. Species richness: measure and measurement. In: Gaston KJ (ed.), *Biodiversity: A Biology of Numbers and Difference*. Oxford: Blackwell, 1996, 77–113.

Glime JM, Vitt DH. The physiological adaptations of aquatic Musci. *Lindbergia* 1984;**10**:41–52.

Hassel K, Kyrkjeeide MO, Yousefi N *et al. Sphagnum divinum* (sp. nov.) and *S. medium* Limpr. and their relationship to *S. magellanicum* Brid. *Journal of Bryology* 2018;**40**:197–222.

Hanssen L, Såstad SM, Flatberg KI. Population structure and taxonomy of *Sphagnum cuspidatum* and *S. viride*. *The Bryologist* 2000;**103**:93–103.

Hedenäs L. On the interdependence of some leaf characters within the *Drepanocladus aduncus–polycarpus* complex. *Journal of Bryology* 1996;**19**:311–24.

Hedenäs L. Molecular variation in *Drepanocladus aduncus* s. l. does not support recognition of more than one species in Europe. *Journal of Bryology* 2008;**30**:108–20.

Hoang DT, Chernomor O, von Haeseler A *et al.* UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 2018;**35**:518–22.

Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 2006;**23**:254–67.

Jassey VEJ, Signarbieux C. Effects of climate warming on *Sphagnum* photosynthesis in peatlands depend on peat moisture and species-specific anatomical traits. *Global Change Biology* 2019;**25**:3859–70.

Johnson MG, Granath G, Tahvanainen T *et al.* Evolution of niche preference in *Sphagnum* peat mosses. *Evolution* 2014;**69**:90–103.

Kalyaanamoorthy S, Minh BQ, Wong TKF *et al.* ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 2017;**14**:587–9.

Kampstra P. Beanplot: a boxplot alternative for visual comparison of distributions. *Journal of Statistical Software, Code Snippets* 2008;**28**:1–9.

Karlin EF, Boles SB, Seppelt RD *et al.* The peat moss *Sphagnum cuspidatum* in Australia: Microsatellites provide a global perspective. *Systematic Botany* 2011;**36**:22–32.

Koutecký P. MorphoTools: a set of R functions for morphometric analysis. *Plant Systematics and Evolution* 2015;**301**:1115–21.

Lane DM. Variation in certain of *Sphagnum* from the Atlantic coastal plain. *Journal of the Hattori Botanical Laboratory* 1981;**49**:169–245.

Li G, Shiqi H, Hou H *et al.* Heterophylly: phenotypic plasticity of leaf shape in aquatic and amphibious plants. *Plants* 2019;**8**:420–32.

Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009;**25**:1754–60.

Li Y, Glime JM, Liao C. Responses of two interacting *Sphagnum* species to water level. *Journal of Bryology* 1992;**17**:59–70.

McQueen CB, Andrus RE. Sphagnaceae. In: Flora of North America Editorial Committee (eds.), *Flora of North America North of Mexico*, Vol. **27**. New York: Oxford University Press, 2007, 45–111.

Minh BQ, Nguyen MAT, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 2013;**30**:1188–95.

Minh BQ, Schmidt HA, Chernomor O *et al.* IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 2020;**37**:1530–4.

Naeem S, Thompson LJ, Lawler SP *et al.* Declining biodiversity can alter the performance of ecosystems. *Nature* 1994;**368**:734–7.

Naeem S, Duffy JE, Zavaleta E. The functions of biological diversity in an age of extinction. *Science* 2012;**336**:1401–6.

Natcheva R, Cronberg N. Maternal transmission of cytoplasmic DNA in interspecific hybrids of peat mosses, *Sphagnum* (Bryophyta). *Journal of Evolutionary Biology* 2007;**20**:1613–6.

Nieto-Lugilde M, Robinson S, Aguero B *et al*. Morphological–molecular incongruence in *S. majus* ssp. *majus* and ssp. *norvegicum*. *The Bryologist* 2022;**125**:294–310.

Oke TA, Turetsky MR, Weston DJ *et al*. Tradeoffs between phenotypic plasticity and local adaptation influence the ecophysiology of the moss, *Sphagnum magellanicum*. *Physiological Ecology* 2020;**193**:867–77.

Parchman TL, Gompert Z, Mudge J *et al*. Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology* 2012;**21**:2991–3005.

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;**155**:945–59.

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, 2022.

Ramasamy RK, Ramasamy S, Bindroo BB *et al*. STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. *SpringerPlus* 2014;**3**:431.

Rastogi A, Antala M, Gąbka M *et al*. Impact of warming and reduced precipitation on themorphology and chlorophyll concentration in peat mosses (*Sphagnum angustifolium* and *S. fallax*). *Scientific Reports* 2020;**10**:8592.

Rice SK, Schuepp PH. On the ecological and evolutionary significance of branch and leaf morphology in aquatic *Sphagnum* (Sphagnaceae). *American Journal of Botany* 1995;**82**:833–46.

Rydin H, Jeglum JK. *The Biology of Peatlands*, 2nd edn. New York: Oxford University Press, 2013.

Såstad SM, Flatberg KI. Leaf morphology of *Sphagnum strictum* in Norway, related to habitat characteristics. *Lindbergia* 1993;**18**:71–7.

Såstad SM, Flatberg KI. Leaf size and shape in the *Sphagnum recurvum* complex: taxonomic significance and habitat variation. *Journal of Bryology* 1994;**18**:261–75.

Såstad SM, Pedersen B, Digre K. Habitat-specific genetic effects on growth rate and morphology across pH and water-level gradients within a population of the moss *Sphagnum angustifolium* (Sphagnaceae). *American Journal of Botany* 1999;**86**:1687–98.

Såstad SM. 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* 1999;**77**: 1–10.

Shaw AJ. A new approach to the experimental propagation of bryophytes. *Taxon* 1986;**35**:671–5.

Shaw B, Terracciano S, Shaw AJ. A genetic analysis of two recently described peat moss species, *Sphagnum atlanticum* and *S. bergianum* (Sphagnaceae). *Systematic Botany* 2009;**34**:6–12.

Shaw AJ, Piatkowski B, Duffy AM *et al*. Phylogenomic structure and speciation in an emerging model: The *Sphagnum magellanicum* complex (Bryophyta). *New Phytologist* 2022;**236**:1497–511.

Shaw AJ, Nieto-Lugilde M, Aguero B *et al*. *Sphagnum diabolicum* n. sp. and *S. magniae* sp. nov.; morphological variation and taxonomy of the '*S. magellanicum* complex'. *The Bryologist* 2023;**126**:69–89.

Spitale D, Petraglia A. *Palustriella falcata* (Brid.) Hedenäs (Amblystegiaceae, Bryopsida) with pluristratose lamina: morphological variability of specimens in springs of the Italian Alps. *Plant Systematic Evolution* 2010;**286**:59–68.

Stenøien HK, Bakken S, Flatberg KI. Phenotypic variation in the *Sphagnum recurvum* complex: a cultivation experiment. *Journal of Bryology* 1997;**19**:731–50.

Stenøien HK, Hassel K, Segreto R *et al*. High morphological diversity in remote island populations of the peat moss *Sphagnum palustre*: glacial refugium, adaptive radiation or just plasticity? *The Bryologist* 2014;**117**:95–109.

Tilman D, Wedin D, Knops J. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 1996;**379**:718–20.

Vanderpoorten A, Jacquemart A-L. Evolutionary mode, tempo, and phylogenetic association of continuous morphological traits in the aquatic moss genus *Amblystegium*. *Journal of Evolutionary Biology* 2004;**17**:279–87.

Wells CL, Pigliucci M. Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics* 2000;**3**:1–18.

Zachos FE. (New) Species concepts, species delimitation and the inherent limitations of taxonomy. *Journal of Genetics* 2018;**97**:811–5.